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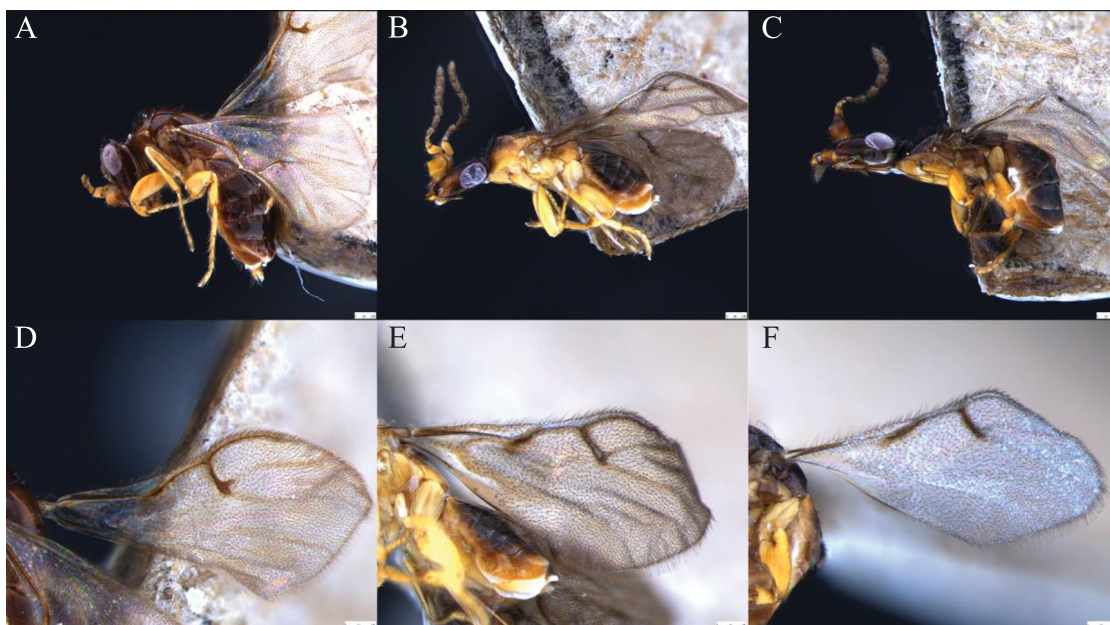


Fig. 1. Habitus, lateral of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Fore wing of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*. Scale bars represent 250 μ m
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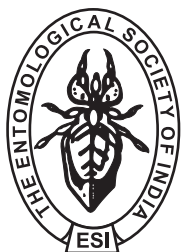
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(Founded 1938)

The Entomological Society of India (ESI) was founded in 1938 as a registered society under the Societies Registration Act 1957 as extended to the Union territory of Delhi under Registration No. S. 2434 of 1963-64 dt. 12.3.1964. It is registered with NITI Aayog under unique ID of VO/NGO-DL.2016/0104219. It is one of the largest professional societies in India serving entomologists and researchers in related disciplines.

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

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Articles submitted for publication must adhere to the Journal's current format and style and the Instructions to authors given in the website of the Society i.e., www.entosocindia.org or in OJMS website indianentomology.org. Each article will require Article Processing Charge (APC) to be made by online payment gateway at entosocindia.org or by NEFT transaction in favour of "Entomological Society of India". NEFT details are given below. The processing fee is non-refundable. It is obligatory that each article is to be submitted online at the online submission/ review platform i.e., indianentomology.org. A PAYNOW link is provided in the website under membership for facilitating online payments which can be used. Article processing charges details are given in website indianentomology.org or entosocindia.org.

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

Bionotes: This publication is privately issued by late Dr R K Varshney from Aligarh. Its online version is hosted by the Society in the website.

Memoirs: Whenever suitable material and finances are available, the Society issues special numbers in the form of Memoirs. Sixteen such Memoirs on different topics have been published so far.

The Bulletin of Entomology: This publication, which was privately issued from erstwhile Madras, had been taken over by the Society from 1967. The Bulletin is an occasional, irregular publication, containing papers on bionomics, taxonomy, morphology etc. Subscription for the Bulletin of Entomology is Rs. 1000/- annually. Any entomologist who wishes to publish lengthy manuscripts can use this.

Indian Entomologist: It is a biannual online magazine published by the Entomological Society of India

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

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EDITORIAL

The objective of the Entomological Society of India, as envisaged in my last editorial is to make entomology purposeful and sensitive to the needs of science and society. This objective is served through the Indian Journal of Entomology which is in its 86th Volume. This Journal is the medium which helps us serving entomology and make it impactful. In the last few years this impact has been making us see insects on a wider canvas and with a different outlook to benefit science and humanity. Yes, I reiterate “Entomology provides multiple opportunities for researchers and scientists in many domains”. This is evident from the multitude of contributors to the Journal- Indian Journal of Entomology, in the recent times, as could be seen from the vast countries from which contributions have been emanating recently. The Journal could be seen providing a valuable and potential platform for this wide diversity of contributors and in all possible frontier areas of entomology with a global outlook. Many intriguing entomological questions are answered in the deliberations of the Journal, and naturally these learnings have emanated from wide variety of sources- some as beginners, and many as excellent practitioners of entomology globally. The Journal serves as mouthpiece of many young and fresh practitioners of entomology, especially those are in the brink of becoming post graduates and doctorates in entomology. These researchers are from many educational institutions of India and abroad, especially from the Indian subcontinent. Huge chunk of papers published in the Journal are emanating from these researchers. These are on the fresh, topical and upcoming entomology aspects as being dealt with by the MSc and PhD students of entomology for their thesis work.

One undisputable purpose of post graduate research, especially entomology is to enable beginners grow as critical thinkers and research frontier areas of entomology both basic and applied. The departments dealing with entomology and crop protection in India, are particularly with the state agricultural universities to a major extent. In many of these outreaches to support the stakeholders especially the farmers are the main thrusts. Hence the objectives of entomology research solely aim towards crop-oriented problems, many a times repetitive and redundant, as there are multiple players reconnoitring around similar problems. A broad analysis of the contributions and their nature reveals many captivating aspects, especially how entomology is practiced towards approaching its frontier areas that require to be explored on priority. Many of these researches as published in the Indian Journal of Entomology, emanating from the postgraduate researchers indicate that there is redundancy in perceiving the major researchable issues. Superfluous approach and repetitive nature are quite common and rampant. There are also discrepancies as to how to deliberate upon these for research in a logical and rational sense as is being followed by the well acclaimable researchers in entomology globally. Therefore, there exists a need to promote and empower these beginners with critical thinking and offset the drawbacks. It is necessary that we bring such researchers together for an objective review of what is going on, what is the current trend, how the researchable are brought to the fore? is there any objectivity exercise underlying these? and how much efforts are getting into it! The Entomological Society of India addressed this through organizing an Entomology Students' Conclave, and brought together hundreds of such researchers on a common platform. An objective review of the contributions and outputs indicates that there is a need to look into how entomology is practiced by the young researchers. There exists lot of gaps and flaws in the thought process of the entire research aspect, especially if one has an urge to thwart the redundancies in the logistics and outputs of research. The spectrum of papers published in the Journal also explicit these, and reveal the reality that many times the objectives are redundant, run of the mill, lack forethoughts and does not promote critical thinking. Critical thinking is the hallmark of objectivity, and it is essential to frame benchmark and touchstones for entomology research towards this. These benchmarks must strive to ameliorate the ills of redundancy. Let us pledge to challenge such redundancy in entomology research and place entomology research in the desired direction.



EFFECT OF REARING SEASON ON QUEEN AND THE REPRODUCTION OF *APIS MELLIFERA CARNICA* AND *A. MELLIFERA LIGUSTICA*

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ABSTRACT

Hybrids of *Apis mellifera carnica* and *Apis mellifera ligustica* queen bees were reared to investigate their physiological and reproductive characters as well as spermatheca semen volume to determine the suitable time for queen rearing under Giza conditions in Egypt. Rearing seasons were found to affect the queens' quality determined based on their weight/ volume of the spermatheca, and quantity and quality of stored sperms in the spermatheca. Results indicated that the Carniolian hybrid gynes were heavier than Italian hybrid ones, with greatest mating success being observed in March and April, while it was worst during June. Spermatheca was less voluminous with less spermatozoa in Italian hybrid queen bees compared to the Carniolian hybrid ones. Thus, under Giza region conditions, it is highly recommended to rear queens during the spring months.

Key words: *Apis mellifera carnica*, *A. mellifera ligustica*, queen, reproductive characters, spermatheca, sperm quantity, spermatozoa

The queen's ability to reproduce is essential to the colony's survival, and thus the queen is the most significant individual within the colony. Analyzing the queens' reproductive potential can reveal the fitness of the colony. A new queen bee is raised either in emergency scenarios (to replace deceased or missing queens) or in superseding (to replace ageing, damaged, or diseased queens) and swarm situations. Queens produced from old larvae are of poor quality in terms of morphology and sperm count, and as a result, they mate with considerably fewer males (Amiri et al., 2017). The start of oviposition, queen acceptance ratio, quantity of spermatozoa in the spermatheca, spermatheca diameter, and laying rate are all significantly influenced by the weight of the queen (Wei et al., 2019). More honey is produced when colonies are regularly re-queened with young ones under a year old compared to older ones. Queen rearing is thus a crucial step in beekeeping (Yi et al., 2020). Requeening colonies led by queen bees whose bees exhibit high disease susceptibility or robust defensive behaviour is a common practice among beekeepers (Chuda-Mickiewicz and Samborski, 2015). Hybrid of *Apis mellifera carnica*, the most spread of the bee races in Giza region, may continue to exist in the area. This might be leading to queens mating with low number of drone and colonies that are unsuitable for commercial apiculture (Elenany and Abdallah, 2016). Commercial beekeepers have to be critical of

the development of queens, and existence of only one queen in a colony is crucial (Adham, 2000; Ahmet and Hakan, 2013). The colony's economic traits depend largely on the caliber of queen (Harris, 2009), and this in turn, depends on the genetic/ environmental factors, the breeding conditions and queen rearing methods (Zedan, 2002; Taha, 2005; John et al., 2011).

The mating of *A. mellifera* queen is influenced by environmental factors (Cengiz et al., 2019), presence of worker brood (Stankus, 2008), age of associate nurse workers (Delaney et al., 2011), drone raising cycle (Rangel et al., 2013) and the number of queens being raised (Koywiwattrakul and Sittipraneed, 2009). Beekeepers either breed their own queens or purchase from others (Wei et al., 2019), and hence many do not know the features of the queens. Adapting the region's hot and dry is an additional problem (Amiri et al., 2017). Local honey bee genotypes can be improved for queen rearing and impending breeding initiatives. The quality of the queen, which is defined by the number of ovarioles in ovaries and the size of spermatheca, is a significant factor in the strength and production of the colony. Producing one's own queen under the right circumstances take long time and also queens thus produced in a year may have varied qualities (Sakla and El-shafeiy, 2022). This study investigates the physiological and reproductive traits of the hybrid

queens of *Apis mellifera carnica* and *A. m. ligustica*. The aim is to find out the traits that could be employed in breeding and selection programmes, as well as to choose the ideal period for producing high-quality queens.

MATERIALS AND METHODS

The study was conducted in the apiary of Faculty of Agriculture, Cairo University during 2020 and 2021. Twenty honey bee colonies (10 each of Carniolian and Italian hybrids) were used and the queens were open mated ensuring that the colonies were equal in strength and exposed to the routine work. The technique used for rearing of the queens in seasons (late winter, spring and summer) is based on the Doolittle larval grafting method. The grafted larvae were <24hr, as taken from the stock colony. Mated queens were removed from the rearings, and made queenless builder colonies. The introduced larval queen cells to the queenless builder colonies were grafted from the same genetic origin of rearing colonies in bee wax cups fixed on wooden bars hanging in the rearing frame. Each frame contained 45 grafted queen cells (Moretto et al., 2004).

The following parameters were recorded for each reared frame for each hybrid: weight (mg) of newly emerged virgin queens (<24 hr) /month/ hybrid (Akyol et al., 2008); number of ovarioles/ queen/ month/ hybrid- both ovaries were delicately extracted after queens were dissected in insect saline. The ovarioles were spread out on a microscope slide and counted; For measuring volume of spermatheca, the queens were placed in the freezer for 4-6 min (- 20°C is sufficient) or until immobilized; euthanize by removing her head and pinning body in a dissecting tray to extract the spermatheca; cut both sides of abdomen using forceps, grasp the stinger and gradually draw it out until the ovaries are visible; carefully remove the spermatheca and lay it on a plate by using a binocular; then the radius was measured ($V = 4/3 \pi r^3$). For count of sperm in mated queen's spermatheca (million), lay spermatheca on a plate with 0.1 ml brine diluent and rupture it with forceps to release sperm; fill a glass petriplate with 0.9 ml water, and using a clean glass pipette mix well around 40 times until everything is combined; and finally the haemocytometer count chamber was used to count the sperm, under 250x magnification- start counts on the gridded section after the sperm have settled (~20 sec), 4 squares at haemocytometer grid each square has 16 squares; and thus sperms were counted from 64 squares and the resulting number is multiplied by 10000 and divided by 0.4 (Woyke, 1979). Measurement of total protein in virgin bee queen's (< 24 hr) haemolymph

was done with Foss Tecator 2100. One-way ANOVA/ independent samples t-tests were used to determine significant differences ($p < 0.05$) using the SPSS 25.00. These differences included samples taken from the inside or entrance of the beehives as well as samples related to the seasons.

RESULTS AND DISCUSSION

The results revealed significant differences in the mean weight of newly emerged virgin queens in two hybrids during the active season (Figs. 1, 2); *A. m. carnica* queens were significantly ($p \leq 0.05$) heavier (219.2 mg) than *A. m. ligustica* queens (179.2 mg), with maximum being during the summer season, and the least during the late winter (152.1 and 135.1 mg for *A. m. carnica* and *A. m. ligustica*, respectively). Figures 3 and 4 illustrates the difference between the number

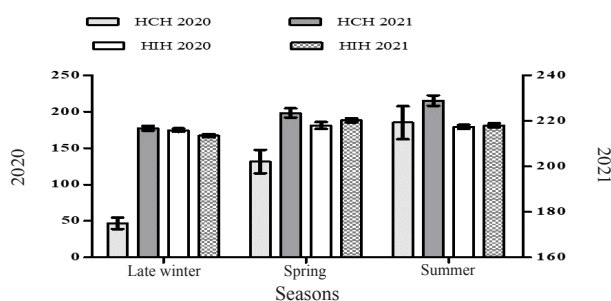


Fig. 1. Weight of heavy queen hybrids of *A. m. carnica* and *A. m. ligustica* (HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)

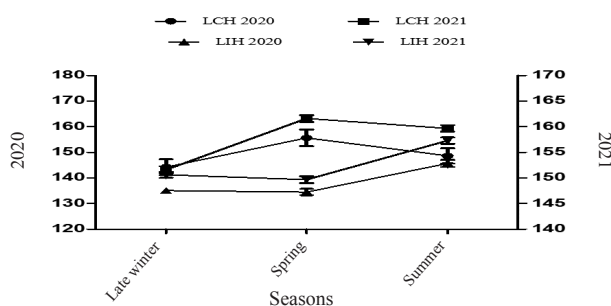


Fig. 2. Weight of light queen hybrids (LCH: Light Carniolian hybrid; LIH: Light Italian hybrid)

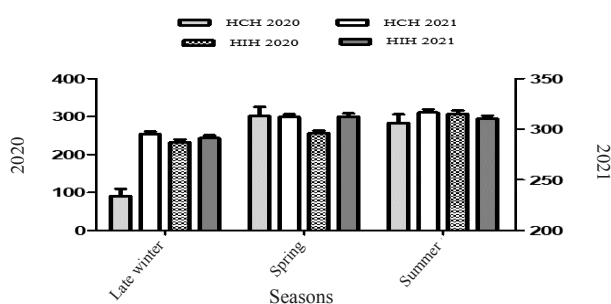


Fig. 3. Ovarioles in queens (HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)

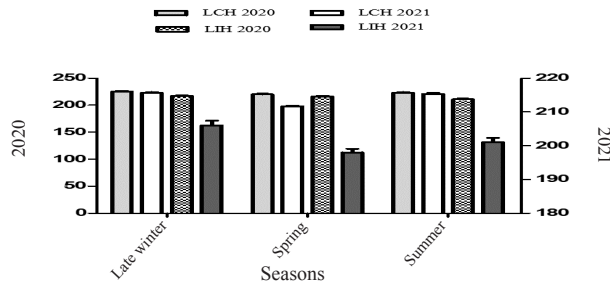


Fig. 4. Ovarioles (LCH: Light Carniolian hybrid; LIH: Light Italian hybrid)

of ovarioles- 313 ovarioles in HCH and 256 in HIH during the spring season; however during late winter, these decreased in both; mean number of ovarioles in hybrids varied significantly during 2020, but without significant differences in 2021. The least spermatheca volume was observed in late winter- 0.05 mm for both hybrids in heavy queens and 0.04 mm in light queens; maximum spermathecal volume was observed during summer season for carniolian hybrid, This was due to the abundance of nectar offering plants. There was no clear significant differences between hybrids for the sperm count in spermatheca; maximum count was during spring (6.2 million) for Italian hybrid, whereas the least was observed during summer (5.8 million) in heavy queens; while in the light queens, the Italian hybrid revealed maximum count in spring (4.6 million) (Table 1). Total protein in haemolymph was maximum

in summer for heavy queen of carniolian hybrid (9.4 KDa) while it was only (7.5 KDa) in the summer for Italian hybrid in light queen (Table 2). The *A. m. carnica* queens were heavier than those of *A. m. ligustica*, and spring-reared queens were noticeably heavier. Such seasonal weight variations across various queens may be due to the workers' effective foraging efforts and better environmental circumstances.

The weight of queens at emergence was reportedly influenced by the rearing season and the origin of the queen (Szabo et al.,1987; Güler et al.,1999). The recipient colonies tended to accept the Carniolian virgin queens more readily than the Italian virgin queens; this might be attributed to the Caniolian bees being more docile (Guzmán-Novova et al., 1998), the mother queen's genetic influence (Moretto et al., 2004), and the presence of elderly workers in the mating nuclei, which can be advantageous because they can prompt the queen to make earlier mating. Additionally, recipient colonies prefer queens with high body weights than those with low body weights (Szabo, 1977; Taranov, 1973). This may account for the increased acceptance rate during the spring, when pollen and nectar are more readily available. These findings are in line with those of Kaftanolu and Kumova (1992), who investigated the impact of queen bee rearing season on quality and discovered that acceptance rates varied from 81.7 to

Table 1. Spermatheca, sperm count in mated queens of *A. m. carnica* and *A. m. ligustica*

Bee hybrids / Seasons	2020				2021			
	HCH	LCH	HIH	LIH	HCH	LCH	HIH	LIH
Spermatheca								
Late winter	0.05± 0.02	0.05± 0.02	0.05± 0.02	0.04± 0.02	0.04± 0.02	0.04± 0.01	0.09± 0.02	0.05± 0.02
Spring	0.17± 0.04	0.09± 0.02	0.21± 0.05	0.08± 0.03	0.18± 0.04	0.12± 0.01	0.11± 0.02	0.10± 0.01
Summer	0.20± 0.05	0.11± 0.03	0.16± 0.04	0.06± 0.02	0.23± 0.06	0.13± 0.04	0.19± 0.02	0.07± 0.03
Sperm count								
Late winter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spring	6.10± 0.35	4.50± 0.15	6.30± 0.37	4.60± 0.16	5.10± 0.27	4.70± 0.14	5.10± 0.22	4.60± 0.15
Summer	6.20± 0.32	4.50± 0.13	5.80± 0.28	4.50± 0.18	5.80± 0.26	5.10± 0.11	5.20± 0.24	4.40± 0.10

(HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)(LCH: Light Carniolian hybrid; LIH: Light Italian hybrid).

Table 2. Total protein (KDa) in virgin bee queen's haemolymph *A. m. carnica* and *A. m. ligustica*

Bee hybrids / Seasons	2020				2021			
	HCH	LCH	HIH	LIH	HCH	LCH	HIH	LIH
Late winter	5.63± 0.41	5.63± 0.43	5.56± 0.41	6.00± 0.50	5.54± 0.42	4.38± 0.38	5.23± 0.41	5.11± 0.42
Spring	6.88± 0.52	5.94± 0.51	7.81± 0.62	6.13± 0.52	5.63± 0.50	4.69± 0.43	5.63± 0.44	5.63± 0.45
Summer	9.38± 0.87	6.56± 0.52	8.75± 0.74	7.50± 0.61	6.25± 0.56	4.81± 0.49	5.63± 0.45	5.31± 0.39

(HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)(LCH: Light Carniolian hybrid; LIH: Light Italian hybrid).

91.4% from April to July but were lower in August (60.0%) and September (58.3%). In contrast to the origin of the queens, the raising season certainly had an impact on mating success. For both honey bee hybrids, the highest meaningful percentages of mating success were observed between March and June. The lowest rates of successful mating occurred in July and August, despite 80% of queens being accepted. The air temperature and the availability of nectar and pollen throughout the spring months appeared to be the main environmental factors impacting the success of mating (Szabo et al., 1987). Most queen losses occur during mating flight (Ruttner, 1983 and Czekoska, 2000) due to bad weather, particularly wind (da Silva et al., 1995), predators, or the queens becoming lost (Hellmich et al., 1986). This explains why queen mortality is so high in the summer and in the late winter. Compared to *A. m. ligustica* queens, *A. m. carnica* queens had a substantially greater spermatheca volume (mm³). Our findings concur with those made public by (Kaftanolu and Kumova, 1992), who discovered that among the ecotypes in Turkey, Caucasian queens had the largest spermatheca. In comparison to other months of our study period, queens reared in May have noticeably larger spermatheca. After July, the worker bees destroyed the majority of the drones due to a lack of pollen and nectar. Due to this circumstance, the amount of sperm in the spermatheca may have fluctuated throughout the course of the year, resulting in variations in spermatheca size.

The amount of sperm in the spermatheca increased in *A. m. carnica* queens due to the bigger spermatheca size. The average sperm count of *A. m. carnica* queens was substantially higher than that of *A. m. ligustica* queens. For *A. m. carnica* and *A. m. ligustica*, the total number of sperm per queen peaked in May and fell to a minimum in September. Throughout June to September, there may have been a decrease in the amount of nectar and pollen flowing in from the field along with a rise in daily temperature. According to research in different fields (Woyke and Jasinski, 1973; Kaftanolu and Peng, 1982; Koeniger and Ruttner, 1989; Kaftanolu and Kumova, 1992), the average quantity of spermatozoa was discovered to be less than the quantities reaching the spermatheca.

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AUTHOR CONTRIBUTION STATEMENT

AG, AE and YE conceived the presented idea. YE and AG developed the theory and performed the computations. YE verified the analytical methods. YE and AE analyzed data. All authors discussed the results and contributed to the final manuscript. YE and AG carried out the experiments. YE and AG wrote the draft manuscript. All authors contributed to the final version, read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECTS OF POPULATION DENSITY ON SURVIVAL, DEVELOPMENT TIME, FECUNDITY AND HATCHABILITY OF *SAMIA CYNTHIA RICINI* BOISDUVAL

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ABSTRACT

Samia cynthia ricini Boisduval (Lepidoptera: Saturniidae) is a lepidopteran insect that has a gregarious behaviour during their larval stage. This research aimed to determine the minimum population limit of larvae that can support the survival of *S. c. ricini* by observing the development time, fecundity, and hatchability of eggs produced by adult moths under different larval densities rearing conditions. The research was conducted by observing the lifecycle and development of *S. c. ricini* at different population densities. The results showed that density had effect on the development time of *S. c. ricini* egg only. Population density also affects the survival and adult fecundity of *S. c. ricini*. A density of 30 is the minimum population required to support the success of *S. c. ricini*'s life. *Samia cynthia ricini* reared at this density had the highest survival rate, faster egg development time, heavier cocoon shell weight and highest fecundity.

Key words: *Samia cynthia ricini*, castor, crowded population, density, development time, eri silkworm, fecundity, gregarious, insect rearing, lifecycle

Population density is an essential factor in the life of organisms including insects. Population density provides an opportunity for intraspecies competition of space and resources, which directly affects survival rate, development time, dispersal, reproduction, behaviour, and even morphology. According to Riddick and Wu (2015), *Coleomegilla maculate* more survived when maintained at a density of 20 compared to other densities. Lazarević et al. (2004) reported that *Lymantria dispar* showed a longer development time at high density compared to low density. Yu et al. (2019) *Diabrotica virgifera virgifera* (LeConte) reported that female adult moth *Diabrotica virgifera virgifera* derived from larvae reared at high density had a further dispersal ability than moths derived from larvae reared at low density. The effect of density on morphology was reported in the migratory locust *Locusta migratoria* (Nijhout and Wheeler, 1982), aphid *Acyrtosiphon pisum* (Tsumuki et al., 1990), and *Anticarsia gemmatilis* (Fescemyer and Hammond, 1986).

Samia cynthia ricini Boisduval (Lepidoptera: Saturniidae) is economically valuable insect for humans (Hani and Das, 2019). The fifth instar caterpillar larva can produce silk fibres that envelop the pupa, which can be utilized as the main material of the textile industry, surgical threads, parachutes, and others. Silkworm fibres have a special feature that has not been replaced

by artificial one to date (Nuraeni and Putranto, 2008). Silkworms have long been cultivated by humans such as mulberry silkworm *Bombyx mori*. However, the *S. c. ricini* silkworm is a new type of silkworm cultivated in Indonesia (Trisnawati and Nurkomar, 2020). The main host plants are castor and cassava as their secondary host plant (Nangia et al., 2000). Although the resulting silk is very different from mulberry silk, the consumer interest in samia silk is quite high.

In our previous pilot study, high mortality was found in solitary-reared early instar larvae, but when *S. c. ricini* were reared at a density of 30, they could live successfully into adulthood (Nurkomar et al., 2022). We hypothesized that the larvae of *S. c. ricini* have a gregarious behaviour during their larval stage. However, there hasn't been information about the minimum larval population that can support the survival of *S. c. ricini*. Research on the effects of *S. c. ricini* population density has been carried out but is limited to the parameters of mortality rates, larval and the resulting cocoons weights. Meanwhile, how population density affects the development and reproductive ability of *S. c. ricini* has never been reported. These parameters are very important for the efficiency of mass rearing purposes. This study aimed to determine the minimum population limit of larvae that can support the survival of *S. c. ricini* by observing the development time, fecundity, and hatchability of eggs produced by adult moths

under various conditions of different larval population densities.

MATERIALS AND METHODS

The most effective diet for supporting the development of *S. c. ricini* was castor bean (*Ricinus communis*) (Devaiah et al., 1985). Leaves were collected from the neighbourhood around campus of Universitas Muhammadiyah Yogyakarta, Bantul, Yogyakarta, Indonesia. The leaves were then dried with sterile tissue paper before usage after being cleaned with tap water. Because each instar has a unique physical trait and feeding behaviour, the food was supplied in varying amounts, depending on the larvae's development stage. For the first and second instar larvae, the diet was administered once daily in the morning; for the third and fourth instar larvae, it was administered twice daily in the morning and afternoon; and for the fifth instar larvae, it was administered three times daily in the morning, afternoon, and evening.

The research was started by preparing eggs according to the population density treatments tested i.e., 1, 5, 10, 20, 30, 40 and 50 eggs. The density used was determined based on the preliminary studies that found that *S. c. ricini* could not be reared solitary. Thus, rearing experiments were carried out under various conditions of different larval population densities. This number was also determined based on the availability of larvae and materials used in rearing. Each treatment was replicated five times. Eggs were kept on a petri dish (86 x 13 mm) until hatching. When hatching, the larvae were transferred to the larval rearing container. To reduce the impact on the environment, identical-sized containers were used. The rearing container used for the first to fourth instar larvae was a tray with a transparent lid (length: 30 cm, width: 11.5 cm, height: 3.5 cm). The larvae were moved into an adult rearing cage when they reached the fifth instar (length: 37 cm, width: 30, height: 33 cm) to facilitate the pupation process. For the moth to emerge with perfect wings, the constructed cocoon was then strung up using thread in a sagittal plane position. Observations were made on the survival rate, the development time of each phase (egg, larva, pupa, and adult), larval body colour, cocoon shell weight, adult fecundity, and hatchability of the egg produced by the adult. The development time was observed daily from the egg until adult died. Observations of the larval body colour were made at each instar change. Cocoon shell was measured after the moth emerged. Adult fecundity was calculated out

by calculating the number of eggs laid by the adult daily until death. Hatchability of eggs was observed from 30 eggs taken randomly from each treatment.

Survival data were analysed with Kaplan Meier's Survival Analysis (May, 2009). The data of the development time, cocoon weight, fecundity, and hatchability of eggs were analysed using the Generalized Linear Model (GLM). The appropriate model used for the related data was determined by using Akaike Information Criteria (AIC) value. The difference in the average value of the data was further tested using Tukey HSD at the level of $\alpha = 5\%$ with Holm's adjustment (Hothorn et al., 2008). Statistical analysis was performed using R Statistics and data visualized using *ggplot* package (Wickham, 2009). Because no larvae grew in the treatment at densities 1, 5, and 10, the observational data were excluded from the analysis. Data analysis was performed using R Statistics version 4.0.2 (R Core Team, 2015).

RESULTS AND DISCUSSION

The results showed that in completing its life cycle, *S. c. ricini* had a gregarious behaviour during the larval stage. It is crucial to know the lowest population density while propagating *S. c. ricini* since it can be employed for mass propagation activities, especially for small-scale farmers and lab-scale research. The results showed that population density affected the survival rate of *S. c. ricini* ($P < 0.0001$, Fig. 1). The highest survival rate was observed in larvae reared at a density 30, with 92% survival rate. The survival rate decreased to 87.5% and 83.2% at a density of 40 and 50, respectively. Meanwhile, the lowest survival rate was observed at a density of 20, which only reached 78%. When the larvae reached the fifth instar, the survival rate generally started to decline. Larvae reared at densities of 1, 5, and 10 died at the beginning of the first instar. The minimum population density for *S. c. ricini* was 20 larvae. However, to obtain optimum survival rate, larval propagation should be carried out at a population density of at least 30 larvae. The amount of density needs to be adjusted to the size of the rearing container used so that the population is not less or excessive because population density can affect the survival rate of larvae. Diamantidis et al. (2020) reported that high larval density decreased the survival rate of larvae and pupae of the fruit fly Tephritidae short-lived biotype. Hooper et al. (2003) also stated that an increase in larval density decreased the survival rate of *Chironomus riparius* larvae. Larval stage is particularly susceptible

to population density. This stage is the main stage in obtaining nutrients from feed. High density increases intraspecific competition that limits resources for each individual so that it can affect the development time as exemplified in the larvae of *Drosophila* fly (Klepsatel et al., 2018).

Population density affected development time of *S. c. ricini* eggs (glm $F_{6,28}=255.39$; $P=2.2e-16$). The development time of *S. c. ricini* eggs reared at a density of 30 was a day faster (6.49 days) than the egg development time in other treatments (Fig. 2). Meanwhile, larvae (lm $F_{3,16}=2.774$, $P=0.075$) developed in relatively the same time. The larva developed in 17-30 days (an average of 19-20 days). It was reported that population density can also affect insect morphology. For example, *Plusia gamma* larvae have little colour variation, which tend to be brightly coloured in solitary conditions and tend to be more diverse with dark colours in gregarious conditions (Long, 1953). In this study, there was no effect of population density on the difference in body size of each larval instar or body colour. However, the larvae of *S. c. ricini* have various body colour. The larvae of the fifth instar of *S. c. ricini* have six different shades of colours, such as plain,

small and large speckled with a basic body colour of white and green tosca, respectively. Natch et al. (2017) mentioned that despite the phenotype variations in the body of *S. c. ricini* larvae, the genetic diversity was quite low. Appearance of colour natural populations may have polymorphisms because of biased mutation, pleiotropy and trade-offs, gene flow, variable selection through time and/or space, and negative frequency-dependent selection that can fend off genetic drift's loss of diversity (Gray and McKinnon, 2007; Majerus, 1998; McKinnon and Pierotti, 2010; Punzalan et al., 2005) where individuals of a rare type (i.e. a rare morph or a rare species).

Population density had no effect on the formation and development time of pupae (glm $F_{3,16}=0.4289$; $P=0.7351$). Pupae developed for ± 16 days at each treatment. The formation and development of pupae in each density treatment tended to be the same since all density treatments examined in this study employed a room with the same temperature and humidity. Temperature and humidity are factors affecting the formation of the pupa of *S. c. ricini* (Vaidya et al., 2014). Hussain et al. (2011) also reported that temperature and humidity affected the formation of *Bombyx mori* pupae and other insects, such as *Aedes aegypti* (Yahya et al., 2019) and *Stomoxys* spp. (Issimov et al., 2020). Nevertheless, all the observed larvae developed into pupae, resulting in cocoons with considerably different weights for each population density examined (lm $F_{3,16}=28.48$; $P=1.187 e-06$). The pupa from the larvae reared at densities of 20 and 30 had a cocoon shell that weighed 0.3 g, while the pupa from the larvae reared at densities of 40 and 50 had a cocoon shell that weighed 0.25 g. (Fig. 3). Cocoon shell weight is a crucial factor in the effective production of silkworms, as it will have an impact on yields and selling prices (Winardi, 2021). Cocoon shell weight is influenced by the ability of the larvae to produce silk fibre-forming proteins during the fourth and fifth instar (Cholifah et al., 2012). The results showed that the heaviest cocoon weights resulted from larvae reared at densities of 20 and 30, with an average cocoon weight of 0.30 g and 0.32 g, respectively. Meanwhile, the density of 40 and 50 resulted in the same average cocoon weight, which was 0.25 g. Cocoon fibres are produced from protein molecules. As was the case with *B. mori*, high density may prevent protein function and cause its weight to decrease. (Putro et al., 2016). In addition, Vaidya et al. (2014) reported that the weight of the *S. c. ricini* cocoon was affected by temperature and humidity in the rearing container.

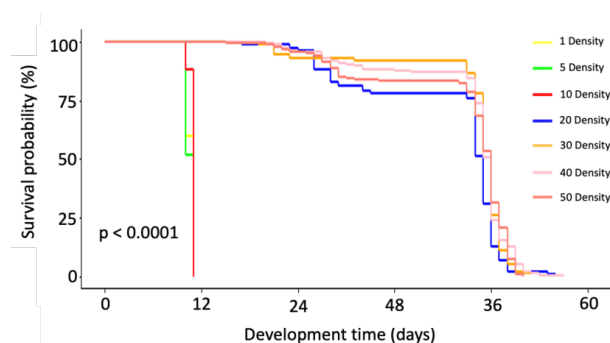


Fig. 1. Survival of *Samia cynthia ricini* at different population densities

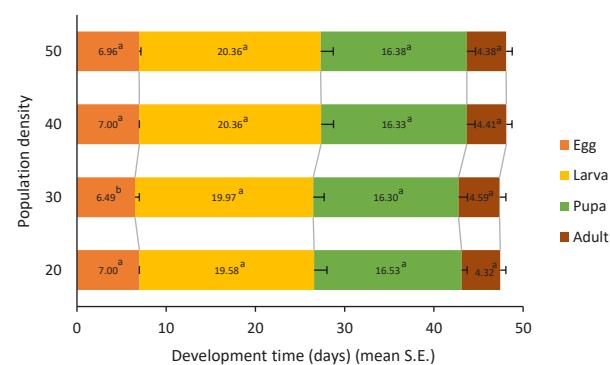


Fig. 2. Developmental time of *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ($\alpha=5$). S.E. Standard Error

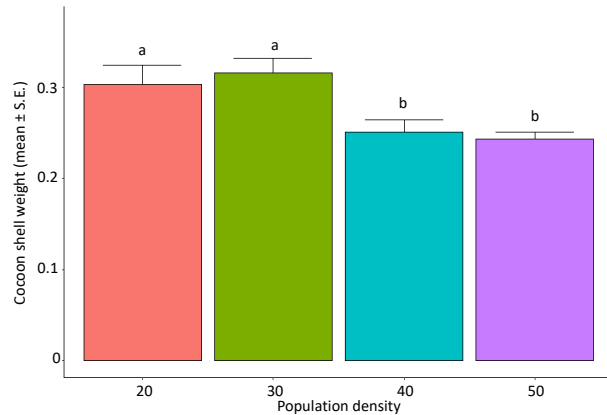


Fig. 3. Cocoon shell weight of *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ($\alpha=5$). S.E. Standard Error

The population density also had no significant effect on adult longevity ($\text{lm } F_{3,16}=3.153$; $P=0.0538$). The entire pupa developed into an adult moth that lived between two and six days. Some adults produced by a density of 50 had damaged wings. This might occur because of the adult not having enough room as it emerges from the cocoon, which affects the wing's irregular shape. However, population density significantly affected the fecundity of adult female moths ($\text{lm } F_{3,16}=3.931$; $P=0.028$). The fecundity of adult females increased as the population density increased. Adult females derived from larvae reared at densities of 30 and 40 could produce more eggs than those reared at a density of 20 or 50 (Fig. 4). Park et al. (2016) also reported similar result in which the fecundity of adult females of *Hermetia illucens* increased as density increased. In contrast, Xing et al. (2021) reported that the fecundity of *Sitobion avenae* decreased along with the decrease in the population density.

The oviposition by adult females was also influenced by the interaction of the population density and females age ($\text{lm } F_{23,96}=4.625$; $P=4.976 \text{ e-}08$). Based on the pattern of daily oviposition, females often exhibit low reproductive rates, with a tiny initial egg production. However, it gradually increases to the maximum (more eggs are laid) and then decreases again (Fig. 5) as a result of physiological degradation at the end of life, or what is known as reproductive senescence (Bradshaw and McMahon, 2018). Adult females descended from larvae reared at a density of 50 did not exhibit this pattern in their daily oviposition, even though fewer eggs were deposited on the final day. Finally, there was no difference in the hatchability of eggs produced by female at all density treatments tested ($\text{lm } F_{3,16}=1.277$; $P=0.3159$). A total of 75-90% of eggs could hatch (Fig. 6).

In conclusion, population density affects the survival of *S. c. ricini*. A density of 30 is the minimum population density to support the success of *S. c. ricini*'s life. *S. c. ricini* reared at a density of 30 had the highest survival rate, faster egg development time, heavier cocoon shell weight, and highest fecundity.

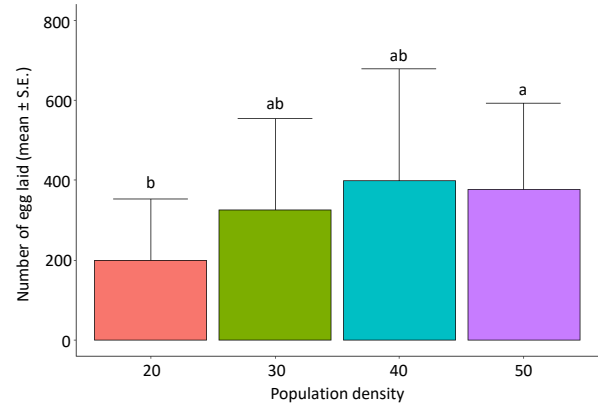


Fig. 4. Adult fecundity of *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ($\alpha=5$). S.E. Standard Error

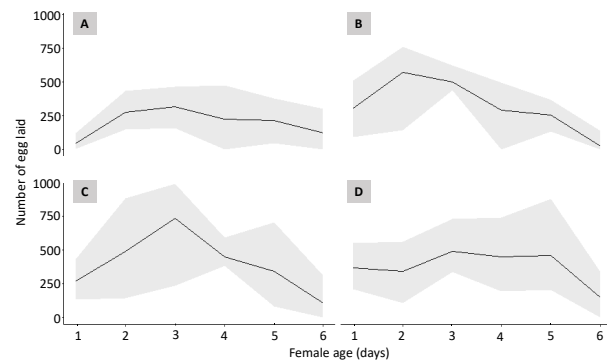


Fig. 5. Adult daily fecundity of *Samia cynthia ricini* at different population densities. A. 20 density, B. 30 density, C. 40 density, and D. 50 density

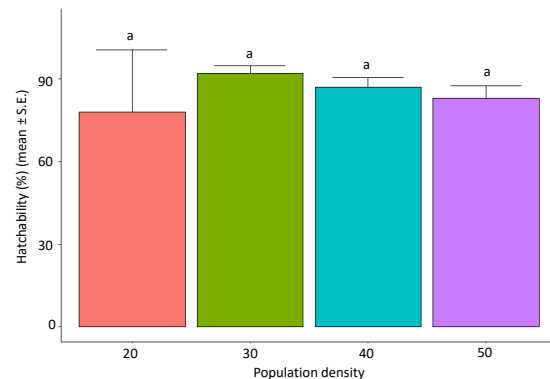


Fig. 6. Hatchability of eggs produced by adult *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ($\alpha=5$). S.E. Standard Error

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AUTHOR CONTRIBUTION STATEMENT

IN and DWT contribute to conceptualization, methodology, data analysis and research supervision. FKW performed the experiments. FKW and IN write the original manuscript, IN and DWT reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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INCIDENCE OF ONION THRIPS *THRIPS TABACI* LINDEMAN IN SOME CABBAGE VARIETIES IN KOSOVO

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ABSTRACT

Onion thrips (*Thrips tabaci* Lindeman) is a serious pest of cabbage worldwide. This study assesses the distribution of this thrips in three cabbage varieties (Proctor, Partula, and Atria) cultivated in open fields in three localities of Kosova (Barilevë, Prishtinë, and Podujevë). The experiment was conducted during 2020 and 2021 in randomized Fisher blocks design with four replications. Observations on the incidence of thrips confirmed with yellow sticky traps measuring 15x 20 cm revealed statistically significant differences with regard to years, localities, and varieties. This study reveals for the first time, the occurrence of *T. tabaci* on cabbage varieties cultivated in Kosova.

Key words: *Thrips tabaci*, cabbage varieties, Atria, Partula, Proctor, incidence, ANOVA, number of thrips, yellow sticky traps, population buildup, variation

Onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a worldwide distributed insect pest of economic importance (Diaz Montano et al., 2011; Fail, 2016). Direct feeding causes economic damage to over 300 host plant species, causing U.S. \$1 billion in crop losses (Balan et al., 2018). Besides causing direct damage, this pest has been known as a vector of two economically significant viral pathogens, tomato spotted wilt virus (TSWV), and Iris yellow spot virus (IYSV). It is estimated that IYSV causes annual losses of U.S. \$90 million to onion production in the western USA alone (Gent et al., 2006), whereas TSWV can cause over U.S \$1 billion in crop losses annually worldwide (Goldbach and Peters, 1995). Onion thrips damage the white cabbage (*Brassica oleracea* L.) outdoors (Wolfenbarger and Hibbs, 1958) and in storage as well (Fox and Delbridge, 1977). Damage can appear as a bronze discoloration and/ or a rough texture on leaves within heads (Shelton and North, 1986), causing injury up to 20 layers deep (Trdan et al., 2005) and reducing marketable yield.

The use of insecticides usually results in poor control of onion thrips on cabbage (Andaloro et al., 1983), and a high amount of insecticides is required because of their high escape capacity, fast development rate, and low sensitivity to the insecticides. On the other hand, few studies have been carried out to evaluate the patterns of resistance of cabbage varieties against onion thrips.

Three different mechanisms of plant resistance to insects have been defined 1) non-preference (antixenosis), 2) antibiosis, and 3) tolerance (Painter, 1941). Based on the number of thrips colonizing the cabbage heads, Fail et al. (2008) reported that only some varieties possess antixenotic resistance. Despite several studies, the resistance mechanisms of cabbage to onion thrips remains unknown (Shelton et al., 2008). Taking into consideration the fact that *T. tabaci* is a serious threat to cabbage production worldwide (Shelton et al., 1983; Giessmann, 1988; Kristof and Penzes, 1984; Kahrer, 1992; Herold and Stengel, 1993; Ellis et al., 1994), the main goal of this work was to assess its buildup and population dynamics in three cabbage varieties grown in open fields in the three localities of Kosova during two cropping seasons (2020–2021).

MATERIALS AND METHODS

Field surveys for *T. tabaci* infesting cabbage in the three most important localities of cabbage production in Kosova (Prishtinë, Barilevë, and Podujevë) were undertaken in the 2020-2021 growing season. The fields were situated in Prishtinë (42°6882', 21°1466', 603 masl), Podujevë (42°8322', 21°2081', 636 masl) and Barilevë (42°7518', 21°1210', 526 masl). Three varieties of cabbage (Proctor, Partula, and Atria) were included, and these were grown under insecticide-free conditions. In May, cabbage seeds were sown in pro

trays filled with vermiculite, one seed per cell, and covered with a polythene sheet until germination. After the seeds were germinated, seedlings were transplanted during June, with spacing of 60x 40 cm. Cultural practices were followed as recommended for cabbage (Filgueira, 2000). Each variety was planted in an experimental block of 1 ha, with plot size of 0.25 ha, forming four plots/ variety in each locality, and these were arranged in a randomized block design.

To assess the thrips abundance and distribution in varieties, yellow sticky traps (BB sticky trap yellow NHS- 25x 10 cm) were placed following Pobozniak et al. (2020). These traps were produced by Biobest Group NV, Belgium, and placed randomly 1 m apart in the middle of each plot, attached to wooden stakes in such a manner that their bottom edges were 10-15 cm above the plant heights. These traps were checked and replaced at 15-day intervals, from the beginning (July) until harvest (September). After the removal of traps, these were wrapped with clear plastic cling film, and provided with the most relevant data (date of sampling, plot number, variety, locality, etc.). These were taken to the laboratory of Plant Protection at the Faculty of Agriculture and Veterinary to identify the thrips and assess their numbers as well. At the laboratory, each trap was examined, and thrips removed from using mineral spirits, before slide mounted in drops of the Heinz solution under a stereomicroscope and identified to species level under a compound microscope, following Mound and Walker (1982). Given that climatic conditions can influence thrips buildup and distribution (Morsello et al., 2008), meteorological data from the Prishtina meteorological station were obtained. The data were statistically processed using the MSTAT-C software packages developed by the University of Michigan version 2.10, and analysed for three-way ANOVA with significance determined using LSD ($p=0.05$ and 0.01).

RESULTS AND DISCUSSION

The observations on the appearance and distribution of *T. tabaci* revealed its occurrence in all cabbage varieties, and their numbers varied over year, localities, and cabbage varieties (Table 1). The incidence started from the beginning of the growing season when the seedlings had just been planted and continued in the entire growing season, with high intensity. Leite et al. (2006) observed that the thrips population tends to increase in the final phase of the plant or reaches a peak in about 40 days after planting when the cabbage head is formed. Voorrips et al. (2008) concluded that

cabbage suffers more damage later in the season. The frequency of appearance in varieties varied over years, with maximum (29881 individuals) during 2020; maximum incidence was in Prishtinë variety. Morsello et al. (2008) observed that temperature and precipitation can influence the thrips incidence. The varieties showed variations; Atria was the most affected, while the least affected was Partula. There were significant differences in the degree of susceptibility of some varieties like Golden Cross, Balashi, Riana, Autumn Queen, 'Leopard', Ama-Daneza', and 'Galaxy' (Fail and Penzes, 2001). Also, it has been reported that there is a large difference in thrips damage among red and white cabbage varieties (Shelton et al., 1983; Stoner and Shelton, 1988; Steene Van and Tirry, 2003; Trdan et al., 2005).

The ANOVA and LSD analyses revealed significant differences in incidence between years, localities, and varieties (Table 1). In 2020, in relation to 2021 (Factor A), where its numbers were 333.2 and 220.2 individuals, respectively; these differences are highly significant. Such differences were observed with regard to localities as well (Factor B), maximum was at Prishtinë (326.2) and the least in Podujevë (246.6), and these variations are highly significant. Amongst the varieties (Factor C) also, highly significant differences were observed- Atria with maximum (392.6) and the least with Partula (157.8). Regarding the interactions of factors, statistically significant differences were found-, concluding that the appearance and distribution of *T. tabaci* varies considerably. With the aim of managing the *T. tabaci* in cabbage, it would be extremely useful to investigate the feasibility of an IPM strategy at the farmer level. Planting varieties with tolerance to thrips damage may serve to protect cabbages without the use of insecticides.

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AUTHOR CONTRIBUTION STATEMENT

Both authors contributed equally.

Table 1. Comparison of onion thrips distribution by localities and varieties during the two growing seasons (ANOVA)

Year (A)	Locality (B)	Variety (C)	Sampling						Sum	ANOVA						
			1	2	3	4	5	6		A	B	C	AB	AC	BC	ABC
2020 A ₁	Prishtinë B ₁	Proktor (C ₁)	415	397	213	918	548	711	3202	A ₁	B ₁	C ₁	A ₁ B ₁	A ₁ C ₁	B ₁ C ₁	A ₁ B ₁ C ₁ 533.7
		Partula (C ₂)	210	132	97	174	49	236	898	333.2**	326.2**	279.6	371.2*	436.2**	365.8	A ₁ B ₁ C ₂ 149.7**
		Atria (C ₃)	625	718	595	627	618	569	3752						B ₁ C ₂	A ₁ B ₁ C ₃ 625.3**
	Barilevë B ₂	Proktor (C ₁)	236	201	317	294	213	322	1583				A ₁ B ₂	A ₁ C ₂	131.4	A ₁ B ₂ C ₁ 263.8
		Partula (C ₂)	126	174	195	245	281	194	1215				166.9	291.4	B ₁ C ₃	A ₁ B ₂ C ₂ 202.5
		Atria (C ₃)	349	387	416	495	410	391	2448						481.4	A ₁ B ₂ C ₃ 408.0
2021 A ₂	Podujevë B ₃	Proktor (C ₁)	215	308	195	240	429	510	1897		B ₂	C ₂	A ₁ B ₃	A ₁ C ₃	B ₂ C ₁	A ₁ B ₃ C ₁ 316.2
		Partula (C ₂)	28	69	155	217	104	319	892		257.1	157.8**	461.4*	271.9	190.6Ns	A ₁ B ₃ C ₂ 148.7
		Atria (C ₃)	308	422	354	293	312	416	2105						B ₂ C ₂	A ₁ B ₃ C ₃ 350.8
	Prishtinë B ₁	Proktor (C ₁)	182	243	287	154	218	104	1188	A ₂			A ₂ B ₁	A ₂ C ₁	206.8Ns	A ₂ B ₁ C ₁ 198.0
		Partula (C ₂)	41	98	117	152	103	168	679	220.2**			188.0	216.2**	B ₂ C ₃	A ₂ B ₁ C ₂ 113.2
		Atria (C ₃)	308	410	295	186	455	371	2026						374.0	A ₂ B ₁ C ₃ 337.5
LSD	Barilevë B ₂	Proktor (C ₁)	39	68	95	152	106	244	704		B ₃	C ₃	A ₂ B ₂	A ₂ C ₂	B ₃ C ₁	A ₂ B ₂ C ₁ 117.3
		Partula (C ₂)	82	118	167	258	213	429	1267		246.6**	392.6**	148.7	222.8	282.4	A ₂ B ₂ C ₂ 211.2
	Podujevë B ₃	Atria (C ₃)	215	295	316	378	349	487	2040						B ₃ C ₂	A ₂ B ₂ C ₃ 340.0
		Proktor (C ₁)	103	255	196	233	410	295	1492				A ₂ B ₃	A ₂ C ₃	135.3	A ₂ B ₃ C ₁ 248.7
		Partula (C ₂)	54	116	92	51	148	270	731				323.7	221.4	B ₃ C ₃	A ₂ B ₃ C ₂ 121.8
		Atria (C ₃)	411	352	301	297	256	145	1762	42.409	62.516	54.161	98.622	85.442	111.081	A ₂ B ₃ C ₃ 293.7
						p=0.01									190.619	
						p=0.05									125.828	

Note: 1-6 represents the terms of onion thrips collected from four repetitions; * significant; ** highly significant; Ns not significant.

CONFLICT OF INTEREST

No conflict of interest.

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A NEW SPECIES OF *CERATOSOLEN* (AGAONIDAE) ASSOCIATED WITH *FICUS HISPIDA* L. AND PROPOSED CHANGES IN STATUS OF TWO OTHER POLLINATOR FIG WASPS

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ABSTRACT

An increasing number of fig trees are known to support more than one species of pollinator fig wasp (Agaonidae). This brings into question the relative importance of co-evolution, radiation and host switching in the development of this mutualistic association. The functionally dioecious *Ficus hispida* has a wide distribution in Asia. It supports at least three morphologically distinct *Ceratosolen* pollinators, one of which we describe as *C. abscondus* Wong & Yu sp. n., based on collections in China and Thailand. The other two taxa were originally described as separate species, but subsequently downgraded to sub-species. The morphological differences between the three taxa are similar in extent to those recorded between other *Ceratosolen* species and we therefore re-instate them as the distinct species, *C. solmsi* (Mayr) and *C. marchali* (Mayr). An identification key is provided to distinguish between them and relationships between *Ficus* species and their *Ceratosolen* pollinators of this and related species are discussed.

Key words: *Ceratosolen abscondus*, Chalcidoidea, coevolution, dioecious, *Ficus* spp., Kradibiinae, morphological differences, mutualism, pollinator specificity, subspecies

The pollinating fig wasps (Hymenoptera: Chalcidoidea: Agaonidae) serve as a model for behavioral, ecological, and evolutionary studies because of their well-known obligate mutualism with fig trees (*Ficus*, Moraceae) across the tropics and subtropics (Weiblen, 2002). The plants and insects are mutually dependent, with the larval fig wasps depending on *Ficus* inflorescences (figs) for food and fig trees depending on adult female fig wasps for pollination (Galil and Neeman, 1977). There are perhaps as many as 1000 species of fig trees, most of which support host-specific pollinator fig wasps (Berg, 1989). For many years it was believed that a 'one-to-one rule' applied, with each species of fig tree supporting a single species of host specific pollinator fig wasp. However, closely related fig wasps are often morphologically similar as adults, especially if they share a host, because their morphology reflects shared selection pressures imposed by the physical structure of their host figs (van Noort and Compton, 1996). Recent studies have revealed that the conclusion of a one-to-one pollinator-plant relationship often resulted from a low sampling intensity, with fig tree pollinators described from one or a small number

of samples collected across a small geographical area (Peng et al., 2008; Compton et al., 2009; Yu et al., 2019). An assumption of strict sense coevolution of the mutualists has therefore been replaced by an appreciation that there is often a less specific form of broad-sense coevolution, involving host switching and radiations of pollinators that better describes the relationship between fig wasps and their hosts (Cook and Segar, 2010).

The Agaonidae are presently divided into four subfamilies based on morphological and molecular evidence (Cruaud et al., 2010; www.figweb.org, accessed December 2021). The subfamily Kradibiinae Saunders contains two genera, *Ceratosolen* Mayr and *Kradibia* Saunders, that are distributed in Africa and Asia. *Ceratosolen* (Chalcidoidea: Agaonidae: Kradibiinae) is monophyletic and was originally placed as a subgenus of *Blastophaga* Gravenhorst before being upgraded to genus level (Mayr, 1906; Cruaud et al., 2010). *Ceratosolen* is a large genus with over 30 described species and many additional species that remain undescribed. The genus displays a strong conservation of host association, being associated

exclusively with *Ficus* subgenus *Sycomorus* (Weiblen and Bush, 2002; Cruaud et al., 2010). There are an increasing number of examples of more than one species of *Ceratosolen* pollinator being associated with a particular species of fig tree (for example Yu et al., 2021). Historically, when two *Ceratosolen* taxa were reared from figs of a single *Ficus* they were described as belonging to different subspecies, rather than species, or if originally described as being distinct species they subsequently had their status downgraded to that of sub-species (Table 1). Definitions vary, but Mayr and Ashlock (1991) defined a subspecies as “an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species.” Geographical distribution was not an explicit consideration when the *Ceratosolen* subspecies were established, but they were described from different countries. However, the full extent of their distributional ranges remains largely uncertain. The assignment of a subspecies rank to the *Ceratosolen* species therefore appears to have reflected an unspoken assumption that a single *Ficus* species could only support a single species of pollinator, even if morphological differences were present between them.

Ficus hispida L. f. is a widespread moderate-sized functionally dioecious tree found throughout much of Asia (Berg and Corner, 2005). It is favored by disturbance and other human activities and has been cultivated for its edible, ethnobotanical and folklore value (Cheng et al., 2020). The tree’s previously described pollinators, currently designated as *Ceratosolen solmsi solmsi* Mayr, 1885 and *C. s. marchali* Mayr, 1906, develop inside the figs on male plants. Pollinated flowers inside figs on female trees develop into seeds. The two *Ceratosolen* taxa were originally described as distinct species, before being synonymized by Wiebes (1963). Wiebes based his decision after carefully considering the extent

of morphological variation within the complex. He concluded that while individuals of most populations could clearly be placed in one or other of the two taxa, there were some populations that were intermediate in terms of the characters that had been originally used to distinguish between them. More recently, molecular analyses have revealed that the extent of differentiation between three *Ceratosolen* taxa associated with *F. hispida* in China is similar in extent to that typically exhibited by different *Ceratosolen* species, suggesting that they represent different species (Deng et al., in preparation). In order to clarify the status of the *Ceratosolen* pollinators of *F. hispida* we examined the morphological variation exhibited by their adult females. Based on clear differences in their morphology, we conclude that three distinct species are present. The original rank of two of the species is restored and a third species is described as new. Species descriptions and a key to distinguish females of the three *Ceratosolen* species are provided and we discuss the nature of their evolutionary relationship with *F. hispida*.

MATERIALS AND METHODS

The specimens of *Ceratosolen* were collected during the years 2010 to 2018 from ten sites distributed over South China and Thailand (Table 2). All the wasps that exited their natal figs were collected and stored in 75% ethanol. The figs were then dissected and the wasps therein were collected. Later, the wasps were dehydrated through an ethanol series (80%, 90%, and 100%) and critical-point dried (LEICA EM CPD300, Leica Microsystems GmbH, Germany) before being mounted on cards following Noyes (1982). A scalable dome was made to cover to the specimens before each photo was taken (Kawada and Buffington, 2016) using a digital camera connected to a stereomicroscope (LIECA M205 FA, Leica Microsystem GmbH, Germany) and processed using the LAS X 3.08.19082 software to

Table 1. Asian fig trees of the *Ficus* subgenus *Sycomorus* that have previously been recorded as being hosts to two different subspecies of *Ceratosolen* pollinator fig wasps (extracted from Wiebes, 1994)

Host <i>Ficus</i>	<i>Ceratosolen</i> current status	Original status
<i>F. septica</i> Burm.f.	<i>C. bisulcatus bisulcatus</i>	<i>Blastophaga bisulcata</i> Mayr, 1885
<i>F. septica</i> Burm.f.	<i>C. bisulcatus jucundus</i>	<i>C. jucundus</i> Grandi, 1927
<i>F. fistulosa</i> Reinw.	<i>C. constrictus constrictus</i>	<i>Blastophaga constricta</i> Mayr, 1885
<i>F. fistulosa</i> Burm.f.	<i>C. constrictus hewitti</i>	<i>C. hewitti</i> Waterston, 1921
<i>F. ribes</i> Reinw.	<i>C. crassitarsus crassitarsus</i>	<i>Blastophaga crassitarsus</i> Mayr, 1885
<i>F. ribes</i> Reinw.	<i>C. crassitarsus gracilis</i>	<i>C. crassitarsus gracilis</i> Wiebes, 1963
<i>F. hispida</i> L.f.	<i>C. solmsi solmsi</i>	<i>C. solmsi</i> Mayr, 1885
<i>F. hispida</i> L.f.	<i>C. solmsi marchali</i>	<i>C. marchali</i> Mayr, 1906

Table 2. Sampling sites of *Ceratosolen* fig wasp species associated with *Ficus hispida* fig trees with names used herein

<i>Ceratosolen</i>	Country	Province	City/ County	Coordinates
<i>C. abscondus</i> sp. n.	China	Hainan	Ding'an	19°40'49.0"N, 110°22'05.0"E
	Thailand	Chiang Mai	Chiang Mai	18°48'00.0"N, 98°54'00.0"E
<i>C. marchali</i> status n.	China	Guangdong	Guangzhou	23°16'48.0"N, 113°34'48.0"E
	China	Guangdong	Huizhou	23°22'12.0"N, 114°22'12.0"E
	China	Guangdong	Zhaoqing	23°09'47.7"N, 112°33'17.7"E
	China	Guangxi	Nanning	22°46'48.0"N, 108°22'48.0"E
	China	Yunnan	Mengla	21°40'48.0"N, 101°25'12.0"E
<i>C. solmsi</i> status n.	Thailand	Chanthaburi	Khao Khitchakut	12°46'26.4"N, 102°05'45.6"E
	Thailand	Tak	Mueang Tak	16°46'55.2"N, 98°55'40.8"E
	Thailand	Trang	Thung Khai	7°28'01.2"N, 99°38'20.4"E

create a stacked image with increased focal depth. Physical characteristics were measured using ImageJ 1.8.0_172 software (National Institutes of Health, USA). Specimen measurements were taken with an accuracy of 0.001 mm, rounded to the nearest 0.01 mm. Specimens were mounted on brass stubs and sputter-coated with gold (Leica EM ACE600, Leica Microsystems GmbH, Germany) before observation and photography using SEM (Jeol JSM-6360LV, Jeol Ltd, Japan). Morphological terminology follows Gibson (1997) and the Hymenoptera anatomy ontology (HAO) Portal (Yoder et al., 2010). The holotype and a group of paratypes are deposited in the Insect Museum of National Taiwan University (25°00'40.4"N; 121°32'26.7"E). Other paratypes and examined materials are deposited in the Plant Science Center, South China Botanical Garden, Chinese Academy of Sciences, China (23°10'48"N; 113°21'8"E).

RESULTS AND DISCUSSION

A. Taxonomic treatment

Ceratosolen Mayr

Type species *Blastophaga* (*Ceratosolen*) *appendiculatus* Mayr, 1885.

Diagnosis: Females. Head generally as long as wide across compound eyes, or a bit longer or shorter. Antenna with 9–11 segments, club 1–3 segmented. Mandibular appendage with four to ten ventral lamellae; maxillary bacilliform process present or absent. Pollen pockets large. Spiracular peritremata variable but usually large.

Distribution and host relationships: Old world. Pollinators of monoecious and dioecious fig trees of *Ficus* subgenus *Sycomorus*, with larvae developing in galled ovules.

1. *Ceratosolen abscondus* Wong and Yu sp. n.

Types: Holotype, ♀, China, Ding'an (Hainan province), 19°40'49.0"N, 110°22'05.0"E, 3 OCT 2010, H. Yu; Paratypes, China: 2♀, Ding'an, 19°40'49.0"N, 110°22'05.0"E, 3 OCT 2010, H. Yu.

Additional material: Thailand: 4♀, Chiang Mai (Chiang Mai province), 18°48'00.0"N, 98°54'00.0"E, 7 JUL 2013, H. Yu.

Description: Color and size. Antennal flagellum yellowish brown, scape yellowish brown; head, mesosoma and metasoma dark brown; mandibles and legs yellowish brown. Body length including head, mesosoma and metasoma 2.76 mm (Fig. 1A). Fore wing finely pubescent, length 2.54 mm, striae radiating from stigmal vein present but indistinct, uncus and stigma distinct; submarginal, marginal, stigmal, and postmarginal veins in ratio 3: 1: 1: 2 (Fig. 1D).

Head. Longer than wide across compound eyes (Fig. 2A); length 0.56 mm, width 0.50 mm. Compound eye as long as gena, with length 0.23 mm and gena length 0.21 mm; face with sparse setae. Three ocelli. Epistomal margin bilobed with a central tooth. Maxilla apically with finger-like projections with long bacilliform process; mandible bidentate, length 0.14 mm with four ventral lamellae; mandibular appendage with six ventral lamellae, with no lamellar teeth developed (Fig. 2D). Distance between toruli smaller than diameter of one torulus; antennal scape robust, a trapezium in outline (Fig. 3A); pedicel with sensilla obscura (Li et al., 2009); hook-shaped apical projection reaching to about middle of fifth antennal segment; eighth flagellomere (tenth antennal segment) longer than ninth flagellomere, with length 0.10 mm, ninth flagellomere length 0.08 mm (Fig. 3D).

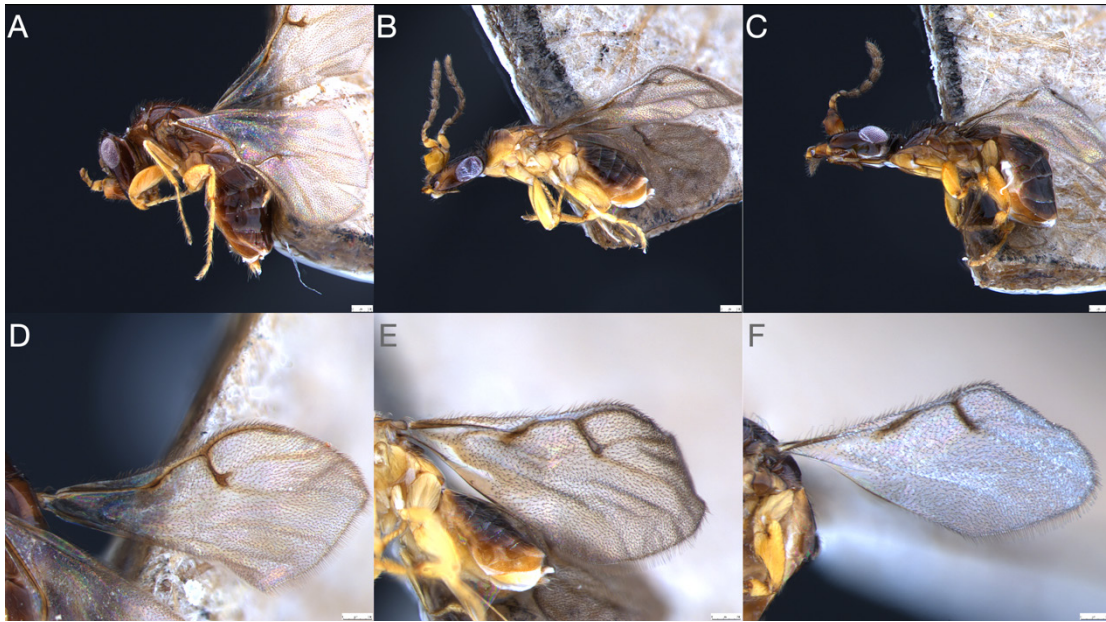


Fig. 1. Habitus, lateral of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Fore wing of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*. Scale bars represent 250 μ m

Mesosoma. Length 0.81 mm (Fig. 4A). Spiracular peritremata large, slipper-like in outline; cilia in spiracle aperture bifurcated (Fig. 4D). Mesosternum without longitudinal median groove (Fig. 5A); pollen pocket nearly closed with 11 spines anteriorly, pocket length 0.12 mm (Fig. 5D). Hind femur length 0.68 mm, tibia length 0.36 mm, with two apical spurs (Fig. 6A).

Metasoma. Length 0.70 mm in specimens extracted from alcohol and without petiole; dorsal surface brown, ventral parts lighter. Ovipositor scarcely projecting. Hypopygium with one row of hyaline setae, cercal bristles long (Fig. 6D).

Males: unknown.

Etymology: The specific name refers to the Latin word “hidden”.

Biology: Presumed ovule galler, in male figs of *F. hispida*.

***Ceratosolen marchali* Mayr, 1906**

Type species *Ceratosolen marchali* Mayr, 1906.

Ceratosolen marchali Mayr, 1906: 155–156.

Ceratosolen solmsi marchali Wiebes, 1963: 68.

Material examined: China: 2♀, Guangzhou, 23°16'48.0"N, 113°34'48.0"E, 9 AUG 17, H. Yu; 2♀, Huizhou, 23°22'12.0"N, 114°22'12.0"E, 17 JUN 18, H. Yu; 2♀, Mengla, 21°40'48.0"N, 101°25'12.0"E,

25 DEC 18, H. Yu; 2♀, Nanning, 22°46'48.0"N, 108°22'48.0"E, 17 SEP 16, H. Yu; 2♀, Zhaoqing, 23°09'47.7"N, 112°33'17.7"E, 17 AUG 18, H. Yu.

Redescription: Color and size. Antennal flagellum yellowish brown, scape yellow; head, mesosoma and metasoma yellowish brown; mandibles and legs yellow. Body length 2.25 mm (Fig. 1B). Fore wing finely pubescent with length 2.67 mm, striae radiating from stigmal vein distinct, uncus less distinct; submarginal, marginal, stigmal, and postmarginal veins in ratio 3: 1: 1: 2 (Fig. 1E).

Head. Female head longer than wide across compound eyes (Fig. 2B); length 0.51 mm, width 0.48 mm. Compound eye as long as gena with length 0.22 mm, gena length 0.18 mm; face with sparse setae. Three ocelli. Epistomal margin bilobed with a central tooth. Maxilla apically with finger-like projections with long bacilliform process; mandible bidentate, length 0.10 mm with four ventral lamellae; mandibular appendage with six ventral lamellae, teeth less distinct on lamellae (Fig. 2E). Distance between toruli smaller than diameter of one torulus; antennal scape robust, trapezoid in outline (Fig. 3B); pedicel with sensilla obscura; hook-shaped apical projection reaching to about middle of fifth antennal segment; eighth flagellomere distinctly longer than ninth flagellomere, eighth flagellomere with length 0.15 mm, ninth flagellomere length 0.07 mm (Fig. 3E).

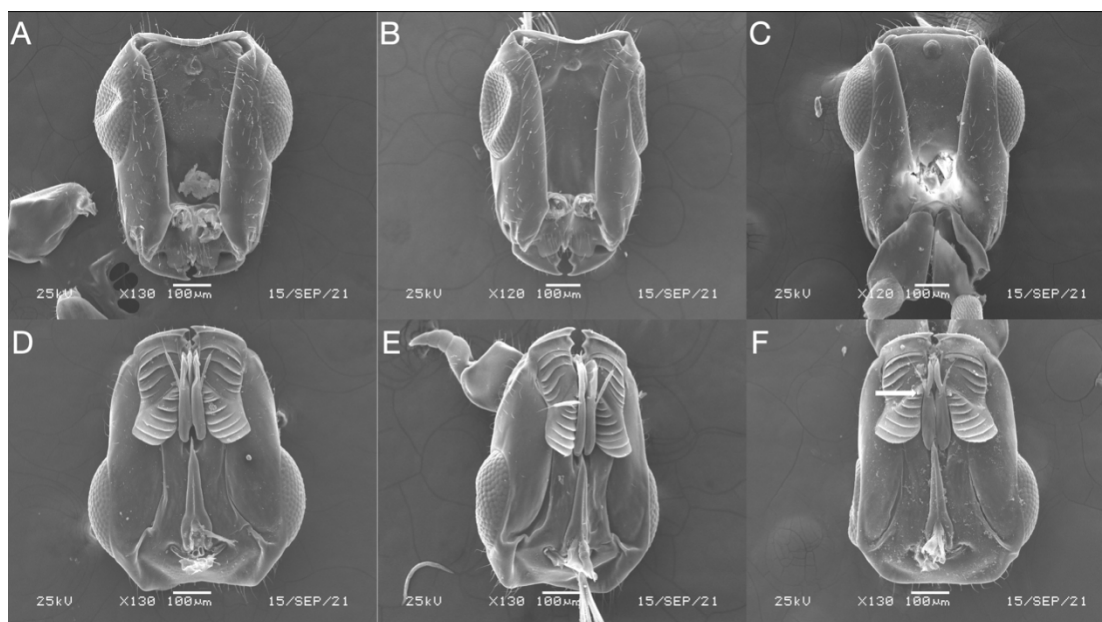


Fig. 2. Head, dorsal view of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Head, ventral view of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*. The tooth-like structure on mandibular lamellae was pointed.

Mesosoma. Length 0.76 mm (Fig. 4B). Spiracular peritremata large, slipper-like in outline; cilia in spiracles bifurcated (Fig. 4E). Mesosternum without longitudinal groove (Fig. 5B); pollen pocket nearly closed with 13 spines anteriorly, pocket length 0.12 mm (Fig. 5E). Hind femur length 0.65 mm, tibia length 0.31 mm, with two apical spurs (Fig. 6B).

Metasoma. Length 0.75 mm and without petiole;

dorsal surface dark brown, ventral parts yellowish brown. Ovipositor scarcely projecting. Hypopygium with one row of hyaline setae, cercal bristles long (Fig. 6E).

Distribution: (from van Noort and Rasplus, accessed 2021): Australia (Queensland), Hong Kong, India, Mainland China (Guangdong, Guangxi, Yunnan provinces), Malaysia, Vietnam.

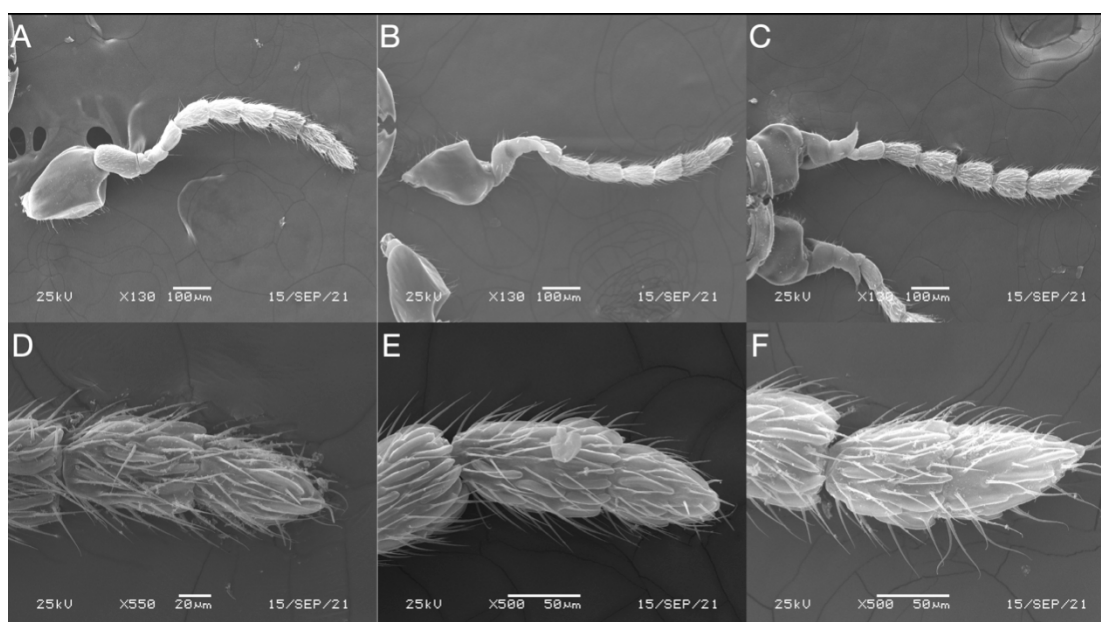


Fig. 3. Antenna of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Eighth and ninth flagellomeres of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*.

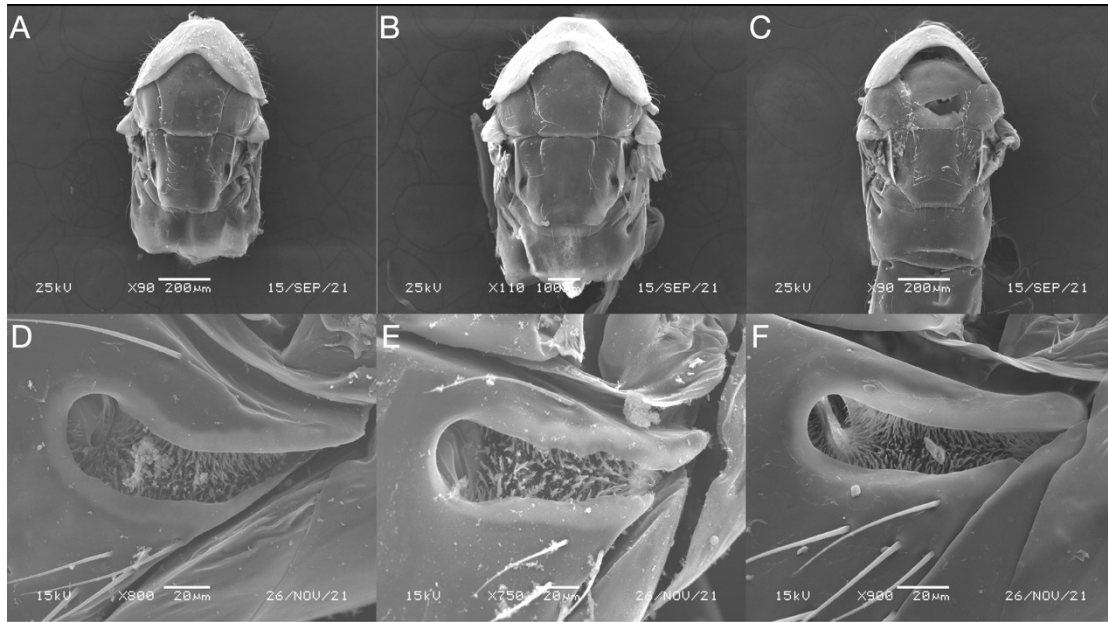


Fig. 4. Mesosoma, dorsal view of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Spiracle of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*. The spiracular cilia in *C. s. abscondus* and *C. s. marchali* were apparently bifurcated.

B. *Ceratosolen solmsi* Mayr, 1885

Type species *Blastophaga (Ceratosolen) solmsi* Mayr, 1885.

Blastophaga (Ceratosolen) solmsi Mayr, 1885: 168-169.

Ceratosolen berlandi Grandi, 1928: 74-79.

Ceratosolen solmsi solmsi Wiebes, 1963: 65-67.

Material examined: THAILAND: 2♀, Khao Khitchakut, 13°28'12.0"N, 98°34'48.0"E, 29 SEP 17, H. Yu; 2♀, Mueang Tak, 16°46'55.2"N, 98°55'40.8"E, 7 JUL 13, H. Yu; 2♀, Thung Khai, 7°28'01.2"N, 99°38'20.4"E, 1 OCT 17, H. Yu.

Redescription: Color and size. Antennal flagellum yellowish brown, scape yellowish brown; head, mesosoma and metasoma dark brown; mandibles and

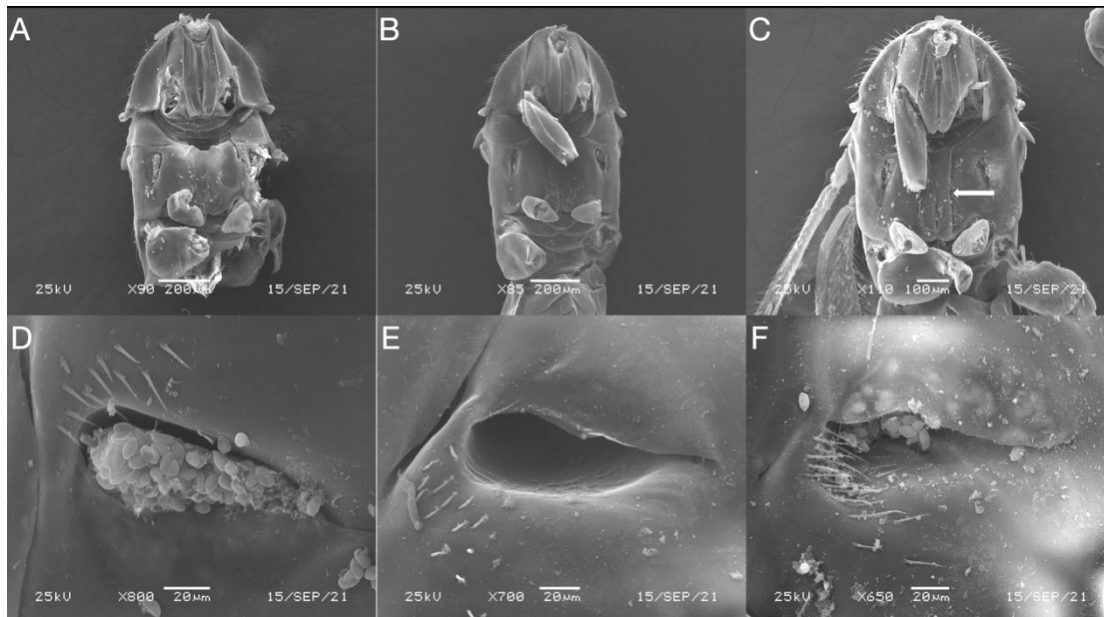


Fig. 5. Mesosoma, ventral view of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Deep groove in mesosternum of *C. s. solmsi* was pointed. Pollen pocket of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*.

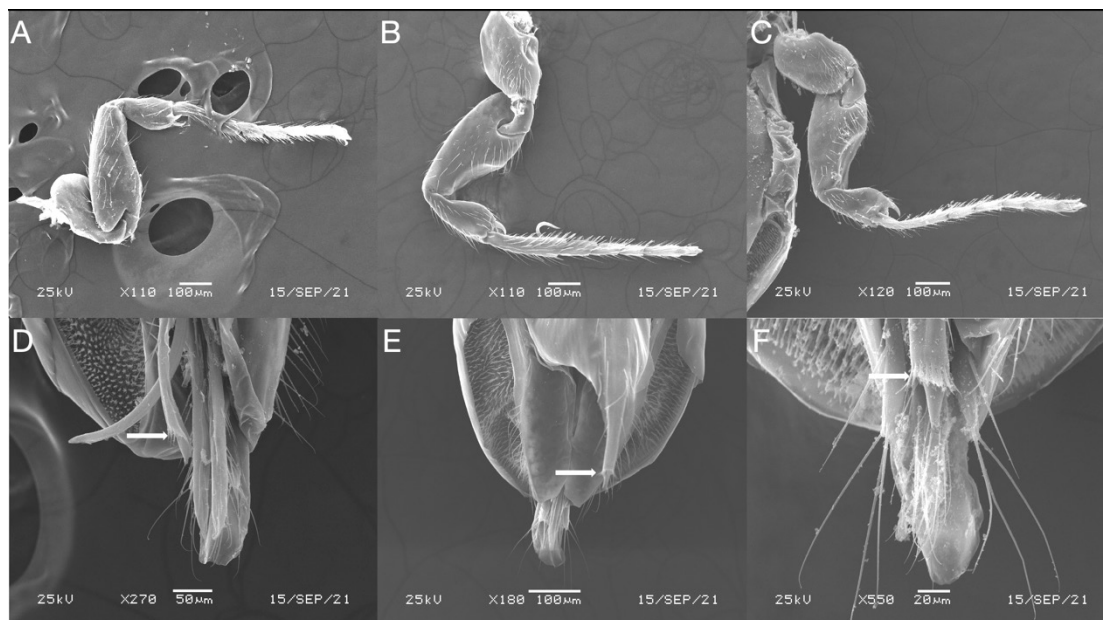


Fig. 6. Hind leg of A: *Ceratosolen abscondus* sp. n.; B: *C. marchali*; C: *C. solmsi*. Hypopygium of D: *C. abscondus*; E: *C. marchali*; F: *C. solmsi*. Hypopygial setae were pointed.

legs yellowish brown. Body length 2.73 mm (Fig. 1C). Fore wing hyaline, finely pubescent with length 2.70 mm; submarginal, marginal, stigmal, and postmarginal veins in ratio 3: 1: 1: 2 (Fig. 1F).

Head. Female head longer than wide across compound eyes (Fig. 2C); length 0.59 mm, width 0.55 mm. Compound eye as long as gena with length 0.26 mm, gena length 0.25 mm. Three ocelli. Epistomal margin bilobed with a central tooth. Maxilla apically with finger-like projections with long bacilliform process; mandible bidentate, length 0.14 mm with five ventral lamellae; mandibular appendage with seven ventral lamellae, teeth present on lamellae (Fig. 2F). Distance between toruli smaller than diameter of one torulus; antennal scape robust, trapezoid in outline (Fig. 3C); pedicel with sensilla obscura; hook-shaped apical projection reaching to about middle of fifth antennal segment; eighth flagellomere as long as ninth flagellomere, eighth flagellomere with length 0.17 mm, ninth flagellomere length 0.15 mm (Fig. 3F).

Mesosoma. Length 0.95 mm (Fig. 4C). Spiracular peritremata large, slipper-like in outline; cilia in spiracles with no or little bifurcation (Fig. 4F). Mesosternum with longitudinal groove (Fig. 5C); pollen pocket nearly closed with 17 spines anteriorly, pocket length 0.12 mm (Fig. 5F). Hind femur length 0.70 mm, tibia length 0.25 mm, with two apical spurs (Fig. 6C).

Metasoma. Length 0.70 mm and without petiole; dorsal surface dark brown, ventral parts lighter.

Ovipositor scarcely projecting. Hypopygium with one row of hyaline setae, cercal bristles long (Fig. 6F).

Distribution (from van Noort and Rasplus, accessed 2021): Australia, India, Indonesia, Malawi, Malaysia, Sri Lanka, Thailand (Chanthaburi, Tak, Trang provinces), Vietnam.

Diagnoses of the three *Ceratosolen* and comparison with other Indomalayan species

The female of *C. abscondus* is close to *C. marchali* but the funicle segments are sub-quadrate rather than rectangular (Table 3). The antennal segments of *C. abscondus* and *C. marchali* are more tightly adpressed than in *C. solmsi*. The facial groove width behind the antennal toruli of *C. abscondus* is similar to *C. marchali* but wider than in *C. solmsi*. The fore wings of *C. abscondus* are paler than *C. marchali* but darker than *C. solmsi* with the striae radiating from the stigmal vein less distinct than in *C. marchali*. The anterior pollen pocket spines of *C. abscondus* are 2x longer than in *C. marchali* but shorter and looser than in *C. solmsi*.

In comparison with *Ceratosolen* species associated with other host plants (Wiebes, 1994), *C. fusciceps* Mayr has a head shorter than wide (0.95). *C. marshalli* Grandi has ahead longer the wide (1.25). *C. internatus* has ten antennal segments. *C. pygmaeus* Grandi has a very short postmarginal vein, one-quarter of the stigmal. *C. adenospermae* Wiebes has a very long postmarginal vein, twice the length of the stigma. *C. bisulcatus* Mayr

Table 3. Morphological characteristics of the *Ceratosolen* fig wasp species associated with *Ficus hispida*

<i>Ceratosolen</i>	Mean body length (mm)	Facial groove behind toruli	Petiolated flagellomere	Eight: ninth flagellomere	Mesosoma color	Mesosoma ventral groove	Spiracular cilia bifurcation	Pocket spines
<i>C. abscondus</i> sp. n. (N=12)	2.76±0.11	Wide	No	1.2: 1	Brown	No	Yes	Loose, long
<i>C. marchali</i> status n. (N=12)	2.74±0.15	Wide	No	1.6: 1	Yellowish brown	No	Yes	Loose, short
<i>C. solmsi</i> status n. (N=10)	2.91±0.37	Narrow	Yes	1: 1	Dark brown	Yes	No	Dense, long

The pollen pockets are associated with active pollination behaviour and are located on the underside of the thorax. MX± SD

has simple maxillae. *C. jucundus* Grandi has very short maxillary bacilliform processes. *C. emarginatus* Mayr has an eye:cheek ratio in 2: 1. *C. solitarius* Wiebes has an eye:cheek ratio in 0.8: 1.

Key to the females of *Ceratosolen* fig wasp species associated with *Ficus hispida*

- 1a. Facial groove behind toruli wider (Fig. 2A, 2B); tooth-like structure absent on mandibular lamellae (Fig. 2D, 2E); cilia in spiracles clearly bifurcated (Fig. 4D, 4E); dark striae radiating from stigmal vein distinct (Fig. 1D, 1E); scape yellowish brown to brown (Fig 1A, 1B) 2
- 1b. Facial groove behind toruli narrower (Fig. 2C); tooth-like structure present on mandibular lamellae (Fig. 2F); cilia in spiracles with no or minimal bifurcation (Fig. 4F); dark striae radiating from stigmal vein less distinct or wing hyaline (Fig. 1F); scape dark brown (Fig. 1C)..... *C. solmsi*
- 2a. Eighth flagellomere >1.5x length of ninth (Fig. 3E); uncus and stigma less distinct (Fig. 1E); pocket spines short (Fig. 5E)..... *C. marchali*
- 2b. Eighth flagellomere <1.5x length of ninth (Fig. 3D); uncus and stigma well-developed (Fig. 1D); pocket spines long (Fig. 5D) ...*C. abscondus* sp. n.

Increasing numbers of *Ficus* species are known to routinely host two or more species of pollinator fig wasps (Cruaud et al., 2012), particularly if the host *Ficus* has a wide continental or multi-island distribution (Yu et al., 2019; Rodriguez et al., 2020). Such breakdowns of one-to-one pollinator-host relationship may result from colonization events followed by pollinator host shifts (Kerdelhue et al., 1999; Cook and Segar, 2010). Evidence for this scenario is provided when two or

more relatively un-related fig wasps share a host. An example is provided by the two *Ceratosolen* species associated with *Ficus sycomorus* in Africa (Compton et al., 1991). Alternatively, two or more pollinator species sharing one host can result from more rapid speciation rates among the fig wasps than their much longer-lived hosts, generating diversification within a fig wasp lineage that is already associated with a particular host in the absence of host diversification (Souto-Vilarós et al., 2019). This pollinator diversification pattern is seen when sister-species share a host. In *F. hirta*, a fig tree with several associated pollinators, one apparent example of the colonization scenario was detected, but the majority of the pollinators represent sister-species (Yu et al., 2019).

Molecular data is required to confirm whether the three fig wasps that we identified in association with *F. hispida* can be considered as sister species, but their specific status, rather than being considered as subspecies, is supported by clear morphological differences between adult females and also unpublished molecular data (X. Deng et al., unpublished). They were described from different countries, but the extent of overlap in their distributions (if any) is unclear, because the sampling density from this and other Asian fig trees remains low. Further samples, especially from elsewhere in the wide range of *F. hispida*, may also detect further associated fig wasps in addition to the three species already detected.

The three species known to be associated with *F. hispida* possess functional pollen pockets and are therefore likely to be active pollinators. *C. abscondus* and *C. solmsi* share a dark body coloration, indicating that they are likely to disperse between trees in daylight, whereas the paler colouration of *C. marchali* suggests

that it may fly at night. Other biological differences between the three species remain unknown, especially whether the species vary in their effectiveness as pollinators.

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AUTHOR CONTRIBUTION STATEMENT

W.D.M. and Y.H.; Data curation, W.D.M. and Y.H.; Formal analysis, W.D.M. and F.S.L.; Methodology, W.D.M. and Y.H.; Validation, W.D.M., F.S.L. and Y.H.; Writing—original draft, W.D.M.; Writing—review and editing, W.D.M., F.S.L. and Y.H. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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mtCO1 ANALYSIS OF THE BUTTERFLY GENUS *PAPILIO*, FROM INDONESIA

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ABSTRACT

The genus *Papilio* is a butterfly that has many subspecies with similar phenotypes. So until now, researchers are still studying the genus *Papilio* based on morphology and genetics. Partial sequences of mitochondrial cytochrome oxidase-I (mtCOI) genes from individuals from 13 species of Papilionidae from North Sulawesi: Indonesia, Papua New Guinea, Malaysia: Negeri Sembilan, India: Assam, South Korea, China, Taiwan: Nantou, and Thailand: Fang, Chiang Mai have been compared in this study. Nucleotide divergences showed that the mean genetic distance between species was 0.023 (2.3%) to 0.723 (72.32%). Phylogenetic analysis revealed that groups of Papilionidae from North Sulawesi: Indonesia formed their own group; Species from Papua New Guinea are closely related to other Papilionidae species from other regions. There are 50 distinct sites or single nucleotide polymorphism (SNP) in the mtCOI sequence of eleven species. These mtCOI sequences can reveal the genetic differentiation of closely related species in the genus *Papilio*.

Key words: Butterfly *Papilio* spp., cytochrome C oxidase-I, North Sulawesi, sequences nucleotide, mtCOI variations, comparisons, phylogenetic analyses, NCB accessions

Butterflies are a group of insects that can be found in tropical and subtropical areas. Butterfly species are insects that have various morphological and colour characteristics that may vary due to environmental habits and geographic location (Simonsen et al., 2012). Due to these butterflies can be used as bioindicators of changes in environmental quality (Basset et al., 2012). The presence of butterfly populations in a habitat ecosystem has begun to decline due to various threats such as uncontrolled exploitation and hunting, as well as land clearing and forest conversion (Basset et al., 2012). Koneri and Maabuat (2016) state that butterfly diversity is very influential in their habitat. Taxonomically, butterflies are included in the order Lepidoptera. Butterflies have an important role in the ecosystem, namely as pollinators of flowers or pollinators (Duara et al., 2014). The classification of insect species is very important for basic and applied research. Classification based on morphological characteristics creates problems for many insect groups due to their small size, and morphological shapes that change over time (Akhilesh and Sebastian, 2014; Tsao and Yeh, 2008). The Papilionidae family, commonly known as swallowtail butterflies, is large and has a variety of colours. This species can be found in tropical regions and can be found on almost every continent. There are an estimated

550 species in this family. In the 1990s, studies of this type of butterfly began to focus on morphological and molecular systematics (Simonsen et al., 2011). Some morphological evidence in the subfamily Baroniinae, Parnassinae and Papilionidae shows no specific differences. (Vane-Wright, 2003). Based on the cladistic method, Kristensen (1976) found that the Papilionidae family has close ties with several family groups such as Pieridae, Nymphalidae Lycaenidae, and Riodinidae.

This genus *Papilio* contains many cryptic species where phenotypic and evolutionary variability has led to misidentification. Apart from that the dimorphism also causes confusion (von-Maria et al., 2011). Taxon identification based on DNA sequences has begun to be used to facilitate identification and even discover new species (Blaxter, 2005; Hebert et al., 2003a, b). DNA sequences from the mitochondrial cytochrome oxidase 1 (mtCO1) gene can serve to identify and analyze the phylogeny (Hebert et al., 2003a, 2004b; Ward et al., 2005; Huang and Ke, 2015), especially the cryptic or similar species in the tropics (Wilcox et al., 1997; Berkov, 2002; Hebert et al. 2004b; Monaghan et al., 2005; Hajibabaei et al. 2006; Bachry et al. 2019) and insects in different places and at various growth stages (Janzen et al., 2005). In Indonesia, substantial studies

on butterfly systematics, morphology, and ecology have been carried out (Tallei et al., 2015; Koneri and Maabuat, 2016; Koneri et al., 2019; Koneri and Nangoy, 2019; Koneri et al., 2020). However, the study of the composition and genetic differences of *Papilio* spp. is still underreported. This study analyzes the mtCOI sequence composition of the *Papilio* spp. from various regions.

MATERIALS AND METHODS

Three sequences were found in this study, namely *P. gigon* from the Talaud archipelago, *P. rumanzovia*, and *P. polytes* from the Sangihe Islands, Indonesia (Table 1; Fig. 1). Each of these samples was analyzed at the Genetics Laboratory, Department of Biology, FMIPA, Sam Ratulangi University. Furthermore, ten sequences of *Papilio* spp. from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) were also used for comparison (Table 1). Extraction of DNA from tissue samples was performed using a commercial kit from Dneasy®

Blood and Tissue Kit no 69504 (50) (Qiagen, Germany) following the manufacturer instructions with some modification (the sample was first crushed, then dried). The target gene was amplified using the polymerase chain reaction using universal primer set LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACC AAAAAATCA-3') (Folmer et al. 1994), producing a 658 bp partial sequence. The total volume of PCR reaction was 25 µl, consisting of 10.8 µl of ddH₂O, 4 µl of Q5 buffer, 5 µl of Q5 enhancer, 1 µl of dNTP, 1 µl of forward primer, 1 µl of reverse primer, 2 µl of DNA template, and 0.2 µ of Taq Hot Start Q5. COI amplification began with an initial pre-denaturation at 94°C for 5 min, followed by denaturation for 35 cycles at 94°C for 30 s, annealing at 54°C for 45 s, elongation at 72°C for 30 s, and a final cycle of extension at 72°C for 5 min. PCR products were purified by electrophoresis through a 1.2% agarose gel using a 1 × TBE (Tris-borate-EDTA acid) buffer. A single band of PCR product was sequenced by a sequencing service

Table 1. Papilionide specimens collected and Genbank accession numbers

Species	Locality (for specimens sequenced in this study)	Specimen ID	Code access NCBI
<i>Papilio gigon</i>	Indonesia; Island Talaud	RK3	MW167784
<i>Papilio rumanzovia</i>	Indonesia; Island Sangihe	RK4	MW167785
<i>Papilio polytes</i>	Indonesia; Island Sangihe	RK5	MW168164
<i>Papilio ulysses</i>	Papua New Guinea	USNM:ENT:00645977	HQ570589
<i>Papilio ambrax</i>	Papua New Guinea	USNM:ENT:00645984	HQ570594
<i>Papilio aegaeus</i>	Papua New Guinea	USNM:ENT:00645996	HQ570605
<i>Papilio euchenor</i>	Papua New Guinea	USNM:ENT:00645995	HQ570604
<i>Papilio demoleus malayanus</i>	Malaysia: Negeri Sembilan	UMKL:JJW0037	KF226558
<i>Papilio bianor gladiator</i>	India: Assam	CBGPFLC_00008	JQ982045
<i>Papilio macilentus</i>	South Korea	SWC-09-5007	GU696025
<i>Papilio noblei</i>	China	FD22	JF747532
<i>Papilio hopponis</i>	Taiwan: Nantou	CBGPFLC_00049	JQ982076
<i>Papilio arcturus</i>	Thailand: Fang, Chiang Mai	CBGPFLC_00003	JQ982040



Fig. 1. Map of the study area in Sangihe Islands and Talaud Islands, North Sulawesi, Indonesia (Google Maps, 2021)

(1st BASE www.base-asia.com) Malaysia. Sequence divergence among species and genera was calculated using the Kimura two-parameter distance model in MEGA 7.0 (Kimura et al., 2016). The Neighbor-Joining method (Saitou and Nei, 1987) was used to reconstruct the phylogenetic tree based on K2P from MEGA7.0 (Kimura et al., 2016). Statistical support for the internodes in the phylogenetic tree was tested by bootstrap % with 1000 replicates (Felsenstein, 1985). Sequence analysis and sample identification were done by inputting the trimmed sequence in NCBI's BLAST tool. A phylogenetic tree was then constructed using MEGA7 software (Kimura et al., 2016).

RESULTS AND DISCUSSION

The result of mtCO1 gene analyses revealed sequence length of 658 bp found at 274 bp (41.64%) conservative sites, 384 bp (58.35%) varied sites, 334 bp (50.75%) parsimony sites, and 50 bp (7.59%) singleton sites (Table 2). The nucleotide composition of the from 13 species showed a higher % of Thymine (T) (38.7%) followed by adenine (A) (32.0%), cytosine (C) (14.9%), and the lowest was guanine (G) (14.4 %). The composition of nucleotides A + T was 70.7% and G + C was 29.3%. Total 50 single nucleotide polymorphism (SNP) for mtCO1 gene sequences were found from 13 species (two species without SNP) of *Papilio* (Table 3). *P. euchenor* had the most SNP (10), compared to other species and *P. Bianor gladiator*

(2 SNP); *P. gigon*, *P. polytes*, and *P. Rumanzovia* (6, 8, and 9 SNP, respectively); *P. ulysses*, *P. ambrax*, *P. demoleus malayanus*, and *P. noblei* (3 SNP) *P. aegeus* and *P. macilentus* (4 SNP). Based on genetic distance analysis with a 2-parameter Kimura model of the interspecies *Papilio* spp. shows the mean value of the genetic distance from 0.023% to 0.723% (Table 4). The difference in genetic distance between *Papilio* spp. is high enough. Based on the phylogenetic tree using the neighbor-joining (NJ) method, it shows three clusters. Black show group I consists of *P. gigon* (MW167784), *P. rumanzovia* (MW167785), and *P. polytes* (MW168164) from North Sulawesi, Indonesia. Red show group II consists of *P. macilentus* (GU696025) from South Korea, *P. ambrax* (HQ570594), and *P. aegeus* (HQ570605) from Papua New Guinea. Green show group III consists of *P. hopponis* (JQ982076) from Taiwan: Nantou, *P. arcturus* (JQ982040) from Thailand: Fang, Chiang Mai, *P. bianor gladiator* (JQ982045) from India: Assam, *P. ulysses* (HQ570589), *P. euchenor* (HQ570604) from Papua New Guinea, *P. demoleus malayanus* (KF226558) from Malaysia: Negeri Sembilan, and *P. noblei* (JF747532) from China.

Papilio spp. from northern Indonesia, showing a single monophyletic group without overlapping with *Papilio* spp. from the Asia Pacific region (Fig. 2). Sekimura et al, (2017) revealed that the phylogenetic relationship of the genus *Papilio* from Japan and the genus *Papilio* from Africa did not show a close

Table 2. Characteristics of the CO1 gene nucleotides of *Papilio* spp.

No.	Species/ access code	Conserve sites	Variables		Total of variable sites-	Base composition (%)			
			Pi	S		T	C	A	G
1	<i>Papilio gigon</i> (MW167784)	274	334	50	384	32.1	13.1	38.1	16.7
2	<i>P. rumanzovia</i> (MW167785)	274	334	50	384	31.9	13.4	37.8	16.9
3	<i>P. polytes</i> (MW168164)	274	334	50	384	31.9	13.5	38.6	16.0
4	<i>P. ulysses</i> (HQ570589)	274	334	50	384	40.3	14.9	31.0	13.8
5	<i>P. ambrax</i> (HQ570594)	274	334	50	384	41.2	14.7	30.2	13.8
6	<i>P. aegeus</i> (HQ570605)	274	334	50	384	39.7	16.1	30.7	13.5
7	<i>P. euchenor</i> (HQ570604)	274	334	50	384	41.5	15.7	28.9	14.0
8	<i>P. demoleus malayanus</i> (KF226558)	274	334	50	384	40.4	15.8	30.2	13.5
9	<i>P. bianor gladiator</i> (JQ982045)	274	334	50	384	41.0	14.9	30.2	13.8
10	<i>P. macilentus</i> (GU696025)	274	334	50	384	40.3	15.5	30.4	13.8
11	<i>P. noblei</i> (JF747532)	274	334	50	384	41.1	15.4	29.8	13.7
12	<i>P. hopponis</i> (JQ982076)	274	334	50	384	41.0	15.0	30.2	13.7
13	<i>P. arcturus</i> (JQ982040)	274	334	50	384	40.4	15.5	30.2	13.8
Avg.						38.7	14.9	32.0	14.4

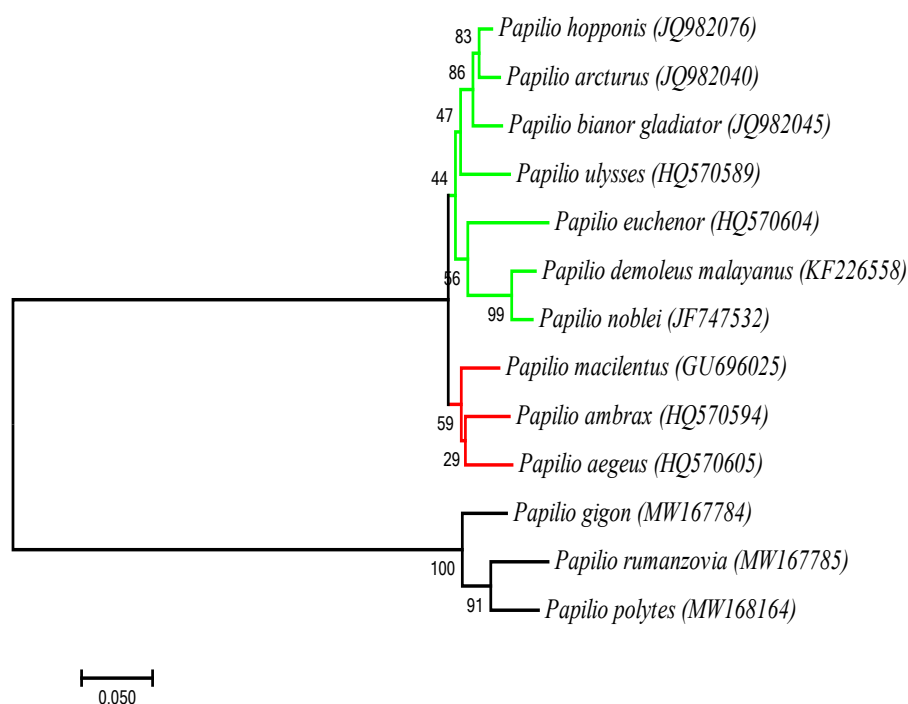
Notes: Avg = Average; Pi= parsimony informative site; s= singleton site; A= Adenine; T= Thymine; G= Guanine; C= Cytosine.

Table 3. Singel nucleotide polymorphisme (SNPn)

Species /code access	Nucleotide base site															
	1	4	6	7	9	1	1	1	1	1	1	2	2	2	2	2
	1	0	7	9	5	4	6	9	9	4	6	7	9	1	1	5
<i>Papilio gigon</i> (MW167784)	A	A	A	A	A	A	A	T	T	T	A	A	A	A	G	A
<i>P. rumanzovia</i> (MW167785)	.	.	.	G	G	.	.	A	.	G	.	.	.	.	T	.
<i>P. polytes</i> (MW168164)	.	.	G	.	.	.	G	A	.	.	.	.	T	.	.	.
<i>P. ulysses</i> (HQ570589)	.	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.
<i>P. ambrax</i> (HQ570594)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
<i>P. aegeus</i> (HQ570605)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.
<i>P. euchenor</i> (HQ570604)	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.
<i>P. demoleus malayanus</i> (KF226558)	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
<i>P. bianor gladiator</i> (JQ982045)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	C
<i>P. macilentus</i> (GU696025)	.	G	.	.	.	.	.	A	C	.	G	.	.	.	.	.
<i>P. noblei</i> (JF747532)	C	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.
<i>P. hopponis</i> (JQ982076)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
<i>P. arcturus</i> (JQ982040)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Continue...																
Species /code access	Nucleotide base site															
	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4
	1	1	2	3	5	6	9	2	3	3	4	5	5	6	6	7
<i>Papilio gigon</i> (MW167784)	3	7	3	9	5	2	2	7	3	6	2	1	4	3	7	2
<i>Papilio gigon</i> (MW167784)	T	G	A	A	T	G	A	T	A	G	T	A	A	T	C	T
<i>P. rumanzovia</i> (MW167785)	.	.	G	.	.	.	C	.	.	A	G	.	.	.	.	.
<i>P. polytes</i> (MW168164)	A	.	.	.	.	.	T	.	.	A	A	T	T	.	.	.
<i>P. ulysses</i> (HQ570589)	.	.	.	.	A	.	.	.	.	A	.	.	.	.	.	.
<i>P. ambrax</i> (HQ570594)	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	C
<i>P. aegeus</i> (HQ570605)	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.
<i>P. euchenor</i> (HQ570604)	.	.	.	.	.	.	.	C	G	T	.	.	.	.	C	.
<i>P. demoleus malayanus</i> (KF226558)	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.
<i>P. bianor gladiator</i> (JQ982045)	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.
<i>P. macilentus</i> (GU696025)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	.
<i>P. noblei</i> (JF747532)	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.
<i>P. hopponis</i> (JQ982076)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
<i>P. arcturus</i> (JQ982040)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
Continue...																
Species /code access	Nucleotide base site															
	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6
	9	0	1	2	2	3	4	5	6	7	8	0	1	3	4	4
<i>Papilio gigon</i> (MW167784)	6	6	9	0	9	1	2	0	5	7	3	1	9	2	0	1
<i>Papilio gigon</i> (MW167784)	G	G	G	A	T	A	C	A	A	G	G	T	T	G	T	T
<i>P. rumanzovia</i> (MW167785)	A	.	T	.	.	T	.	.	.	A	A	.	.	A	.	.
<i>P. polytes</i> (MW168164)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
<i>P. ulysses</i> (HQ570589)	A	.	.	.	.	T	.	.	T	A	A	.	.	.	.	.
<i>P. ambrax</i> (HQ570594)	A	.	.	T	.	T	.	.	.	A	A	.	.	.	.	.
<i>P. aegeus</i> (HQ570605)	A	.	.	.	.	T	.	.	.	A	A	C	C	.	.	.
<i>P. euchenor</i> (HQ570604)	A	A	.	.	C	T	T	T	.	A	A	.	.	.	.	C
<i>P. demoleus malayanus</i> (KF226558)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	C	.
<i>P. bianor gladiator</i> (JQ982045)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
<i>P. macilentus</i> (GU696025)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
Continue...																
Species /code access	Nucleotide base site															
	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6
	9	0	1	2	2	3	4	5	6	7	8	0	1	3	4	4
<i>P. noblei</i> (JF747532)	6	6	9	0	9	1	2	0	5	7	3	1	9	2	0	1
<i>P. noblei</i> (JF747532)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
<i>P. hopponis</i> (JQ982076)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
<i>P. arcturus</i> (JQ982040)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.

Table 4. Pairwise distance between of *Papilio* spp.

Sample code	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Papilio gigon</i> (MW167784)													
<i>P. rumanzovia</i> (MW167785)	0.096												
<i>P. polytes</i> (MW168164)	0.081	0.074											
<i>P. ulysses</i> (HQ570589)	0.713	0.740	0.724										
<i>P. ambrax</i> (HQ570594)	0.701	0.741	0.732	0.079									
<i>P. aegaeus</i> (HQ570605)	0.702	0.733	0.721	0.086	0.064								
<i>P. euchenor</i> (HQ570604)	0.723	0.754	0.754	0.096	0.110	0.114							
<i>P. demoleus malayanus</i> (KF226558)	0.702	0.734	0.729	0.103	0.098	0.098	0.105						
<i>P. bianor gladiator</i> (JQ982045)	0.699	0.713	0.717	0.062	0.079	0.077	0.086	0.098					
<i>P. macilentus</i> (GU696025)	0.681	0.716	0.704	0.077	0.059	0.064	0.125	0.107	0.084				
<i>P. noblei</i> (JF747532)	0.706	0.738	0.737	0.101	0.100	0.094	0.101	0.031	0.091	0.103			
<i>P. hopponis</i> (JQ982076)	0.678	0.697	0.701	0.057	0.075	0.077	0.092	0.084	0.031	0.070	0.082		
<i>P. arcturus</i> (JQ982040)	0.695	0.725	0.713	0.064	0.074	0.072	0.092	0.093	0.041	0.079	0.089	0.023	

Fig. 2. Phylogeny tree construction of *Papilio* spp. based on a 2-parameter Kimura model with a 1000-loop bootstrap. Black show group I, Red show group II, and Green show group III

relationship. The mtCO1 gene is highly effective in distinguishing closely related and morphologically similar species (Hebert et al., 2004b). In addition, phylogenetic results also prove that *Papilio* spp. from Talaud Island reveal that the origin of the island of Sangihe is closely related to form a subgroup. Akhilesh and Sebastian (2016) reported that between and intraspecies *P. polytes* from the Calicut region, Kerala, India, have shown genetic divergence based on the mtCO1 gene. *Papilio* spp. from the territory of Indonesia formed a monophyletic group. *P. euchenor* (HQ570604) had the highest SNP site, and the lowest SNP site was *P. bianor gladiator* (JQ982045). Meanwhile, three types of species originating from the territory of Indonesia showed a relatively high genetic variation. The divergences between the *Papilio* subspecies from Taiwan, Hong Kong, and China based on the mtCO1 sequence were low (Tsao and Yeh, 2008). These nucleotide variations provide evidence that groups of species originating from different regions have specific nucleotide site markers. SNPs of the mtCO1 gene found in *Papilio* spp. can be used to differentiate species. Hebert et al. (2004a) revealed that the mtDNA CO1 gene sequence variation in the 648 bp area could be a barcode for animal species. Waugh (2007) stated that each species has a specific nucleotide sequence for the mtCO1 gene. Vandewoestijne et al. (2004) revealed no differences in mtCO1 in the subspecies *Aglaia urticae* (Lepidoptera: Nymphalidae) from the entire Palaearctic region.

The results of the analysis of the genetic distance in *Papilio* spp. ranged from 0.023 (2.3%) to 0.723 (72.32%) (Table 4). Differences in the genetic distance in *Papilio* spp. from northern Indonesia with *Papilio* spp. in other Asia Pacific regions showed a relatively significant difference. Tsao and Yeh (2008) found differences in genetic distance between swallowtail butterfly subspecies of 1.7 to 11.6% and between genera within the same family from 6.7 to 17%. Geographical distance affects the genetic distance between species. Where gene flow in isolated species will change along with environmental conditions. The factor of geographical area differences causes these species to adapt to the environment, such as habitat, temperature, and type of feed. *Papilio* spp. widespread in Africa and Madagascar show divergences ranging from 0% -1.2% (Zakharov et al., 2004). Brower and Jeansonne (2004) reported that the difference in mtCO1 between Nymphalidae populations in the *Danaus plexippus* species from North and South America was <0.8%. *Papilio* spp. group from North Sulawesi, Indonesia shows its own group. The interspecies genetic distance

is quite high. There is a single nucleotide polymorphism which is a feature of the *Papilio* spp. from each region. The nucleotide composition shows a higher % of A + T than G + C.

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AUTHOR CONTRIBUTION STATEMENT

All authors, Roni Koneri, Syamsul Bachry and Beivy Jonathan Kolondam: our article is original from research result.

CONFLICT OF INTEREST

No conflict of interest.

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OVERVIEW OF PREDATORS IN SHALLOTS PLANTATION IN PEATLAND, LANDASAN ULIN SOUTH BORNEO

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ABSTRACT

Species abundance and diversity of predatory arthropods are important information for developing biological pest control strategies. A study that aims to determine the abundance of predators (Coccinellidae and Araneae) in peatlands planted with shallots in Tegal Arum Village, Landasan Ulin District, Banjarbaru City, South Borneo has been carried out. The method used is purposive sampling method. Selecting plots with uniform growth and plant height of shallots in one plot or beds measuring 3 x 20 m and six plots were observed using three methods: direct sampling, swing nets, and pitfall traps. Observations on species abundance of spiders and predatory coccinellids were carried out to describe the number of species and the abundance of insects in shallot cultivation on peatlands. Predators from the order Coleoptera of the Coccinellidae family observed belong to (*Menochilus sexmaculatus*, *Micrapsis* sp, *Coccinella novemnotata* and *Scymnus* sp. with 143 individuals and the highest population was found by *Micrapsis* sp (128 individuals). Meanwhile, there were 6 species of spiders (Araneae) from 5 different families (Oxyopidae, Tetragnatidae, Lynphiidae, Thomisidae, and Lycosidae) with the most individuals being *Oxyopes variabilis* (Araneidae: Oxyopidae). The distribution index of species diversity (E) shows $E > 0.6$, indicating that the evenness of species on the land is uneven

Key words: Abundance, araneae, coccinellidae, coleoptera, diversity, families, peatland, predator, shallots, species

Plant pest population management is carried out by paying attention to the agro-ecosystem. Especially the biotic factors which consist of insect pests, natural enemies, both predators, and parasitoids (Chakravarthy et al., 2016). The approach to studying the structure of the agroecosystem is to study the biodiversity of the cultivated land, in this research the land used is peatland for shallot cultivation in South Kalimantan. Coleoptera predators are biological control agents that are found in various types of agricultural land. This insect has a large number of species with a cosmopolitan distribution (Efendi et al., 2016). These Coccinellidae is a well-known beetle family, consist of seven families, i.e. Epilachninae, Coccinellidae, Chilocorinae, Coccidulinae, Ortaliinae, Scymninae, and Sticholotidinae (Pope, 1988). Except for the mycophagous Coccinellinae (Halyziini and Tythaspis) and the phytophagous Epilachninae, all remaining coccinellids are predators of hemipteran insects from the suborder Sternorrhyncha (e.g. aphids, scales, psyllids and whiteflies), mites eventually other insect larvae and insect eggs (Amir, 2002; Dixon, 2000; Damayanthi, 2016). Fiaboe et al., (2007) stated that as many as 5200 species of predatory Coccinellidae have been identified. Foltz (2002) estimates that there are 5000 species of

predatory Coccinellidae worldwide, while according to Vandenberg (2009); Kundoo and Khan (2017) predatory Coccinellidae is 6000 species and are found in mountainous areas, agricultural areas, coastal areas to urban areas and 90% predatory. Coccinellidae has fairly high diversity, it is estimated that there are 5000 species worldwide, while in Indonesia it is estimated that more than 300 species are widespread (Nelly et al., 2015). Many types of predatory Coccinellidae in Indonesia have great potential in controlling the population of various types of plant pests (Aprila et al., 2019). The Coleoptera represent one of the most abundant animal groups (Sushko, 2018) and important natural enemies of many insect pests in terrestrial ecosystems such as peatlands. They are important members of food webs, providing protein for species at higher trophic levels (Sushko 2012; Sushko 2017). In Soraya's research (2016), nine species of predatory Coccinellidae were found in eggplant plantations, i.e. *Epilachna vigintioctopunctata*, *E. borealis*, *Illeis flava*, *Harmonia consformis* and *H. testudinaria*. Meanwhile, Efendi et al. (2016) found ten predatory Coccinellidae viz., *Chilocorus melanophthalmus*, *Coelophora maculata*, *C. inaequalis*, *C. reniplagiata*, *C. bisellata*, *Ropalonedea decussate*, *Verania discolor*. In eggplant and chili

three species were found *Coccinella transversalis*, *Menochilus sexmaculatus*, and *Verania lineata*. This study explores abundance of Coccinellidae predators and spiders as natural enemies.

MATERIALS AND METHODS

This research was conducted in Tegal Arum Village (-3°25'44" S, 11°44'69" E, 10.8 m, 284°), Landasan Ulin District, Banjarbaru City, South Borneo, Indonesia. The research was conducted from August to October 2020. Species diversity was analyzed using the Shannon-Wiener index (H'), the Simpson Domination Index (D), and Evenness (E) using the Magurran handbook (Magurran 1988). If the value of $H' < 1$ then the community is less stable, if the value of H' is between 1-2 then the community is stable, and if the value of $H' > 2$ is said to be very stable (Kent and Paddy, 1992). The criteria for the level diversity based on this index are high if the value of $H' > 3.5$, moderate if $H' = 1.5 - 3.5$, and low if $H' < 1.5$. Odum (1993) stated that the criteria for dominance if the C value approaches 0 (< 0.5), then no species dominate and If the C value approaches 1 (≥ 0.5), then there is a dominant species. Observations of Predator. Using purposive sampling method, by selecting plots with uniform growth and plant height of shallots in one plot or beds measuring 3 x 20 m. The observed 6 plots. Observations were made using three methods: direct sampling, sweeping nets, and pitfall traps. The determination of the point for trapping is done deliberately. 3 traps were placed in the front, middle and back of the plot with a distance of 5 meters between the traps, placed in the center of the plot. Observations were made at the age of 0, 28, 42, 56 dd. Installation of pitfall traps for 24 hrs, at the same time as other observations. Sampling was done by direct collection. Hand picking on the sample plots. Sweeping net. Insect sweeping nets were used with 10 double swings across the plot area vertically and three captures were made for each observation. Pitfall trap were used to observe ground-level arthropods made of plastic cups with a diameter of 50 mm and a depth of 100 mm. This trap was filled with a solution of water and four drops of liquid detergent (10:1). This trap is filled with a solution of water and four drops of liquid detergent (10:1), the solution being filled with only one third of the total volume. Then placed in a hole that has been provided according to the size of the plastic cup, placed for 24 hours on the ground. The traps are cleaned and bottled for identification. Predator identification was carried out by observing insect specimens, especially the Coccinellidae, Coleoptera and spiders

(Family Araneidae, Araneae). Predatory Coccinellidae specimens obtained in the field were identified to species level by matching the specimens with pictures and descriptions from the identification key book. Identification of specimens using morphological characteristics of the wings, antennae, and thorax. Predatory Coccinellidae specimens obtained in the field were identified to species level using the identification key of Khan et al. (2006), Stephens and Losey (2004), Kalshoven (1981), McAlpine, et al. (1987), and Barrion and Litsinger (1995). To identify spiders using Wegner, (2009), Jocqué and Anna (2007), and Heisswolf, S., et al. (2010). Species composition data and the number of individual predators of Coccinellidae and spiders were used to analyze abundance and evenness. The measure of abundance used is the value of the Shannon-Wiener species diversity index (H') and Evenness (E) using Magurran's (1988) book.

RESULTS AND DISCUSSION

The land for shallot cultivation in the Tegal Arum is a peatland that has just been cleared and is starting to be planted with Citrus, sugar cane, chilies, and shallots. Based on the results of observations that have been made, 217 individual predators were collected. The results showed that the predators of the order Coleoptera family Coccinellidae were 4 species with 143 individuals and the highest population was found by *Micrapsis* sp (128). Meanwhile, there were 6 species of spiders with the most individuals being *Oxyopes variabilis* (22) Araneidae: Oxyopidae and the lowest was *Lycosidae* sp (Araneidae: Lycosidae) with 4 individuals (Table 1).

Predators from coccinellidae have more individuals than Aranea but the number of Aranea taxa are Coccinellids function in complex food webs as predators, as non-prey food consumers, and as prey or host of natural enemies. In the life stage of the coccinellids, the eggs are particularly susceptible to predation, and the coccinellids are behaviorally adapted to reduce egg predation from heterospecific predators (Weber and Jonathan, 2009).

Several papers have discussed the positive and negative effects of these two predators in new environments (Camacho-Cervantes et al., 2017; Kenis et al., 2017; Koch and Costamagna, 2017; Riddick, 2017). The second interaction affects community structure and predator-prey dynamics in various ecosystems, including agroecosystems. Thus, the expansion of the range of these predatory species has both negative and

Table 1. Diversity and abundance of predators in shallot peatland agroecosystems
(Tegal Arum Village, Landasan Ulin District, Banjarbaru City)

No.	Taxon	Ordo	Family	Number of insects caught				Total
				Plant age				
				0	28	42	56	
1	<i>Menochilus sexmaculatus</i>	Coleoptera	Coccinellidae	8	1	0	0	9
2	<i>Micrapsis</i> sp	Coleoptera	Coccinellidae	43	45	30	10	128
3	<i>Coccinella novemnotata</i>	Coleoptera	Coccinellidae	2	1	0	0	3
4	<i>Scymnus</i> sp	Coleoptera	Coccinellidae	1	1	1	0	3
								143
1	<i>Oxyopes variabilis</i>	Araneidae	Oxyopidae	4	9	4	5	22
2	<i>Oxyopes</i> sp	Araneidae	Oxyopidae	3	2	0	0	5
3	<i>Tetragnatha</i> sp	Araneidae	Tetragnathidae	4	2	4	1	11
4	<i>Linyphiidae</i> sp	Araneidae	Lyniphiidae	10	6	2	0	18
5	<i>Vitia misumena</i>	Araneidae	Thomisidae	5	6	1	2	14
6	<i>Lycosidae</i> sp	Araneidae	Lycosidae	0	2	2	0	4
								74
Number of taxa				9	10	7	4	10
Total				80	75	44	18	217

positive ecological effects on biodiversity and levels of biological control. Although many studies examine the spatial and temporal patterns among predatory coccinellidae and predatory aranaea. However, the mechanisms involved in the coexistence of this species are still limited (Hongran et al., 2021). *Micrapsis* sp had the highest individual among the others. It is suspected that this species is a species whose natural habitat is in peatlands. This is supported by research by Soedijo et al. (2012) and Samharinto et al. (2011). *Micrapsis* sp was found in large numbers from three research sites on peatland planted with rice in South Kalimantan. When viewed from the length of the life cycle, *Micrapsis* sp were higher than the males in longevity and predation rate. Longer than other species, which ranged from 1-2 weeks to develop from eggs to adults. Chaudhary et al. (2015) in their research stated that female *Menochilus sexmaculatus* (Fabricius) consumed higher than other Coccinellidae. However, Hillaing et al. (2017) stated otherwise, the predation rate of 1st instar grub was 37.5 ± 4.6 and 4th instar grub was 51.7 ± 9.8 . The male predation rate (51.1 ± 16.1) and female predation rate (57.8 ± 16.4) were observed under the same conditions. It is possible that this is the basis for this species to dominate in onion fields. In observations this type is found in grasses, shrubs. Meanwhile, *Menochilus sexmaculatus*, *Coccinella novemnotata* and *Scymnus* sp were not found. According to Puspasari et al. (2016), *Menochilus sexmaculatus* is more commonly found in peanuts, soybeans, beans, corn, eggplant and vegetables.

Coccinella novemnotata in chili plants attacked by *Bemisia tabaci* and *Scymnus* sp was more commonly found in plants infected with mealybugs (*Paracoccus marginatus*) (Tairas et al., 2015). In the research area, eggplant, chili were planted, while banjar siam oranges and guava were attacked by mealybugs. It is possible that the three predators apart from this *Micrapsis* sp, have already gotten their food from these plants, so they are rarely found in shallot plantations.

Spiders are common predators that can eat a wide variety of prey (Foelix, 2011) and are very effective in managing pests and reducing crop damage. Spiders are very abundant in agriculture and if conserved and the population is plentiful, they can control insect pests (Basnet and Mukhopadhyay, 2014). At the initial observation, in the uncultivated land found various types of leafhoppers and grasshoppers. It is suspected that this is a supporting factor why many types of spiders are found. The ability of spiders to prey on the main insect pests of brown planthoppers, their ability to prey on nymphs and imago ranges from 5-15 tails/day and can also become cannibals for fellow species or different species (Soedijo and Indar, 2015). It is also suspected that the clearing of land for shallot plantations causes spiders to lose their habitat so that their population also decreases and food decreases. Each spider species has a different active time and prey species, so it is very important to manage the diversity of spider species in an area (Wayan and Hery, 2013). So that the time

of observation in the field also affects the number of individual spiders caught in the observation.

The highest individual in Araneae is *Oxyopes* sp. ratter (lynx spider) and *Lycosidae* sp. (wolf spider) was the lowest among the Araneae. Lynx spiders are roving hunters commonly associated with the shrubby and grassy understory and are small and slender. This is in contrast to the larger wolf spiders and fast-moving ground hunters. The research of Memah et al. (2018) states, the preying ability of *Oxyopes* sp. on imago is the highest compared to others. The families Agelenidae, Araneidae, Tetragnathidae, and Thomisidae are spiders that are nocturnal or nocturnal. On the other hand, Lyniphiidae is a family of spiders that are active during the day or diurnal. Costello and Daane (2005) also found that most members of the Araneidae and Lycosidae are nocturnal, while all Salticidae are diurnal. *Oxyopidae* is more in the field because this species was active during the day and night or 24 hrs.

There is a tendency for the predatory population of Coccinellidae and Araneidae to decrease with the increasing age of shallot plants. Successively, at 0 days to 56 days after planting the population was 80, 75, 44, 18 predators. There was a decrease in species diversity and the number of individuals along with the age of the shallot plants. The land used in this study is peatland, where this land is formed from organic materials, such as leaves, stems, branches, and plant roots. Likewise, according to Sugiyarto (2007), organic matter which is difficult to decompose will function as a ground cover so that it can be used as a good source of energy for the insect community. Therefore, trophic composition of predator assemblage can play an important role in determining the nature of the relationship between predator diversity and ecosystem function (Finke and Denno, 2005). Organic matter has an important role to support plants and soil organisms so that if the soil organic matter content decreases, the soil's ability to support the productivity of plants and soil organisms also decreases. Naturally, the number of insects in this study predators Coccinellidae and spiders is more at the beginning of the observation.

The population of pest predators at the beginning of planting (0 days) was more diverse and the numbers were more numerous plus there were still weeds and bushes. On the land, grasses, shrubs and shrubs were found such as *Leersia hexadra*, *Hymenachne acutigulma*, *Brachiaria* sp. *Pennisetum purpureum*, *Panicum* sp, *Paspalum* sp. *Stenochlaena palustris*, *Chromolaena*

odorata, *Xyris indica* and *Melastoma malabathricum*. Wild plants or weeds are important agro-ecosystem components because they can positively influence the biology and dynamics of natural enemies (Altieri and Nicholls, 2004; Puspasari et al., 2016). This plant is an alternative host, a place to hide if predators or natural enemies experience bad environmental conditions (Van Emdem, 1991; Altieri and Nicholls, 2004). The cropping pattern used also affects the diversity and population of insects in a land. This study used a shallot monoculture pattern. According to Semiun and Stefanus (2016) and Melhanon and Dewi (2020), the diversity and abundance of monoculture farming systems of arthropods are lower than that of polyculture systems.

Besides, the growth phase of the shallot also affects the abundance of these two predators. This is supported by Kaleb et al. (2015) which states that the height and low level of individual insects as natural enemies of the crown and soil surface indicate that the availability of available food sources is closely related, i.e. conformity to the growth phase of plants that provide a source of food for the growth and development of natural enemy insects, the reduction of pests that have the potential to serve as a food source for natural enemy insects.

At the beginning of soil cultivation or 0 days, the diversity index showed a moderate and stable category (1.57), along with land cultivation and cleaning of weeds and planting into monocultures (shallots), the diversity index decreased to a low (<1.5) (Table 2). This is thought to be due to land clearing and weed clearing, loss of housing for various types of insects, both pests and predators. Many types of planthoppers (zig-zag planthoppers, white-backed planthoppers, brown leafhoppers, green leafhoppers, leafhoppers, white and yellow rice stem borers and grasshoppers were found before land clearing. All these are food for spiders and coccinelid beetles. Changes occur in the food web or trophic transfer. Yang et al. (2018) observed the effects of predator diversity on prey biomass and

Table 2. Diversity index, evenness index of predatory (Coccinellidae and Araneida species) in shallot crop ecosystems on peatlands in South Kalimantan

Observation (days)	Index value	
	Diversity (H')	Evenness (E)
0	1.57	0.71
28	1.43	0.62
42	1.15	0.59
56	1.09	0.78

trophic transfer efficiency (using the predator/prey biomass ratio). Higher prey diversity increases predator diversity and biomass, as well as trophic transfer efficiency, which may result from a more balanced diet and/or increased niche complementarity due to higher prey diversity. This effect of prey diversity affects predator-prey interactions in natural ecosystems. Weber and Jonathan (2009) and Wilby et al. (2005) stated that the effects of species diversity appear in natural enemy assemblages depending on the context; they depend not only on the characteristics of the predatory species but on the identity of the species they prey on. This effect of prey diversity affects predator-prey interactions in natural ecosystems.

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AUTHOR CONTRIBUTION STATEMENT

Salamiah and Helda conceived and designed research. Samharinto and Muhammad conducted experiments. Pramudi and Lyswiana contributed analyzed data. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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DETECTION AND PHYLOGENETIC ANALYSIS OF *WOLBACHIA* IN ONION THRIPS (*THRIPS TABACI* LINDEMAN)

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ABSTRACT

Wolbachia is a group of bacteria that is known to infect many arthropods and nematodes. It is one of the most common parasitic microbes and is possibly the most common reproductive parasite in the biosphere. The bacterium is best known for its ability to manipulate host reproductive biology where it can induce cytoplasmic incompatibility, parthenogenesis, feminization and male-killing. In the present study, onion thrips (*Thrips tabaci* Lindeman) were collected from 9 locations in India along with melon thrips (*Thrips palmi* Karny) and chilli thrips (*Scirtothrips dorsalis*). From the molecular level detection by using 16s rDNA, the *Wolbachia* infection has been detected in the onion thrips collected from 6 locations out of 9. Melon thrips and chilli thrips samples were also found to be infected by *Wolbachia*. The phylogenetic analysis revealed that all detected *Wolbachia* samples showed that all were distantly related to the previously known *Wolbachia* samples.

Key words: *Wolbachia pipientis*, *Thrips tabaci*, 16s rDNA, phylogeny, melon thrips, chilli thrips, reproductive parasite, *Allium cepa*, geographical distributions, male-killing

The *Wolbachia* is a group of bacteria that are known to infect a wide range of arthropods (Gomes et al. 2022). Earlier the infection of *Wolbachia* in insects was estimated to be approximately up to only 20% (Werren and Windsor, 2000; Jiggins et al., 2001), but the later progression in the study has shown that *Wolbachia* is very commonly known to infect a majority of arthropods (up to 70% of all insects) (Stouthamer et al., 1999). *Wolbachia* was first described in the year 1924 when M. Hertig and S. B. Wolbach observed certain bacteria in the cells of a mosquito (*Culex pipientis*) which were later named *Wolbachia pipientis* in 1936 by Hertig. The actual study of *Wolbachia* started gaining momentum when Yen and Barr (1971) discovered that due to the presence of *W. pipientis* in the maternal cytoplasm of mosquitoes, it is transmitted from the parent to the offspring. This association had caused cytoplasmic incompatibility in certain intraspecific crosses within *Culex* mosquitos which lead to the cause of the death of mosquito eggs when sperm of infected males fertilized the eggs of *Wolbachia*-free females. The major fascination of *Wolbachia* infection is that it is been thought to cause unusual manipulations in the reproductive system of its host organism by inducing parthenogenesis, cytoplasmic incompatibility, feminization of genetic males, and male-killing (Werren, 1997). The different

modes of reproduction in thrips are thelytoky (females produced from unfertilized eggs), arrhenotoky (males produced from unfertilized eggs and females produced from fertilized eggs) and deuterotoky (females and males produced from unfertilized eggs) (Nault et al., 2006). The infection of *Wolbachia* has been reported in both thelytokous and arrhenotokous populations of thrips species (Kumm and Moritz., 2008). In India, *Wolbachia* has been reported from *Thrips palmi* (Saurav et al., 2016), *Sciothrips cardamomi* (Jacob et al., 2015), *Plicothrips apicalis* (Ambika and Rajagopal, 2018) and onion thrips (*Thrips tabaci* Lindeman) (Gawande et al., 2019). Many species of thrips infest a variety of crops (Ullman et al., 2002). *T. tabaci* has been first described in 1888 by Lindeman. *T. tabaci* has become a global pest of increasing concern in onion (*Allium cepa* L.) (Diaz-Montano et al., 2011). Thrips ability to develop resistance to insecticides, ability to transmit plant pathogens and frequency of producing more generations at high temperatures makes it more concerning. *T. tabaci* feeds directly on leaves, causing blotches and premature senescence as well as distorted and undersized bulbs. The yield loss due to *T. tabaci* can go up to 50% and it can be even more problematic when it transmits Iris yellow spot virus (family *Bunyaviridae*, genus *Tospovirus*, IYSV) (Diaz-Montano et al., 2011).

T. tabaci feeding can reduce onion bulb weight (Kendall and Capinera 1987; Fournier et al., 1995; Rueda et al., 2007; Diaz-Montano et al., 2010). In thrips, *Wolbachia* was discovered by Pintureau et al. (1999) in *Heliothrips hemorrhoidalis* and *Hercinothrips femoralis* (Reuter), by Arakaki et al. (2001) in *Frankliniopsis vespiformis* (Crawford) and Cano-Calle et al. (2021) in *Frankliniella* sp. and *Scirtothrips hansonii* found in avocado (*Persea americana*). It has been observed that *Wolbachia* induce thelytokous reproduction in *F. vespiformis* (Arakaki et al., 2001).

To study the phylogenetic relationship between different *Wolbachia*, in various host organisms in which *Wolbachia* has been proven to infect, the 16S ribosomal DNA sequence is used since the initial times and is still being used even in recent times. Later Baldo et al., (2006) developed a standard Multilocus Sequence Typing (MLST) system for characterization of different *Wolbachia* strains. The MLST system uses a combination of five housekeeping conserved genes (*ftsZ*, *gatB*, *coxA*, *hcpA*, and *fbpA*) for more efficient detection of *Wolbachia* and analysis of its diversity in various hosts. Gawande et al. (2019) conducted a study on microbiome profiling of the *T. tabaci*, to study the bacterial communities associated with *T. tabaci* in India. It was first time the presence of the genus *Wolbachia* in *T. tabaci* was reported. Hence, to carry forward this study further, we tested the presence of *Wolbachia* infections in *T. tabaci* that were collected from several locations across the country. By using the molecular approach, particularly PCR amplification of the targeted 16S rRNA gene, the presence of *Wolbachia* was confirmed. Further, phylogenetic analysis was also conducted based on the sequences obtained by PCR amplification of the 16S rRNA gene.

MATERIALS AND METHODS

Onion thrips were obtained from nine locations across India; 1. Junagadh Agricultural University (JAU); 2. Indian Agricultural Research Institute (IARI), New Delhi; 3. CSK Himachal Pradesh Agricultural University, Palampur; 4. Punjab Agricultural University (PAU), Ludhiana; 5. Kotputli, Rajasthan; 6. Tamil Nadu Agricultural University, Tamil Nadu; 7. National Horticultural Research and Development Foundation (NHRDF), Karnal; 8. Srinagar; and 9. ICAR-DOGR, Rajgurunagar, Pune. In addition to that chilli thrips (*Scirtothrips dorsalis*) and melon thrips (*Thrips palmi* Karny) were collected from ICAR- Indian Agricultural Research Institute, Regional Station, Pune. The collected samples were preserved in absolute ethanol

and kept at 4°C until further use. Fifty individual thrips from a population (location-wise) were used for DNA isolation. Extraction of the genomic DNA was done by using the DNeasy Blood and Tissue Kit (Qiagen, cat. no. 69504) from thrips by following the manufacturer's protocol and the DNA was then stored at -20 °C until further use. In this study, PCR screening was done for testing the presence of *Wolbachia* infections in thrips. For this, we targeted the 16S rRNA gene (Forward primer 5' TTGTAGCCTGCTATGGTATAACT 3' and Reverse primer 5' GAATAGGTATGATTTTCATGT 3') which was amplified under the following conditions: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 7 min. The PCR products were electrophoresed on 1% agarose gels and stained by Ethidium Bromide. Thrips DNA yielding amplicons of the expected size (900bp) were scored as positive for *Wolbachia*.

Extraction of the PCR product from the agarose gel was performed using Mini Elute Gel Extraction Kit (Qiagen) by following the recommendations of the manufacturer. The eluted PCR product was further subjected to ligation reaction which was performed by using CloneJET PCR Cloning Kit (ThermoFisher) by following the manufacturer's protocol. After ligation of the PCR product with pJET 1.2/ blunt Cloning Vector (ThermoFisher), the ligated product was then transformed and cloned into DH10β competent *E. coli* cells. Antibiotic marker selection technique was used for screening and selection of positive colonies. Further, colony PCR was done for confirming the colonies that were containing the plasmid along with the desired sequence. Bacterial colonies containing the positive plasmid were transferred to Luria Bertani broth medium and were grown overnight. Finally, the plasmid DNA was isolated from an overnight bacterial culture by using the GeneJET™ Plasmid Miniprep Kit (ThermoFisher). This plasmid DNA was subjected to Sanger sequencing and the resulting sequences were compared with the sequence database at the National Centre for Biotechnology Information using BLAST program. 16S rRNA gene sequences (900bp) representative of *Wolbachia* strains from different host organisms were selected from the NCBI database (<https://www.ncbi.nlm.nih.gov>) and used to classify *Wolbachia* strains detected in our thrips samples. Sequence alignments were carried out using ClustalX 1.8344. Maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic analysis.

RESULTS AND DISCUSSION

Wolbachia has been first discovered by M Hertig and S B Wolbach from the reproductive tissues of mosquitoes. In thrips, *Wolbachia* was discovered by Pintureau et al. (1999) in *Hercinothrips*. Gawande et al. (2019) reported the presence of the genus *Wolbachia* for the first time in *T. tabaci*. In this study, the presence of *Wolbachia* infection in *T. tabaci* from different locations throughout India has been shown along with the phylogenetic analysis. Previous studies in insects and arthropods have shown a high level of *Wolbachia* infection ranging from 40 to 90% depending on their geographical distributions (Jeyaprakash and Hoy 2000; Chai et al., 2011). Recent microbiome profiling experiment on fungivorous thrips *Hoplothrips carpathicus* showed 69.95% *Wolbachia* population out of a total bacterial population (Kaczmarczyk et al., 2018). In the present study, PCR amplification reactions were carried out by *Wolbachia* 16S rDNA specific primers. The 16S rDNA gene amplified fragment of 900 bp was obtained on 1% Agarose gel (Fig. 1A). Out of nine locations (Fig. 2A), thrips from 6 locations (DOGR, Tamil Nadu, Karnal, Gujarat, Punjab, Himachal Pradesh) were found to have *Wolbachia* in them while remaining three were found to be negative for *Wolbachia* infection. In addition, *S. dorsalis* and *T. palmi* collected from Pune were also found to be positive for *Wolbachia* infection (Fig. 1B). The level of *Wolbachia* infection in *T. tabaci* was found to be 66%. In addition, *Wolbachia* infection was also found in *T. palmi* as previously reported by Saurav et al. (2016). The *S. dorsalis* samples collected from Pune were also found to be positive for *Wolbachia* infection. This is the first report of *Wolbachia* infection in *S. dorsalis*. In conclusion, the results showed high infection rates of *Wolbachia* in *T. tabaci* populations from different geographical locations in India.

The amplified fragments were sequenced by 16S rDNA specific primers and then compared with the 16S ribosomal RNA sequence of *W. pipientis* (Sequence ID: AF501664.1) that exists in the GenBank. As a result, the amplified sequences of 16S rDNA from DOGR, Karnal, Punjab, Gujarat, Tamil Nadu, TP 1 (*T. palmi*), ST 1 (*S. dorsalis*) showed 98.89, 99.44, 98.67, 99.55, 99.55, 97.88, and 97.27% nucleotide identity, respectively. While thrips samples collected from Himachal Pradesh 16S rDNA showed 98.66% nucleotide identity with *Wolbachia* sp. MSebKT1 gene for 16S ribosomal RNA (Sequence ID: AB795345.1). The obtained sequences of samples from all locations in India were submitted to NCBI database (GenBank accession number: OL702843, OL702844, OL702845, OL702846, OL702847, OL702848, OL702849, OL702850).

Phylogenetic analysis between the *Wolbachia* samples collected from a different locations in present study showed that the *Wolbachia* sample from ICAR-DOGR and *S. dorsalis* were closely related to each other (Fig. 2). Similarly, the *Wolbachia* sample from *T. palmi* showed homology with *T. palmi* (KM393213) sequence in GenBank. The rest of the *Wolbachia* samples showed to have a common ancestor but were distantly related. Phylogenetic analysis of *Wolbachia* sequence from present study along with *Wolbachia* from other hosts showed that samples from present study were distantly related to the ones which were previously discovered (Fig. 3). Moreover, *Wolbachia* samples from Tamil Nadu, Gujarat, Karnal, Himachal Pradesh Punjab, *T. palmi* from a clade, separating *S. dorsalis* and DOGR sample from them. The phylogenetic analysis showed that all the *Wolbachia* sequences from thrips collected from different locations in India showed no similarity with the previously known *Wolbachia* from other hosts. This indicated the horizontal transfer between

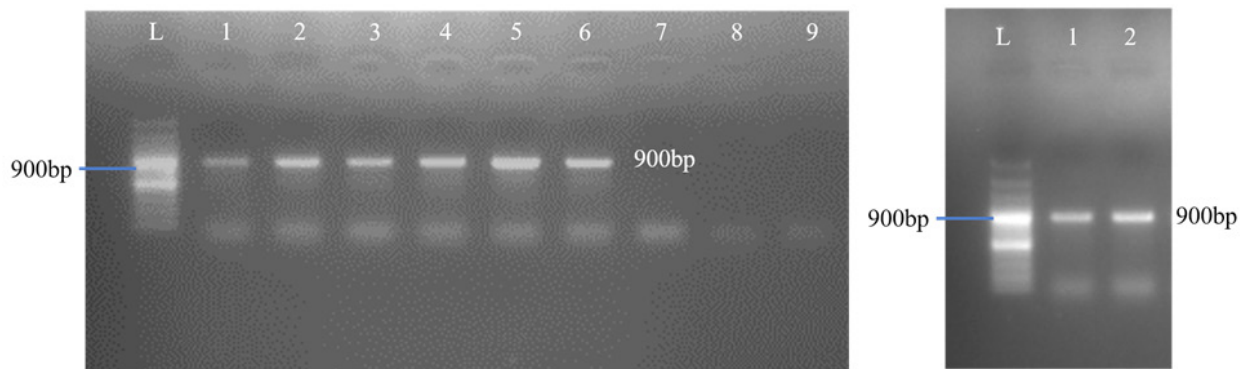


Fig. 1. Detection of *Wolbachia* via amplification of 16S rDNA gene fragment from thrips collected from locations in India. *Wolbachia* detection with forward and reverse primers for a fragment (900 bp) of the 16S rDNA gene. (A) L: 100bp Ladder, 1: DOGR, 2: Tamil Nadu, Lane 3: Karnal, Lane 4: Gujarat, Lane 5: Punjab, Lane 6: Himachal Pradesh, 7: Jammu Kashmir, 8: Rajasthan, 9: New Delhi, (B) L: 100bp Ladder, 1: *Thrips palmi* (melon thrips), 2: *Scirtothrips dorsalis* chilli thrips

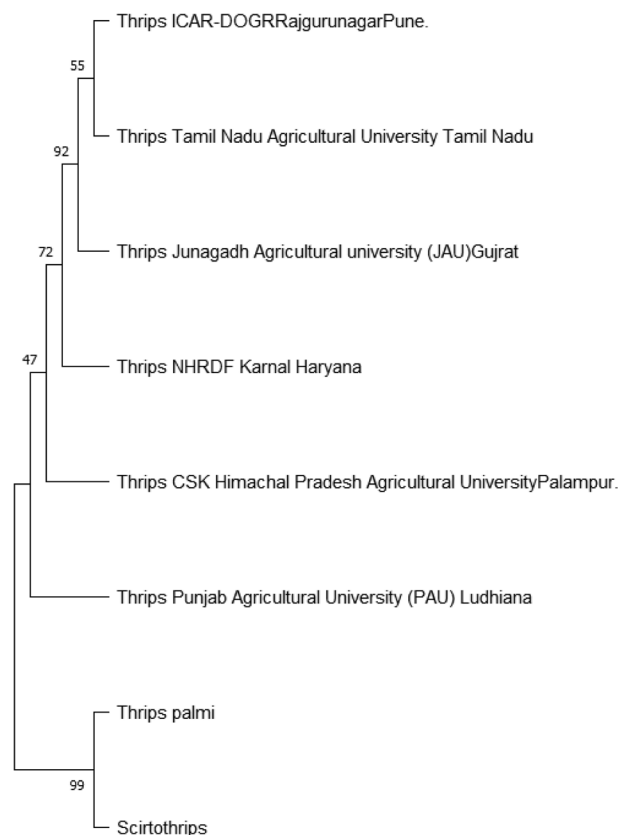


Fig. 2. The phylogenetic tree of *Wolbachia* were detected in onion, chilli and melon thrips based on their 16S rDNA sequences. Trees inferred from Maximum likelihood and Bayesian inference (BI) methods using MEGA 11.0 program

arthropods species as reported previously (O'Neill et al., 1992; Werren et al., 1995; Huigens et al., 2004; Klasson et al., 2009; White et al., 2017). For instance, closely related strains of *Wolbachia* are found in diverse hosts as flies, beetles, and wasps (Werren et al., 1995). Moreover, the horizontal transmission of *Wolbachia* has been proven to occur in spiders (Rowley et al., 2004). However, the mechanism of horizontal transmission of *Wolbachia* in nature is poorly understood. These findings can help us to gain the basic information related to *Wolbachia*'s evolution in various hosts as well as to analyse its diversity and its behavioural pattern with its host in the prior stages of the study. In the advanced stage as thrips are known as potential vectors for transmission of viral diseases in plants, the study of *Wolbachia* can unravel novel methods for controlling pest populations, by modifying the insect's ability to transmit disease as well as enhancing the mass production of beneficial insects used for biological control.

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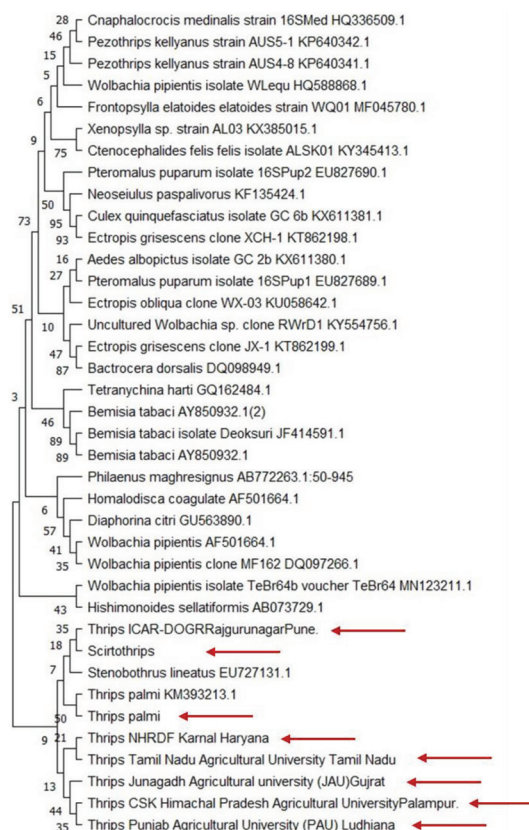


Fig 3. The phylogenetic tree of *Wolbachia* from different arthropods. Tree inferred from Maximum likelihood and Bayesian inference (BI) methods using MEGA 11.0 program. The arrows indicate the thrips samples used in this study

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AUTHOR CONTRIBUTION STATEMENT

SG conceived and supervised research, PR, PK, IB conducted experiment, KC provided chilli and melon thrips, KK, PK and PR wrote draft of MS and all authors reviewed and approved the manuscript

CONFLICT OF INTEREST

No conflict of interest.

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ELECTROPHYSIOLOGICAL AND BEHAVIOURAL RESPONSES OF *ANOMALA DIMIDIATA* TO THREE HOST PLANT'S VOLATILES

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ABSTRACT

The electrophysiological and behavioural responses of *Anomala dimidiata* (Coleoptera: Rutelinae) were studied for its host kiwi plant's (*Actinidia deliciosa*) three volatiles, (Z)-3-Hexen-1-ol, Linalool, and (-)- α -Pinene. This experiment comprises electroantennographic assay using EAG and field testing in the Kiwi Horticulture field using synthetic compounds. In EAG assays, all three test compounds elicit significant responses with $55.66 \pm 3.41\%$ for (Z)-3-Hexen-1-ol, $44.97 \pm 1.08\%$ for Linalool, and $53.75 \pm 0.33\%$ for (-)- α -Pinene in male beetles and $54.82 \pm 3.52\%$ for (Z)-3-Hexen-1-ol, $44.97 \pm 0.30\%$ for Linalool and $53.75 \pm 4.69\%$ for (-)- α -Pinene in females beetles as compare to reference compound. In the field testing, (Z)-3-Hexen-1-ol baited traps caught maximum beetles while (-)- α -Pinene-baited traps had significantly less number of beetles attracted to them. Linalool-baited traps caught 60.1% fewer beetles than (Z)-3-Hexen-1-ol baited traps but 62.7% more beetles than (-)- α -Pinene- baited traps. This study concludes that (Z)-3-Hexen-1-ol is a potential kairomone for *A. dimidiata*.

Key words: Allelochemical, electroantennogram, leaf-chafer, kairomone, kiwi fruit, plantation, phytophagous, Rutelinae, white grub, baited trap, attraction

Actinidia deliciosa (Kiwi) has recently become an important cash crop in Arunachal Pradesh, India (Singh et al., 2008). The leaf chafer beetles of the subfamily Rutelinae (Coleoptera: Scarabaeidae) consist of phytophagous beetle and is commonly included in the white grub pest family (Sushil et al., 2006). One of these white grub species, *Anomala dimidiata* (Hope), is observed to be infesting the leaves of the kiwi tree. This species is economically significant, with the adults mostly defoliating the leaves of different fruit trees and the larvae feeding on various ground crops (Sharma et al., 2021). They are widely distributed throughout the Indian subcontinent, including the Northeastern state of Arunachal Pradesh (Chandra and Gupta, 2012; Devanda et al., 2022). *Anomala* species are highly diversified generalist pests, and their adults are known to feed on the foliage of various trees and shrubs (Sushil et al., 2006; Rahman et al., 2017). For any generalist insect herbivore, locating its host plants successfully in a complex environment with different arrays of volatiles in the air is crucial yet complex for feeding or oviposition. Unlike specialist species, the polyphagous species utilize hundreds of plant species across multiple plant families for the same (Bernays & Chapman, 1994; Forister et al., 2015). Insect herbivores

possess the olfactory mechanisms necessary to detect their host-plant volatile cues, as demonstrated by the mechanisms by which all generalist and specialist herbivores can optimize their foraging for suitable hosts. Previous experiences also enhance host location finding with olfactory and visual cues.

Due to an insect's limited neural capacity to process multiple sensory inputs, it is usually believed that generalist herbivores may find it challenging to choose among alternative host plants (Agrawal, 2001; Carrasco et al., 2015). Thus, competing sensory inputs from the potential host make it slower to decide than the specialist (Bernays and Funk, 1999). Despite this fact, the generalist species like *A. dimidiata* occurs in nature and florist to an enormous extent, even causing substantial economic losses in agriculture fields. However, it is still challenging to ascertain how these herbivores recognize and locate their hosts (Bruce and Pickett, 2011). Many phytophagous chafer species have been explored for sex pheromones and allelochemicals with conformation, and few have been promoted for biomonitoring and pest management in agro-ecosystems (Klein et al., 1981; Leal, 1998; Ruth et al., 2005). Leaf-derived allelochemicals (kairomones)

have been identified for several *Anomala* species (Vuts et al., 2014), but none are available in the literature for *A. dimidiata*. This study used synthetic versions of three volatile compounds present in its important host plant (*A. deliciosa*). We tested these compounds for their attractant potential to *A. dimidiata* using Electroantennography and Field application techniques, which can be of huge importance in its management in agroecosystem.

MATERIALS AND METHODS

Adult *Anomala dimidiata* were collected from their infested host plant kiwi trees (*Actinidia deliciosa*) for EAG assay from the field study site in the Old Ziro geographical locality (27°34'19.9" N and 93°48'19.0" E), District Lower Subansiri, Arunachal Pradesh, India during June 2022. For identification purposes, referred specimens to APRC ZSI (Zoological Survey of India), Itanagar, examined by Chandra and Gupta (2012) and pinned specimens were deposited at the same. The electrophysiological assay was done in a 2-channel Signal Acquisition interface IDAC-2, Syntech, Netherlands. Sample preparation for EAG recording was done as suggested by Olsson and Hansson (2013), and Du et al. (2019). It measures the difference in electrical potential between the proximal and distal portions of the antennal flagellum brought on by olfactory stimulation. It counts the total electrical potentials generated by olfactory sensory neurons (OSNs) that are active on an insect's antennae (Roelofs, 1984). Beetles were treated with cold anaesthesia before having an antenna cut off at the base with a dissection scissor. A small amount of electrode gel was used to quickly attach the antenna to both sides of a two-pronged electrode "fork" recording probe (base in the ground position) for EAG recordings. A stimulus air controller (Type CS-55, Syntech, Netherlands) was used to deliver the stimulus. With a supplemental air supply running at 4 m/s connected to the stimulus applicator, the constant air flow was kept at 4-5 m/s. For the assay, the test compounds (Z)-3-Hexen-1-ol (99%), Linalool (99%), and (-)- α -Pinene (99%) was dissolved in hexane, and aliquots of 10 μ l were made, pipetted onto a strip of filter paper (1 cm in diameter), and then put into a fresh, disposable Pasteur pipette for each recording. Hexane alone treated filter strip served as control, and Anethol, a known attractant of *Anomala* spp., was used as a reference. 20 μ l (70%) dosage per filter paper strip was chosen as the optimum dose for all recordings after dose-response trials revealed that it

elicited a good response. Each recording was carried out independently in 3 replicates, with alternate intervals of 40 seconds to allow for antennal receptor recovery. Signal acquisition and data processing were done using the Syntech IDAC-2 signal acquisition system and EagPro signal acquisition software. For EAG data sorting, the magnitude of depolarization elicited by test compounds was subtracted from the amplitude of the reference response. The % values of the mean reactions to the reference stimuli were converted from millivolt responses for all other stimuli and represented in a graph (Jyothi et al., 2002).

The field study was conducted for three months, from June to August 2022, to evaluate the effect of the volatiles as a potential attractant of *A. dimidiata*. Since the study field is a hilly slope and the plantation is very close to the ground, the baited pitfall trapping method was used with a similar design used for ground beetles (Hoekman et al., 2017) but with few modifications. The plastic cup used in the trap was 20 cm in height and 15cm in diameter. The experiments were done in a kiwi field ($\approx$ 1.4 acres) at Keliya locality, Old Ziro, Arunachal Pradesh, India (27°34'19.9" N and 93°48'19.0" E). One trap was baited only with the attractant for *Anomala* spp. Anethol (0.1 ml) was considered a positive control; another trap was left unbaited, and the other three traps were baited with test compounds (Z) 3-Hexen-1-ol, Linalool and (-)- α -Pinene (0.1 ml each) respectively. These 5 traps were set out in an experimental field in 3 rows with the alternating position of positive control bait, unbaited and baited with test compounds (all in three replicates each). Traps were spaced 20 m apart in a 3 $\times$ 5 grid within the experimental field. 0.1 ml of 99% test compound in a vial with the pierced cap was taped to the interior wall of a trap to allow evaporation of the chemical. The vials were refilled every week. 5 cm water was added to each trap to preserve captured insects. Insects were collected weekly and identified based on their characteristic morphological tibial features as well as anatomical features of genitalia using a LEICA S8 APO B stereomicroscope (Leica Microsystems CMS GmbH, Wetzlar, Germany). The data were checked for normality using the Kolmogorov-Smirnoff test. EAG responses were analyzed for analysis of variance, followed by Tukey's post hoc test to compare the mean at a significance level of $p < 0.05$. Similarly, the field trapping experiment was analyzed by analysis of variance followed by Dunnett's multiple comparisons test. All these tests were performed using GraphPad Prism 8.4.0. (GraphPad Software Inc., California, USA).

RESULTS AND DISCUSSION

The EAG responses of *A. dimidiata* (Fig. 1), its antennae to all three test compounds are substantial for both male and female beetles (Fig. 2). For every puff of volatile, a typical negative waveform was produced, indicating a rapid depolarization of the antenna shown in negative mV. It was followed by a slower recovery phase, back to the standing potential within a few milliseconds. EAG negative amplitudes were expressed as the mean ($\pm$ standard error) of the three replicate readings. The two-way analysis of variance showed a significant difference in the mean responses of antennae of *A. dimidiata* to 3 different test compounds as compared to the reference ($F(11,11)=5.549, P<0.01$). However, no significant difference was observed in the mean responses of antennae of male and female beetles to 3 different test compounds ($F(1,11)=0.2176, P>0.05$). The responses to Linalool are most significant in males and females compared to the reference (Tukey's *post hoc* test; $P<0.05$). The relative responses show a small range of distinction in EAG profiles for 3 test compounds, with overall (-)- α -Pinene having the highest percentage of response intensity and Linalool being the least. The percentage magnitude of depolarization elicited by the test compound compared to the reference amplitude was found to be 55.66 ± 3.41 for (Z)-3-Hexen-1-ol, $44.97 \pm$



Fig. 1. *Anomala dimidiata* A. Male dorsal view B. lateral view

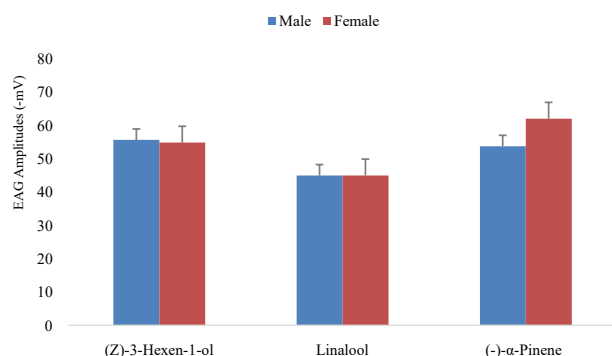


Fig. 2. EAG responses of *A. dimidiata* to three host's volatile compounds. Two-way ANOVA followed by Tukey's Post Hoc Test: ** $p < 0.05$

1.08 for Linalool and 53.75 ± 0.33 for (-)- α -Pinene in males; 54.82 ± 3.52 for (Z)-3-Hexen-1-ol, 44.97 ± 0.30 for Linalool and 62.00 ± 4.69 for (-)- α -Pinene in females (Fig. 3). In the field testing, one-way ANOVA showed that a significantly greater number of *A. dimidiata* were caught in (Z)-3-Hexen-1-ol baited traps as compared to Linalool and (-)- α -Pinene baited (male $F(2,114)=66.04, P<0.0001$; female $F(2,114)=19.31, P<0.0001$). The mean number of beetles also caught significantly differed for three different baited traps in both male and female cases by Dunnett's multiple comparisons test ($\alpha=0.05$). The mean number of male beetles caught was found to be 0.179 ± 0.009 in unbaited traps, 6.59 ± 0.06 in Anethol baited traps (positive control), 6.85 ± 0.07 in (Z)-3-Hexen-1-ol baited traps, 2.69 ± 0.06 in Linalool baited traps and 1.28 ± 0.04 in (-)- α -Pinene baited traps; and the mean number of female beetles caught was found to be 0.051 ± 0.006 in unbaited traps, 6.97 ± 0.06 in Anethol baited traps (positive control), 6.07 ± 0.08 in (Z)-3-Hexen-1-ol baited traps, 3.79 ± 0.07 in Linalool baited traps and 2.33 ± 0.05 in (-)- α -Pinene baited traps during June, July and August 2022 (Fig. 4). The weekly mean number of beetles caught during three months are projected in figure 5 (Fig. 5).

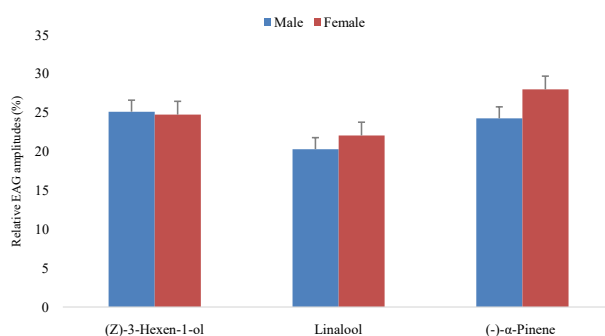


Fig. 3. Relative EAG amplitudes (%) of different test compounds against *A. dimidiata*. Values are the mean ($\pm$ SEM) of three EAG responses expressed as % responses relative to the reference.

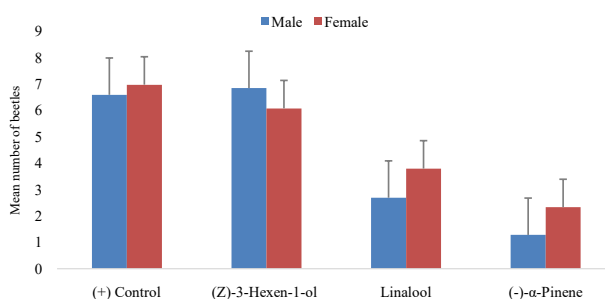


Fig. 4. Mean ($\pm$ SEM) number of adult *A. dimidiata* caught in different traps from June to August 2022 in Kiwi Field, Ziro, Arunachal Pradesh. Both male and female means differ significantly at $p < 0.05$ (Dunnett's Multiple Comparisons Test).

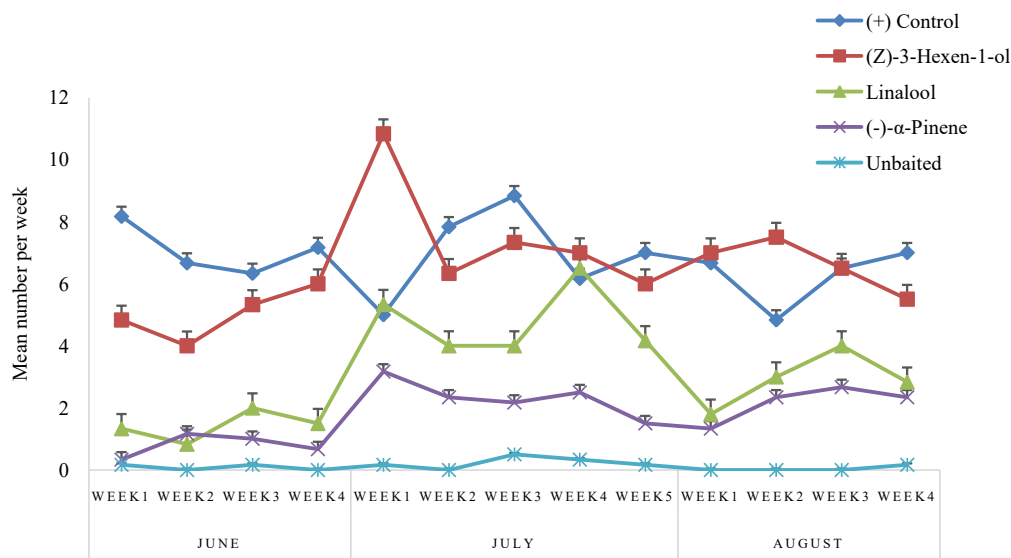


Fig. 5. Weekly Mean ($\pm$ SEM) number of adult *A. dimidiata* trapped in different baited traps from June to August 2022 in Kiwi Horticulture Field, Ziro, Arunachal Pradesh. (Week1= 1st Sunday of that month)

Our results demonstrated that this economically significant leaf chafer beetle in laboratory experiments responds well to the synthetic version of 3 volatiles present in its host plant, *A. deliciosa*. The GCMS profile of this host plant showed that it contains these three test compounds (Unpublish observation). Our EAG experiments indicate that (-)- α -Pinene elicits a maximum electrophysiological response compared to Linalool and (Z)-3-Hexen-1-ol. Though EAG responses are generally treated as qualitative rather than quantitative indicators of olfactory reception (Olsson and Hansson, 2013), the amplitude elicited by (-)- α -Pinene is equivalent to a reference compound Anethol which is a well-known attractant for many *Anomala* spp. (Leal et al., 1993; Cherry et al., 1996). So far, no literature reports are available conforming (-)- α -Pinene as an attractant to *Anomala* spp. On the other hand, (Z)-3-Hexen-1-ol also has more or less similar attractant potential in EAG as (-)- α -Pinene. Moreover, (Z)-3-Hexen-1-ol is reported as allelochemical with an attraction effect for *Anomala corpulenta* (Motschulsky) and *Anomala exoleta* (Falderman) (Li et al., 2013). Our field observations showed that (Z)-3-Hexen-1-ol baited traps caught the highest number of adult beetles. This common leaf alcohol has been widely explored for its role in the plant-insect interaction of many leaf chafer species. However, no study has been projected in context to *A. dimidiata*. The possible explanation for the attraction of *A. dimidiata* to (Z)-3-Hexen-1-ol may lie in the fact that many related generalists leaf chafer species attract a common allelochemical compound as part of their host detection, which could be the result of host plants

overlapping, unlike specialist species. Contrasting to (-)- α -Pinene, monoterpenoid alcohol, Linalool has a few reports of semiochemical roles in *Anomala*'s related leaf chafer beetle species, acting as sex pheromone of *Holotrichia parallela* Motschulsky (Leal et al., 1993) which belonging to the subfamily Melolonthinae and as an attractant of *Phyllophaga congrua* (Melolonthinae) (Crocker et al., 1999) and *Popillia japonica* Newman (Rutelinae) (Tumlinson et al., 1977). Nevertheless, Linalool-baited traps caught 60.1% fewer beetles than (Z)-3-Hexen-1-ol baited traps, but it caught 62.7% more beetles than (-)- α -Pinene, which is in contrast to electrophysiological results. Interestingly, the other similar adult leaf chafer species, such as *Phyllophaga* sp., *Holotrichia* sp., *Mimela* sp. and *Lepidiota* sp., were also observed to be sometimes caught in the baited traps along with *A. dimidiata*, which indicates that these test compounds possess attractant potential to those species as well, but further investigation is required.

This study mainly aimed to determine whether any of the three host-leaf volatiles have any field attraction to this important pest species in the kiwi field. Since the attractant activity of (Z)-3-Hexen-1-ol to *A. dimidiata* in the lab experiment and the field test has been demonstrated significantly, it will be prudent to research effective release rates and optimum concentration levels and the impact of using this compound in the agroecosystem. (-)- α -Pinene also showed good potential in EAG responses, but the contradicting result in the field study gave an opinion that it is not a major attractant like (Z)-3-Hexen-1-ol to *A. dimidiata*. Linalool,

though described as allelochemical with an attraction effect to related leaf chafer species, does not seem to have a major effect but rather a mild attractant to *A. dimidiata*. Exploiting such attractant compounds with potential bioactivity is important for their utilization in field monitoring, mass trapping, and pest management (Murali-Baskaran et al., 2018; Weber et al., 2022).

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AUTHOR CONTRIBUTION STATEMENT

J C, N M and Y R conceived the idea and designed the study. N M collected the samples, performed the experiments, analyzed the data and provided the first draft. N M, J C, and Y R wrote and edited the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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CHECKLIST OF SPIDER (ARACHNIDA: ARANEAE) FAUNA OF KASHMIR

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ABSTRACT

A checklist of spiders from Kashmir, India, is provided which indicates the occurrence of 185 species under 100 genera and 25 families. The checklist is based primarily on the published records of spiders of Kashmir from 1885 to 2023 (up to January). These faunistic records reveal that the most diverse spider families in this region are Salticidae, Linyphiidae, Araneidae, Gnaphosidae, Thomisidae, Lycosidae and Theridiidae while about 11 families (Cheiracanthiidae, Clubionidae, Ctenidae, Filistatidae, Liocranidae, Mimetidae, Oxyopidae, Pimoidae, Scytodidae, Sicariidae and Uloboridae) are represented by a lone genus. The checklist includes taxa that have been identified upto species level. This checklist compiles the available regional faunistic records to serve future research.

Key words: Spiders, Kashmir Himalaya, North Western Himalaya, Arachnids, Araneae, families, genera, list, biodiversity hotspot

Spiders are one of the most omnipresent and a considerably diverse group of arachnids. At present there are 50,871 identified species worldwide (World Spider Catalog, 2023). In India, there are currently 1929 known species (Caleb and Sankaran, 2023). Numerous unrecorded or unknown species, especially from the north-western parts of India remain, where only a few studies have been undertaken some of which are archaic. The work carried out by Pickard-Cambridge (1885) is antiquated as it is more than a century old. Compilation of Caporiacco (1934 and 1935) is outdated at present due to several synonymies, zoogeographical reorganisation and taxonomic regroupings as the study was carried in the entire Himalayan belt of which, now parts are governed by India, Pakistan and China separately. A lion's share of the species have been inadequately described outside the context of any revisionary work, and there has been little comparative research. Therefore, such works are in need of a thorough revision. The work carried out by Dayal (1942), Tikader and Bal (1981), Zabka (1981), Tikader (1982), Sadana (1983), Thaler (1987), Gajbe (1988), Majumder and Tikader (1991) and Hormiga (1994) is highly relevant to the present study. Recent contributions focused on systematic accounts and species enumeration include those of Khan (2009, 2011a and b, 2012a and b, 2013 a and b), Khan and Rather (2012), Chakrabarti (2013), Khan and Khan (2013), Lone et al. (2015), Punjoo and Bhat (2015), Shah and Buhroo (2022) and Singh et al.

(2023). Although the spider fauna of Kashmir is highly diverse, the spider taxonomy remains understudied resulting in the status of many taxa being dubious, since very few detailed research efforts to identify spiders based on their genitalia have ever been undertaken from Kashmir.

MATERIALS AND METHODS

Kashmir is a territory in the northern part of India which covers a total area of 15948 km² with an altitude range from 1600 masl (Srinagar) to 5420 masl (Kolahoi peak, Ganderbal) lying in the north-western Himalaya (33°20' - 34°54' N and 73°55' - 75°35' E). This region is known for its diverse geography and climatic conditions, supporting a rich fauna. Its political boundaries include 10 districts (Anantnag, Bandipora, Baramulla, Budgam, Ganderbal, Kulgam, Kupwara, Pulwama, Shopian and Srinagar). However, the present checklist records species from 9 out of 10 districts of Kashmir as no species has ever been recorded from 1 district (Kulgam). The checklist of spiders is based primarily on thorough literature search and published records available. Every entry in the checklist was verified for its consistency with current taxonomic literature, the one present in the World Spider Catalogue (2023). Regional and global distributional data for each species was collected from the published records of the taxa published between 1885 and 2023 and the World Spider Catalogue (2023).

RESULTS AND DISCUSSION

S.No.	Family/ scientific name	Distribution in Kashmir (District)	Reference	Global distribution
I	Agelenidae C. L. Koch, 1837			
	<i>Agelena labyrinthica</i> (Clerck, 1757)	Srinagar	Caporiacco, 1935	Europe to Central Asia, Mongolia, China, Korea, Japan
2.	<i>Coelotes stylifer</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935	India
II	Araneidae Clerck, 1757			
3.	<i>Agalenatea redii</i> (Scopoli, 1763)	Srinagar	Caporiacco, 1934	Azores, Europe, Turkey, Caucasus, Russia (Europe to South Siberia), Iran, Central Asia, China
4.	<i>Araneus anantnagensis</i> Tikader and Bal, 1981	Anantnag	Tikader and Bal, 1981; Tikader, 1982; Khan, 2011b	India
5.	<i>Araneus diadematus</i> Clerck, 1757	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Middle East, Turkey, Caucasus, Russia (Europe to Far East), Iran, Central Asia, China, Japan. Introduced to North America
6.	<i>Araneus ellipticus</i> (Tikader and Bal, 1981)	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, Bangladesh, China, Thailand, Laos
7.	<i>Araneus gibber</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
8.	<i>Araneus pahalganensis</i> Tikader and Bal, 1981	Srinagar	Tikader, 1982; Khan, 2009; Khan and Rather, 2012; Punjoo and Bhat, 2015	India, China
		Budgam	Khan and Rather, 2012	
		Anantnag	Tikader and Bal, 1981; Khan and Rather, 2012; Punjoo and Bhat, 2015	
9.	<i>Araneus panchganiensis</i> Tikader and Bal, 1981	Srinagar	Khan, 2009; Khan and Rather, 2012	India
		Budgam	Khan and Rather, 2012	
		Anantnag	Khan and Rather, 2012	
10.	<i>Araneus panniferens</i> Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
11.	<i>Araneus peltax</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
12.	<i>Araneus pontii</i> Caporiacco, 1934	Srinagar	Caporiacco, 1934	India
13.	<i>Araneus praedatus</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
14.	<i>Araneus trifolium</i> (Hentz, 1847)	Baramulla	Khan, 2011a; Khan, 2012a	USA, Canada, India
		Srinagar	Khan, 2011a; Khan, 2011b; Khan, 2012a; Khan and Rather, 2012	
		Pulwama	Khan, 2011a	

15.	<i>Araniella cucurbitina</i> (Clerck, 1757)	Baramulla	Caporiacco, 1934; Khan, 2011a; Khan, 2012a	Europe, Turkey, Russia (Europe) to Central Asia, China, Korea
		Srinagar	Caporiacco, 1934; Khan, 2009; Khan, 2011a; Khan, 2012a; Punjoo and Bhat, 2015	
		Pulwama	Khan, 2011a; Khan, 2012a	
		Anantnag	Punjoo and Bhat, 2015	
		Ganderbal	Pickard-Cambridge, 1885	
		Shopian	Khan, 2012a	
16.	<i>Araniella nympha</i> (Simon, 1889)	Srinagar	Sadana, 1983; Punjoo and Bhat, 2015	Pakistan, India, China
		Anantnag	Sadana, 1983; Punjoo and Bhat, 2015	
17.	<i>Chorizopes congener</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
18.	<i>Chorizopes stoliczkae</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
19.	<i>Cyclosa conica</i> (Pallas, 1772)	Srinagar and Anantnag	Punjoo and Bhat, 2015	
20.	<i>Cyclosa elongata</i> Biswas and Raychaudhuri, 1998	Baramulla	Khan, 2011a; Khan, 2012a	Bangladesh, India
		Srinagar	Khan, 2009; Khan, 2011a; Khan, 2011b; Khan, 2012a; Punjoo and Bhat, 2015	
		Budgam	Khan, 2011b Khan and Rather, 2012	
		Pulwama	Khan, 2011; Khan, 2012a	
		Anantnag	Khan and Rather, 2012; Punjoo and Bhat, 2015	
		Shopian	Khan, 2012a	
21.	<i>Cyclosa kashmirica</i> Caporiacco, 1934	Ganderbal	Caporiacco, 1934	North America, Europe, Turkey, Caucasus, Russia (Europe to Far East), Iran, Central Asia, China
22.	<i>Cyrtarachne pallida</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
23.	<i>Neoscona achine</i> (Simon, 1906)	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, China
24.	<i>Neoscona mukerjei</i> Tikader, 1980	Baramulla	Sadana, 1983; Khan, 2011; Khan, 2012a	Pakistan, India, Bangladesh
		Srinagar	Sadana, 1983; Khan, 2009; Khan, 2011a; Khan, 2011b; Khan, 2012a; Khan, 2012b; Khan and Rather, 2012; Punjoo and Bhat, 2015	
		Budgam	Khan, 2011b Khan and Rather, 2012	
		Pulwama	Khan, 2011a; Khan, 2012a	
		Anantnag	Khan, 2011b; Khan and Rather, 2012; Punjoo and Bhat, 2015	
		Shopian	Khan, 2012a	
25.	<i>Neoscona pavida</i> (Simon, 1906)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Pakistan, India, China

26.	<i>Neoscona shillongensis</i> Tikader and Bal, 1981	Baramulla	Khan, 2011a; Khan, 2012a; Khan, 2013b	Pakistan, India, China
		Srinagar	Khan, 2009; Khan, 2011a; Khan, 2012a Khan and Rather, 2012	
		Pulwama	Khan, 2011a; Khan, 2012a; Khan, 2013b	
27.	<i>Neoscona theisi</i> (Walckenaer, 1841)	Baramulla	Khan, 2011a; Khan, 2012a	Cyprus, Israel, Caucasus (Georgia, Russia), Iran, Pakistan, India, Nepal, Philippines, China to Indonesia, Japan, Australia. Introduced to Seychelles, Pacific Is.
		Srinagar	Khan, 2009; Khan, 2011a; Khan, 2012a; Punjoo and Bhat, 2015	
		Budgam	Khan, 2011b Khan and Rather, 2012	
		Pulwama	Khan, 2011a, Khan, 2012a	
		Anantnag	Khan, 2011b; Punjoo and Bhat, 2015	
		Shopian	Khan, 2012a	
28.	<i>Neoscona vigilans</i> (Blackwall, 1865)	Srinagar and Anantnag	Khan, 2013a; Punjoo and Bhat, 2015	Africa, Asia (without Russia)
29.	<i>Plebs himalayaensis</i> (Tikader, 1975)	Srinagar	Sadana, 1983; Punjoo and Bhat, 2015	India
		Anantnag	Punjoo and Bhat, 2015	
	<i>Singa chota</i> Tikader, 1970	Baramulla and Srinagar	Sadana, 1983	India
31.	<i>Zygiella x-notata</i> (Clerck, 1757)	Srinagar	Caporiacco, 1934	Europe, Turkey, Caucasus, Iran? Introduced to North America, Chile, Uruguay, Argentina, China, Japan, Réunion
III	Cheiracanthiidae Wagner, 1887			
32.	<i>Cheiracanthium approximatum</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	Pakistan, India, Myanmar, Laos to China, Taiwan, Philippines
33.	<i>Cheiracanthium himalayense</i> Gravely, 1931	Baramulla	Khan, 2012a; Khan, 2013b	India
		Srinagar	Khan, 2009; Khan, 2011a; Khan, 2012a	
		Pulwama	Khan, 2011a; Khan, 2013b	
		Shopian	Khan, 2012a	
34.	<i>Cheiracanthium kashmirens</i> Majumder and Tikader, 1991	Srinagar	Majumder and Tikader, 1991	India
IV	Clubionidae Wagner, 1887			
35.	<i>Clubiona deletrix</i> O. Pickard- Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
36.	<i>Clubiona drassodes</i> O. Pickard- Cambridge, 1874	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, Bangladesh, China
37.	<i>Clubiona japonicola</i> Bosenberg and Strand, 1906	Baramulla	Khan, 2011a; Khan, 2012a	Russia (Far East), Korea, Japan, China, Taiwan, Philippines, Indonesia
		Srinagar	Khan, 2009; Khan, 2011a; Khan, 2011b; Khan and Rather, 2012	
		Budgam	Khan, 2011b; Khan and Rather, 2012	
		Pulwama	Khan, 2011a; Khan, 2012a	
		Anantnag	Khan, 2011b; Khan and Rather, 2012	

V	Ctenidae Keyserling, 1877			
38.	<i>Bowie cochinensis</i> Gravely, 1931	Srinagar	Khan, 2009; Khan and Khan, 2013	India
39.	<i>Bowie himalayensis</i> Gravely, 1931	Baramulla Srinagar Pulwama Shopian	Khan, 2012a Khan, 2009; Khan, 2011a; Khan, 2012a Khan, 2011a; Khan, 2012a Khan, 2012a	India
40.	<i>Bowie indicus</i> Gravely, 1931	Baramulla Srinagar Pulwama Shopian	Khan, 2011a; Khan, 2012a Khan, 2009; Khan, 2011a; Khan, 2012a Khan, 2011a; Khan, 2012a Khan, 2012a	India
41.	<i>Bowie meghalayaensis</i> Tikader, 1976	Srinagar	Khan, 2009; Khan and Khan, 2013	India
42.	<i>Bowie sikkimensis</i> Gravely, 1931	Srinagar	Khan, 2009; Khan and Khan, 2013	India
VI	Dictynidae O. Pickard-Cambridge, 1871			
43.	<i>Archaeodictyna consecuta</i> (O. Pickard-Cambridge, 1872)	Ganderbal and Srinagar	Caporiacco, 1934	Europe, Caucasus, Russia (Europe to South Siberia), Iran, Central Asia, China
44.	<i>Lathys sindi</i> (Caporiacco, 1934)	Ganderbal and Srinagar	Caporiacco, 1934	India
45.	<i>Nigma puella</i> (Simon, 1870)	Srinagar	Caporiacco, 1934	Azores, Madeira, Canary Is., Europe
VII	Filistatidae Simon, 1864			
46.	<i>Zaitunia rufa</i> (Caporiacco, 1934)	Srinagar	Caporiacco, 1934	Pakistan, India
VIII	Gnaphosidae Banks, 1892			
47.	<i>Berlandina drassodea</i> (Caporiacco, 1934)	Ganderbal	Caporiacco, 1934	India
48.	<i>Berlandina plumalis</i> (O. Pickard-Cambridge, 1872)	Ganderbal and Srinagar	Caporiacco, 1934	West Africa, Mediterranean to Central Asia, Iran
49.	<i>Drassodes carinivulvus</i> Caporiacco, 1934	Srinagar	Tikader, 1982	India
50.	<i>Drassodes dispulsus</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	China (Yarkand), India, Tajikistan or Afghanistan
51.	<i>Drassodes himalayensis</i> Tikader and Gajbe, 1975	Srinagar Ganderbal	Tikader, 1982 Gajbe, 1988	India
52.	<i>Drassodes involutus</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
53.	<i>Drassodes parvidens</i> Caporiacco, 1934	Ganderbal Srinagar	Caporiacco, 1934 Caporiacco, 1934; Tikader, 1982	India, Pakistan
54.	<i>Drassodes rubicundulus</i> Caporiacco, 1934	Ganderbal	Caporiacco, 1934	India, Pakistan
55.	<i>Gnaphosa stoliczkai</i> O. Pickard-Cambridge, 1885	Kashmir	Tikader, 1982	Mongolia, China, India
56.	<i>Haplodrassus dalmatensis</i> (L. Koch, 1866)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, North Africa, Turkey, Caucasus, Middle East, Russia (Europe) to Central Asia

57.	<i>Haplodrassus omissus</i> (O. Pickard-Cambridge, 1872)	Ganderbal	Caporiacco, 1934	Canary Is., Morocco, Mediterranean
58.	<i>Haplodrassus signifer</i> (C. L. Koch, 1839)	Ganderbal	Caporiacco, 1934	North America, Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Israel, Iran, Central Asia, China, Korea
59.	<i>Micaria dives</i> (Lucas, 1846)	Ganderbal	Caporiacco, 1935	Europe, Turkey, Israel, Caucasus, Russia (Europe to Far East), Iran, Central Asia, India, China, Korea, Japan
60.	<i>Sergiolus montanus</i> (Emerton, 1890)	Srinagar and Anantnag	Punjoo and Bhat, 2015	North America, India
61.	<i>Talanites tibialis</i> Caporiacco, 1934	Srinagar	Caporiacco, 1934; Tikader, 1982	India, Pakistan
62.	<i>Zelotes desioi</i> Caporiacco, 1934	Srinagar	Caporiacco, 1934; Tikader, 1982	India
63.	<i>Zelotes pseudopusillus</i> Caporiacco, 1934	Kashmir	Tikader, 1982	India
64.	<i>Zelotes sindi</i> Caporiacco, 1934	Ganderbal	Caporiacco, 1934	India, Pakistan
IX	Liocranidae Simon, 1897			
65.	<i>Apostenus annulipes</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	India
X	Linyphiidae Blackwall, 1859			
66.	<i>Erigone dentipalpis</i> (Wider, 1834)	Ganderbal	Caporiacco, 1935; Pickard-Cambridge, 1885	Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, Iran, Central Asia, China. Introduced to Canada
67.	<i>Erigone pseudovagans</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935	India
68.	<i>Erigone rohtangensis</i> Tikader, 1981	Srinagar, Budgam and Anantnag	Khan, 2011b; Khan and Rather, 2012	India
69.	<i>Gongylidiellum confusum</i> Thaler, 1987	Baramulla, Ganderbal and Anantnag	Thaler, 1987	Pakistan, India
70.	<i>Heterolinyphia tarakotensis</i> Wunderlich, 1973	Baramulla	Thaler, 1987	India, Nepal
71.	<i>Himalaphantes martensi</i> (Thaler, 1987)	Anantnag	Thaler, 1987	India, Nepal
72.	<i>Indophantes digitulus</i> (Thaler, 1987)	Baramulla, Ganderbal and Anantnag	Thaler, 1987	Pakistan, India, Nepal
73.	<i>Lepthyphantes allegrii</i> Caporiacco, 1935	Bandipora	Caporiacco, 1935	India
74.	<i>Lepthyphantes bhudbari</i> Tikader, 1970	Baramulla Srinagar and Pulwama Shopian	Khan, 2011a Khan, 2011a; Khan, 2012a Khan, 2012a	India
75.	<i>Lepthyphantes leprosus</i> (Ohlert, 1865)	Srinagar and Anantnag	Punjoo and Bhat, 2015	North America, Europe, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan. Introduced to North America, Chile

76.	<i>Lepthyphantes lingsoka</i> Tikader, 1970	Baramulla and Anantnag	Sadana, 1983	India
77.	<i>Lepthyphantes peramplus</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
78.	<i>Linyphia consanguinea</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	Pakistan, India
79.	<i>Linyphia triangularis</i> (Clerck, 1757)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Caucasus, Russia (Europe to Far East), Iran, Kazakhstan, China. Introduced to Canada, USA
80.	<i>Megalepthyphantes nebulosus</i> (Sundevall, 1830)	Ganderbal	Caporiacco, 1934	North America, Europe, Turkey, Caucasus, Russia (Europe to Far East)
81.	<i>Microlinyphia pusilla</i> (Sundevall, 1830)	Bandipora	Caporiacco, 1934	North America, Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, Iran, Central Asia, China, Mongolia, Japan
82.	<i>Neriere birmanica</i> (Thorell, 1887)	Srinagar	Caporiacco, 1935	India, Myanmar, Laos, China, Indonesia
83.	<i>Neriere clathrata</i> (Sundevall, 1830)	Anantnag	Thaler, 1987	North America, Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Iran, Central Asia, China, Korea, Japan
84.	<i>Neriere radiata</i> (Walckenaer, 1841)	Srinagar	Chakrabarti, 2013	North America, Europe, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, China, Korea, Japan
85.	<i>Mitrager globiceps</i> (Thaler, 1987)	Anantnag	Thaler, 1987	India
86.	<i>Oedothorax dubius</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	India
87.	<i>Piniphantes himalayensis</i> (Simon, 1884)	Ganderbal	Thaler, 1987	Pakistan, Nepal, India
88.	<i>Scotargus pilosus</i> Simon, 1913	Anantnag	Thaler, 1987	Europe, Algeria, Caucasus, Russia (Europe to Far East), Kazakhstan, Central Asia, Nepal
89.	<i>Tiso megalops</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935	India
90.	<i>Troxochrota kashmirica</i> (Caporiacco, 1935)	Ganderbal and Srinagar	Caporiacco, 1935	India
XI	Lycosidae Sundevall, 1833			
91.	<i>Evippomma evippiforme</i> (Caporiacco, 1935)	Ganderbal and Srinagar	Caporiacco, 1935	India
92.	<i>Hippasa agelenoides</i> (Simon, 1884)	Srinagar and Anantnag	Punjoo, 2015	Pakistan, India, Myanmar
93.	<i>Hippasa flavicoma</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	India
94.	<i>Hogna rubromandibulata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Caporiacco, 1935; Pickard-Cambridge, 1885	India
95.	<i>Pardosa algoides</i> Schenkel, 1963	Srinagar Baramulla Srinagar, Budgam, Anantnag Pulwama	Caporiacco, 1935 Khan, 2011a; Khan, 2012a Khan and Rather, 2012 Khan, 2011a	Pakistan, India, Bangladesh, China

96.	<i>Pardosa altitudis</i> Tikader and Malhotra, 1980	Baramulla	Khan, 2011a; Khan, 2012a Khan, 2013b	India, China
		Srinagar	Khan, 2011a; Khan, 2011b; Khan, 2012a; Khan and Rather, 2012; Khan, 2013a	
		Budgam	Khan, 2011b; Khan and Rather, 2012	
		Pulwama	Khan, 2011a Khan, 2013b	
		Anantnag	Khan, 2011b; Khan and Rather, 2012	
		Shopian	Khan, 2012a	
97.	<i>Pardosa flavisterna</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935; Shah and Buhroo, 2022	Pakistan, India
		Srinagar	Caporiacco, 1935; Punjoo and Bhat, 2015; Shah and Buhroo, 2022	
		Anantnag	Punjoo and Bhat, 2015:711; Shah and Buhroo, 2022	
		Pulwama, Baramulla, Bandipora, Kupwara and Budgam	Shah and Buhroo, 2022	
98.	<i>Pardosa fortunata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India, China (Yarkand), Tajikistan or Afghanistan
99.	<i>Pardosa fuscula</i> (Thorell, 1875)	Srinagar and Anantnag	Punjoo and Bhat, 2015	USA, Canada, India
100.	<i>Pardosa hydaspis</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	India
101.	<i>Pardosa stellata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Caporiacco, 1935	China (Yarkand), Tajikistan or Afghanistan, India
102.	<i>Pardosa tridentis</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935	India, Nepal
103.	<i>Pardosa vindicata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Caporiacco, 1935	Pakistan, China (Yarkand), India
104.	<i>Schizocosa concolor</i> (Caporiacco, 1935)	Ganderbal	Caporiacco, 1935	India
105.	<i>Trochosa propinqua</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
106.	<i>Trochosa terricola</i> Thorell, 1856	Ganderbal	Caporiacco, 1935	North America, Europe, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, Iran, Central Asia, China, Japan
XII	Mimetidae Simon, 1881			
107.	<i>Mimetes laevigatus</i> (Keyserling, 1863)	Srinagar	Caporiacco, 1934	Mediterranean to Central Asia, India
XIII	Oxyopidae Thorell, 1869			
108.	<i>Oxyopes birmanicus</i> Thorell, 1887	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, China to Indonesia
109.	<i>Oxyopes elegans</i> L. Koch, 1878	Srinagar and Anantnag	Punjoo and Bhat, 2015	Australia, India

110.	<i>Oxyopes hindostanicus</i> Pocock, 1901	Baramulla Srinagar Budgam Pulwama Anantnag Shopian	Khan, 2011a Khan, 2009; Khan, 2011a; Khan, 2011b; Khan, 2012a; Khan and Rather, 2012 Khan, 2011b; Khan and Rather, 2012 Khan, 2011a; Khan, 2012a Khan, 2011b; Khan and Rather, 2012 Khan, 2012a	
111.	<i>Oxyopes javanus</i> Thorell, 1887	Baramulla Srinagar Budgam Pulwama Anantnag Shopian	Khan, 2011a Khan, 2009; Khan, 2011a; Khan, 2011b; Khan, 2012a; Khan and Rather, 2012 Khan, 2011b; Khan and Rather, 2012 Khan, 2011a; Khan, 2012a Khan, 2011b; Khan and Rather, 2012 Khan, 2012a	India, Nepal, Bangladesh, Thailand, Indonesia, Philippines, China
112.	<i>Oxyopes jubilans</i> O. Pickard-Cambridge, 1885	Srinagar Ganderbal	Caporiacco, 1935 Pickard Cambridge, 1885	India
113.	<i>Oxyopes lineatus</i> Latreille, 1806	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Caucasus, Russia (Europe to Central Asia), Middle East, Central Asia, Nepal
114.	<i>Oxyopes praedictus</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
115.	<i>Oxyopes ramosus</i> (Martini and Goeze, 1778)	Srinagar and Anantnag	Punjoo, 2015	Europe, Turkey, Caucasus, Russia (Europe to South Siberia), Kazakhstan, Korea, India
116.	<i>Oxyopes rejectus</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
XIV	Philodromidae Thorell, 1870			
117.	<i>Philodromus cinereus</i> O. Pickard-Cambridge, 1876	Srinagar	Caporiacco, 1935	Egypt, India
118.	<i>Thanatus albescens</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
119.	<i>Thanatus coloradensis</i> Keyserling, 1880	Srinagar and Anantnag	Punjoo and Bhat, 2015	North America, Europe, Turkey, Russia (Caucasus to Far East), Kazakhstan, China
120.	<i>Thanatus vulgaris</i> Simon, 1870	Ganderbal	Caporiacco, 1935	Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Middle East, Iran, Kazakhstan, Central Asia, China, Korea, Japan. Introduced to North America, South Africa, Australia
XV	Pholcidae C. L. Koch, 1850			
121.	<i>Crossopriza lyoni</i> (Blackwall, 1867)	Ganderbal	Caporiacco, 1934	Probably native to Africa and/or Asia. Introduced to the Americas, Belgium, Germany, Australia, Micronesia

122.	<i>Pholcus phalangioides</i> (Fuesslin, 1775)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Western Asia. Introduced to both Americas, Europe, Africa, Asia, Australia, New Zealand and numerous islands.
123.	<i>Physocyclus globosus</i> (Taczanowski, 1874)	Srinagar and Anantnag	Punjoo and Bhat, 2015	North America. Introduced to Africa, Czechia, Iran, Sri Lanka, China, India, Japan, Philippines, Indonesia, Australia, Pacific islands, Galapagos
XVI	Pimoidae Wunderlich, 1986			
124.	<i>Pimoida gandhii</i> Hormiga, 1994	Anantnag	Hormiga, 1994	India
XVII	Pisauridae Simon, 1890			
125.	<i>Perenethis dentifasciata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
126.	<i>Pisaura mirabilis</i> (Clerck, 1757)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Middle East, Caucasus, Russia (Europe to Middle Siberia), Central Asia, China, India
127.	<i>Stoliczka affinis</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	Pakistan, India
XVIII	Salticidae Blackwall, 1841			
128.	<i>Afraflacilla flavipes</i> (Caporiacco, 1935)	Srinagar	Caporiacco, 1935	Turkmenistan, Pakistan, India
129.	<i>Attulus diductus</i> (O. Pickard-Cambridge, 1885)	Srinagar	Caporiacco, 1935	Pakistan, India
130.	<i>Bianor albobimaculatus</i> (Lucas, 1846)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Africa, Mediterranean to Russia (Europe), Azerbaijan, Kazakhstan, Iran, Central Asia, Afghanistan, Pakistan, India, Nepal
131.	<i>Brettus anchorum</i> Wanless, 1979	Srinagar	Chakrabarti, 2013	India, Nepal
132.	<i>Chalcoscirtus glacialis</i> Caporiacco, 1935	Ganderbal	Caporiacco, 1935	USA (Alaska), Russia (Central Asia to Far East), Kazakhstan, Mongolia, India
133.	<i>Cyrba algerina</i> (Lucas, 1846)	Srinagar	Caporiacco, 1935	Canary Is. to Central Asia, India
134.	<i>Dendryphantus caporiaccoi</i> Roewer, 1951	Srinagar	Caporiacco, 1935	India
135.	<i>Euophrys evae</i> Zabka, 1981	Baramulla	Zabka, 1981	India
136.	<i>Evarcha jucunda</i> (Lucas, 1846)	Ganderbal	Caporiacco, 1935	Canary Is., Mediterranean. Introduced to Belgium, Germany, India
137.	<i>Hasarius adansoni</i> (Audouin, 1826)	Ganderbal	Pickard-Cambridge, 1885	Africa, Middle East. Introduced to both Americas, Europe, India, Laos, Vietnam, China, Taiwan, Japan, Australia, Pacific Is.
138.	<i>Myrmaplata platyleoides</i> (O. Pickard-Cambridge, 1869)	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, Sri Lanka, China, Southeast Asia
139.	<i>Myrmarachne corpuzarrosae</i> Barrios, 1981	Srinagar	Khan, 2009	Philippines, India
140.	<i>Myrmarachne hidaspis</i> Caporiacco, 1935	Srinagar	Caporiacco, 1935	India

141.	<i>Myrmarachne himalayensis</i> Narayan, 1915	Baramulla Srinagar Budgam Pulwama Anantnag Shopian	Khan, 2011a Khan, 2011a; Khan, 2012a; Khan and Rather, 2012 Khan, 2011b; Khan and Rather, 2012 Khan, 2011a; Khan, 2012a Khan, 2011b; Khan and Rather, 2012 Khan, 2012a	India
142.	<i>Myrmarachne melanocephala</i> MacLeay, 1839	Srinagar and Anantnag	Punjoo and Bhat, 2015	Pakistan to Indonesia
143.	<i>Nandicius pseudoicioides</i> (Caporiacco, 1935)	Srinagar	Caporiacco, 1935	India
144.	<i>Pellenes allegrii</i> Caporiacco, 1935	Ganderbal and Srinagar	Caporiacco, 1935	Bulgaria, Ukraine, Russia (Europe), Kazakhstan, Central Asia, India
145.	<i>Plexippus paykulli</i> (Audouin, 1826)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Africa. Introduced to both Americas, Europe, Middle East, Nepal, southern Asia, Australia, Pacific Is.
146.	<i>Salticus beneficus</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
XIX	Scytodidae Blackwall, 1864			
147.	<i>Scytodes thoracica</i> (Latreille, 1802)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, North Africa, Turkey, Iran, temperate Asia to China, Korea, Japan. Introduced to North America, Argentina, South Africa, India, Australia, New Zealand
XX	Sicariidae Keyserling, 1880			
148.	<i>Loxosceles rufescens</i> (Dufour, 1820)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Southern Europe, northern Africa to Iran, Afghanistan. Introduced to USA, Mexico, Peru, Macaronesia, South Africa, India, China, Japan, Korea, Laos, Thailand, Australia, Hawaii
XXI	Sparassidae Bertkau, 1872			
149.	<i>Heteropoda venatoria</i> (Linnaeus, 1767)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Tropical Asia. Introduced to Pacific Is., North, Central and South America, Macaronesia, Europe, Africa, India
150.	<i>Olios rossettii</i> (Leardi, 1901)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Pakistan, India, Nepal
XXII	Tetragnathidae Menge, 1866			
151.	<i>Leucauge celebesiana</i> (Walckenaer, 1841)	Baramulla Srinagar Budgam Pulwama Anantnag Shopian	Khan, 2011a; Khan, 2012a Khan, 2009; Khan, 2011a; Khan, 2012a; Khan and Rather, 2012; Khan, 2013a; Punjoo and Bhat, 2015 Khan, 2011b; Khan and Rather, 2012 Khan, 2011a; Khan, 2012a Khan, 2011b; Khan and Rather, 2012; Punjoo and Bhat, 2015 Khan, 2012a	Russia (Far East), Korea, India to China, Vietnam, Laos, Japan, Indonesia (Sulawesi), New Guinea

152.	<i>Leucauge fastigata</i> (Simon, 1877)	Srinagar	Chakrabarti, 2013	India to Philippines, Indonesia
153.	<i>Mesida culta</i> (O. Pickard-Cambridge, 1869)	Srinagar and Anantnag	Punjoo and Bhat, 2015	India, Sri Lanka
154.	<i>Orsinome vethi</i> (Van Hasselt, 1882)	Baramulla and Srinagar	Sadana, 1983	India, China, Vietnam, Laos, Malaysia, Indonesia
155.	<i>Tetragnatha extensa</i> (Linnaeus, 1758)	Ganderbal and Srinagar	Caporiacco, 1934; Pickard- Cambridge, 1885	North America, Greenland, Europe, Turkey, Caucasus, Russia (Europe to Far East), Iraq, Iran, Kazakhstan, Central Asia, China, Korea, Japan
156.	<i>Tetragnatha javana</i> (Thorell, 1890)	Srinagar and Budgam	Khan, 2011b; Khan and Rather, 2012	Africa, Asia
157.	<i>Tetragnatha keyserlingi</i> Simon, 1890	Srinagar Budgam Anantnag	Khan, 2009; Khan, 2011b; Khan, 2012b; Khan and Rather, 2012; Punjoo and Bhat, 2015 Khan, 2011b Khan and Rather, 2012 Khan, 2011b; Khan and Rather, 2012; Punjoo and Bhat, 2015	Central America, Caribbean, Brazil, Africa, Korea, China, India to Philippines, New Hebrides, Polynesia
158.	<i>Tetragnatha montana</i> Simon, 1874	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, Iran, Central Asia
XXIII	Theridiidae Sundevall, 1833			
159.	<i>Euryopsis sagittata</i> (O. Pickard-Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
160.	<i>Euryopsis venutissima</i> (Caporiacco, 1934)	Srinagar	Caporiacco, 1934	India
161.	<i>Nesticodes rufipes</i> (Lucas, 1846)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Central and South America. Introduced to Europe, Macaronesia, North Africa, Middle East, China, India, Japan, Sri Lanka, Indonesia, New Zealand, Pacific Is.
162.	<i>Phylloneta sisypbia torandae</i> (Strand, 1917)	Ganderbal	Pickard-Cambridge, 1885	India
163.	<i>Rugathodes instabilis</i> (O. Pickard-Cambridge, 1871)	Ganderbal	Pickard-Cambridge, 1885	Europe, Russia (Europe to West Siberia)
164.	<i>Steatoda albomaculata</i> (De Geer, 1778)	Ganderbal and Srinagar	Caporiacco, 1934	North America, Europe, North Africa to Israel, Russia (Europe to Far East), Caucasus, Kazakhstan, Iran, Central Asia, China, Korea, Japan
165.	<i>Steatoda grossa</i> (C. L. Koch, 1838)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Russia (Europe to Far East), Caucasus, Iran, Kazakhstan, Central Asia, China, Korea, Japan. Introduced to North and South America, Macaronesia, Africa, New Zealand, Hawaii
166.	<i>Steatoda triangulosa</i> (Walckenaer, 1802)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Turkey, Caucasus, Russia (Europe to Far East), Kazakhstan, Iran, Central Asia, China, Korea. Introduced to Canada, USA, Canary Is., South Africa
167.	<i>Theridion expallidatum</i> O. Pickard-Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India

168.	<i>Theridion latisternum</i> Caporiacco, 1934	Baramulla	Caporiacco, 1934	India
169.	<i>Theridion manjithar</i> Tikader, 1970	Srinagar	Khan, 2013a	India
170.	<i>Theridion melanurum</i> Hahn, 1831	Srinagar	Caporiacco, 1934	Macaronesia, North Africa, Europe, Turkey, Caucasus, Russia (Europe to Middle Siberia), Middle East. Introduced to USA
171.	<i>Theridion spinosissimum</i> Caporiacco, 1934	Srinagar	Caporiacco, 1934	India
XXIV	Thomisidae Sundevall, 1833			
172.	<i>Ebrechtella tricuspidata</i> <i>concolor</i> (Caporiacco, 1935)	Srinagar	Caporiacco, 1935	India
173.	<i>Misumena oblonga</i> O. Pickard- Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
174.	<i>Ozyptila trux</i> (Blackwall, 1846)	Srinagar and Anantnag	Punjoo and Bhat, 2015	Europe, Caucasus, Russia (Europe to Far East), Japan. Introduced to Canada
175.	<i>Psammitis xysticiformis</i> (Caporiacco, 1935)	Ganderbal	Caporiacco, 1935	Central Asia, China, India
176.	<i>Runcinia insecta</i> (L. Koch, 1875)	Baramulla Srinagar Pulwama	Khan, 2012a Khan, 2009; Khan, 2012a Khan, 2011a; Khan, 2012a	Africa, Asia. Introduced to Australia
177.	<i>Thomisus nirmali</i> Saha and Raychaudhuri, 2007	Srinagar and Anantnag	Punjoo and Bhat, 2015	India
178.	<i>Thomisus whitakeri</i> Gajbe, 2004	Baramulla, Srinagar and Pulwama Shopian	Khan, 2011a; Khan, 2012a Khan, 2012a	India
179.	<i>Tmarus dejectus</i> (O. Pickard- Cambridge, 1885)	Ganderbal	Pickard-Cambridge, 1885	India
180.	<i>Tmarus stellio</i> Simon, 1875	Srinagar	Caporiacco, 1935	Southern, Central and Eastern Europe, Turkey, Caucasus, Iran, India
181.	<i>Xysticus cristatus</i> (Clerck, 1757)	Ganderbal Srinagar Anantnag	Caporiacco, 1935 Punjoo and Bhat, 2015 Punjoo and Bhat, 2015	Europe, Turkey, Caucasus, Russia (Europe to South Siberia), Kazakhstan, Iran, Central Asia, Nepal, China, Korea, Japan. Introduced to Canada, USA
182.	<i>Xysticus maculiger</i> Roewer, 1951	Bandipora	Caporiacco, 1935	Pakistan, India
183.	<i>Xysticus mundulus</i> O. Pickard- Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India
184.	<i>Xysticus pseudobliteus</i> (Simon, 1880)	Srinagar	Caporiacco, 1935	Russia (Central Asia to Far East), Kazakhstan, Mongolia, China, Korea
XXV	Uloboridae Thorell, 1869			
185.	<i>Uloborus albescens</i> O. Pickard- Cambridge, 1885	Ganderbal	Pickard-Cambridge, 1885	India

The analysis of the faunistic records reveal the occurrence of 185 species under 100 genera and 25 families. The most diverse families of spiders in this area are Salticidae and Linyphiidae (16 genera each) followed by Araneidae (10 genera), Gnaphosidae and Thomisidae (8 genera each). 2 families (Lycosidae and Theridiidae) have been represented by 6 genera each. Family Tetragnathidae is represented by 4 genera. 3 families (Dictynidae, Pholcidae and Pisauridae) are represented by 3 genera each. 3 families (Agelenidae, Philodromidae and Sparassidae) are represented by 2 genera each. 11 families (Cheiracanthiidae, Clubionidae, Ctenidae, Filistatidae, Liocranidae, Mimetidae, Oxyopidae, Pimoidae, Scytodidae, Sicariidae and Uloboridae) have only a single representative genera reported from this area. The checklist includes species reported from 9 out of 10 political districts of Kashmir as no spider species has ever been recorded from one district (Kulgam). Spiders from most families were recorded from Srinagar (23 families) followed by Ganderbal and Anantnag (17 families each), Pulwama (10 families), Shopian (9 families), Baramulla (8 families), Budgam (7 families), Bandipora (2 families) and Kupwara (1 family).

The checklist includes taxa that have been identified upto species level. 4 families (Amaurobiidae, Corinnidae, Desidae, Eresidae) have also been reported from Kashmir (Singh et al., 2023) but have not been included in this checklist as no species has yet been identified from these families. Genera like *Argiope*, *Argyroneta*, *Dictyna*, *Callilepis*, *Setaphis*, *Atypena*, *Milleriana*, *Obscuriphantes*, *Alopecosa*, *Arctosa*, *Evippa*, *Lycosa*, *Marpissa*, *Phidippus*, *Zygoballus*, *Sparassus*, *Eucta*, *Achaearana*, *Anelosimus*, *Coleosoma*, *Enoplognatha*, *Episinus*, *Latrodectus*, *Phoroncida* and *Theridula* have also been reported from Kashmir (Singh et al., 2023) but have not been included in this checklist as species level identification has not been carried out yet. Some spider species vis-a-vis *Araneus trifolium*, *Cyclosa elongata*, *Nigma puella*, *Haplodrassus omissus*, *Sergiolus montanus*, *Piniphantes himalayensis*, *Pardosa fuscus*, *Pardosa vindicata*, *Mimetes laevigatus*, *Oxyopes elegans*, *Oxyopes ramosus*, *Philodromus cinereus*, *Physocyclus globosus*, *Pisaura mirabilis*, *Stoliczka affinis*, *Afraflacilla flavipes*, *Attulus diductus*, *Cyrra algerina*, *Evarcha jucunda*, *Myrmarachne corpuzarrosae*, *Heteropoda venatoria*, *Nesticodes rufipes*, *Psammitis xycticiformis*, *Tmarus stellio* and *Xysticus maculiger* have been reported from India but do not reflect in the distributional records of World Spider Catalogue (2023). However, such species have been

included in the present checklist after critical review of the published data.

The checklist indicates that Kashmir has a rich spider diversity, despite harsh climatic conditions. There is an immense need to record the existing biodiversity in the biodiversity hotspots like Kashmir Himalaya. As spiders of this area are not studied thoroughly, it is anticipated that many new taxa could be identified from this hotspot.

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AUTHOR CONTRIBUTION STATEMENT

NZ contributed in data mining, data handling and prepared the manuscript. MY edited the manuscript. All authors read and approved the manuscript for submission.

CONFLICT OF INTEREST

No conflict of interest.

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MORPHOMETRICS OF MALE GENITALIA IN RICE YELLOW STEM BORER *SCIRPOPHAGA INCERTULAS* POPULATIONS FROM TELANGANA

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ABSTRACT

Morphometric characterization of male genitalia is an important aspect for studying intraspecific variation in Lepidoptera. In this study, size variation in the male genitalia of rice yellow stem borer (YSB) *Scirpophaga incertulas* (Walker) collected from nine locations through sex pheromone traps was examined. Measurements of male genitalial parts like uncus length (UL), uncus width (UW), valva length (VL), valva width (VW), saccus length (SL), saccus width (SW), aedeagus length (AD) were considered. Significant size variation was observed among the populations, and the measurements SL, SW, VL, VW, UL, UW and AD contributed to about 16% to location variability. The results showed that all characters except valva width was significantly different ($p \leq 0.05$) among the nine populations.

Key words: Rice *Scirpophaga incertulas*, male genitalia, intraspecific variations, uncus, valva, saccus, aedeagus, morphometrics, principal, components, cluster analysis

Rice (*Oryza sativa* L.) is an ancient and the most genetically diversified cereal crop. Rice harbours more than 100 species of insect pest. Among various stem borers reported from rice, yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) shares >70% of stem borer pests (Gururaj Katti et al., 2011). YSB is majorly distributed in Southeast Asia, China, Afghanistan and India. Grain yield losses of 3 to 95% due to YSB damage had been reported in India (Muralidharan and Pasalu, 2006). It is the most destructive and widely occurring insect pest of rice that attacks all stages of the crop from the seedling stage to maturity (Bandong and Litsinger, 2005). The adult moths are always observed resting on the rice leaf blade in a field in the early hours of the day. It was also observed that there is a variation in the size of the adult moths. Hence, a study on the morphometrics in understanding the intraspecific variations is necessary. Morphometrics has become a widely used technique in taxonomy, with a special emphasis to geometric morphometrics (Rohlf and Marcus, 1993; Adams et al., 2004; Zelditch et al., 2004; Mutanen et al., 2007; Tóth et al., 2014). Geometric morphometrics proved to be suitable to uncover and quantify small intra- or interspecific differences (Garnier et al., 2005; Tóth and Varga, 2011). Insect genitalia are important for species identification and are key traits for the evolution of reproductive isolation. Among the many parameters,

morphometric characterization of male genitalia is an important aspect for studying intraspecific variation in Lepidoptera. Male genitalia are widely recognized as the most stable and informative of all morphological structures. Male genitalia are considered one of the most important diagnostic traits in insect systematics (Tuxen, 1970). Carlos Cordero and Joaquín Baixeras (2015) opined that in Lepidoptera, male genitalia have been generally considered more informative in taxonomical terms than female genitalia. This study on the morphometrics of the male genitalia of adult moths of *S. incertulas* includes six populations collected from Telangana state, and one each from Palakkad, Kerala; Raichur, Karnataka; and Ludhiana, Punjab.

MATERIALS AND METHODS

Adult male moths of *S. incertulas* were collected from nine locations viz., Medak (MDK), Warangal (WGL), Nizamabad (NZD), Jagtial (JGL), Rangareddy (RGD), Mehabubnagar (MBR), Palakkad (PKD), Raichur (RCR) and Punjab (PJB) using sex pheromone traps (YSB Detector^R Pheromone chemicals, Hyderabad) installed in the rice fields. Abdomen from adult male moths was detached by pressing it slightly with the help of forceps. Then, it was placed in 10% potassium hydroxide (KOH) for digestion overnight at room temperature. After digestion, the abdomen was

transferred to a cavity block filled with distilled water and softly scrubbed hairs, scales, and the debris from the abdomen using camel hair brush. Then, the abdomen was placed into a watch glass with 20% ethanol and cleaned again. The abdomen was then placed into a cavity block containing 70% ethanol for final cleaning and genitalia were separated from the abdomen. For separation of male genitalia from the abdomen, inter segmental membrane of last abdominal segment was cut and the aedeagus was removed from the genitalia by holding the base of the valva with a pair of forceps and detached the aedeagus with another pair. The detached genitalia was shifted to a cavity block containing absolute ethanol. Genitalia were kept in proper position and valva were stretched and spread apart by using fine needle and forceps. Valva were held in a stretched position for 1-2 min and then, glass chips were placed over it for 2 hr. The stretched genitalia were mounted in Canada balsam using clean microscope slide and covered with cover slide. The slides of male genitalia (N=10 from each location) were prepared and measurements of genitalial characters like uncus length (UL), uncus width (UW), valva length (VL), valva width (VW), saccus length (SL), saccus width (SW) (Fig. 1a) and aedeagus length (AD) (Fig. 1b) were taken with LYNX ISH500 5.0 MP camera mounted on Lawrence and Mayo Stereozoom microscope. Descriptive statistics was calculated using MS-EXCEL for all chosen traits. The analyses viz., univariate and multivariate ANOVA and principal component analysis (PCA) were done to determine intraspecific variation. All these tests were carried out using R package 'ggpubr'.

RESULTS AND DISCUSSION

The morphometrics was analysed from the nine field populations. The measurements of seven parameters pertaining to male genitalia were observed and given in Table 1 and Fig. 1. Descriptive and univariate analysis were conducted for each character. Tukey's Honest Significant Difference (HSD) Test was carried out to test differences among the means of locations. The results showed that all characters except valva width was significantly different ($p \leq 0.05$) among the nine populations. The length and width of uncus, length of valva, length and width of saccus and aedeagus length differed significantly among nine locations. The UL ranged from 604.80 (MBR) to 699.40 μm (RCR). RCR population showed highest mean uncus length (699.40 μm), uncus width (378.20 μm) and saccus length (294.80 μm). The mean UW of MDK, WGL, JGL, RGD, MBR and PJB populations were not significantly different from each other, but different from RCR and JGL populations. The highest and lowest mean UW were reported from RCR (378.20 μm) and MDK (283.60 μm) populations, respectively. The mean VL of populations ranged from 810.40 (JGL) to 949.40 μm (NZD). There were no significant differences among the populations in case of VW. The mean value of VW ranged from 443.40 to 497.20 μm . Saccus length varied from 224 μm (JGL) to 294.80 μm (RCR). Aedeagus length varied from 1171.60 μm (JGL) to 1590.20 μm (NZD). The study on *Drosophila mojavensis* supports a role for aedeagus size in the male-female mating interaction, with a secondary role for aedeagus shape.

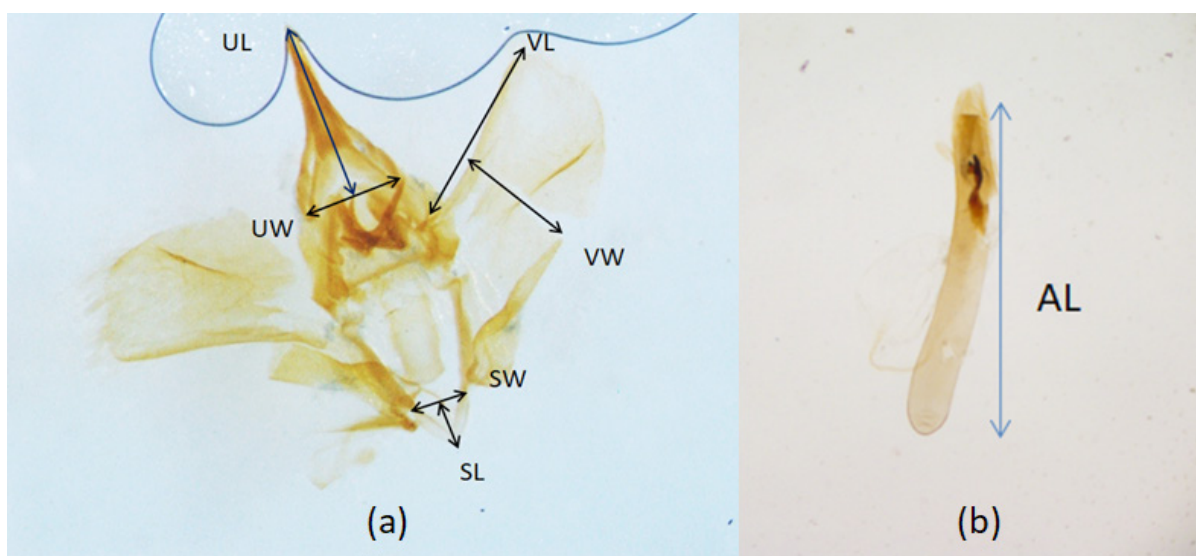


Fig. 1. *S. incertulas*. a. Male genitalia of. B. Aedeagus

(UL-uncus length, UW-uncus width, V-valva length, VW-valva width, SL-saccus length, SW-saccus width, AD-aedeagus length)

Table 1. Morphometrics of the male genitalia from populations of *S. incertulas*

Location with its Geographical Coordinates	Measurement (μ M)						
	UL	UW	VL	VW	SL	SW	AD
MDK 17.5192° N, 78.2784° E	624.80 ^{bc}	283.60 ^b	890.20 ^{abc}	443.40	231.80 ^{bc}	150.80 ^{ab}	1242.40 ^{dc}
WGL 18.0122° N, 79.5990° E	687.20 ^{ab}	311.00 ^b	917.80 ^{ab}	469.80	242.00 ^{bc}	185.20 ^{ab}	1344.00 ^c
NZD 18.67°N 77.9°E	650.20 ^{abc}	322.80 ^{ab}	949.40 ^a	478.80	237.80 ^{bc}	154.60 ^{ab}	1590.20 ^a
JGL 8.7895° N 78.9120°E	640.40 ^{abc}	300.80 ^b	810.40 ^c	448.60	224.00 ^c	151.20 ^{ab}	1171.60 ^c
RGD 17.3201° N 78.3939° E	641.20 ^{abc}	319.60 ^b	946.20 ^a	497.20	243.40 ^{bc}	161.60 ^{ab}	1455.60 ^b
MBR 17.2731° N 81.844° E	604.80 ^c	316.80 ^b	868.80 ^{abc}	480.60	249.00 ^{abc}	161.60 ^{ab}	1254.20 ^d
PKD 10.8088° N 76.1894° E	699.20 ^a	331.80 ^{ab}	835.80 ^{bc}	479.80	277.80 ^{ab}	168.60 ^{ab}	1222.60 ^{dc}
RCR 16.2044° N 77.3341° E	699.40 ^a	378.20 ^a	938.80 ^a	489.20	294.80 ^a	184.60 ^a	1378.00 ^{bc}
PJB 30.9041°N, 75.8066° E	678.00 ^{ab}	317.80 ^b	943.60 ^a	483.40	236.40 ^{bc}	147.20 ^b	1382.60 ^{bc}
Min	604.80	283.60	810.40	443.40	224.00	147.20	1171.60
Max	699.40	378.20	949.40	497.20	294.80	185.20	1590.20
Mean	658.36	320.27	900.11	474.53	248.56	162.82	1337.91
SD	32.06	24.32	48.90	16.85	21.63	13.34	123.93
CV	0.05	0.08	0.05	0.04	0.09	0.08	0.09
F value	4.87*	4.36*	7.96*	0.72 NS	4.35*	3.64*	58.70*

Means with same alphabets within a column not significantly different; *Significant at ($p \leq 0.05$); (UL-uncus length, UW-uncus width, V-valva length, VW-valva width, SL-saccus length, SW-saccus width, AD-aedeagus length)

In natural populations, mating failure based on aedeagus size could serve as an important reproductive isolating mechanism resulting in failed insemination attempts after both the male and female show a willingness to mate (Richmond, 2014). The effect of location on the male genitalia morphometric characters was tested with MANOVA. The results registered that the effect of location was highly significant (Pillai's Trace 2.759, $p=5.237e-09$; Hotelling's -Lawley Trace 23.653, $p=2.2e-16$; Roy's Largest Root 18.39, $p=2.2e-16$; Wilks' Lambda 0.0032425, $p=2.2e-16$) on the seven measured parameters of male genitalia suggesting significant differences between nine locations (Table 2).

PCA plots of male genitalia morphometric variables of first two dimensions with linear projections were made to see the major contributing characters for variability among male moth populations occurring in nine different locations. SL, SW, VL, VW, UL, UW and AD contributed about 16 percent to location variability in male moths (Fig. 2). Valval length, valval width and aedeagus length were highly correlated to each other. On the other hand, saccal length and saccal width were also correlated with uncus length and uncus width but correlation was weak as compared to valval and aedeagus correlation. VL, VW and AD were not correlated with SL, SW, UL and UW. Male

Table 2. MANOVA table for location effects on male genitalia morphometrics

Effect		Value	F Value	Num DF	Den DF	Pr (>F)
Locations (8)	Pillai's Trace	2.759	2.927	56	252	5.237e-09***
	Hotelling's Trace	23.653	11.947	56	198	< 2.2e-16***
	Roy's Largest Root	18.390	82.754	8	36	< 2.2e-16***
	Wilk's Lambda	0.003	5.657	56	166.87	< 2.2e-16***

Signif. codes 0 '***'; 0.001 '***'; 0.01 '**'

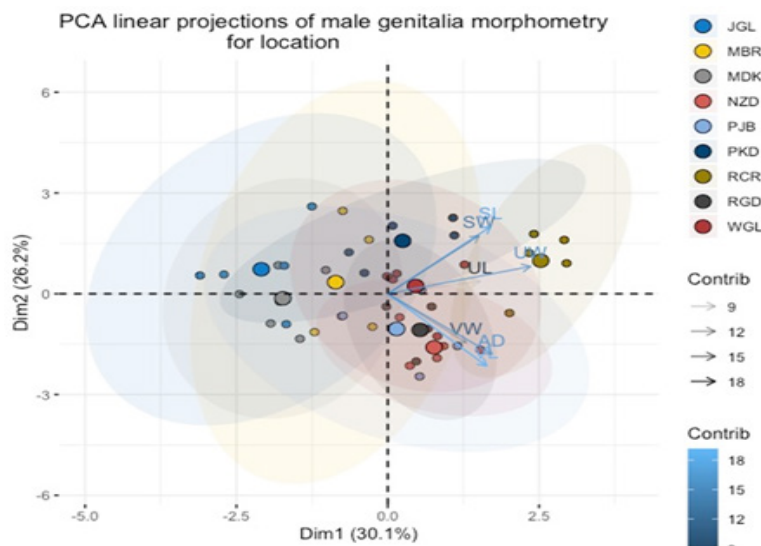


Fig. 2. PCA linear projections of male genitalia morphometry for locations

genitalia morphometry had very strong influence on the variability of male moth populations of WGL, NZD, RGD and PKD locations while the male populations of MBR, MDK and JGL were less influenced by the male genitalia morphometry. The saccal morphometry had high influence in variability of populations of PKD whereas uncus morphometry had high influence on populations of WGL. Contrarily, valval and aedeagus morphometry had high influence in variability of populations of WGL, NZD and PJB. Populations of JGL, MBR and MDK were completely devoid of any influence by male genitalia morphometry to their location.

The significant differences observed in the size of the male genitalia parameters were taken into consideration. Parts of male genital exhibited geographic variability, but it is not related to the geographic distance. It is also noted that there were no structural differences in male genitalia of *S. incertulas* populations from nine locations under study. Generally, the genital characters are known to be more stable and informative in taxonomic aspect than the wing patterns (Shapiro and Porter 1989; Mutanen, 2005). During the past half of

the century, morphometrics has become a widely used method in taxonomy, with special respect to geometric morphometrics (Mutanen et al., 2007). Geometric morphometrics proved to be suitable to uncover and quantify small intra- or interspecific differences. Until now, no study has reported quantitative comparison genital traits of rice yellow stem borer. Some authors have concluded that variability may occur in the genital structures, mainly of the males, due to their complexity, which attains great importance for the taxonomy of a species (Singh-Pruthi, 1926; Lent and Jurberg, 1966). Due to possible correlation between size and shape, species with remarkable variation in overall size may consequently exhibit larger genital variability. Mutanen and Kaitala (2006) opined that genital shape rather than size that tends to evolve rapidly. So size explorations should not be ignored in genital variation studies. The results suggest that genital morphology is not constant and should therefore be used with caution in lepidopteran taxonomy, agreeing with the observations of Mutanen et al. (2007) on the male genital variation in a moth *Pammene luedersiana* (Lepidoptera: Tortricidae). Several studies show that genitalia vary a lot within species (Goulson, 1993; Eberhard et al., 1998; House

and Simmons, 2003). Intraspecific variation in genital morphology reflects the outcome of the interaction between genes and environment (Willmore et al., 2007).

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AUTHOR CONTRIBUTION STATEMENT

APK and TU conceived and designed research. AR conducted experiments. APK, TU facilitated collection of populations, contributed new reagents and analytical tools. AR, APK and AK analyzed the data. AR and APK wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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COMPARATIVE STUDY ON ECTOPARASITIC TICK ABUNDANCE AND DIVERSITY ON GOATS AND COWS

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ABSTRACT

Ectoparasitic infestation is one of the threats to the growth of the livestock industry. Ticks, one of the most notorious groups of ectoparasites on livestock, create issues for cattle rearers by causing a wide range of diseases like babesiosis, theileriosis, anaplasmosis, KFD, CCHF, etc. In addition, conditions like tick worry, metabolic debilitation, secondary infections, and loss of large quantities of blood due to parasitic feeding also pose severe health issues. The current study analyzed the ectoparasitic tick infestation on goats and cows, the common domesticated animals in south India. Gaining from rearing these animals support the economic status of the rural peoples of the region. The study discussed the abundance, diversity, and host selectivity of the ticks recorded from the area. Additionally, the role of each species in transmitting various diseases in animals and humans is also discussed.

Key words: Domestic animals, cows, goats, livestock, ectoparasites, ticks, diversity, abundance, host specificity, tick-borne diseases, seasonal variation, *Haemaphysalis bispinosa*, *Rhipicephalus (Boophilus) annulatus*, *Rhipicephalus haemaphysaloides*, *Hyalomma anatolicum*, *Boophilus* sp.

In the developing world, domestic animals have their position as the source of energy, food, raw material, and manure mainly for rural farmers. This is especially true for India which is known to be a land of agriculture, where cattle wealth determines the socio-economic status of millions of rural people. Even though cattle farming has not evolved as a major industry in a small state like Kerala, the culture of maintaining a few animals like cows and goats in small stables of the household backyards is still a practice in the rural belts of the state. Low space requirements and maintenance costs, ease in management, and high demand for their products even in urban markets have made these small-scale cattle rearing a major contributor to the economic stability of the middle-class farming community. Ectoparasitism is the major issue faced by the cattle industry all over the world, which severely affects animal health, growth rate, and production (Ajith et al., 2017; Puvarajan, 2017). Among the common ectoparasites on cattle, ticks are one of the major pests causing serious health issues in the host. They cause transmission of tick-borne diseases by bacteria, rickettsiae, protozoa, spirochaetes, and viruses more than any other arthropod vector. Tick-borne diseases like babesiosis, theileriosis, anaplasmosis, and many more are considered major limitations on livestock productivity (Monfared, 2015; Waskel and Gaur, 2015; Riaz, 2017). Tick worry, metabolic debilitation, tick

toxicosis, secondary infections, anaemia, weight loss, dermatitis, sweating sickness, and even death due to draining large quantities of blood are the other major deleterious impacts of severe tick infestation (Eyo et al., 2014; Monfared et al., 2015; Soundararajan et al., 2018). From an economic point of view, they are causing a great reduction in milk production and weight gain in cattle (Kumar et al. 2018). The issue of tick infestation is on the high rise hitting the cattle industry of the state. The continued presence of ectoparasites is forcing many small-scale farmers to stop cattle rearing due to increased financial burden, reduced production, and high maintenance costs. The current study aims at analyzing the abundance, diversity, and host specificity of common tick species infesting cows and goats in the Palakkad district of Kerala state in India.

MATERIALS AND METHODS

A survey of tick species infesting cows and goats was carried out for a period of six months from September 2019 to February 2020, from Pallatheri, a rural village in Elappulli panchayat, Palakkad district (Latitude 10.7570°N, 76.7134°E). The study period includes two seasons, post-monsoon and pre-summer. Random sampling was done from cows (*Bos taurus*) and goats (*Capra aegagrus hircus*) above two years of age, reared in the residential backyards

of the study area. Collections were made by hand picking method (Debbarma et al., 2018). Collected specimens were transferred into Tarsons vials (PVC) containing 70% alcohol. The specimens were taken to the laboratory at St. Josephs College (Autonomous), Devagiri, and identified up to the species level with the identification keys provided by (Sen, 1938; Trapido, 1964; Geevarghese, 1997). The monthly data of tick species obtained were subjected to statistical analysis by using PAST software. The tick abundance, diversity, and evenness concerning the hosts were analysed. Variation in the abundance of tick species on the two hosts was done with one factor ANOVA and variation in season-wise abundance in the two hosts was analysed with the Kruskal Wallis test followed by Wilcoxon- Mann Whitney test.

RESULTS AND DISCUSSION

The study recorded a total of 202 ticks from 80 cows and 369 ticks from 80 goats. The ticks identified are *Haemaphysalis bispinosa*, *Boophilus* sp., *Rhipicephalus* (*Boophilus*) *annulatus*, *Rhipicephalus haemaphysaloides*, *Hyalomma anatolicum*, and one unidentified species. Out of the five identified species, all species were found to infest cows, whereas only two species were recorded on goats namely, *H. bispinosa* and *R. haemaphysaloides*. The percentage of tick infestation in cows was 35.38% and in goats was 64.62%. Regarding the overall species-wise abundance of the ticks in the locality, *H. bispinosa* (67.08%) was recorded as the prevalent species followed by *R. haemaphysaloides* (13.66%), *Boophilus* sp. (11%) and *R. (B) annulatus* (6.13%) respectively. *H. anatolicum* was recorded as the least abundant species in the area (1.04%). While considering the percentage of infestation in different animal hosts *H. bispinosa* was the abundant species in cows (47%) followed by *Boophilus* sp. (31%) and *R. (B) annulatus* (17%). *H. anatolicum* (3.9%) and *R. haemaphysaloides* (0.5%) recorded low abundance. In goats, *H. bispinosa* (78%) was the most abundant species followed by *R. haemaphysaloides* (20.87%). *Boophilus* sp., *R. (B) annulatus*, and *H. anatolicum* were completely absent in goats. *Rhipicephalus* was the predominant genus recorded from the locality, with three species followed by *Haemaphysalis* genus with a single species.

Species diversity and evenness of ticks were high for cows. Statistical analysis showed that overall tick abundance on the host varied between the two hosts namely goat and cow ($p = 0.002308$, $F = 10.17$),

and was recorded to be highest on goats than on cows. Significant variation was observed in the abundance of individual tick species that were present on both hosts. *H. bispinosa* ($p = 0.00$, $F = 21.14$) and *R. haemaphysaloides* ($p = 0.00$, $F = 28.44$, Table 1) recorded the highest abundance in goats than in cows. Despite the low diversity of tick species, overall tick abundance was high in goats compared to cows. Goats with longer hairs and thicker coats create a microclimate that favours tick survival (Fraga et al., 2003) which supported the high abundance of individual tick species on goats. Cows with comparatively shorter hairs and thinner fur coats are intensively maintained in the region with a practice of regular bathing and grooming, daily cleaning of cow sheds, and use of plant-based or chemical compounds to regulate the ectoparasitic population. Such practices lead to the continuous removal of ticks from the animal body resulting in a low abundance of pest species (Shyma et al., 2013). The absence of hygienic measures along with favourable habitat conditions supported the population build-up of the ticks on goats. The higher tick diversity in cows indicates the ability of the host to maintain multiple populations of ectoparasites on them.

Haemaphysalis bispinosa being the most abundant species on both hosts marks its importance as the predominant ectoparasitic tick in the region. *H. bispinosa* is one of the prevalent tick species in Kerala (Prakasan and Ramani, 2007; Shyma et al., 2013; Balasubramanian et al., 2019). It is a three-host hard tick attributed with lower host specificity and ability to infest a wide range of animals including all domestic animals (Prakasan and Ramani, 2007; Geevarghese and Mishra, 2011). This provides *H. bispinosa* with higher chances of existence on different hosts widely in the region. All life stages have been found to inhabit the grooves and crevices in the cattle shed for about 3 to 8 feet above the floor, and they can reproduce quickly and occur in clusters on the walls of the cattle shed (Chhabra, 1992; Geevarghese and Mishra, 2011). This phenomenon helps them escape from the regular tick control measures practiced by the farmers providing higher survival rates. *Haemaphysalis bispinosa* transmits diseases like Theileriosis and Bartonellosis in cattle by transmitting pathogens like *Theileria orientalis* and *Bartonella bovis* respectively. (Kho et al., 2015; Kakati et al., 2015). Additionally reports on the presence of *H. bispinosa* in areas endemic to the Kyasanur Forest Disease (KFD) outbreak and the ability of the animal to act as a reservoir for the virus (Balasubramanian et al., 2019; Prakasan, 2015) show that the tick species have a role in

the transmission of this disease. Hence the prevalence of the species in the region is of great concern, as they can affect the health status of the host animals and humans associated with cattle rearing.

Rhipicephalus haemaphysaloides is a common tick species of south India that shows comparatively low host specificity and can infest a wide range of animals including domestic and wild animals (Prakasan and Ramani, 2007). It was recorded as the second most abundant species in goats whereas in cows it showed less abundance. They act as a vector of benign *Theileria ovis* (Chhabra, 1992). KFD virus was isolated from the wild-caught species of *R. haemaphysaloides* (Kumar et al., 2014) which shows its potency to spread the pathogen. Ticks under the *Rhipicephalus* (*Boophilus*) genus including *Rhipicephalus* (*Boophilus*) *annulatus* act as vectors of *Babesia bigemina* and *Babesia bovis* causing babesiosis in a wide range of animals and humans (Antunes et al., 2012) they also transmit *Anaplasma marginale* causing rickettsia (Bovine anaplasmosis or gall sickness). Nair et al., 2013 reported *Anaplasma marginale* in samples collected from cows in Palakkad and other Malabar districts. The absence of subgenus *R.* (*Boophilus*) in goats is notable despite its occurrence as the second major pest in cows. The exclusion of goats as a host is suspected not due to their high host specificity on cattle as the species have been reported earlier from goats from Kerala (Prakasan and Ramani, 2007). Additionally, the members of this subgenus

are reported to be able to complete their life cycle on goats (Nyangiwe and Horak, 2007). According to Daemon et al., 1998 and Nyangiwe and Horak, 2007, goats act as alternative hosts for many species, playing a role in maintaining infestations of ticks on other animals. Hence it is concluded that goats might be acting as an alternative host for the species in the region. The abundance of the preferred hosts like cows might have caused the reduction in the population of *Rhipicephalus* (*Boophilus*) sp. on alternative hosts like goats. *Hyalomma anatolicum* is a multi-host hard tick that is a common member of the genus in India (Shyma et al., 2012). Even though a lower abundance of *H. anatolicum* in cows and its complete absence in goats were recorded in the region, the tick species have been reported from both these hosts in other regions (Islam et al., 2006; Choubdar et al., 2019). Hence *H. anatolicum* remains as a minor ectoparasitic tick on cows.

While looking into the relationship with tick abundance and seasonality, Kruskal-Wallis test showed a significant variation in the season-wise abundance of ticks on goats (Table 1). Tick abundance in goats during post-monsoon (15.87 ± 7.87) is higher than in pre-summer (8.73 ± 6.69). Among the two tick species recorded on goats, only *H. bispinosa* showed significant variation in season-wise abundance where the highest abundance was recorded in post-monsoon (12.40 ± 7.40) than in the pre-summer (6.80 ± 5.85) season. No

Table 1. Diversity of the ticks on two hosts- goat and cow

Abundance and infestation				
Tick species recorded	% of infestation		Abundance (Mean± SD)	
	Goat	Cow	Goat	Cow
<i>H. bispinosa</i>	78.05%	47.03%	9.60± 7.15	3.17± 2.78
<i>Boophilus</i> sp.	0.00%	31.19%	0.00± 0.00	2.10± 2.06
<i>R. (B) annulatus</i>	0.00%	17.33%	0.00± 0.00	1.17± 2.05
<i>R. haemaphysaloides</i>	20.87%	0.50%	2.57± 2.60	0.03± 0.18
<i>Hyalomma anatolicum</i>	0.00%	3.96%	0.00± 0.00	0.27± 1.01
Unidentified species	1.08%	0.00	0.13± 0.51	0.00± 0.00
Diversity analyses				
	Cows	Goats		
Dominance_D	0.3501	0.6528		
Shannon_H	1.176	0.5695		
Evenness_e^H/S	0.6483	0.5891		
Seasonal variation				
Host	Kruskal Wallis test		Significant variation in abundance between the	
	P	H	two seasons (Mann Whitney test)	
Cow	0.2306	1.427		
Goat	0.01347	6.091	0.01428	

(Kruskal Wallis test, followed by Mann Whitney test for parameters showing significant variation)

significant variation was noted in the overall abundance of ticks on cows. Among the three tick species on cows, only *Boophilus* sp. showed significant variation in season-wise abundance. Here the abundance is higher in pre-summer (2.93 ± 2.34) than in post-monsoon (1.27 ± 1.33). The higher abundance of *H. bispinosa* in the post-monsoon season than during pre-summer, which is also the prevalent species on goats, is the major contributor to the overall tick abundance in goats during the post-monsoon season. Of the three species reported on cows, only *Boophilus* sp., the second prevalent species, showed significant variation in seasonal abundance which is more abundant during pre-summer than in post-monsoon. Hence it is thought that the seasonal variation in tick abundance is directly proportional to the seasonal abundance of the prevalent tick species.

The recorded high tick abundance on goats and high tick diversity on cows in the study confirms the role of cows and goats as important hosts of the ixodid ticks in the region. The occurrence of *H. bispinosa* and *R. haemaphysaloides* as the dominant ectoparasitic tick pest on both the hosts and their ability to serve as a vector in transmitting several tick-borne pathogens is a clear indicator of the possibility of the spread of various tick-borne diseases in the hosts of the region. The prevalence of the vectors of KFD in the study area on the domesticated hosts further show the risks posed by these ectoparasites to animals and humans.

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AUTHOR CONTRIBUTION STATEMENT

Princy and Aswathi conceived and designed

research. Sample collection and preliminary sorting done by Nandakrishnan. Species level Identification done by Princy. Data analysis, interpretation and writing part done by Princy and Aswathi. All authors read and approved the manuscript.

CONFLICT OF INTERST

No conflict of interest.

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PREFERENCE AND PERFORMANCE OF *BEMISIA TABACI* (GENNADIUS) ON DIFFERENT HOST PLANTS

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ABSTRACT

Host preference and development biology of *Bemisia tabaci* (Gennadius) on brinjal, soybean, cucumber, cotton, tomato and *Solanum nigrum* L. was studied during 2020-21 at PAU, Ludhiana. Under free choice test conditions, cucumber was the most preferred host which attracted maximum number of adults followed by soybean. The other host plants in decreasing order of preference were cotton > brinjal > tomato > *S. nigrum*. Brinjal and cotton were the most suitable hosts having shorter development period, higher survival and fecundity. The survival of immature stages on soybean was 50% as compared to 67.5-72.5% on the most suitable hosts i.e. cotton and brinjal. Thus, soybean is a preferred host for adult feeding and oviposition but less suitable for immature stages of the *B. tabaci*. Hence, soybean may be exploited as a trap crop for the management of *B. tabaci*.

Key words: *Bemisia tabaci*, biology, cotton, development period, host preference, management, oviposition preference, soybean, trap crop, whitefly

Whitefly *Bemisia tabaci* (Gennadius) is one of the most important pests of vegetables, ornamentals and field crops in the tropics and subtropics of the world (De Barro, 2011). In India, *B. tabaci* was first time reported in 1905 on cotton and it becomes a serious pest of cotton in the late 1920s and early 1930. Both nymph and adults suck the cell sap from ventral surface of leaves, resulting in loss of plant vigour and yield (Attique et al., 2003). Apart from causing direct losses, *B. tabaci* also causes damage by transmitting many viral diseases and secreting honeydew, on which black mould grows, resulting in reduced photosynthetic activities (Jones, 2003; Kranthi, 2014). Depending on the host plants, cultivar and environmental conditions, *B. tabaci* can cause heavy yield losses. Insecticides application is the main control strategy for this pest, though effective spraying is complicated because of the insect's preference for the abaxial side of the leaf (Simmons, 1994). Moreover, *B. tabaci* developed resistance to many active ingredients (Horowitz et al., 2005; Naveen et al., 2017) and sole reliance on insecticides is expected to increase tolerance to insecticides, in addition to the adverse effects on non-target organisms (Palumbo et al., 2000). Therefore, it is necessary to develop ecological based management strategies for *B. tabaci*.

Trap cropping is a promising pest control method that involves growing another crop to attract pests from main crop to reduce damage to the main crop (Shelton and Badenes, 2006). However, for successful pest

control, the trap plants cultivated within agricultural fields should either retain high densities of pest to prevent insect pest dispersal to the main crop or increasing pest mortality on the trap crop (Holden et al., 2012). The selection of trap crop is based on the fact that herbivores show a distinct preference to certain plant species and cultivars (Cook et al., 2007), which need to be studied for devising ecological based pest management strategy. *Bemisia tabaci* shows dissimilar preference for oviposition, host suitability, adaptation, and efficiency of virus transmission (De Barro et al., 2005). It has a wide host range of about 1000 plant species in 74 plant families, including cotton, brinjal, tomato, soybean, cucumber and weed host, *Solanum nigrum* L. (Abd-Rabou and Simmons, 2010; Kedar et al., 2018). The effective management of such a polyphagous pest in diverse, year-round agricultural systems is often a challenge. The studies of host preference and developmental rates on different host plants are important tools to identify a potential trap crop for whitefly management (Muniz, 2000). Although a number of investigations have examined the performance of *B. tabaci* on various host plants, few studies have compared the differences in fitness of *B. tabaci* on different hosts under North Indian conditions. Therefore, the present study was carried out to investigate the host preference and suitability based on oviposition, developmental time and survival of *B. tabaci* on six preferred hosts of *B. tabaci* under Punjab conditions. The aim of the study was to identify

a potential trap crop for whitefly management in cotton.

MATERIALS AND METHODS

The survey conducted in the cotton growing area of Punjab during 2019 (data not presented) revealed that brinjal, cucumber, cotton, soybean, and tomato were the preferred cultivated host of *B. tabaci*. Ovipositional preference and developmental biology of *B. tabaci* on soybean (var. SL 958), tomato (var. Punjab Gaurav), brinjal (var. PBHR 41), cotton (var. RCH 773) and cucumber (var. Punjab Naveen) was evaluated along with a perennial weed host i.e. *makoh*, *Solanum nigrum*. The experiments were conducted under screen house condition during 2020-21 and experimental design was completely randomized design (CRD). Oviposition preference of *B. tabaci* adults was studied under free choice test. The plants were sown/ transplanted in the earthen pots (12 cm height and 10 cm diameter) and kept under nylon cage (40 mesh) to avoid infestation by any other pest. The experiment was performed when plants were of four leaves stage. Potted plants of each species were placed at a uniform distance in nylon mesh cage (90 x 90 x 60 cm) and approximately 200 adults were released in the centre of the cage. The numbers of whitefly adults settled on each plant were recorded after 24, 48, 72 and 96 hours, while eggs deposited on each host plant were counted after 96 hours under stereo zoom binocular microscope. There were four replications and data was subjected to analysis of variance (ANOVA) for comparison.

The developmental biology of *B. tabaci* on different host plants was also studied under screen house at Entomological Research Farm. One generation of *B. tabaci* was reared on each host (brinjal, cotton, cucumber, soybean, tomato and *S. nigrum*) and adults that emerged were used to conduct the experiment when test plants were of four leaf stage. Newly emerged adults were separated on sex basis. A pair of adults was released in a cup cage on under surface of the leaves on 20 plants to obtain fresh eggs, each plant representing one replication. After 24 hr, the adults were removed and leaves were examined for eggs with the help of compound microscope. The eggs bearing leaves were tagged at petiole region and five eggs on each leaf were retained to observe the duration of different development stage and remaining were removed with the help of a fine brush. Cup cage were again attached to the leaves on selected leaves to avoid further infestation of whitefly or other insects. The time period between

laying of eggs and appearance of the crawler were taken as incubation period. The observations on hatchability, nymphal and pupal survival and duration of different stages were recorded at 24 hr interval. To study the adult longevity and fecundity, the freshly emerged pair of male and female was released in cup cage on under surface of leaf and confined till the death of the adult. The mortality of adults in each cage was recorded daily and longevity of each sex was calculated. To calculate pre-oviposition, oviposition, post-oviposition period and fecundity, different set of experiments were performed. Freshly emerged adult pair was confined in the cup cage and numbers of eggs laid on leaf covered under cup cage was recorded daily and different periods and fecundity was calculated. The data was subjected to analysis of variance (ANOVA) for comparison of treatments.

RESULTS AND DISCUSSION

Feeding and oviposition preference: The results revealed significant differences in feeding and oviposition preference of *B. tabaci* under free choice test conditions (Table 1). The data recorded at 24, 48, 72 and 96 hr after release of whitefly adults, showed that cucumber was the most preferred host of *B. tabaci* for feeding and oviposition, followed by soybean. The relative preference of whitefly adults increased for cotton, whereas decreased in case of *S. nigrum* and tomato over time, as depicted in observations recorded at 24, 48, 72 and 96 hr. Mean number of whitefly adults were significantly highest on cucumber (36.57 adults/ leaf) followed by soybean (20.04 adults/ leaf), which was on par with cotton (16.92 adults/ leaf). *S. nigrum* was least preferred host which attracted minimum number of whitefly adults (2.49 adults/ plant) under free choice conditions. The mean number of whitefly adults on brinjal and tomato were on par with each other having mean value of 14.65 and 12.34 adults/ plant, respectively. Similar trend of relative preference for oviposition was observed in data recorded at 96 hr after release of whitefly adults. Significantly maximum eggs were recorded on cucumber (42.80 eggs/ three leaves) and minimum on *S. nigrum* (5.93 eggs/ three leaves).

These results revealed that when six host plants of *B. tabaci* were placed together, cucumber was the most preferred host for adult feeding and oviposition. The other host plants in decreasing order of preference were soybean, cotton, brinjal, tomato and *S. nigrum*. These results are in consonance of Sharma and Budha (2015), who also observed cucumber as the most preferred

Table 1. Host preference of *B. tabaci*

Incidence						
Host plant	Mean ($\pm$ SE) number of whitefly adults/ plant					Number of eggs / three leaves
	24 hrs	48 hrs	72 hrs	96 hrs	Mean	96 hrs
Brinjal	13.39 $\pm$ 1.04 (3.79)	15.71 $\pm$ 1.22 (4.08)	14.45 $\pm$ 0.64 (3.93)	15.06 $\pm$ 1.20 (4.01)	14.65 $\pm$ 0.49 (3.96)	22.73 $\pm$ 1.58 (4.87)
Cotton	15.33 $\pm$ 1.09 (4.04)	17.11 $\pm$ 1.18 (4.25)	16.72 $\pm$ 0.97 (4.21)	18.51 $\pm$ 1.69 (4.41)	16.92 $\pm$ 0.65 (4.23)	24.20 $\pm$ 1.33 (5.02)
Cucumber	31.33 $\pm$ 3.42 (5.67)	36.61 $\pm$ 2.34 (6.12)	38.11 $\pm$ 2.15 (6.25)	40.22 $\pm$ 2.51 (6.41)	36.57 $\pm$ 1.90 (6.11)	42.80 $\pm$ 3.49 (6.60)
Soybean	19.56 $\pm$ 2.03 (4.52)	21.72 $\pm$ 1.26 (4.76)	19.83 $\pm$ 1.14 (4.56)	19.06 $\pm$ 1.20 (4.47)	20.04 $\pm$ 0.58 (4.58)	28.40 $\pm$ 2.03 (5.41)
<i>S. nigrum</i>	4.61 $\pm$ 0.48 (2.36)	1.78 $\pm$ 0.24 (1.66)	2.17 $\pm$ 0.75 (1.75)	1.39 $\pm$ 0.34 (1.54)	2.49 $\pm$ 0.73 (1.83)	5.93 $\pm$ 1.45 (2.60)
Tomato	17.39 $\pm$ 1.03 (4.28)	12.22 $\pm$ 1.22 (3.63)	9.67 $\pm$ 1.21 (3.25)	10.06 $\pm$ 0.87 (3.32)	12.34 $\pm$ 1.78 (3.62)	19.27 $\pm$ 1.50 (4.49)
CD (p= 0.05)	(0.61)	(0.46)	(0.42)	(0.41)	(0.47)	(0.54)
Durations of immature stages						
Host plant	Mean ($\pm$ SE) duration (days)					Total development
	Eggs	1 st instar	2 nd instar	3 rd instar	4 th instar	
Brinjal	4.20 $\pm$ 0.18	3.90 $\pm$ 0.14	3.40 $\pm$ 0.11	3.65 $\pm$ 0.11	4.20 $\pm$ 0.17	19.35 $\pm$ 0.36
Cotton	4.10 $\pm$ 0.19	4.15 $\pm$ 0.15	3.30 $\pm$ 0.11	3.50 $\pm$ 0.11	4.60 $\pm$ 0.18	19.65 $\pm$ 0.18
Cucumber	4.25 $\pm$ 0.14	4.35 $\pm$ 0.11	3.75 $\pm$ 0.18	4.05 $\pm$ 0.15	4.65 $\pm$ 0.17	21.05 $\pm$ 0.41
Soybean	4.55 $\pm$ 0.14	4.40 $\pm$ 0.13	3.90 $\pm$ 0.14	4.20 $\pm$ 0.12	5.05 $\pm$ 0.15	22.10 $\pm$ 0.39
<i>S. nigrum</i>	4.30 $\pm$ 0.15	5.05 $\pm$ 0.17	4.40 $\pm$ 0.18	4.25 $\pm$ 0.16	5.15 $\pm$ 0.18	23.15 $\pm$ 0.49
Tomato	4.50 $\pm$ 0.11	4.42 $\pm$ 0.11	4.00 $\pm$ 0.16	4.55 $\pm$ 0.14	5.75 $\pm$ 0.16	23.22 $\pm$ 0.42
CD (p= 0.05)	NS	0.39	0.42	0.38	0.48	1.14
Survival of immature stages						
Host	Survival (%)					
	Eggs	1 st instar	2 nd instar	3 rd instar	4 th instar	Egg to adult
Brinjal	92.50 $\pm$ 2.24	83.89 $\pm$ 2.62	90.20 $\pm$ 2.80	96.43 $\pm$ 3.20	100.0 $\pm$ 0.50	67.50 $\pm$ 2.24
Cotton	95.00 $\pm$ 2.59	86.94 $\pm$ 2.09	90.97 $\pm$ 2.71	100.0 $\pm$ 0	96.88 $\pm$ 2.80	72.50 $\pm$ 2.24
Cucumber	92.50 $\pm$ 2.24	81.75 $\pm$ 4.14	88.10 $\pm$ 2.56	92.26 $\pm$ 3.59	97.50 $\pm$ 4.79	62.50 $\pm$ 3.66
Soybean	91.88 $\pm$ 2.49	81.39 $\pm$ 2.05	83.48 $\pm$ 3.59	88.10 $\pm$ 4.03	90.83 $\pm$ 4.79	50.00 $\pm$ 4.88
<i>S. nigrum</i>	85.00 $\pm$ 2.59	70.00 $\pm$ 3.66	67.56 $\pm$ 3.47	82.50 $\pm$ 5.34	77.08 $\pm$ 7.07	32.50 $\pm$ 5.64
Tomato	84.62 $\pm$ 2.08	81.05 $\pm$ 3.54	80.71 $\pm$ 3.03	80.42 $\pm$ 1.77	83.75 $\pm$ 4.97	43.70 $\pm$ 2.31
CD (p= 0.05)	NS	8.94	7.96	8.71	10.45	8.15
Adult longevity and fecundity						
Host	Mean ($\pm$ SE) duration (days)					Fecundity (mean $\pm$ SE)
	Pre oviposition period	Oviposition period	Post oviposition period	Female longevity	Male longevity	
Brinjal	1.10 $\pm$ 0.10	5.80 $\pm$ 0.27	1.20 $\pm$ 0.22	8.10 $\pm$ 0.31	5.29 $\pm$ 0.19	28.32 $\pm$ 3.44
Cotton	1.74 $\pm$ 0.14	7.21 $\pm$ 0.22	1.11 $\pm$ 0.18	10.05 $\pm$ 0.29	5.83 $\pm$ 0.26	33.74 $\pm$ 4.11
Cucumber	1.37 $\pm$ 0.16	3.89 $\pm$ 0.20	1.16 $\pm$ 0.21	6.42 $\pm$ 0.33	4.94 $\pm$ 0.27	26.53 $\pm$ 2.16
Soybean	1.53 $\pm$ 0.16	3.63 $\pm$ 0.15	0.89 $\pm$ 0.25	6.05 $\pm$ 0.26	4.72 $\pm$ 0.22	24.58 $\pm$ 2.37
<i>S. nigrum</i>	1.32 $\pm$ 0.24	3.05 $\pm$ 0.10	1.11 $\pm$ 0.25	5.47 $\pm$ 0.23	4.28 $\pm$ 0.17	19.37 $\pm$ 2.37
Tomato	1.47 $\pm$ 0.22	2.68 $\pm$ 0.18	0.95 $\pm$ 0.26	5.11 $\pm$ 0.34	4.06 $\pm$ 0.19	22.63 $\pm$ 3.43
CD (p= 0.05)	NS	0.47	NS	0.68	0.44	5.91

Figures in parentheses square root transformed values; SE=Standard error

host plant of *B. tabaci* for feeding and oviposition in free choice among brinjal, tomato and capsicum. Lanjar et al. (2012) observed higher *B. tabaci* adult counts on cucurbits than on non-cucurbit hosts and their results revealed that cucumber was most preferred host followed by melon, brinjal, cluster bean, Indian squash, cotton, ridge gourd, sesame, pumpkin, water melon, okra, bitter gourd, chillies and cowpea. Foda (2000) studied the feeding and oviposition responses of *B. tabaci* on four host plants and reported that cotton and tomato attracted highest whitefly adults, whereas cantaloupe and cucumber were preferred for oviposition. However, the results of Morales and Cermeli (2001) were in contradiction to our findings, which showed that *B. tabaci* adults preferred cucumber over tomato. Present study also showed non-significant difference in preference of whitefly for brinjal and tomato, but these results disagree to findings of Khan et al. (2011), who reported that number of adults and eggs were higher on brinjal in comparison to tomato. Hossain et al. (2018) also reported that adults and egg counts of whitefly were higher on brinjal than tomato, which indicated its suitability for oviposition and feeding in a free choice experiment. This disparity was probably due to the differences in varieties of tested host plants.

Development and survival: The data pertaining to influence of host plants on development and survival of immature stages of *B. tabaci* are presented in Table 1. The incubation period varied from 4.10 to 4.55 days on different host plants, but differences were non-significant. The first instar nymphs lasted for 3.90 to 5.05 days on different host plants and significantly maximum duration was recorded on *S. nigrum*. The shortest duration of first instar nymph on brinjal (3.90 days) was on par with cotton (4.15 days). Similar trend was observed during 2nd instar nymphs, the development period was significantly longer on *makoh* (4.40 days), while shortest period was registered on cotton (3.30 days), followed by brinjal (3.40 days). The third instar nymphs lasted for longest period on tomato (4.55 days), which was statistically on par with soybean (4.20 days) and *makoh* (4.25 days). The similar trend was observed in fourth instar nymphs, the mean duration varied from 4.20 to 5.75 days on different host plants. The total development period (egg to adult) varied from 19.35 to 23.22 days and shortest duration was registered on brinjal, which was statistically on par with cotton (19.65 days). Significantly longer development period was recorded on tomato (23.22 days) followed by *S. nigrum* (23.15 days). The data also showed significant variations in survival of immature stages

of *B. tabaci* on different host plants (Table 1). The egg hatchability varied between 84.62 and 95.00%, being highest on cotton, and lowest on tomato. The survival of first instar, second instar, third instar and fourth instar varied from 70.00 to 86.94, 67.56 to 90.97, 80.42 to 100.0 and 77.08 to 100.0%, respectively. The overall survival of immature stages (from egg to adult) ranged between 32.50 to 72.50% on different hosts and it was significantly lowest in *makoh* followed by tomato (43.70%). The highest mean survival on cotton (72.50%) was on par with brinjal (67.50%), which was further on par with cucumber (62.50%). The perusal of the survival data revealed that cotton and brinjal were most suitable hosts of *B. tabaci* supporting its growth and development. The other hosts in decreasing order of suitability for development of whitefly was cucumber, soybean, tomato and *S. nigrum*.

Adult longevity and fecundity: The female longevity varied among different host plants and it ranged between 5.11 to 10.05 days (Table 1). Significantly longer longevity of female adults was recorded on cotton (10.05 days), followed by brinjal (8.10 days). The female adults lived for a shortest period on tomato (5.11 days), which was on par with *S. nigrum* (5.47 days). Preoviposition period varied from 1.10 to 1.74 days and differences among host plants were non-significant. However, oviposition period was significantly longer on cotton (7.21 days), followed by brinjal (5.80 days). The post oviposition period varied from 0.89 to 1.20 days, but host plants differed non significantly. The longevity of male ranged between 4.06 and 5.83 days, being longest on cotton and shortest on tomato. The data pertaining to fecundity of *B. tabaci* completed one generation on different hosts showed significant differences, being maximum of 33.74 eggs in case of cotton, which was on par with brinjal (28.32 eggs). The lowest fecundity on *S. nigrum* (19.37 eggs per female) was on par with soybean (24.58 per female) and tomato (22.63 eggs per female).

Scrutiny of the data revealed that brinjal and cotton were the most suitable host of *B. tabaci* having shorter development period, higher survival and fecundity among tested host plants. These results disagree with findings of Foda (2000), who reported that cotton and tomato was preferred host for adults feeding, but cucumber and cantaloupe were more suitable hosts than cotton or tomato in term of growth and development of *B. tabaci*. However, our results were in conformity with the reports of Kakimoto et al. (2007), who studied the growth, development and survival of *B. tabaci* on

four vegetable hosts and observed that development time was shortest on brinjal (21.8 days), followed by cucumber (22.4 days), sweet pepper (22.7 days) and tomato (25.6 days). They also reported higher survival of immature stages and fecundity on brinjal than other crops and study concluded that brinjal was most suitable host among cucumber, tomato, and sweet pepper. Fekrat and Shishehbor (2007) studied the development of *B. tabaci* on three host plants and recorded shortest life cycle on potato (14.2 days) followed by brinjal (14.9 days) and longest on tomato (20.0 days). Similarly, Khan et al. (2010) examined host preference and survival of *B. tabaci* on brinjal, tomato and chilli and registered lower whitefly attack on other host plants in the presence of brinjal. Later, Fekri et al. (2013) studied biological parameters of *B. tabaci* on different tomato varieties and stated that short development period and higher survival on a suitable variety will result in more number of generations/ year. The present results derive support from Shah and Liu (2013), who stated that eggplant was suitable host for development of immature stages of *B. tabaci*, where cucumber and tomato were rated as marginal hosts. The present study concluded that host plants had significant effect on growth and reproduction of *B. tabaci*. The cucumber was the most preferred host for oviposition by *B. tabaci* and soybean ranked second in the order of preference. The survival of immature stages on soybean was lower as compared to the most suitable hosts of cotton and brinjal. Thus, soybean may be exploited as a trap crop for management of *B. tabaci*.

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AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST

No conflict of interest.

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JUVENILE HORMONE-INDUCED ABNORMALITY OF *PERIPLANETA AMERICANA* (L.)

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ABSTRACT

Application of juvenile hormone III (JH III) and a JH analogue, methoprene to last instars of *Periplaneta americana* suppressed adult emergence and caused morphological abnormalities such as twisted wings and asymmetry of appendages resulting in formation of either nymphoids or giant supernumerary nymphs and adultoids. The effects of JH III and methoprene were concentration dependent in inducing aberrations in morphogenetic moult as well as in significantly lowering the body mass (BM) and lipid reserves (LR) in *P. americana*.

Key words: *Periplaneta americana* growth hormone, JH analogue, methoprene, morphology, appendages, body, lipid content, nymphoids, giant nymphs, adultoids

The American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae) is the largest of the common peri-domestic cockroaches, adults measuring 39.43 to 43.30 mm in length (Borah and Hazarika, 2019a). The species is ubiquitous (Schal et al., 1984; Mudoï et al., 2019) and adults live and actively feed for over 300 days. As a domestic pest, the *P. americana* causes economic losses to stored products and food and poses public health concerns in houses, hotels and public transports (Bell and Adiyodi, 1982; Bell et al., 2007). To manage this pest, synthetic insecticides ("blatticides," Wilson, 2007) are extensively applied as spray, dusts or baits, which may be an economic burden and cause environmental contamination (Schal and Hamilton, 1990; Mudoï et al., 2019). Therefore, there is a dire need to investigate alternatives and insect hormones and their analogues could be promising candidates (Alamer and Hoffman, 2013). Hormones that regulate physiological, morphogenetic, developmental and reproductive processes in insects are of particular interest. This paper reports aberrations in morphogenetic moults caused by Juvenile Hormone III (JH III) and methoprene, a synthetic JH analogue that serves as an insect growth regulator.

One of the target sites of JH is the fat body, an organ analogous to vertebrate liver and adipose tissues; fat body constitutes 50% of the fresh BM, and is the major metabolic centre (Gilbert and Chino, 2001; Arrese and Soulages, 2010) integrating hormonal and nutritional signals centrally to regulate reproduction

(Arrese and Soulages, 2010), growth, development and metamorphosis, body size, circadian rhythms, diapause, longevity and feeding behaviour, partially under the neuroendocrine direction of the brain (Hazarika and Gupta, 1987; Colombani et al., 2005; Adamo, 2012; Li et al., 2019). Lipids accumulated in the fat body during active feeding stages, and LR accumulate and are used in an age-specific manner in insects (Anand and Lorenz, 2008). The process of mobilization of fat body energy reserves is essentially regulated by the action of hormones like adipokinetic hormones, JH, ecdysteroids and various neurohormones (for recent review see Li et al., 2019). We are also interested to understand how exogenous application of hormones brings about changes in the LR and consequently upon the BM of the *P. americana*.

MATERIALS AND METHODS

The experiment was conducted from 2013-2017 at Physiology Laboratory, Department of Entomology, Assam Agricultural University, Jorhat, Assam, India. The cockroaches used in these experiments were raised in the Physiology Laboratory, Department of Entomology at 28±3°C, 70 to 85% relative humidity and 12:12 L:D cycle (light was switched on at 0500 h and switched off at 1700 h) in wooden rearing cages (90×65×60 cm) fixed with nylon netting on four sides and the top. Rearing procedures were described by Borah and Hazarika (2019a) and Mudoï et al. (2019). Laboratory cockroaches were age-synchronized by

moving freshly emerged adult males and females within one hour after the final moult (designated as 1 day old) into new glass jars, each measuring 32 cm tall and 22 cm diameter (Borosil Glass Works Ltd., Mumbai, India) with the open top covered with a piece of muslin cloth held in place with a rubber band.

To study the effect of hormones on adult BM and LR, 30 adult cockroaches of a specific age were selected randomly, and then subjected to lipid estimation by the method described by Folch et al. (1957). After weighing the sample, an extract was made by homogenizing the insect in chloroform-methanol (2:1 V/V) using a grinder. The homogenate was filtered through a sintered glass filter to a separating funnel. The residue was washed with 20 ml of chloroform-methanol. The residue collected from the filter paper was oven dried at 80°C to a constant mass for determining the lean dry sample mass. The pooled chloroform-methanol sample filtrate was purified by washing with 20 ml of 3% NaCl solution and the chloroform layer containing the lipids was withdrawn. The remaining aqueous layer was washed two times with 10 ml chloroform and put in a pre-weighed beaker. Anhydrous sodium sulphate was added to the combined layers to remove water. The chloroform was allowed to dry in an oven and finally the total mass of the lipids was taken as (weight of beaker + lipid) – weight of beaker.

To study the effect of JH III and methoprene, experiments were conducted using a Completely Randomized Design (CRD) with five replications and four treatments (three 1-day-old 6th instars per treatment) including the untreated control. Three concentrations (1, 3 and 5 ppm) of JH-III (Sigma-Aldrich) and methoprene (Sigma-Aldrich) were prepared in physiological saline (using double-distilled water) using ethanol as solvent. JH III was less soluble in water. JH III is the most common compound of the JH family in insects. JH III or methoprene were either topically applied or injected. Topical application was performed onto the dorsal surface of the abdomen of the insect during day time (Alamer, 2013). Injection of the hormone was administered into the cervix region of the test insect individually with the help of a Hamilton Microsyringe (25µl capacity) that delivered 1 µl to each insect. Control insects were treated with physiological saline alone. Mortality and deformities of appendages, effects of hormones on BM and LR and reproduction in the control and treated insects were recorded at 7, 15 and 30 days after treatment (DAT). All data were subjected to CRD analysis of variance (ANOVA) (Panse and

Sukhatme, 1985). The difference in hormone-induced deformities of the body parts between untreated and treated insects were separated using Student's T test (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

We observed morphological deformities (demorphogenesis) in the form of intermediate forms, i.e. nymphoids (Fig. 1a), giant nymphs (Fig. 1b) and adultoids (Fig. 1c) in a dose-dependent manner as a result of exogenous application of JH III and methoprene. These results were similar to those reported by other workers (Das and Gupta, 1974; Kawada et al., 1989; Fathpour et al., 2007) on the German cockroach. As stated above, amongst the four doses of JH III and methoprene tested, 5 ppm was the most effective. Comparison of morphometrics, such as body length, head width, and pronotum width of the control and the treated individuals (giant nymph) is presented in Table 1, which revealed that all the body parameters were significantly different between the treated and the control insects. Borah and Hazarika (2019) reported morphometrics of the *P. americana* in untreated individuals. Exogenous application of JH and JHA during the sensitive period (King and Bennet, 1991) would lead to production of another larval stage by altering the gene expression and deactivating the PTG for ecdysteroids synthesis (Lanzrein et al., 1985; Hazarika and Gupta, 1986; Chapman, 2004). Kawada et al. (1989) also found that exposure of nymphs to pyriproxyfen (a JHA) suppressed adult emergence and caused morphological abnormalities in emerged adults.

The effect of various concentrations of JH III and methoprene on the appendages of male and female *P. americana* are represented in Fig. 1d with 1i. Besides demorphogenesis of the body, appendages were also affected. The morphological abnormalities were recorded on nymphoids, giant nymphs and adultoids were asymmetry of antennae, wings and cercus (Fig. 1d with 1g) as well as “amputation” of antennae, legs and cerci (Fig. 1h and 1i). Twisted wings were observed as was earlier reported by Lim and Yap (1996). Since exogenous application of JH and JHA might have caused reduction of ecdysteroid titre in the haemolymph of the *P. americana*, which might have caused amputation of the appendages as was earlier reported in *Rhodnius prolixus* Stal (Hemiptera: Reduviidae) due to temporary reduction of ecdysteroid level in the hemolymph (Hackney and Cherbas, 2014). Nevertheless, few works have been reported abnormal

effects of JHAs on appendages (Hazarika and Baishya, 1997; Wheeler and Gupta, 1988; Fathpour et al., 2007).

We also observed that females treated with methoprene and JH III were unable to produce oothecae (NB, personal observation). Previously, Alamer (2013) reported that methoprene treatment of females resulted

in prevention of ootheca formation or complete resorption of the ootheca, depending upon the time of treatment. Sterility might be related to effect of these hormones on the process of development of internal reproductive organs, sex pheromone production or on mating. These compounds might have affected chemoreceptors on the antennae as a result of which the male might have failed to detect pheromones needed



Fig. 1a-i. Effect of Methoprene and JH III on *P. americana* a. resultant nymphoid (dorsal and ventral view); b. giant nymph; c. adultoid showing distinct twisted tagmina; nymphoid of *P. americana* due to application of methoprene showing asymmetrical development of d. antennae; e. wings; nymphoid of *P. americana* due to application of JH III showing f-g. breakage of hind legs and asymmetrical growth of cercus (dorsal and ventral view); h. "amputation" of antennae and legs; i. "amputation" of legs and cercus.

Table 1. Comparison of morphometrics (Mean $\pm$ SEM) between normal and giant nymphs of *P. americana*

Nymph	Body length (mm)	Head width (mm)	Pronotum length (mm)	Pronotum width (mm)	Body mass (g)
Normal	39.03 $\pm$ 1.78	4.05 $\pm$ 0.17	7.96 $\pm$ 0.06	9.00 $\pm$ 0.17	1.21 $\pm$ 0.09
Giant	43.00 $\pm$ 6.48*	5.02 $\pm$ 0.15*	9.45 $\pm$ 0.57*	10.21 $\pm$ 1.06*	1.39 $\pm$ 0.08*

Sample size = 30; *Significant at p=0.05

for mating. This needs to be researched in more detail in the future.

The effect of topical application and injection of various concentrations of JH III and methoprene on BM and LR of *P. americana* are presented in Tables 2, 3, 4 and 5. The BM and LR of the treated individuals were significantly lower than those of the controls and the effect was age- as well as dose-dependent, because both parameters varied with age or days after treatment (DAT). Method of application also influenced the

results, as the lowest BM was recorded with 5 ppm methoprene treated insects (0.31 g and 0.29 g of topical and injection, respectively on 30 DAT) and 5 ppm JH III treated insects (0.35 g and 0.26 g of topical and injection, respectively on 30 DAT). Irrespective of doses, it was found that injection of JH III and methoprene suppressed the BM and the LR more compared with those individuals that were topically applied. Similarly, both hormones were also found to be equally effective in lowering the LR of the treated insects (Table 4 and 5). Methoprene reduced LR in *Dicladispa armigera*

Table 2. Effect of JH III on body mass (g) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	1.01	1.14	1.23	1.27	0.97	1.09	1.12	1.14
1	1.19	1.03	0.84	0.75	1.05	0.94	0.84	0.64
3	1.10	0.86	0.81	0.63	1.05	0.68	0.45	0.41
5	1.01	0.39	0.38	0.35	1.03	0.34	0.29	0.26
S.Ed. ($\pm$)		0.12	0.10	0.11		0.11	0.11	0.12
CD _{0.05}	NS	0.28	0.22	0.25	NS	0.25	0.25	0.27
CV (%)		16.92	13.02	14.97		14.87	15.24	17.12

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 3. Effect of methoprene on body mass (g) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	1.06	1.11	1.20	1.22	1.04	1.16	1.23	1.27
1	0.96	0.93	0.61	0.59	0.97	0.82	0.71	0.62
3	1.10	0.87	0.50	0.49	1.06	0.52	0.50	0.47
5	1.07	0.53	0.33	0.31	0.95	0.32	0.30	0.29
S.Ed. ($\pm$)		0.09	0.14	0.13		0.12	0.12	0.11
CD _{0.05}	NS	0.20	0.31	0.29	NS	0.27	0.26	0.26
CV (%)		11.50	19.08	18.28		16.75	16.26	16.11

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 4. Effect of JH III on lipid reserves (mg) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	326.16	328.21	331.41	332.10	323.88	324.50	325.55	330.98
1	324.44	323.98	321.10	318.76	323.87	311.22	304.08	294.42
3	327.98	325.02	322.22	317.22	325.27	294.92	284.12	271.73
5	330.80	312.78	308.30	306.20	324.72	283.30	263.40	247.78
S.Ed. ($\pm$)		5.05	7.96	7.81		7.52	17.88	12.99
CD _{0.05}	NS	11.01	17.35	17.02	NS	16.40	38.97	28.32
CV (%)		2.21	3.51	3.46		3.50	8.59	6.42

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 5. Effect of methoprene on lipid reserves (mg) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	324.68	329.50	330.70	332.30	328.28	329.10	332.55	336.18
1	324.57	319.43	314.63	308.89	329.77	319.80	309.64	287.02
3	325.53	310.39	305.87	300.57	328.53	315.60	294.12	265.52
5	326.28	307.00	302.42	300.12	330.28	308.94	278.82	240.98
S.Ed. (±)		6.94	5.98	5.14		7.03	18.63	17.31
CD _{0.05}	NS	15.12	13.05	11.21	NS	15.33	40.61	37.73
CV (%)		3.10	2.70	2.34		3.12	8.67	8.67

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

(Olivier) (Coleoptera: Chrysomelidae) (Baishya and Hazarika, 1996), *Gryllus firmus* Scudder (Orthoptera: Gryllidae) (Zera and Zhao, 2004), mosquito pupae (Downer et al., 2012) by substantially decreasing *in vivo* biosynthesis of total lipid and triglycerides.

We conclude that different concentrations of a juvenile hormone and its analogue (JHA) caused morphological and reproductive disorders and lowered the BM and LR of the insect. Thus insect growth regulators can be successfully utilized for its management. Moreover, JH or JHA act on the ultimate instar during the critical period only when endogenous JH is absent in the haemolymph, resulting in an additional nymph or nymphoid and adultoid development. Since application of JH and JHA was done on the ultimate instar i.e. 6th instar, only those insects which were at the critical period were affected, as a result 20-30% tested individuals developed abnormalities. However, sterility was evident in the remaining 70-80% due to occurrence of abnormalities that they failed to produce oothecae.

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AUTHOR CONTRIBUTION STATEMENT

LKH conceived and designed the experiment. NB conducted experiments, analysed data and wrote manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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TOXICITY OF *SARGASSUM WIGHTII* TO THE MALARIA VECTOR *ANOPHELES STEPHENSI* LISTON (DIPTERA: CULICIDAE)

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ABSTRACT

Seaweed based pesticides exhibit mosquitocidal properties and are found to be effective, biodegradable and non-toxic to non-target organisms. *Sargassum wightii* Greville butanol and ethanol extracts were tested for its toxicity against the second and third instar larvae of *Anopheles stephensi* Liston in the present study. One hundred percent larval mortality was observed in the second instar of *An. stephensi* by the ethanol extract at the highest concentration of 500 mg/l after 24 hours of exposure. The LC_{50} values of *S. wightii* butanol and ethanol extracts were 107.03 and 89.67 mg/l, against the second instar of *An. stephensi*; and 132.56 and 92.00 mg/l against the third instar of *An. stephensi* respectively. Phytochemical analysis of *S. wightii* extracts revealed presence of alkaloids, carbohydrates, fatty acids, flavonoids, phenols, proteins, tannins and terpenes. Larval mortality of second and third instars of *An. stephensi* can be attributed to the major compounds present in *S. wightii* revealed by GC-MS analysis, viz., ethyl salicylate, methyl salicylate, ethyl palmitate, palmitic acid, oleic acid, phytol and diethyl phthalate. The present study accentuates *S. wightii* butanol and ethanol extracts to cause lethal effects to the second and third instar larvae of *An. stephensi*.

Key words: *Sargassum wightii*, brown seaweed, phytochemical constituents, *Anopheles stephensi*, larvicide

Malaria continues to be a significant public health problem, and the recent reports of WHO state that nearly 219 million people are living in malaria risk areas (WHO, 2019), and nearly half of the world's population are exposed to malaria (WHO, 2021). *Anopheles stephensi* is the major malaria vector known to transmit human malaria parasites *Plasmodium falciparum* and *P. vivax* in India (Das et al., 1990; Korgaonkar et al., 2012). Vector control is the most important public health objective, and the successful method of reducing the occurrence of vector-borne diseases is through the control of the disease transmitting vector mosquitoes (Manikandan et al., 2022). Chemical insecticides to control mosquitoes cause harmful side effects on non-target organisms, humans and to environment as well (Kaushik et al., 2019; Siam et al., 2022). Further, problems associated with extensive usage of synthetic compounds have increased substantially which makes mosquito/vector control more difficult over the years (Demirak and Canpolat, 2022). Hence, an outcry is exhibited against the use of pesticides due to their hazardous effects. In this situation, ecofriendly alternatives are important for safer mosquito management. Traditionally, plant-based products have constituted an important source of insecticides against

Anopheles mosquitoes (Muhammed et al., 2022). Current research community is paying more attention towards marine natural products (Yasmeen et al., 2018). Bioinsecticides from marine algal extracts represent safe, alternatives for synthetic pesticides for mosquito management, which negatively affect the environment and health (Elbanna and Hegazi, 2011). The field of seaweed based pesticides steadily progresses as they offer a natural source of compounds to the development of insecticides from marine algae which has become more rigorous in recent years with calls for more standardization, especially against mosquitoes as they display their mosquitocidal properties (Thangam and Kathiresan, 1991a,b; Yu et al., 2014, 2015; Deepak et al., 2019; Suganya et al., 2019; Haleem et al., 2022), and are found to be effective, biodegradable and non-toxic to non-target organisms. In addition to the direct use of seaweed extracts for mosquito control, biosynthesized nanoparticles using seaweeds has gained momentum too (Murugan et al., 2015, 2018). Nevertheless, crude seaweed extracts still dominate in combating mosquitoes. Extracts of the brown seaweed, *Sargassum wightii* has been reported for larvicidal activity against *Aedes aegypti*, *An. stephensi*, *An. sudaicus*, *Culex quinquefasciatus* and *Cx. tritaeniorhynchus* (Manilal

et al., 2011; Kumar et al., 2012; Poonguzhali and Nisha, 2012; Murugan et al., 2018; Suganya et al., 2019). However, few reports present the larvicidal activity of its ethanol extracts (Suganya et al., 2019), and no report has been published on its butanol extract. Hence, the toxicity of *S. wightii* butanol and ethanol extracts were assessed for its toxicity against the second and third instar of *An. stephensi* larvae in this study.

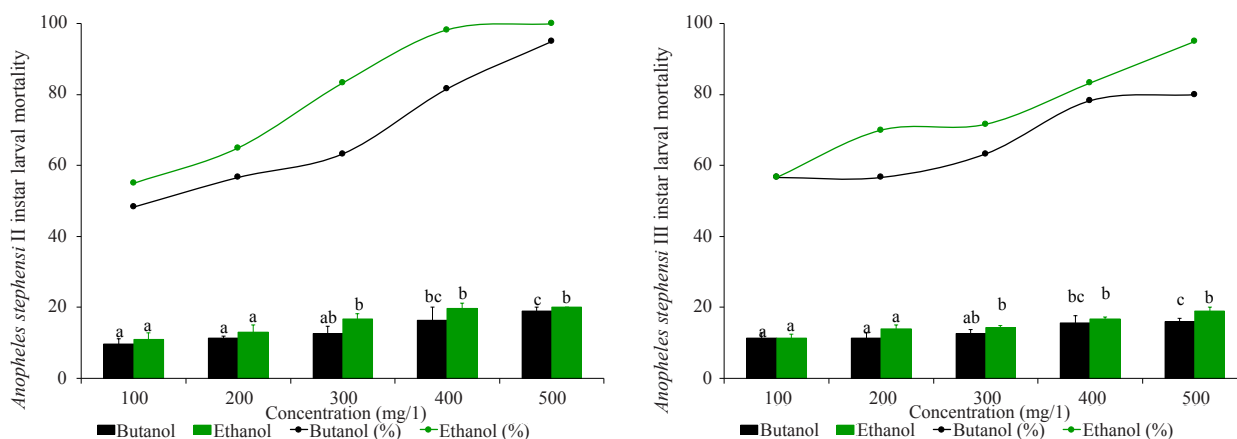
MATERIALS AND METHODS

S. wightii (Fig. 1) collected by hand picking from the intertidal zone of Rameswaram, Tamil Nadu, India (8° 46' N, 78° 9' E and 9° 14' N, 79° 14' E), was immediately rinsed in water to remove all kinds of epiphytes and other particles (sand, molluscs, and sea grasses), and kept in sterilized ziplock bags, and transferred to laboratory for further studies. Taxonomical identification and confirmation of the collected seaweed was done at the Marine Algal Research Station, Mandapam, Tamil Nadu, India with the aid of morphological key characters and identification manual (Dhargalkar and Kavlekar, 2004; Rao, 2012). The cleaned macroalgae was shade dried at room temperature, and powdered with the aid of a mixer grinder. The powdered sample (250g) was suspended in butanol and ethanol (750 mL) each separately, and transferred to soxhlet apparatus for eight hours for extraction (Vogel, 1978). Thereafter, the extracted sample was filtered using Whatman No.1 filter paper, and the filtered sample was centrifuged at 5000rpm for 10 minutes at 4°C, and the supernatant was collected in a separate flask. The butanol and ethanol extract each was then concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland).

The final concentrated butanol and ethanol crude extract was stored in sterile air tight amber coloured bottle, and kept in a refrigerator until further use. Prior to this, the percentage of yield of extraction of these two crude extracts were calculated. The phytochemical profile of *S. wightii* extracts was performed to identify the plant chemicals (Harborne, 1978), followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The spectrum of components obtained were compared with the database of spectrum of known components stored in the GC-MS-National Institute for Standards and Technology library.

Anopheles adults collected from cattle sheds of Madurai, Tamil Nadu, India with an aid of an aspirator were transferred to an one feet mosquito cage, and transported to laboratory. The adults on emergence were identified and species confirmed prior to rearing (WHO, 2020). Subsequently, their cyclical generations were provided a blood meal, and was maintained in two feet mosquito cages with an average room temperature of $27 \pm 2^\circ\text{C}$ and a relative humidity of 70-80% inside an insectary. Ovitrap inside the mosquito cages collected the oviposited eggs which were shifted to the larval rearing room in enamel larval trays, and the larvae on hatching were provided with larval food (yeast and dog biscuits of ratio 1:3). The larvae on turning into pupae were moved to another mosquito cage in enamel bowls for adult emergence.

World Health Organization (WHO) protocol was adopted for the study with minor modifications (WHO, 2005). Serial dilution of 1.0% stock solution of the crude seaweed extracts yielded requisite test concentrations



Data in bar represent larval mortality as Mean±Standard deviation; and data in line denotes percent larval mortality. Different superscript alphabets on the bar indicate statistical significant difference in larval mortality between concentrations at $P < 0.05$ level by One way ANOVA followed by Tukey's test performed. Similarity in superscript alphabets on the bar indicate no significant variation.

Fig. 1. Larval mortality of *An. stephensi* on exposure to *S. wightii* extracts

(100, 200, 300, 400, and 500 mg/L) and amount of test solution. Early second and third instar larvae obtained from the laboratory colonized F_1 generation formed the choice as the test instar. The early second and third instars numbering twenty five each were added separately into glass beakers (250 mL) holding distilled water and test concentration for each replicate apiece trial. Distilled water (250 mL), and Tween 80 (1.0 mL) dissolved in distilled water (249 mL) maintained separately and run simultaneously served as positive and negative control, respectively. Larval mortality after 24 hrs was confirmed when the moribund larvae showed no signs of movement when prodded by a needle on their respiratory siphon, and were scored dead. A total of five replicates and a negative and positive control were run concurrently for every trial, and overall five trials were run. Larval mortality (%) and corrected mortality where control mortality ranged from 5-20% (Abbott, 1925) was calculated. Statistical analysis of all mortality data were subjected to probit analysis. Chi-square and regression analysis were performed. One-way analysis of variance and Tukey's honestly significant difference post hoc tests were used to determine if the mortality in treated bioassays significantly differed from that of controls, and at which dosages in specific. The differences were considered as significant at $P \leq 0.05$ level. All statistical analysis of data were carried in IBM SPSS Statistics v22 with significance set at 95% confidence (SPSS, 2010).

RESULTS AND DISCUSSION

The percentage yield of *S. wightii* butanol and ethanol extract was 0.98% and 2.45%, respectively, and its phytochemical screening revealed presence of alkaloids, carbohydrates, fatty acids, flavonoids, phenols, proteins, tannins and terpenes. This was correlated with the results of Begum and Hemalatha (2017). The major phytochemical compounds present in butanol and ethanol extracts revealed by GC-MS analysis were ethyl salicylate, methyl salicylate,

ethyl palmitate, palmitic acid, oleic acid, phytol and diethyl phthalate. The mortality of second and third instar larvae of *An. stephensi* exposed to various concentrations of *S. wightii* butanol and ethanol extracts are presented in Fig. 1. No larval mortality was observed in both controls. One hundred percent mortality was observed in *An. stephensi* second instar larvae at the highest concentration on exposure to ethanol extract. *S. wightii* butanol and ethanol extracts reported LC_{50} values of 107.03 and 89.67mg/L; and 132.56 and 92.00mg/L against the second and third instar larvae of *An. stephensi* respectively. One Way ANOVA, comparing treated and control group, with a significance level established at $P < 0.05$ showed that *S. wightii* concentrations significantly influenced the mortality of *An. stephensi* larvae (Table 1). Overall results indicated the ethanolic extract of *S. wightii* to be slightly more toxic on both the tested larval instars of *An. stephensi*, and amongst the two instar stages tested, the second instar was found to be more susceptible than the other. Thangam and Kathiresan (1996) classified seaweeds with larvicidal activity less than LC_{50} 100 mg/L as effective larvicide, LC_{50} between 100 and 200mg/L as less effective larvicide, and LC_{50} more than 200mg/L as ineffective larvicide. The results of the present study revealed that *S. wightii* ethanol and butanol extracts were effective and less effective larvicides based on their LC_{50} values and classification of Thangam and Kathiresan (1996). The susceptibility of mosquito larvae to seaweed insecticides depends in general on the seaweed used, the solvent used for extraction, and the mosquito species tested. A thorough knowledge on the phytochemical profile of the seaweed used should be drawn, prior to selection of solvents, in order to get a potent extract as there exists a relationship between the extract effectiveness and solvent polarity. The bioactivity of a seaweed compound is related to the compound's chemical structure and chemical reactions (Barbosa et al., 2012). Marine algal extracts possess mosquito larvicidal activity since they contain bioactive

Table 1. Probit analysis and other associated statistical inferences for *S. wightii* extracts

Solvent	LC_{50} (mg/L)	LC_{90} (mg/L)	Chi-square	Regression equation	R^2	F	P
<i>An. stephensi</i> II instar							
Butanol	107.03	327.62	18.38*	$Y=2.71+0.04x$	0.86	5.19	<0.04*
Ethanol	89.67	573.90	8.01*	$Y=3.02+0.03x$	0.82	4.07	<0.04*
<i>An. stephensi</i> III instar							
Butanol	132.56	647.64	24.17*	$Y=2.09+0.03x$	0.87	4.30	<0.05*
Ethanol	92.00	748.08	9.21*	$Y=2.90+0.03x$	0.77	4.28	<0.05*

*Values significant at $p < 0.05$ level

compounds (Manilal et al., 2009; Ravikumar et al., 2011), rich in polyphenolic and triterpene compounds, causing larval mortality, as they inhibit the protein responsible for the cholesterol transportation during the larval development (Blunt et al., 2011; Bibi et al., 2020). Extracts of brown, green and red seaweeds have strong larvicidal activity against mosquito larvae (Ahmad et al., 2016), and brown algae under which *Sargassum* genus is housed are rich in alkaloids, glycosides, quinones, saponins, tannins and terpenoids (Suganya et al., 2019).

Secondly, the choice of solvent is influenced by what is intended with the extract, as it targets the compounds to be extracted (Shaalán et al., 2005; Ghosh et al., 2012). Variation of the larvicidal potential of *S. wightii* changed with different solvents used and tested against different species of mosquito larvae, and the results of the present study were comparable with the earlier reports of *S. wightii* against mosquito larvae in view of the aforementioned factors. *S. wightii* methanol extracts showed LD₅₀ values of 87.09 µg/ mL and 107.15 µg/ mL against *Cx. quinquefasciatus* and 84.82 µg/ mL and 97.28 µg/ mL against *Ae. aegypti* second and third instars, respectively, which was due to presence of tannins (Manilal et al., 2011); LC₅₀ values of 0.88%, 0.73%, 1.34% and 1.56% against first, second, third and fourth instar of *An. sundaicus*, respectively due to the action of dioctyl phthalate (Kumar et al., 2012); LC₅₀ values of 424.57 mg/ L (methanol extract), 363.14 mg/ L (acetone extract) and 435.24 mg/ L (benzene extract) against *An. stephensi* (Poonguzhali and Nisha, 2012). Rare reports have been documented on the butanol extracts for its toxicity to the second and third instars of *An. quadrimaculatus*, *Ae. aegypti*, *Cx. pipiens* and *Cx. quinquefasciatus* larvae (Spielman and Lemma, 1973; Kumar et al., 2014; Raveen et al., 2017), and on the other hand, Samuel et al. (2018) provided an exhaustive review on the list of ethanolic extracts reported for mosquito larvicidal property. *S. wightii* extracts has been reported for its toxicity against *An. stephensi* second and third instar larvae in this study. Mosquito larvicidal effectiveness of the ethanolic extracts from *S. wightii* and *Halimeda gracilis* against *Ae. aegypti*, *An. stephensi*, and *Cx. tritaeniorhynchus* have been reported (Suganya et al., 2019). Ali et al. (2012) reported larvicidal activities of ethanolic extract of seagrasses, *Syringodium isoetifolium* and *Cymodocea serrulata* against *Ae. aegypti*. Butanol and ethanol has the property to extract alkaloids, flavonoids, phenols, saponins, sterols, tannins, terpenes and terpenoids. All

these phytochemicals are toxic to immature stages of mosquito (Shaalán et al., 2005).

Suganya et al. (2019) reported that *S. wightii* ethanolic extract caused shrinkage and disintegration of midgut epithelial cells, and outer cuticle leading to larval death in *Ae. aegypti*. This would have been the cause of larval death too in the present study. Rey et al. (1999) reported the larvicidal mode of action by phytochemicals on mosquito larvae affects the midgut epithelium, gastric caeca and the malpighian tubules. Hexadecanoic acid methyl ester from leaf extracts of *Heliotropium indicum* and *Mukia maderaspatana* have been reported for its larvicidal property against *Ae. aegypti* (Krishnaveni and Ramamurthy, 2014). *Sargassum* species possess oleic, linoleic, linolenic, palmitic, and stearic acids and their respective methyl esters, were found to affect the metabolism and morphology of midgut along with the fat body of the fourth instar of larvae of *Cx. quinquefasciatus* (de Melo et al., 2018). The same can be corroborated to the present study, wherein, mortality to *An. stephensi* larvae can be attributed to the presence of its ethyl salicylate, methyl salicylate (phenols), ethyl palmitate (fatty acid ester), palmitic acid (sterol), oleic acid (fatty acid), phytol (terpene) and diethyl phthalate in *S. wightii* extracts, for the reason that phytochemicals work as stomach poison in mosquito larvae, as they enter the larvae body through the digestive tract, and lowers surface tension of the mucosa membrane in the digestive tract, making it easier to be damaged, and functions as a stomach toxin that disrupts digestion, nutrition absorption, ion transport, and osmoregulation (Sina and Shukri, 2016). Further, the abnormal behaviour of *An. stephensi* larvae in the present study can be correlated to the toxicity of this seaweed in affecting the nervous system and motor coordination. Nonetheless, further investigations are needed to confirm the neurological effect of seaweed extract that act as a nerve poison. Finally, selection of mosquito species for testing is also of fundamental importance since great variations exist in responses between the genera and species. The susceptibility of different mosquito species toward seaweed treatment varies (Ali et al., 2013). Manilal et al. (2011) reported *Ae. Aegypti* larvae to be more susceptible (with lower LC₅₀ values) towards treatment of seaweed extracts. However, in the present study Anophelines larvae too were susceptible when exposed seaweed extracts which exhibited low LC₅₀ values.

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AUTHOR CONTRIBUTION STATEMENT

Vaithiyanathan Selvi prepared the extracts, performed the bioassay experiments, and carried out GC-MS analysis. Subramanian Arivoli contributed to literature review and manuscript drafting. Samuel Tennyson wrote the manuscript and statistically analysed the data. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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ASSESSMENT OF ELECTROSTATIC SPRAYER AGAINST CITRUS PSYLLA *DIAPHORINA CITRI* KUWAYAMA IN KINNOW MANDARIN

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ABSTRACT

Field experiments were conducted to evaluate and standardize the efficiency of electrostatic sprayer for checking citrus psylla *Diaphorina citri* population in kinnow mandarin orchards using the recommended insecticide imidacloprid @ 0.0071% and compared to standard knapsack sprayer and tractor mounted power sprayer. The results revealed that the use of electrostatic sprayer was significantly efficient in reducing >30% spray volume, covered significant more area (4.8-5.6 ha/ day), is labour saving (1 labour per operation) and much effective in reducing *D. citri* population by >80% after 14 days of spray in kinnow mandarin orchards.

Key words: Citrus, kinnow mandarin, *Diaphorina citri*, seasonal incidence, spray technology, electrostatic sprayer, management, imidacloprid, knapsack sprayer, spray volume, labour

In Punjab, Kinnow mandarin occupies a prominent place in terms of acreage and production although other citrus cultivars viz. mandarin, sweet orange, grapefruit, lime and lemons are also gaining importance in the region. Many of the biotic stresses (diseases and insect-pests) contribute to the qualitative and quantitative losses in citrus fruit crop. Among the major insect-pests attacking this crop citrus psylla, *Diaphorina citri* Kuwayama is the most serious as it inflicts damage in multifarious ways i.e. it devitalizes the plant by sucking cell sap, secretes honeydew that captivate sooty mould fungus growth and impairs photosynthesis. Moreover, it vectors the fastidious bacterium, responsible for citrus greening disease that ultimately contributes to citrus decline. To avoid these undue pest losses, various spray operations are done. Traditionally, the high volume knapsack sprayers and tractor mounted power sprayers are been used in kinnow orchards which are simple, cost-effective and exhibit easy operability (Bateman and Jessop 2008 and Roten et al., 2013) but lacks in terms of their targeted delivery, distribution and penetration to the target host in tree crops which contributed in over application of pesticides and reduced pest control (high does, frequent applications etc). Moreover, their energy use efficiency in terms of manpower and spray coverage is also less. These are labour-intensive, time consuming and exhibit high application costs. Significant revolution in spray technology is marked with the application of electrostatic sprayers the use of which overall increases the bio-efficacy and deposition efficiency of pesticides (Hoffmann et al., 2007; Patel et al., 2015; Ukran et

al., 2016 and Patel 2016). These electrostatic sprayers are effective in controlling the pest with impending reduction of over usage of insecticides (Kabashima et al., 1995 and Gossen et al., 2008). Under Punjab field conditions, the comparative evaluation of electrostatic sprayer over the traditional sprayers demonstrated that the electrostatic sprayer was significant in terms of bio-efficacy in cotton crop which resulted due to improved droplet density, spray deposition, and higher area coverage (Patel et al., 2016 and Patel et al., 2017). The application of electrostatic spray technology has already been demonstrated in many crops showing significant enhanced efficiency as discussed above. In citrus orchards, the introduction/procurement of these electrostatic sprayers for application of pesticides has been undertaken in recent years by different farmers in Punjab. However, their efficiency and delivery parameters are not quantified and standardized. Hence, this study was undertaken to compare the efficiency of electrostatic sprayer with the conventionally used sprayers in delivering the spray solutions and managing the in kinnow mandarin.

MATERIALS AND METHODS

The experiments were carried out at the experimental farm of the Punjab Agricultural University Regional Research Station, Abohar, India (30°08'N; 74°12'E and 185.78 amsl). The area is semi-arid, characterized by hot and dry weather and receives 75-300 mm of annual rainfall most of which befalling between July to September months. The study was conducted on

a 15 year old Kinnow mandarin orchard planted at a spacing of 20 x 20 feet maintained as per recommended practices of Punjab Agricultural University, Ludhiana (Anonymous 2016). To study the population dynamics of *D. citri*, weekly observations were recorded from fifteen marked kinnow mandarin plants. No hard insecticide spray was given for any of the insect-pest during the season. The number of *D. citri* adults was counted from 10 leaves from each direction of the plant, while *D. citri* nymphal count was taken from 10 cm apical twig from all the directions.

For the management of *D. citri* there were a total four experiments conducted during March and August months, respectively in randomized block design with three replications/ treatment. The plot size taken was 400 m². Three different sprayers viz. manually operated knapsack sprayer, tractor mounted power sprayer and tractor mounted electrostatic sprayer was compared to find out the best effective spray technology for pest management in citrus orchards. The treatment with manually operated knapsack sprayer was kept as check (general practice of spraying in orchards) to compare of efficiency of modern spray technology. Commercial formulation of imidacloprid (Confidor 17.8% SL, Bayer Crop Science) procured from local market was used to find out the efficacy of the insecticide with the three different sprayers against citrus psylla in kinnow mandarin orchards. Sampling was carried out one day before and 3, 7, 10 and 14 days after the spray application. From each replication five kinnow mandarin plants was randomly selected for taking *D. citri* nymphal counts. Five twigs of 10 cm length covering all the directions of tree were randomly picked and number of *D. citri* live nymphs counted. The corrected efficacy percentage was calculated as per Henderson and Tilton's (1955). The (%) data were subjected to arc sine transformation for statistical analyses. The data generated was pooled and subjected to ANOVA to evaluate the treatment effects and the means separated (LSD p=0.05).

RESULTS AND DISCUSSION

The detailed specifications of the different sprayers used during the experiment are depicted in Table 1. The seasonal incidence of *D. citri* nymphs and adults on kinnow mandarin plants during the experiment is presented in Fig. 1. During the study period the population of *D. citri* was ranged from 0.0 - 58.70 nymphs/ 10 cm apical twig and 5.33 - 51.55 adults/ 10 leaves from SMW 1 to SMW 52 (Table 1). The incidence

of *D. citri* was found to be severe between SMW 7-16 and 6-15 for nymphs and adults, respectively, during this period it inflicts maximum damage to kinnow plants. However, the population of *D. citri* peaked during 9th and 11th SMW for the adults and nymphs, respectively (Fig. 1). Two peaks of *D. citri* population were observed annually, first between SMW 7-15 and the second between SMW 31-35. The damage of *D. citri* on kinnow mandarin plants was at peak in between mid-February to first week of April in comparison to the second peak (August-September). In south western Punjab *D. citri* became a major threat to citrus cultivation causing huge economic losses to farmers every year. The adults of *D. citri* remain active in unmanaged citrus orchards as well as on other alternate host throughout the year and shift immediately to the citrus as the new flush start emerging. While plotting the peak activity period of *D. citri* to major abiotic factors, a temperature ranged between 21.02 – 37.73 °C (Tmax), 10.17 – 20.49 °C (Tmin) along with 47.16 - 77.5% RH was found favourable for the population buildup and further spread of this insect.

The comparison of electrostatic sprayer with the standard operating sprayers was quantified in terms of efficacy in reducing *D. citri* population in kinnow mandarin, volume of water used per acre and area covered per day. In terms of insecticide efficacy as revealed from the experiment data, both electrostatic sprayer and tractor mounted power sprayer were at par in reducing the population of *D. citri* nymphs in kinnow mandarin by more than 80% after 14 days of spray application. The major differences observed were water use efficiency, area covered/day and manpower used (number of persons involved). The electrostatic sprayer was proved to be more efficient as compared to power operated gun sprayer as it reduces more than 30% of spray solution, covers more area per day and is labour saving (Table 1). The tractor mounted electrostatic sprayer in kinnow orchards utilizes around 1125 l of spray volume to cover 1 ha of orchard in comparison to power sprayer (Average: 1500l/ ha) thus it will significantly able to reduce the insecticide usage in orchards. Also the manpower involved in spray application was reduced with electrostatic sprayer (1 labour used for handling and spraying) whereas the tractor mounted power sprayer engaged 3 individuals (1 tractor driver and 2 labourers for handling spray guns). Because of ease in handling the electrostatic sprayer covers about 4.8 - 5.6 ha/ day for spray application of chemicals in orchards. It has substantial potential on application of different spray formulations in

Table 1. Sprayers used their performance assessment against *D. citri* in kinnow mandarin

Detailed specifications of the sprayers											
Particulars		Details of sprayers									
		KS		TMPS		TMES					
Prime mover	Type	Manual operated		Tractor PTO		Tractor PTO					
	Power, kW	0.075-0.100		20-30		20-30					
Pump	Type	Diaphragm		Hydraulic		Hydraulic triple action piston					
	Horse power, kW	0.075-0.100		5.0		5.0					
	Pump, rpm	-		600		900-1200					
	Discharge l/ min	1.0		60-80		50					
	Pressure developed, kPa	4.0		35.00		35.0					
Fan	Diameter of blade, mm	-		-		612					
Nozzle	Type	Hollow cone		Gun hollow		Hollow cone (Electrostatic)					
	Type of energy used to atomize the liquid	Hydraulic		Hydraulic		Gaseous					
	Pressure at nozzle tip, kPa	3.00		7.00		3.00					
	Flow rate of nozzle, l/min	0.80-1.00		20.00		20					
	Number of nozzle	one		one							
	Nozzle spacing	-		-							
Tank	Materials	PVC		PVC		PVC					
	Main Capacity lit	16		500		600					
	Hand wash capacity, lit					10.0					
Hose pipe	Material	HDVC		HDVC		-					
	Length, m	1.5		60		-					
Evaluation of efficiency (pooled data)											
S. No.	Treatments	Insecticide used	Dosage ml/ l	Area covered/day (ha)	Spray volume used ℓ/ ha	Pre-treatment count of <i>D. citri</i> nymphs/ 10 cm twig	% reduction in <i>D. citri</i> nymphs population			No. of persons involved	
							3 DAT	7 DAT	10 DAT	14 DAT	
1	KS	Imidacloprid	0.4	0.3 – 0.4	1375	39.60	72.25 (56.12)	78.94 (62.55)	83.87 (64.78)	74.59 (56.29)	1
2	TMPS	Imidacloprid	0.4	4.0 – 4.8	1500	41.40	75.63 (58.47)	84.78 (66.74)	90.02 (70.58)	82.25 (62.33)	3
3	TMES	Imidacloprid	0.4	4.8 – 5.6	1125	40.90	78.31 (59.52)	86.89 (67.56)	91.54 (72.71)	83.11 (62.27)	1
CD (p=0.05)							(3.37)	(4.43)	(5.03)	(4.64)	

*DAT: days after treatment; KS: Knapsack Sprayer; TMPS: Tractor Mounted Power Sprayer; TMES: Tractor Mounted Electrostatic Sprayer

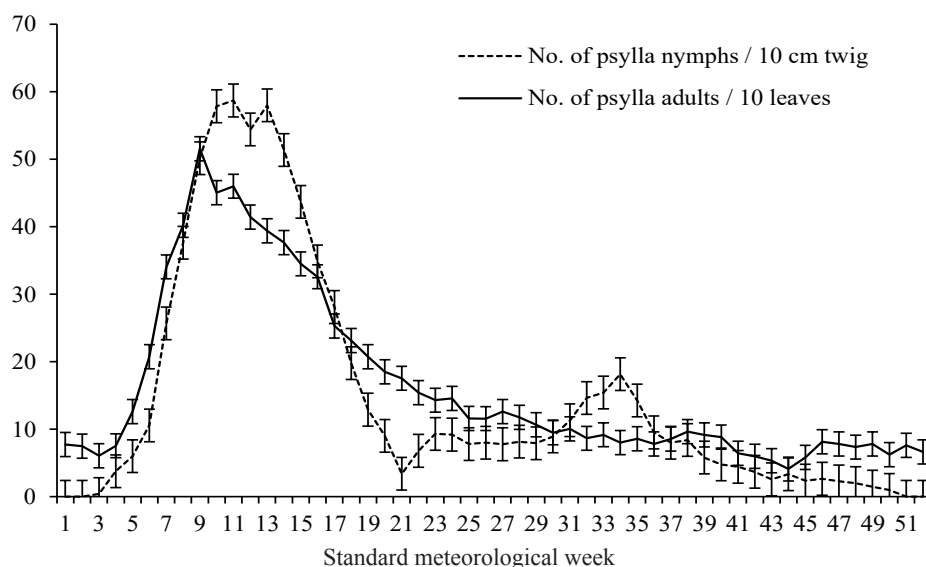


Fig. 1. Seasonal dynamics of *Diaphorina citri* nymphs and adults in kinnow mandarin orchards (pooled)

agricultural crops as the charged particles can execute uniform coverage of spray on the crop canopy with noticeably less quantity and better efficacy. Derksen et al. (1991) also supported that electrostatic sprayers have a better application efficiency of roughly 80% with 50% less spray volume used which corroborates our findings. Comparison of air-assisted electrostatic pesticide application techniques with conventional sprayer revealed that these sprayers showed improved performance against green peach and melon aphids as electrostatic application provided significant control over the conventional sprayers while using 40 times less water in an equivalent area (Derksen et al., 1991). Electrostatic spray technology resulted in significant gain in spray deposition in the order of 1.6 to 2.5 times, and reduced application rates, ultimately contributing to higher application efficiency has been reported (Kabashima et al., 1995). Another study revealed that the use of electrostatic sprayers for the management of insect-pests in fruit crops (Patel et al., 2016) and cotton (Kumar et al., 2016) enhance the deposition efficiency of insecticide thereby reducing the usage of chemicals to about 30 to 40% compared to air-assisted sprayer. According to a study conducted by Aneesha et al. (2020), the energy use efficiency of electrostatic sprayer was 1.5 and 2 times higher than that of mist blower and air compression sprayers, respectively, alongwith reduced chemical usage as compared to knapsack sprayer.

It can be concluded from the study that the lower

spray volume witnessed through the electrostatic sprayer resulted in corresponding lower volume of active ingredient, while accounting the significant higher efficacy against increasing population of *D. citri* in kinnow mandarin orchards over the knapsack sprayer. Better deposition, more area coverage equipped with single manpower highlighted the significance of this sprayer over the conventional sprayers and in long run this type of sprayers can play a pivotal role in improvising the spray technology in orchards. Advancement in pesticide application has been made by the commercialization of electrostatic sprayers and by making these sprayers available to farmers on custom hiring basis will improve the overall efficiency of spray in orchards. It is also emphasized that there is a need to standardize the technology for reduced dosage of active ingredient of insecticide implying to decrease input cost of insecticide to achieve significant control of insect-pests in orchards.

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AUTHOR CONTRIBUTION STATEMENT

PKA conceived and designed research. MP and PKA conducted experiments. MP and PKA analyzed data. MP and US wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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DIVERSITY OF POLLINATORS ON SUNFLOWER

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ABSTRACT

Sunflower crop was grown during rabi 2021 and summer 2022 at the M S Swaminathan School of Agriculture, Centurion University of Technology and Management, Gajapati, Odisha. Investigation has been made on diversity of different pollinating agents associated with sunflower by observing their abundance, diversity, percentage contribution following the statistical diversity indices. The present study revealed eighteen different pollinators associated with sunflower. The Indian honey bee, *Apis cerana indica* (F) was recorded as the most dominant hymenopteran pollinator followed by the rock bee *Apis dorsata* (F) from family Apidae. The foraging activity of *A. c. indica* was observed to be maximum during mid flowering stage (1.72 ± 1.23 and 2.78 ± 1.81 bees/ capitulum/ min in 1st and 2nd season, respectively) with highest activity during 01:00-02:00 PM (3.02 bees/ capitulum/min.) and 10:00 to 11:00 AM (4.42 bees/ capitulum/ min) during first and second seasons respectively. The Apidae family members' i.e. *A. c. indica* and *A. dorsata* have attained eudominant and other pollinators were belonging mostly to the recedent and subrecedent classes based on dominance status. The diversity indices showed mid flowering stage attracted the most diversified group of pollinators.

Key words: *Apis cerana indica*, *Apis dorsata*, *Tetragonula iridipennis*, butterflies, relative abundance, dominance status, diversity index, ecology, Simpson's index, Shannon-Weiner Index (H')

Pollinators can be of any means of transferors such as bees, butterflies, birds, bats and other insects that play a crucial role in ecosystems and agriculture by facilitating the transmission of pollen from the male to the female parts of a flower. This phenomenon is known as pollination and results in several significant benefits for both the environment and human society. The diversity of pollinators refers to the wide variety of animal species that participate in the pollination which leads to fertilization and subsequent seed and fruit production. This process is essential for the reproduction and survival of many plant species, including those that are vital for human food production and ecosystem health. The diversity of pollinators provides several advantages and benefits to ecosystems, plants, and human societies. The presence of diversified pollinators maintains the resilience and stability of ecosystems. Apart from this, pollinators contribute to genetic diversity within plant populations. The relative abundance of different pollinators is proportional representation or frequency of various pollinator species within a given ecosystem or area. It describes the numerical or population density of different pollinators and their respective contributions

to the pollination process. Understanding the relative abundance of pollinators is important for assessing the overall health and functioning of pollination networks. Among all of the pollinators, honey bees are regarded as the best because they not only help in pollination but also stores honey which can be utilized by human beings.

Insect pollination contributes 9.5% of economic value of agricultural production used directly for human food according to Gallai et al. (2009). The western honey bee has been widely used as pollinators since the application of pollination services began according to Valido et al. (2019). Honey bees are considered as the excellent pollen vectors as majority of the foragers land on the outer ring of the florets and then move to inner rings where fresh pollen is available, before flying to next head. In addition, their movements between capitula are always indiscriminate which enhanced pollen movement according to Toit and Holm (1992). Honey bees along with birds and bats contribute around 35% yield towards agriculture production (F.A.O., 2023). Sunflower *Helianthus annuus* (L) is an important oilseed and a valuable crop. It has bright

and showy flowers that produce large amounts of nectar and pollen, making them highly attractive to pollinators, particularly bees and butterflies. Apart from this, the sunflower's inflorescence, which is made up of multiple tiny flowers arranged in a large disk, provides a generous landing platform for pollinators. Therefore, an investigation has been made on diversity of pollinating agents associated with sunflower by observing their abundance, diversity, and contribution following the statistical diversity indexes.

MATERIALS AND METHODS

The present study has been undertaken during two seasons i.e. first season (September-December, 2021) and second season (January-April, 2022) in the Experimental Station of Entomology located in the upland area of Experimental Research Field, M S Swaminathan School of Agriculture, Paralakhemundi, Centurion University of Technology and Management, Odisha. Apart from this, three other areas were also used for research. Those were the farmers' field of Kashinagar block, Gumma Block and Goshani block of Gajapati District of Odisha where three villages from each location were selected. The MSSSoA was coming under block Goshani. Keen observation has been taken on diversity of pollinators, their abundance, and contribution and dominance status. Following methods were used- Data has been collected on composition and abundance of pollinators from different agroecosystems of Gajapati district through visual observation at 05 days interval during the two cropping seasons during 2021-23. The identification of the pollinators was done from a large number of samples following fixed plot survey in selected experimental sites. The collected adult insects were killed by using chloroform and dry preserved through pinning/pointing in the laboratory of Department of Entomology, MSSSoA. The specimens were identified referring the identified specimen maintained in collections of AICRP on Honey bees and pollinators, OUAT, Bhubaneswar. The common name, scientific name, family, order, habitat of the specimens were recorded with their foraging behaviour. The dominance status of various taxa of the pollinators were described on the basis of relative abundance which determines the % of specimens of a given species in the total number of organism collected and the index of dominance was described following classes of dominance (Jakiewicz, 2003). For statistical analysis of diversity of pollinators, Simpson's index of diversity (Simpson, 1949) and Shannon-Weiner index (Shannon-Weiner Index, 1949) were used.

RESULTS AND DISCUSSION

Eighteen different pollinators were found foraging on sunflower in Gajapati district of Odisha. The present study conducted at MSSSoA, CUTM, Goshani block, in the first season revealed that the crop sunflower is visited by pollinators were belonging to order Hymenoptera and Lepidoptera. The adult lepidopteran pollinators were coming towards the plants as a source of nectar. According to Table 1, sunflower crop is visited by three species of honey bees, two species each of leaf cutter bees and wasps. Apart from the hymenopteran pollinators, eight lepidopteran adult pollinators were also found visiting sunflower. Studies on pollinator diversity in the second season revealed that the crop sunflower is visited by pollinators belonging to only Hymenoptera. Sunflower crop is visited by similar hymenopteran pollinators observed in first season with addition to *Apis florea* (F) and *Amegilla zonata* (L).

The data from two other blocks of Gajapati district. that the pollinators were similar revealed in case of Gumma block where European bees, *Apis mellifera* (F) was observed which was not observed in other two blocks (Table 1). Nayak et al. in 2021 at Bhawanipatna, Odisha recorded two honey bee species i.e. *A. dorsata* and *A. c. indica* and one bumble bee (*Xylocopa* sp). Yasmeen et al. in 2021 found a total of eight species of pollinators. A total of 14 pollinator insects species was recorded on sunflower belonging to the orders Hymenoptera and Lepidoptera (Adeoye and Pitan, 2020). Similarly, Mehmood et al. (2018); Basak and Mandal (2018) identified 7 and 18 different major insect pollinators, respectively. Bhowmik and Bhadra (2015) identified seventeen pollinators. Hussain et al. (2015) at Swabi Khyber Pakhtunkhwa, North Western Pakistan recorded fifteen species of pollinators belonging to 11 genera, 8 families and three orders. Rasheed et al. (2015) in Islamabad identified twelve different species of insect pollinators. Krishna et al. (2014) at Parbani, Maharashtra also revealed that *A. mellifera*, *A. c. indica* and *A. florea* were the major pollinators associated with sunflower. Goswami et al. (2013) at Pantnagar, Uttarakhand recorded 12 species of insect pollinators visiting sunflower. Jadhav et al. (2011) at Tirupati identified twenty species of insect pollinators associated with sunflower. Nderitu et al. (2008) at Eastern Kenya observed 14 insects species visiting sunflower floral heads. Kasina et al. (2007) at Makueni district, Eastern Ken recorded 14 insect species associated with sunflower. Mahavir (1999) at Haryana, Swaminathan and Bharadwaj (1982) at

Table 1. Pollinators' diversity on sunflower in Gajapati District, Odisha (2021-2022)

Sl. No.	Common name	Scientific name	Block Goshani		Block Kashinagar, 2022	Block Gumma, 2022
			First season, 2021	Second season, 2022		
Family: Apidae; Order: Hymenoptera						
1	Indian hive bee	<i>Apis cerana indica</i> F.	1	1	1	1
2	Rock bee	<i>Apis dorsata</i> F.	2	2	2	2
3	European bee	<i>Apis mellifera</i> L.	-	-	-	3
4	Stingless bee	<i>Tetragonula iridipennis</i> Smith	3	3	3	4
5	Little bees	<i>Apis florea</i> F.	-	4	-	-
6	Carpenter bee	<i>Xylocopa latipes</i> Drury	4	5	4	5
7	Carpenter bee	<i>Xylocopa aestuans</i> L.	5	6	-	-
8	Digger bee	<i>Amegilla zonata</i> L.	-	7	-	-
Family: Vespidae; Order: Hymenoptera						
9	Oriental hornet	<i>Vespa orientalis</i> L.	6	8	-	-
10	Wasp	<i>Vespa tropica</i> L.	7	-	-	-
Family: Nymphalidae; Order: Lepidoptera						
11	Tawny coster	<i>Acraea terpsicore</i> L.	8	-	-	-
12	Common crow	<i>Euploea core</i> Cramer	9	-	-	-
13	Grey pansy	<i>Junonia atlites</i> L.	10	-	-	-
14	Blue glassy tiger	<i>Idiopsis vulgaris</i> Butler	11	-	-	-
15	African monarch	<i>Danaus chrysippus</i> L.	12	-	-	-
16	Lemon pansy	<i>Junonia lemonias</i> L.	13	-	-	-
Family: Pieridae; Order: Lepidoptera						
17	Common/Lemon emigrant	<i>Catopsilia pomona</i> F.	14	-	-	-
Family: Crambidae; Order: Lepidoptera						
18	Cucumber moth	<i>Diaphania indica</i> Saunders	15	-	-	-

Udaipur, Rajasthan, Satyanarayan and Seetharam (1982) at Bangalore, Karnataka and Arya et al. (1994) at Hisar, Haryana identified 15, 10, 21 and 20 pollinators, respectively.

The studies conducted at MSSSoA during first season revealed that the order Lepidoptera (53.33%) was having more diversified species as compared to order Hymenoptera (46.67). From Lepidoptera, family Nymphalidae (40%) consisted highest number (6) followed by Pieridae (6.67%) and Crambidae (6.67%). From Hymenoptera, Apidae (33.33%) was the dominant one (5) followed by Vespidae. Whereas, in the second season one and only order Hymenoptera were found visiting the sunflowers on majority basis. Family Vespidae constituted 14.29% while family Apidae found the major by contributing 85.71% of pollinators. In the first season, Indian honey bees, *Apis cerana indica* constitute 30.81% of the total foragers and it remained the most dominant among the bee species visiting sunflower flower followed by *Apis dorsata* (Fabricius) (23.65%), *Tetragonula iridipennis* (19.56%), *Xylocopa latipes* (Drury) (5.68%), *Acraea terpsicore* (Linnaeus) (2.64%), *Junonia lemonias* (L.) (2.44%), *Xylocopa*

aestuans (2.44%), *Catopsilia pomona* (1.99%), *Vespa orientalis* (1.64%), *Junonia atlites* (1.59%), *Idiopsis vulgaris* (1.54%), *Diaphania indica* (1.54%), *Vespa tropica* (1.54%), *Danaus chrysippus* (1.49%) and *Euploea core* (1.44%) were also observed. In the second season also Indian honey bees, *A. c. indica* remained the most dominant among the bee species visiting sunflower flower constituting 33.94% followed by *A. dorsata* (30.38%), *T. iridipennis* (22.51%), *A. zonata* (8.49%) *X. latipes* (1.63%), *V. orientalis* (1.60%) and *X. aestuans* (1.46%).

Yasmeen et al. (2021) observed *A. mellifera*, *A. dorsata*, *A. c. indica*, *T. iridipennis*, *Vespa tropica* and Hesperidae as the major sunflower visiting species. Adeoye and Pitan (2020) revealed that the hymenopterans belonged to five families namely: Apidae, Halictidae, Anthophoridae, Megachilidae and Vespidae while the families Nymphalidae, Danaidae and Erebididae were the lepidopterans. The most common pollinators were *Trigona* sp (61.00%), *A. mellifera* (15.58%), *Dactylurina staudine* (13.23%) and *Acreazetes* (4.13%). The results showed that Hymenoptera (93.38%) were the most dominant insects

in the study, followed by Lepidoptera (6.62%) which is in line with the present investigation. Basak and Mandal (2018) revealed *A. dorsata* as the most dominant pollinator followed by *A. c. indica*.

Present results revealed that the population of *A. c. indica* was the maximum with 1.72 bees/ capitulum/ 5 min similar results were obtained. In the second season also, *A. c. indica* remained major pollinator 2.78 bees/ capitulum/5 min followed by *A. dorsata* (2.49 bees/capitulum/5 min) and *T. iridipennis* (1.84 bees/ capitulum/ 5 min). Other pollinators also visited with very less foraging activity (Table 2); *X. latipes* was very less in numbers during early and mid flowering stages but more during late flowering stage; *A. c. indica* was found to be more prevalent during 01:00-02:00 PM in the first season (3.02 bees/capitulum/ 5 min) whereas it was more abundant during 10:00-11:00 AM in the second season (4.42 bees/capitulum/ 5 min). *A. dorsata* was found to be more prevalent during 10:00-11:00 AM in the first season (2.34 bees/ capitulum/ 5 min) whereas it was more abundant during 10:00-11:00 AM in the second season (3.69 bees/ capitulum/ 5 min). *T. iridipennis* shown higher tendency during 10:00-11:00 AM in both of the seasons i.e. 1.67 bees/capitulum/5 min and 2.74 bees/ capitulum/ 5 min in first and second season respectively. Hemanth et al. (2020) with *A. cerana* showed peak activity time from 11.00 to 12.00; followed by *A. cerana* (2.87 bees/ capitulum/ 5 min); when 51- 75% of disc florets opened the foragers of *A. cerana* (3.73 bees/ capitulum/ 5 min) was seen at mid flowering. During full bloom, *A. cerana* foragers recorded mean number of foragers with 4.25 bees/ capitulum/ 5 min. In general the number of *A. cerana* foragers increased with disc florets opening/ capitulum. During the present investigation, similar rising trend of *A. c. indica* count was observed with increase in number of disc florets.

Fifteen pollinators were observed which can be categorized in to five out of six classes of dominance. In the first season, majority of the pollinators came under recedent constituted 10 numbers (*A. terpsicore*, *E. core*, *J. atlites*, *I. vulgaris*, *D. chrysippus*, *J. lemonias*, *C. pomona*, *D. indica*, *V. orientalis* and *V. tropica*) followed by subrecedent (*X. latipes* and *X. aestuans*) and dominant (*A. c. indica* and *A. dorsata*) each constituting two numbers followed by sub dominant (*T. iridipennis*) having only one species covering four classes of dominance classes during early flowering stage. During mid flowering stage majority of the 12

numbers of pollinators came under recedent (*X. latipes*, *X. aestuans*, *A. terpsicore*, *E. core*, *J. atlites*, *I. vulgaris*, *D. chrysippus*, *J. lemonias*, *C. Pomona*, *D. indica*, *V. orientalis* and *V. tropica*) followed by 2 dominant (*A. dorsata* and *T. iridipennis*) and eudominant having only one species covering three classes of dominance status. During late flowering stage majority of the 7 numbers of pollinators came under subrecedent (*E. core*, *J. atlites*, *I. vulgaris*, *D. chrysippus*, *J. lemonias*, *D. indica* and *V. tropica*) followed by 4 numbers of recedent (*X. aestuans*, *A. terpsicore*, *C. pomona* and *V. orientalis*), 3 numbers of subdominant (*A. dorsata*, *T. iridipennis* and *X. latipes*) and eu-dominant having only one species i.e. *A. c. indica* covering four classes of dominance classes. It is further observed that *A. c. indica* is having eudominant class status in both mid flowering and late flowering stages.

In the second season, seven pollinators were observed categorized into five out of six classes of dominance. Majority came under subrecedent (*X. latipes*, *X. aestuans* and *V. orientalis*) followed by eudominant (*A. c. indica* and *A. dorsata*) followed by dominant (*T. iridipennis*) and recedent (*A. zonata*) covering four classes of dominance status in the early flowering stage. During mid flowering stage majority came under subrecedent (*X. latipes* and *X. aestuans*) and dominant (2 numbers) (*A. c. indica* and *T. iridipennis*); followed by eudominant (*A. dorsata*), subdominant (*A. zonata*) and recedent (*V. orientalis*). During late flowering stage majority under recedent were (*T. iridipennis*, *X. latipes*, *X. aestuans*, *V. orientalis* and *A. zonata*) followed by subdominant and eudominant (*A. dorsata* and *A. c. indica*, respectively). In the first season, diversity of pollinators estimated through the Simpson's index of diversity during various flowering stages (45-84 DAS) revealed that the number of species varies between 5- 14 with the Simpson's Index, Simpson's index of diversity and Simpson's reciprocal Index ranging from 0.13 to 0.26, 0.78 to 0.87 and 3.80 to 7.51, respectively. The diversity during various timing of a day (i.e. 07:00 hr, 10:00 hr, 13:00 hr and 17:00 hr) revealed that the number of species varies between 7- 15 with Simpson's index of diversity ranging from 0.77-0.81 and diversity at all early, mid and late flowering (0.77-0.84). In the second season, diversity during various flowering stages (48-88 DAS) revealed that the number of species varies between 3-7 with the Simpson's Index, Simpson's index of diversity and Simpson's reciprocal Index ranging from 0.25 to 0.37, 0.63 to 0.75 and 2.70 to 4.87, respectively. The

diversity during various timing of a day (*i.e.* 07:00 hr, 10:00 hr, 13:00 hr and 17:00 hr) varies between 6-7 species with Simpson's index ranging from 0.73-0.80 and at all early, mid and late flowering (0.69-0.74). In general the diversity was almost uniform because of the evenness of the species.

The Shannon-Weiner Index values during various flowering days (45-84 DAS) ranges from 1.43 to 2.24 in the first season and 1.06-1.56 during different flowering durations (48-88 DAS) in the second season. The diversity of pollinators during various timing of a day (*i.e.* 07:00 hr, 10:00 hr, 13:00 hr and 17:00 hr) was with Shannon-Weiner Index ranging from 1.64 to 1.96 and 1.42 to 1.63 in the first and second season, respectively. The diversity during flowering stages varied from 1.82 to 2.13 and 1.24 to 1.49 in the first and second seasons respectively. Shannon-Weiner Index at 70 DAS (1.57) and 68 DAS (1.32), 01:00 PM (1.64) and 10:00 AM (1.42) and mid flowering stage (1.82 and 1.24) have shown more diversity in first and second season, respectively. Hymenoptera and Lepidoptera were evenly distributed within the experimental field with a value range of 0.90 - 0.96 (Adeoye and Pitan, 2020). Thus, a wide variety of insect species, notably those belonging to order Hymenoptera are extremely attracted towards sunflower capitulum. *Apis cerana indica* was the dominant one followed by *A. dorsata*. *Apis cerana indica* and *A. dorsata* have attained eudominant status whereas others mostly having recedent and subrecedent status.

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AUTHOR CONTRIBUTION STATEMENT

DP, CRS and SB conceived and designed research. DP conducted experiments. DP and CRS analyzed data. DP wrote the manuscript. CRS and SB reviewed the manuscript and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF SPERMIDINE SUPPLEMENTATION ON TESTICULAR AND OVARIAN DEVELOPMENT IN SEX-LIMITED AND NON-SEX-LIMITED BIVOLTINE SILK WORM BREEDS OF *BOMBYX MORI* L

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ABSTRACT

Silk worm *Bombyx mori* L is a holometabolous, lepidopteran model for investigating the effect of various molecules. The sex-limited character of cocoon colour is useful for the separation of male and female pupae during commercial seed production. Fecundity is the major limitation of cocoon colour sex-limited breeds. Spermidine is a polyamine, present in all living cells involved in the growth and reproduction of many organisms. The effect of spermidine on testicular and ovarian development in the selected bivoltine sex-limited breed; APS27SL and non-sex-limited hybrid; APS45 x APS12 is studied in the present work. Results showed that foliar feeding of spermidine significantly increased pupal growth, testicular and ovarian growth, number of ovarioles and ovules in the sex-limited breed. Moreover, increased expression of Bm-tektin was observed in the testes of spermidine fed groups. Thus, the study suggests that foliar feeding of spermidine can be used to overcome the limitation of reproductive potential in sex-limited breeds.

Key words: *Bombyx mori*, fecundity, spermidine, sericulture, sex-limited breed, reproduction, polyamine, bivoltine, growth, tektin, cocoon colour, sex linked character, testis, ovarioles

The silk worm *Bombyx mori* L has been intensively studied for a long time because of its economic value. It feeds on mulberry and gives silk, which has various traditional and medical applications. In sericulture, rearing, and egg production are two important factors that contribute immensely to silk production. Fecundity is the most important factor and a major challenge to be considered to improve the economic and mechanical parameters of sex-limited breeds and in the preparation of double hybrids (Dayanand et al., 2011; Subhra et al., 2011). Studies revealed that double hybrids produced by CSRTI (Central Sericulture Research and Training Institute) showed better fecundity compared to single hybrids. Many approaches have been used to boost the reproductive rate. The exposure of gamma radiation to *B. mori* eggs caused an increase in fecundity (Salam and Mahoud, 1995). The suitable rearing conditions, environment, and nutrition during the larval period may lead to higher fecundity by silkworm moths (Miller, 2005; Malik and Reddy, 2007; Hussain et al., 2011). A 1% concentration of ascorbic acid increased the number of eggs in the silkworm (Chauhan and Singh 1992). Further studies showed that fecundity and hatchability were improved by the application

of 20-hydroxyecdysone on the larvae in various concentrations (Prasad and Upadhyay, 2012). Mulberry leaves fortified with Zinc increased fecundity when fed to silkworms (Wani et al., 2018). APS27SL is a bivoltine cocoon colour sex-limited breed, male spin yellow cocoons and female spin white cocoons. Spermidine supplementation (Spd) increased the fecundity of this breed in previous studies (Madhavi et al., 2023). APS45 x APS12, a hybrid was developed by Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, under the collaborative project entitled "Popularization of authorized silkworm hybrids among the farmers of South India", funded by Central Silk Board (CSB), Bangalore. It is characterized by bluish-white plain larvae, white coloured hybrid cocoons with medium grains. The hybrid is quantitatively and qualitatively superior and suitable for rearing under favorable seasons resulting in yielding consistent cocoon crops.

The major constraint of sex-limited breeds was low fecundity (Suresh Kumar et al. 2008). Studies revealed that double hybrids produced by CSRTI showed better fecundity compared to single hybrids (APS45 x APS12),

with respect to growth and reproductive potential. Spd is a biologically important polyamine that serves as a key regulator of processes like DNA stability, protein synthesis, cell proliferation, differentiation, and apoptosis (Eisenberg et al., 2009; Minois et al., 2014; Morselli et al., 2011). Spermine was essential for spermatogenesis in rats (Weiner KX et al., 1992). Infertile men have low spermine and spermidine levels (Calandra RS et al., 1996). Studies show that polyamines increase sperm motility and capacitation in humans (Morales ME et al., 2003; Rodriguez-Paez et al., 2021). Spd concentrations in the testis were reported to be associated closely with the maturation of the testis as well as germ cell differentiation (MacIndoe and Turkington, 1973). In the newt testis, Spd and spermine levels were low in the winter, increased in the spring, and reached a peak in the summer during active spermatogenesis (Matsuzaki et al., 1981). During spermatogenesis in roosters, the ratios of spermine and Spd/DNA were relatively constant throughout spermatogenesis, whereas the ratio of putrescine/DNA increased in elongated spermatids. The cellular contents of putrescine, Spd, and spermine decreased markedly in mature spermatozoa of roosters (Oliva et al., 1982). These data suggest the importance of polyamines in testicular maturation and spermatogenesis. The Micromolar concentrations of polyamines increased the growth and economic parameters of *B. mori* (Lattala et al., 2014). Oral supplementation of Spd in hybrid (CSR2 x CSR4) *B. mori* boosted testicular development and egg production (Mysarla et al., 2016). The current study is focused to check and compare the effect of Spd on testicular and ovarian development in bivoltine cocoon color sex-limited breed (APS27SL) with non-sex-limited bivoltine promising hybrid (APS45 x APS12).

MATERIALS AND METHODS

Standard polyamine Spd free base (RM5438) was purchased from Hi Media Chemicals. The bivoltine *B. mori* cocoon colour sex-limited breed APS27SL (the male and female spin white and yellow cocoons respectively) and non-sex-limited promising bivoltine hybrid APS45 x APS12 were selected. The disease-free layings of both the breeds were released from the schedule and reared under standard rearing conditions (26-28°C, 65- 85% RH), up to the 4th moult with superior cultivar, V₁ mulberry leaves as feed. The 5th instar larvae on day 1 were divided into two groups, one group was fed with Spd (50 µM) swabbed leaves till the day of spinning. Three replications for each breed (25 male and female each) were maintained with batches fed on the

mulberry leaves. The matured larvae of both the control and treated groups were allowed to spin cocoons. After harvesting cocoons, the male and female were separated easily based on their colour in the sex-limited breed, and sex separation was carried out manually in the non-sex-limited breed. Testis and egg samples were collected from the male and female pupa at different time points during pupal development in control and Spd-supplemented groups of both sex-limited and non-sex-limited breeds and analyzed. The cocoons were cut open and the weight of the male and female pupa was taken (at the beginning, middle, and at end of the pupal stage; days 13, 16, and 19) till emergence and recorded. Pupae were dissected at different time points of the pupal development (days 13, 16, and 19), testes and ovaries obtained from pupae were weighed. Weighments were taken from randomly selected pupae. Three biological replications were performed and three samples were analyzed from each replication (n = 3). Testes and ovaries were weighed individually and averages with mean error values were recorded.

For tissue sample preparation the testes and ovaries were dissected out from the pupae at different time points during pupal development (days 13, 16, and 19) from treated and control groups using insect Ringer's solution (0.68% NaCl) and transferred into a fresh microfuge tube and stored at -80 °C till use. The expression of the Bm-tektin and RP49 genes was carried out by RT-PCR (reverse transcription polymerase chain reaction). Total RNA was isolated from 0.4 g of the testes (n=3 pooled) of control and Spd-treated groups of sex-limited and non-sex-limited breeds on day 19 of pupal development by the TRIzol method. The RNA samples (3 µg) were reverse transcribed into cDNA using SRL-RT-PCR Kit (First Strand cDNA Synthesis Kit) 94837-(BOS001). Three replicates of PCR were performed with each sample. In each run of PCR, the housekeeping gene RP49 was used as the reference. 5'- AGCGAATGCTTTCTCTGGAA -3' and 5'- TGTCGCTCCAATCAAATTCA -3' were used as forward and reverse primers for the Bm-tektin gene amplification, and 5'- CAGGCGGTTCAAGGGTCAATAC -3' and 5'- TGCTGGGCTCTTTCCACGA -3' primers were used for RP49 gene amplification (control). The densitometric analysis of Bm-tektin and RP49 was carried out in the control and Spd-treated APS27SL and APS45 x APS12 using Image Lab software in GelDoc^{XR} by BioRad. Statistical analysis was carried out by taking the measurements of pupal weights, egg weights and testes weights in triplicates and compared

between the control and treated groups (Spd). The experimental data was subjected to statistical analysis by t-test; two samples assuming equal variances ($T < t$, one tail; $p = 0.05$).

RESULTS AND DISCUSSION

Results showed that the effect of Spd was more prominent in the sex-limited *B. mori* breeds when compared to the non-sex-limited breed. This study for the first time showed foliar supplementation of 50 μ M Spd during 5th instar larvae enhanced growth and development of pupa, testis and ovary of cocoon colour sex-limited breeds and APS45 x APS12. The weights of the pupae were recorded on days 13, 16, and 19 of the control and Spd treated groups. Spd-treated male pupae showed a significant increase in weights on day 13 and day 16 of APS27SL and on day 13, 16 and 19 of APS45 x APS12 compared to the control group (Fig. 1A). Spd-treated female pupae showed a significant increase in weight on days 13, 16, and 19 of APS27SL

compared to the control group, the Spd-treated APS45 XAPS12 showed increase in female pupal weights compared to control (Fig. 1B). These results were in accordance with the earlier studies which showed the role of Spd in the growth and sexual reproduction of *Fusarium graminearum* (Tang Guangfei et. al., 2021). Spd treated mice showed an increment in body weight (Wang et al., 2022). Larvae of strain CSR2 X CSR4 fed with Spd showed increased male and female pupal weights than the control (Mysarla et al., 2016).

The testicular and ovular weights were recorded on days 13, 16 and, 19. Day 19 is considered the final day as moths emerge on day 20. The weight of Spd-treated APS27SL testes showed a significant increase on days 13 and 19 compared to a control group (Fig. 1C). The results obtained matched with the earlier findings of Spd treatment. Testicular weights were shown significantly higher in Spd-treated groups than in the control groups of CSR2×CSR4 cross-breed silkworms (Mysarla et al., 2016). Spd treated mice showed improved testicular

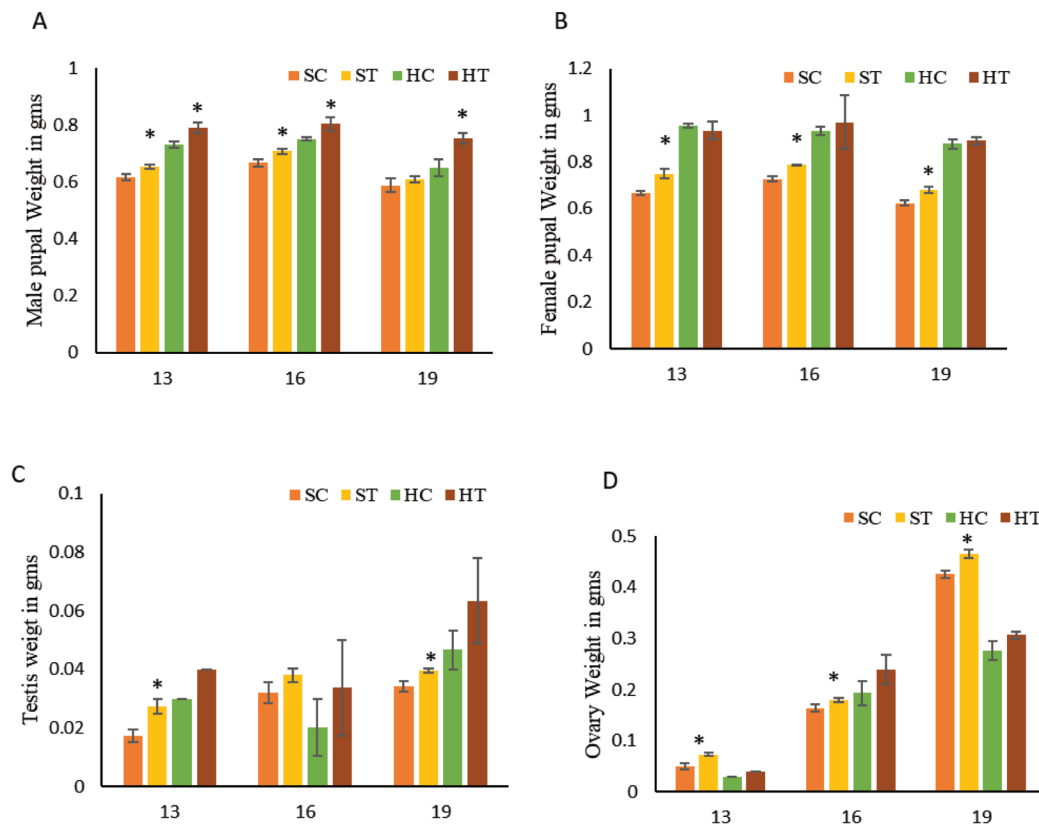


Fig. 1. Effect of Spd on the growth of APS27SL and APS45×12 pupae A. male pupal weights B. Female pupal weights C. Testes weights D. Egg weights, X-axis represents different days of pupal development and Y-axis represents average weight in grams (gms). SC and ST represent the control and Spd treated groups of the sex-limited breed, HC and HT represent the control and Spd-treated groups of the non-sex-limited breed. * represents ($p \leq 0.05$)

function, with an increment in the testis weight (Wang et al., 2022). Spd-treated ovary weights showed a significant increase on days 13, 16 and 19 of APS27SL when compared to the control groups and an increase in ovary weight was observed Spd-treated compared to control groups of APS45 x APS12 (Fig. 1D). In vitro, studies in Kiwi fruit showed the positive effect of Spd on ovary growth by reduction in necrosis (R. Biasi et al., 1997). The increased ovarian weights in Spd supplemented groups could be due to decreased cell death. The Spd group of both sex-limited and non-sex-limited breeds showed 5 ovarioles whereas, 4 ovarioles were observed in control groups (Fig. 2A and B). Spd protected the ovarian function by increasing antioxidants in mice (Jiang D et al., 2023). The size of the testes increased in Spd-treated sex-limited and non-sex-limited breeds compared to the control. The distinct tubular formation can be noticed in Spd-treated sex-limited and non-sex-limited breeds compared to the control (Fig. 2). The exogenous supplementation of Spd enhanced the growth of testis in mice (Qi Zhao et al., 2021). The Spd supplementation enhanced the testicular and egg production in the CSR2×CSR4 breed of *B. mori* (Mysarla et al., 2016).

To understand the molecular levels differences, expression analysis of Bm-tektin on day 19 testicular

homogenates was performed with sex-limited and non-sex-limited breeds. Cloning and characterization of testis-specific tektin in *B. mori* was done earlier (Ota et al., 2002). The Bm-tektin gene is responsible for the flagellar activity of sperm which in turn facilitates the motility of the sperm., which plays a major role in reproduction. RP49 gene was used as an internal control to normalize the expression of Bm-Tektin. Results showed elevated expression of Bm-tektin in the Spd-treated group of the sex-limited breeds compared to the control group (Fig. 3A). The fold change in the Spd-treated group was calculated by normalizing the Bm-tektin expression with RP49. Results showed a 0.5 fold increase in the Spd-treated sex-limited breed when compared to the control (Fig. 3B). Tektin 3 and Tektin 4 were shown to be essential for sperm motility in mice (Roy et al., 2009). Tektins found abundance in centrioles and axonemal microtubules (Steffen et al., 1988). The transcriptome analysis of the testis of the silkworm, *B. mori* was carried out and the testis-specific genes were found to be accumulated more on the Z chromosome (Arun Kumar KP et al., 2009). Thus the foliar supplementation of Spd during 5th instar larvae resulted in a significant increase in pupal weight testicular and ovarian growth in the sex-limited breed compared with the non-sex-limited breed. Further

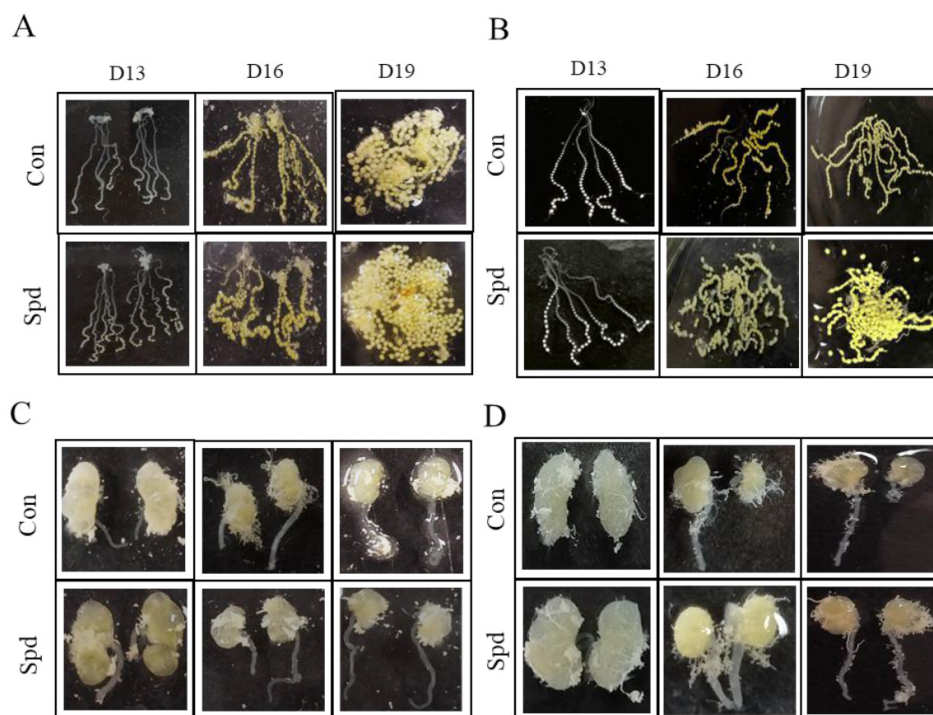


Fig. 2. Effect of Spd on testicular and ovular morphology of sex-limited and non-sex-limited pupae in control and treated groups (A) ovary of (B). ovary of non-sex-limited breed. (C). testis of sex-limited breed (D). testis of non-sex-limited breed.

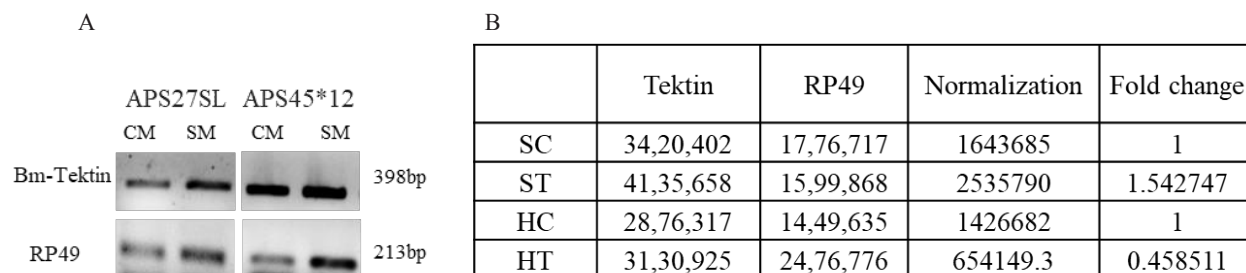


Fig. 3. Expression analysis of Bm-tektin. (A) RT-PCR of Bm-tektin and RP49 genes in testicular homogenates of *B. mori*. (B) Relative expression of Bm-tektin was quantified and normalized with RP49 of control and Spd-treated testes of APS27SL and APS45×12 on day 19.

studies are required to understand the role played by Bmtektin in the growth and reproduction of *B. mori*.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed. MK carried out growth analysis. BGLD carried out RT-PCR analysis. SJ and PJR provided rearing facilities and checked rearing performance experiments. AM conceptualized the idea and supervised the project work.

CONFLICT OF INTEREST

No conflict of interest.

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RECORD OF *DOWNSIOMYIA ALBOLATERALIS* (THEOBALD) (DIPTERA: CULICIDAE) FROM THE HIMALAYAN PLAINS OF WEST BENGAL

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ABSTRACT

The present study was carried out with the aim to characterize a mosquito species that was documented near about a century ago from Darjeeling district of northern West Bengal, India. Mosquito larvae were collected from the bamboo stumps or holes of sub-Himalayan plains of Darjeeling district, West Bengal, India. Collected immatures were reared upto adults maintaining standard protocol. Larva and adult specimens were investigated for morphological identifications upto generic and species level. In the present study, the Niveus species *Downsiomyia albolateralis* (Theobald, 1908) is a newer addition to the mosquito fauna in the region and it will update the mosquito checklist for the knowledge of vector and non-vector species.

Key words: *Downsiomyia albolateralis*, Culicidae, new record, morphology, mosquito, Niveus subgroup, rediscovery, taxonomy, vector, Darjeeling, West Bengal

The Niveus subgroup members are identified as important vectors of dengue virus and the human lymphatic filariasis parasite, *Wuchereria bancrofti* in the countries of South and Southeast Asia (Shriram et al., 2014; Choochote, 2001). A recent finding has estimated that 30 species of the Genus *Downsiomyia* Vargas, 1950 including the Niveus subgroup are found throughout the Oriental and adjoining Palearctic and Australian regions (Harbach, 2022). In India, according to Tyagi et al. (2015), there are currently six species of the genus, namely- *Do. albolateralis* (Theobald, 1908), *Do. albonivea* (Barraud, 1934), *Do. mohani* (Knight, 1969), *Do. nivea* (Ludlow, 1903), *Do. niveoides* (Barraud, 1934) and *Do. novonivea* (Barraud, 1934). The type species of Niveus subgroup *Downsiomyia nivea* Ludlow, 1903 is the only established vector of Diurnally subperiodic *Wuchereria bancrofti* (DspWB) form of filariasis restricted in the Nicobar district of Andaman and Nicobar Islands, India (Shriram et al., 2014).

Downsiomyia albolateralis Theobald, 1908 was first recorded from Assam hills, India by Theobald in the year 1908 (Theobald, 1908). Later the species was recorded from two northern districts of sub-Himalayan West Bengal, India, namely- Darjeeling in 1922, 1928 and Jalpaiguri in 1923 (Barraud, 1934). The species was also recorded from several other North-eastern (Mahanta et al., 2010; Rajput and Singh, 1992; Rajput and Kulkarni,

1991), North-western Himalayan states (Debi and Jauhari, 2007) and the Eastern and Western Ghat ranges of South India (Selvan et al., 2016; Munirathinam et al., 2014). Believably, *Do. albolateralis* is native to South and Southeast Asian countries e.g., India (Barraud, 1934), Pakistan (Jabeen et al., 2019), China (Maquart et al., 2021), Nepal (Darsie Jr. et al., 1993), Bangladesh (Irish et al., 2016), Sri Lanka (Gunathilaka, 2018), Myanmar, Cambodia (Maquart et al., 2021), Thailand (Choochote, 2001), Malaysia (Colless, 1958), Singapore (Colless, 1958) and Papua New Guinea (Maquart et al., 2021). In the current investigation by Zoological Survey of India, *Do. albolateralis* has been recorded from the western Himalayan hills although the eastern Himalaya, including sub-Himalayan plains of West Bengal, still awaits documentation about its occurrence (Banerjee et al., 2018). Recently 50 different mosquito species, without *Do. albolateralis*, belonging to 8 different genera have been documented and compiled in a study from this northern part of West Bengal (Saha and Saha, 2021) justifying its absence from the entire West Bengal for nearly a century, i.e., precisely after 1920s.

This species commonly breeds and lays their eggs in the forest tree holes, bamboo holes or stumps, leaf axils and rock holes at an elevation of about 300–3000 meters (Munirathinam et al., 2014; Mahanta et al., 2010; Debi and Jauhari, 2007; Rajput and Singh, 1992; Rajput and Kulkarni, 1991). Though Niveus

subgroup members are generally regarded as zoophilic but this species is a day biter, bites both humans and cattle (Rajput and Kulkarni, 1991; Choochote, 2001). Laboratory investigations in Thailand reported that, *Do. albolateralis* is 9.43% susceptible to bancroftian filariasis and 100% susceptible to DEN-2 virus as a competent vector of these pathogens (Choochote, 2001). Medical importance of the Genus *Downsiomyia*, especially the species *Do. albolateralis* has not been still widely studied likely due to its evasive behaviour but also because its identification is difficult with the existing keys and poor literature descriptions.

MATERIALS AND METHODS

Field surveys were conducted in the sub-Himalayan plains of Darjeeling district, the northern most part of West Bengal state, India (26°26'50"-26°58'00"N, 88°06'13"-88°31'03" E). The study area receives annual mean rainfall of 225 to 275 cm between June to September with an average temperature extension of 13.8 to 28.6°C giving the zone a tropical or sub-tropical climatic conditions. The study area also shares international borders with neighbouring nations, like- Nepal in the west and Bangladesh in the south-east. The meteorological conditions of the region and also its great vegetation with diverse habitats render the area highly vulnerable to mosquito breeding. Immature stages of mosquitoes were collected from the natural breeding habitats of mosquitoes, like- bamboo holes or stumps and sticks. Mosquito sampling was conducted in five locations: Naxalbari, Matigara, Khoribari, Phansidewa and Sukna, in the plains of Darjeeling district between September and November, 2022. All the immatures were collected in 500 ml plastic containers, brought to the laboratory and reared upto adults (F_0) in separate rearing cages for each sampling site. Rearing was done maintaining $25 \pm 2^\circ\text{C}$, and 70-80% RH according to the standard protocol as described by Clemons et al. (2010). Fourth-instar larva and adult mosquito specimens were thoroughly investigated under a stereoscopic binocular microscope, Magnus MS-24 for morphological identifications. Standard identification keys were used for systematic identification of the specimens up to genus (Tyagi et al., 2015; Rueda, 2004) and species level (Colless 1958; Barraud 1934; Theobald 1908). Genitalia of the morphologically identified adult male mosquito specimens were dissected out and slide mounted according to the methodology described by Anuradha et al. (2022) and Rattanarithikul (1982). Male genitalia were investigated under Olympus OIC (708139) microscope.

RESULTS AND DISCUSSION

A total of 77 individuals were identified as *Do. albolateralis* among all the collected samples from the sub-Himalayan plains of Darjeeling district (Table 1); of the five sampling sites, four sites were found to be positive breeding habitats (PBH) for this species with other mosquitoes in mixed population; 43 were male and 34 were female. Majority (45 out of 77) of individuals were found from the bamboo stumps of Matigara. 19 individuals were yielded from Sukna, 12 individuals from Naxalbari area and a single individual was found from Khoribari area. Phansidewa area was found to be a negative breeding habitat (NBH) for the species in the present study (Table 1).

***Downsiomyia albolateralis* (Theobald, 1908)** (Fig. 1, 2)

Taxonomic account: Superfamily- Culicoidea Malloch, 1917; Family- Culicidae Meigen, 1818; Subfamily- Culicinae Meigen, 1818; Tribe- Aedini Neveu-Lemaire, 1902; Genus- *Downsiomyia* Vargas, 1950. Recent phylogenetic investigations on the classification of the tribe Aedini postulated that the species of Niveus Subgroup of the subgenus *Finlaya* Theobald, 1903 of the genus *Aedes* Meigen, 1818 has been instated in the genus *Downsiomyia* Vargas, 1950 (Natarajan, 2022; Reinert and Harbach, 2006). Therefore, *Aedes (Finlaya) albolateralis* has been reclassified and is currently known as *Downsiomyia albolateralis*.

Diagnosis: *Downsiomyia albolateralis* is a species-member of Niveus Subgroup, mainly distinguished by the male genitalia as females are sometimes undifferentiated. Like other species of the subgroup, this species has flat silvery-white scales on head and scutellum. A single, large, distinct silvery-white patch on front of mesonotum. Thorax with a broad silvery-white patch on each side in front. Legs dark, basal $\frac{2}{3}$ of hind-femur white. Wings dark, almost 3 mm. In male, gonocoxite having leaf-like long lanoeolate scales at its base. In larva, comb scales 8-11 and siphon slightly wider in middle than the either ends.

Description: Female (Fig. 1A-J). Medium-size mosquitoes. Head. Proboscis (P) slender, straight and black; maxillary palp (MPlp) short and black with few white scales at apex (Fig. 1B); with a thin border of silvery scales around eye-margins, a wider white patch of flat scales in the middle on front of vertex on head (H) (Fig. 1C); Thorax. Single large roundish

Table 1. Sampling sites, and other details of *D. albolateralis* (September-November, 2022)

Sampling sites	Representative coordinates	Habitat type	<i>Do. albolateralis</i> (n)	Sex	Population shared with
Naxalbari	26°43'4.3968"N 88°20'38.5764"E	Bamboo stumps (peri-domestic)	12	M-11 F-1	<i>Aedes albopictus</i> , <i>Culex quinquefasciatus</i> , <i>Cx. fuscocephalus</i> <i>Toxorhynchites</i> sp.
Matigara	26°44'10.9068"N 88°22'52.6332"E	Bamboo stumps (Tea Garden)	45	M-19 F- 26	<i>Ae. albopictus</i> , <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>
Khoribari	26°32'18.042"N 88°7'14.484"E	Bamboo stumps (Forest patch)	1	M-0 F- 1	<i>Ae. albopictus</i> , <i>Armigeres</i> sp.,
Phansidewa	26°40'24.4632"N 88°22'43.3884"E	Bamboo stumps (peri-domestic)	Nil	Nil	<i>Ae. albopictus</i> <i>Cx. quinquefasciatus</i> <i>Armigeres</i> sp.
Sukna	26°47'22.0416"N 88°21'55.5228"E	Bamboo stumps (Forested area)	19	M-13 F-6	<i>Ae. albopictus</i> <i>Cx. quinquefasciatus</i>

**Ae.* = *Aedes*; *Cx.* = *Culex*; *Do.* = *Downsiomyia*; F = Female, M = Males

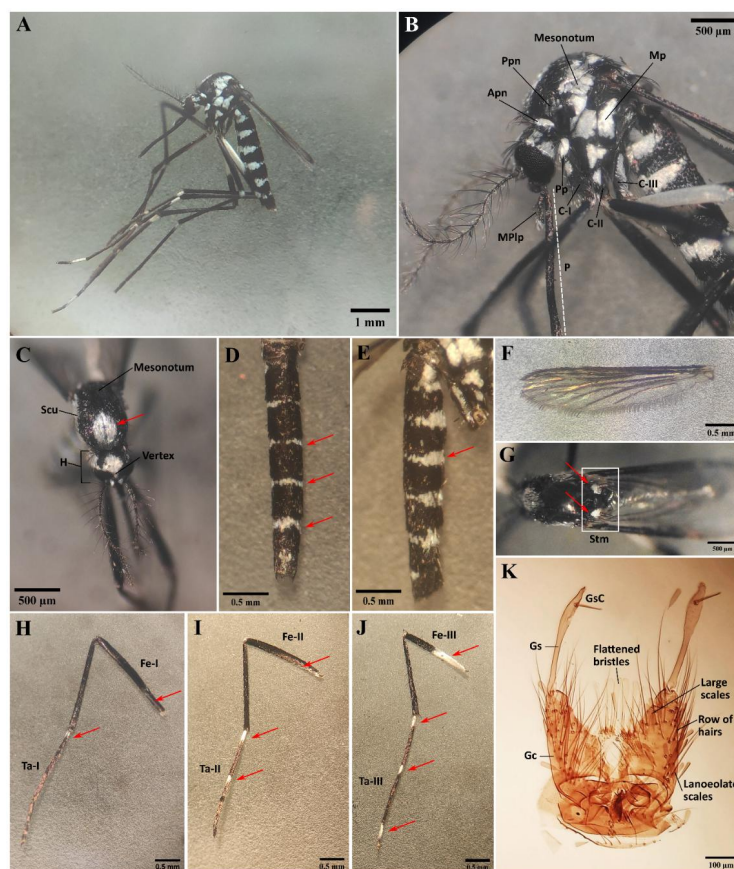


Fig. 1. *Downsiomyia albolateralis*- Adult female: A, Whole mosquito specimen; B, Lateral view of thorax; C, Dorsal view of head and scutum; D, Dorsal view of abdomen; E, Lateral view of abdomen; F, Wings; G, Dorsal view of mesonotum and scutellum; H, Fore leg; I, Mid leg; J, Hind leg; K, Dorsal view of male genitalia. Apn, antepronotum; C, coxa; Fe, femur; Gc, Gonocoxite; Gs, Gonostylus; GsC, Gonostylar claw; H, head; Mp, mesepimeron; MPlp, Maxillary palp; P, proboscis; Pp, propleura; Ppn, postpronotum; Scu, scutum; Stm, scutellum; Ta-tarsi (red coloured arrow showing pale/white streak, white band or white scale patch on different structures of the figures accordingly).

silvery-white patch of scales in front of mesonotum (Fig. 1C, showed by red arrow); Mesonotum having a large silvery-white scaling on its either side, forming the large lateral patches (Fig. 1B); Mesepimeron (Mp) largely covered by an elongated patch of silvery-white scales, another one silvery patch is in front of it and the other one below (Fig. 1B); Small silvery-patches on coxa (C), propleura (Pp), post-pronotum (Ppn) and ante-pronotum (Apn) (Fig. 1B); Scutellum (Stm) trilobed, lobes of either side have white flat scales (Fig. 1G); Abdomen. Abdomen dark-black, basal bands of large white scales form wedge-shaped markings on lateral side but not extended to dorsum (Fig. 1D); basal markings form narrow bands on Vth to VIIth abdominal segments, band on VIIIth segment quite large (Fig. 1E, showed by red arrow); Legs. Posterior $\frac{2}{3}$ or more of hind-femur (F-III) is entirely white, hind-tarsomeres (Ta-III) with 3 white bands (Fig. 1J); Mid-femur (F-II) anteriorly black and basal $\frac{1}{3}$ of it more extensively pale, some silvery scaling on anterior surface, tibia black and tarsi (Ta-II) paler with 2 white bands (Fig. 1I); Fore-femur (F-I) anteriorly black and basal $\frac{1}{3}$ of it has a pale streak beneath, tibia and tarsi (Ta-I) entirely black or brownish-black, paler at anterior end (Fig. 1H); Wings. Wings with dark scales and about 3 mm long (Fig. 1F).

Male (Fig. 1K). Males are little lighter than females or brownish. Smaller in size in comparison to females. Head. Head scales creamy or light brownish in colour; Maxillary palp black having a single white scale-band at anterior part near apex; palpi almost similar or a little shorter than proboscis in length. Antennal plumes dense and brown. Thorax. Anterior $\frac{2}{3}$ of mesonotum covered with white scales and other markings are very similar to those of ♀. No white scales above wing-

roots. Legs. Legs are similar to that of ♀. Abdomen. Abdomen- slender, ninth abdominal segment modified into hypopygial structure. Hypopygium (Fig. 1K). The gonocoxite is narrower and conical; A pair of submedian lobes placed together closely, each with 4 strong flattened bristles; the gonostylus (Gs) is almost equal to the length of its gonocoxite (Gc), long gonostylar claw (GsC) joints ventrally near the expanded apex of gonostylus; Gonocoxite having a group of large scales on ventral border, dorsal border with a row of hairs and leaf-like long lanceolate scales at its base (Fig. 1K).

Larva, 4th instar (Fig. 2). Number of hairs having few short, fine branches on thorax and abdomen; Lateral hairs of abdomen- large, 2-3 branched; Comb scales 8 to 11 in number, largely pointed, arranged in a single row (Fig. 2A, marked with white box); Sub-siphonal tuft with 6 branches (Fig. 2A); Siphon. Slightly wider in middle than the either ends, length >3x than that of its base (Fig. 2B); Pecten with 15 small teeth, each with one large and one or two small denticles, pecten covers $\frac{1}{2}$ of the tube from base, a hair-tuft with 3 branches at middle of the tube (Fig. 2B, marked with white box); Saddle almost encloses the anal segment (Fig. 2B).

Distribution in India: The species is distributed over all the seven states of North-eastern India, Garhwal region of Western Himalayas, Bastar district of Madhya Pradesh, Darjeeling district (Sukna and Kurseong) and Jalpaiguri dooars of northern West Bengal, Western Ghat hill ranges, and Nicobar district of Andaman and Nicobar Islands. But among the above-mentioned regions, *Downsiomyia albolateralis* has not been reported from Darjeeling and Jalpaiguri districts of the northern West Bengal. The species was lastly recorded

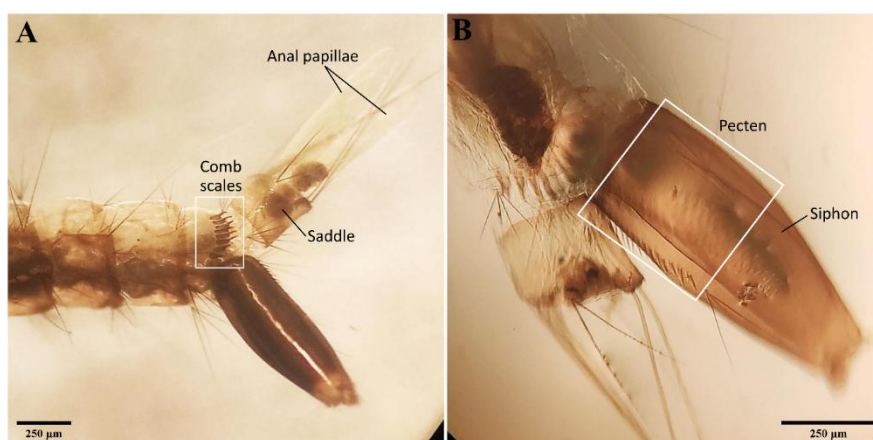


Fig. 2. *Downsiomyia albolateralis*. Fourth-instar larva: A, Posterior abdominal segments; B, Siphon (white-coloured boxes are used for denoting different minute structures on the figures).

from Sukna and Kurseong of Darjeeling and dooars region of Jalpaiguri district during 1920s, about a century ago.

Bionomics: Immature stages of *Do. albolateralis* were found from the bamboo stumps of PBHs in the study area. Adults were emerged in the laboratory after collection. Immatures of the species were co-existing with the other mosquito species, like- *Aedes aegypti* Linnaeus, 1762, *Ae. albopictus* Skuse, 1895, *Armigeres* sp. Theobald, 1901, *Culex fuscocephalus* Theobald, 1907, *Cx. quinquefasciatus* Say, 1823 and *Toxorhynchites* sp. Theobald, 1901 in the mixed population. Bionomics of the species is not known in greater details.

The description given here about *Do. albolateralis* is based on our present study over 34 adult females, 43 males with their male genital characters and a number of fourth-instar larval specimens collected from the sub-Himalayan plains of Darjeeling district. Majority of characters of the species *Do. albolateralis* described by Theobald (1908) and Barraud (1934) are found to be similar to that of our described specimens. The major characters include: in adult females, a wider white patch in the middle on front of vertex on head; a single large roundish silvery-white patch in front of mesonotum; posterior $\frac{2}{3}$ of hind-femur is entirely white; wings with dark scales. In the male genitalia, dorsal border of gonocoxite with a row of hairs and leaf-like long lanoeolate scales at its base. In the fourth instar larva, comb scales largely pointed, 8 to 11 in number, arranged in a single row; Pecten with 15 small teeth and wider in the middle region. *Do. albolateralis* females show slight differences from the nominotypical member of *niveus* subgroup, *Do. nivea* which possess a larger solid white area on front of mesonotum covering $\frac{2}{3}$ area of it and all scutellar scales are black. *Do. nivea* males have coxite devoid of scales at the base but have long hairs in that position. *Do. albolateralis* larva also differ from *Do. nivea* in comb scale number, siphon length and the number of pecten teeth. Other *Niveus* species namely- *Do. alboniveus*, *Do. niveoides* and *Do. novoniveus* are largely indistinguishable from *Do. nivea* adult but show distinguishable differences in adult female characters and larval structures of *Do. albolateralis*. Females of these species do not show any external differences from *Do. nivea* where the white spot on mesonotum is rather wider and complete. In *Do. albolateralis*, the white-spot is distinct, roundish and restricted only on front of mesonotum. Adults of this subgroup are almost identical and only can be distinguished by the male

genital structures. Presence of large leaf-like lanoeolate scales at the basal area of gonocoxite in males, is the distinguishing character of this species from other members of the subgroup.

However, there are few minor characters that differing from the literature descriptions (Theobald 1908; Barraud 1934; Colless 1958) in having distinct white colour bands on hind-tarsi (Ta-III) and mid-tarsi (Ta-II), a collection of few pale-white scales in postpronotum (Ppn) and few white scales at the apex of maxillary pulp (MPlp) in female and a single white scale-band at anterior part (near apex) of maxillary pulp in male. Although few minor characters differing from those described by Theobald (1908) and Barraud (1934), major characters of adult females, males (male genitalia) and fourth instar larva examined are seemed to be sufficient to recognise them as *Do. albolateralis* Theobald. Globally, there is no morphology-based identification key available that describes the present species. Thus, the species level identification is very difficult with the existing keys and inadequate literature descriptions. The only illustrated description was made by Barraud (1934) published under the world-famous book- 'The Fauna of British India including Ceylon and Burma' series where the species was reported from Sukna (1922) and Kurseong (1928) in Darjeeling district more than a century ago. The first record of mosquitoes from the sub-Himalayan foothills when published in 1970, it reported 18 culicine species but did not mention *Do. albolateralis* (Varma and Mahadevan, 1970). In later studies also, the species has not been reported from this region (Saha and Saha, 2021; Rudra and Mukhopadhyay, 2010; Aditya et al., 2006; Malakar et al., 1995). Now, reporting of the species in the present study simply depicts it re-occurrence after about 100 years from this region and the first record of the species- *Do. albolateralis* Theobald from the northern part of West Bengal after Indian independence in 1947. The present study cites and compiles all the possible available data and descriptions best known to date for the species.

The *Niveus* species *Do. albolateralis* was found to be a potential vector for NspWB and DENV in laboratory but their role as natural vector for these two diseases is still unknown in India (Choochote, 2001). One of its close relatives *Do. nivea* and some members of this group (also including *Do. albolateralis*) have been implicated in the transmission of DspWB among the Nicobarese of Andaman and Nicobar islands (Shriram et al., 2015) and NspWB in Thailand (Gould

et. al., 1982). Now *Do. albolateralis* is resurging in the sub-Himalayan Darjeeling district which is among the prevalently dengue endemic districts of northern West Bengal (Roy et al., 2022; Bharati et al., 2021; Taraphdar et al., 2010). The two adjacent districts, namely-Cooch-Bihar is endemic for nocturnally periodic *W. bancrofti* (NpWB) form of filariasis (Saha and Saha, 2021) and Jalpaiguri is another prevalently dengue endemic districts of northern West Bengal (Bharati et al., 2021). The species is found here in association with the established primary vectors of dengue namely- *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse and principal lymphatic filariasis vector, *Culex quinquefasciatus* Say in the mixed populations (Table 1). Therefore, occurrence of this Niveus species in the region that has a potentiality for acting as an important vector elsewhere, has made a serious concern in the context of public health implications of the area. It may be a possible vector for these diseases in changing environmental conditions of upcoming days in absence of primary vectors. Rapid transformation of peripheral forest areas and tea plantations into urban set-ups in this region has resulted in the deterioration of the natural landscape in and around these urban centers. In earlier studies over India, the species have reported from the rural, semi-forested, hilly area having certain elevations but in the present study they are yielded from the urbanised, peri-domestic settlements of plains also (Table 1). These ultimately results into indiscriminate exposure of large population towards these jungle mosquitoes and increases the chances of zoonosis. The species documentation is an essential component of biodiversity study and helps to design the conservation programme of genetic resources as well as vector and pest control measures. The study will be helpful for better understanding of the bionomics and illustrated descriptions with greater details which were not previously documented well enough for this species. It will also be an addition to the mosquito faunal diversity and update the mosquito checklist of this area.

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AUTHOR CONTRIBUTION STATEMENT

Ratnadeep Sarkar. Roles. Data curation, Formal analysis, Investigation, Methodology, Resources, Writing- original draft and Writing- review and editing; Abhirup Saha. Roles. Data curation, Formal analysis, Investigation, Methodology, Resources and Writing- review and editing; Subhajit Das. Roles. Resources, Software and Visualization; Prapti Das. Roles. Formal analysis, Investigation and Writing- review and editing; Dhiraj Saha. Roles. Conceptualization, Supervision, Validation and Writing- review and editing

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF THIACTOPRID EXPOSURE ON HONEY BEES *APIS MELLIFERA* F.

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ABSTRACT

An evaluation of the effect of thiacloprid (21.7% SC), an N-cyanoamidine neonicotinoid exposure on honey bees *Apis mellifera* F. was done under field conditions. Study on foraging behaviour, mortality and food stores were carried out with 500, 325, 250, 125, 62.50, and 31.25 ppm, while 0 ppm concentration served as control. Of these, a significant effect was observed with 500 and 325 ppm as could be observed from the foraging behaviour. Thus, thiacloprid is found to be safe at lower concentrations <250 ppm.

Key words: *Apis mellifera*, thiacloprid, neonicotinoid, foraging, behaviour, mortality, honey stores, pollen stores, exposure, 250 ppm, safety

Honey bees account for 35% of the global food production and hence are important for food security (Genersch, 2010). In the United States alone, *Apis mellifera* F. pollinates \$15 to \$20 billion worth of crops and around \$200 billion worldwide (Van Engelsdorp and Meixner, 2010). A serious ailment of honey bees is Colony Collapse Disorder (CCD) in which the worker bees fly off leaving the colony starve to death leading to serious losses (Underwood and Van Engelsdorp, 2007; Dainat et al., 2012). In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. But these have shown indirect or sublethal effects on honey bees which may include effect on behaviour like foraging, learning, cleaning, navigation. Being symptomatic such as uncoordinated movements of bees, tremors; these effects greatly impact the overall growth of the colonies (Buszewski et al., 2019). Neonicotinoids have shown a wide range of effects detected through their acute toxicity tests as shown by Cresswell (2011), and on gut microbiota by Alaux et al (2010). High rate of mortality from *Nosema* infections was caused as a result of imidacloprid and thiacloprid exposure (Vidau et al., 2011); and detrimental effect on behaviour, learning and memory as studied by Aliouane et al. (2008). Thiacloprid has profound effect on sucking or biting insects and even nematodes like *Meloidogyne incognita*. The LD₅₀ value of thiacloprid was found to be as low as 14600 ng/ bee (quite less) as against imidacloprid, clothianidin, thiamethoxam, dinotefuran, and nitenpyram (Iwasa et al., 2004) Residues of various neonicotinoids are found in bee products like honey, propolis, pollen, nectar (Tanner

and Czerwenka, 2011). Residue analysis revealed, the presence of thiacloprid in the most of honey samples when compared with the other three neonicotinoids, acetamiprid, thiamethoxam and imidacloprid (Birdi et al., 2018). In addition, thiacloprid has an effect on the flight pattern of honey bees with a slowed down flight speed compared to imidacloprid and clothianidin treated honey bees (Fischer et al., 2014). Queen failure and subsequent colony loss is another disturbing case (Brandt et al., 2017); and thiacloprid and clothianidin lead to the failure of immune defense response. Jammu and Kashmir holds a high potential for beekeeping industry in India (Mutto, 1952). In Jammu division of J&K, beekeeping is practiced on traditional as well as commercial level, with 81,000 bee colonies (*A. mellifera*) with honey production of 860 mt. Information on the impact of thiacloprid on bee health is limited, and hence this study was taken up to study the effects of thiacloprid on behaviour and food stores.

MATERIALS AND METHODS

Study was conducted during February-March, 2021 for which 21 full frame hives uniform in terms of adult, brood and food stores of the bees were selected to account for seven treatments with three replications including control. Various concentrations of thiacloprid 21.7% SC were prepared. Sugar candies were prepared using 100 g sugar and 10 ml of the treatment concentration. For control, candies were prepared using sugar powder and distilled water (0 ppm of thiacloprid). A total of three feedings were given at weekly intervals. The day 2nd was chosen for the observations because thiacloprid has quite a fast rate

of metabolism and the waiting period is of five days in brinjal. For recording number of foraging bees getting in and out from the entrance of the hive for a period for five min was done. Mortality on one hr basis was recorded by counting the number of dead bees fallen on the top cover kept below the entrance of the hives every 2nd day after the exposure to treatments. Food stores (honey and pollen) were evaluated by quantifying the area of the frame. This was done on 5th day after every exposure. For this a transparent sheet was divided into squares of equal size using a black marker and placed over the frame and the area having honey and pollen was quantified by counting the number squares on the sheet covered (Delaplane et al., 2012) and % reduction over control was calculated. The means of data were analysed after square root transformation with statistical tests performed with SPSS16.0 for MS Windows.

RESULTS AND DISCUSSION

The results for foraging behaviour showed a significant difference between the number of incoming and outgoing *A. mellifera* only with the higher concentrations, namely 500 and 325 ppm (Table 1). On 2nd day after first feeding, the outgoing bees outnumbered the incoming bees in both 500 and 325 ppm concentrations. This may be attributed to the loss of memory, orientation (Yang et al, 2008; Henry et al., 2012). For the rest of the concentrations, the difference between the incoming and outgoing bees was insignificant, as thiacloprid, being a cyanoamidine neonicotinoid is safer (Tison et al., 2016). A slight increase in the difference between incoming and outgoing was observed, with an overall decrease in both incoming and outgoing bees after 2nd and 3rd feeding. This may be due to the cumulative effect of feedings one after the other but again significant difference was only seen with 500 and 325 ppm concentration. Pyke (2022) showed that consumption of neonics impaired ability to navigate and thus time spent foraging. Yang et al. (2008) while working with different concentrations of imidacloprid, viz., 50, 100, 200, 400, 600, 800 and 1200 µg/ l observed abnormal behaviour only with concentrations >50 µg/ l. The effect of nitro substituted neonics was much adverse than thiacloprid on honey bee foragers (Marimuthu et al., 2022). Long-term exposure to thiacloprid showed greater effect on learning and memory abilities than a single administration (Shi et al., 2023).

The results on mortality of *A. mellifera* in 1 hr revealed maximum mortality at 500 ppm followed

by 325 ppm, both being at par with each other (Table 1); mortality at 325 ppm (recommended dose) may be attributed to the direct feeding of thiacloprid as treated sugar candy. These present observations agree with those obtained in the laboratory study done by Laurino et al. (2011). However, the present study shows mortality at the recommended concentration of 325 ppm also which is significantly different from that of the control. This contrast might be due to the difference in the experimental conditions and observation times, the present study is a field study, with observations taken on 2nd day of feeding the treated candy. Woodcock et al. (2017) observed that neonicotinoids interacting with environmental factors can amplify the losses of worker bees. Also, even the field realistic concentrations of thiamethoxam and clothianidin reduced the honey bee drone survival by 51% (Straub et al., 2020). Siede et al. (2017) observed that sublethal doses of thiacloprid administered to honey bees could not cause any significant effect on their mortality even after an exposure for two long years; these results are in agreement with present study, where except 500 and 325 ppm, there was no effect seen on the survival. Also, present results are in line with those of Tison et al. (2017), in which no mortality was seen with the concentrations starting from 0.5, 5, 50 ppm up to 200 ng/ bee which were later labelled as the sublethal. Liu et al. (2019) revealed that significant mortality was observed when honey bees were fed with sucrose solution mixed with different concentrations of thiacloprid viz., 0, 0.2, 0.6 and 2.0 mg/ l for about 2 weeks.

Since thiacloprid impacted foraging activity, a reduction in the food stores of was also recorded; maximum was seen at 500 ppm followed by 325 ppm (Table 1). These results are in line with those of Ohlinger et al. (2021) on imidacloprid. Further, on 5th day after 2nd and 3rd feeding, a slight increase in % reduction was seen which can be attributed to the cumulative effect of thiacloprid on the foraging activity; reduction with 500 and 325 ppm were found to be at par, but significantly different from rest of the concentrations. Rumkee et al. (2017) observed that with lower doses of pesticides were received by larvae when there were less number of foraging bees and higher doses were received with foraging bees increasing. Wu-Smart and Spivak (2016) observed that number of cells containing honey and pollen were significantly reduced in the hives which received higher concentrations of imidacloprid- > 20 ppb as against 10 ppb. Meikle et al. (2022) found that food stores in colonies decreased significantly in spite of

Table 1. Effect of thiacloprid on foraging, mortality, honey and pollen stores of *Apis mellifera*
Foraging behaviour and mortality on 2nd day after feeding

Conc. (ppm)	No. of bees (foraging behaviour)						No. of dead bees (mortality)		
	1 st feeding		2 nd feeding		3 rd feeding		1 st feeding	2 nd feeding	3 rd feeding
	Incoming	Outgoing	Incoming	Outgoing	Incoming	Outgoing			
500	175.00 (13.22± 0.30)	206.33 (14.35± 0.43)	155.33 (12.45± 0.20)	187.67 (13.69± 0.13)	135.33 (11.63± 0.11)	169.00 (12.99± 0.13)	106.67 (10.32± 0.08)	109.17 (10.44± 0.10)	111.67 (10.56± 0.10)
325	185.67 (13.62± 0.16)	217.00 (14.72± 0.23)	170.33 (13.04± 0.39)	200.33 (14.14± 0.32)	143.67 (11.98± 0.15)	175.00 (13.22± 0.13)	105.00 (10.24± 0.07)	105.83 (10.28± 0.04)	106.67 (10.32± 0.04)
250	267.00 (16.33± 0.10)	273.00 (16.52± 0.06)	252.33 (15.88± 0.21)	258.33 (16.07± 0.17)	229.33 (15.14± 0.19)	235.33 (15.33± 0.24)	73.33 (8.56± 0.09)	76.67 (8.75± 0.09)	78.33 (8.84± 0.09)
125	273.00 (16.52± 0.04)	279.00 (16.70± 0.10)	272.67 (16.51± 0.11)	279.00 (16.70± 0.05)	231.33 (15.20± 0.18)	237.67 (15.41± 0.12)	71.66 (8.46± 0.09)	76.66 (8.75± 0.09)	76.67 (8.75± 0.09)
62.5	288.00 (16.96± 0.10)	294.00 (17.14± 0.17)	273.00 (16.52± 0.06)	279.00 (16.70± 0.05)	245.67 (15.67± 0.09)	251.67 (15.86± 0.05)	71.00 (8.42± 0.12)	73.33 (8.56± 0.09)	76.67 (8.75± 0.09)
31.25	298.00 (17.26± 0.10)	304.00 (17.43± 0.05)	279.33 (16.71± 0.17)	285.33 (16.88± 0.22)	247.00 (15.71± 0.06)	253.33 (15.91± 0.05)	67.50 (8.20± 0.22)	70.00 (8.36± 0.11)	71.66 (8.46± 0.09)
control	299.00 (17.29± 0.07)	306.00 (17.49± 0.03)	285.56 (16.90± 0.05)	292.00 (17.08± 0.05)	250.33 (15.82± 0.06)	255.67 (15.98± 0.01)	66.67 (8.15± 0.25)	68.33 (8.26± 0.10)	71.66 (8.46± 0.07)

Reduction over control in honey and pollen stores after 5th day after feeding

Concentration (ppm)	% reduction over control					
	Honey stores			Pollen stores		
	1 st feeding	2 nd feeding	3 rd feeding	1 st feeding	2 nd feeding	3 rd feeding
500	30.98 (0.31± 0.005)	32.01 (0.32± 0.003)	37.97 (0.39± 0.005)	32.89 (0.33± 0.004)	39.27 (0.40± 0.004)	44.36 (0.46± 0.002)
325	30.25 (0.30± 0.001)	31.63 (0.32± 0.005)	37.11 (0.38± 0.007)	31.65 (0.32± 0.004)	38.93 (0.39± 0.003)	43.68 (0.45± 0.002)
250	15.12 (0.15± 0.023)	16.52 (0.16± 0.019)	17.07 (0.17± 0.026)	16.89 (0.16± 0.010)	17.50 (0.17± 0.003)	19.02 (0.19± 0.026)
125	12.83 (0.12± 0.009)	15.47 (0.15± 0.01)	16.11 (0.16± 0.008)	14.85 (0.14± 0.025)	16.62 (0.16± 0.008)	17.61 (0.17± 0.003)
62.50	10.42 (0.10± 0.009)	11.45 (0.11± 0.01)	12.78 (0.12± 0.010)	14.19 (0.14± 0.021)	15.71 (0.15± 0.008)	16.49 (0.16± 0.008)
31.25	10.00 (0.10± 0.023)	10.07 (0.10± 0.01)	10.26 (0.10± 0.01)	12.04 (0.13± 0.021)	12.94 (0.13± 0.02)	15.71 (0.15± 0.008)

Figures in parenthesis are sine transformed values ± SE.

higher brood production when exposed to imidacloprid. Thus, it can be concluded that thiacloprid is safe to honey bees at lower concentrations. The recommended concentration of 325 ppm showed an effect which was not significantly different from the effect due to 500 ppm. This is in conformity with the study of Cabirol and Haase (2019) which showed that field realistic doses vary from place to place and have different uptake pathways.

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AUTHOR CONTRIBUTION STATEMENT

Pratibha Angral and R S Bandral conceived and designed research. Pratibha Angral conducted experiments, analyzed data and wrote the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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AMELIORATING POTENTIAL OF CURCUMIN AND ASCORBIC ACID AGAINST HEPATOTOXICITY CAUSED BY SYNERGISTIC EFFECT OF HEAVY METALS IN MALE ALBINO RATS

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ABSTRACT

The ameliorative role of combination of curcumin and ascorbic acid (low and high doses) was determined against biochemical and histopathological changes in liver caused due to synergistic effects of heavy metals (As, Cd and Pb) present above permissible limits in drinking water for 60 and 90 days in 84 male albino rats. The histology of liver in rats treated with a mixture of low as well as high doses of heavy metals showed mild alternations after 60 days and intense damage after 90 days. The content of biochemical parameters like proteins and lipids were significantly decreased and cholesterol, phospholipids and fatty acids were significantly increased after 60 and 90 days of treatment. The rats treated with curcumin and ascorbic acid showed restoration of histological damage and content of biochemical components indicated amelioration of hepatotoxicity effectively up to 60/90 days depending upon the dose of heavy metals, curcumin and ascorbic acid.

Key words: Ascorbic acid, amelioration, curcumin, hepatotoxicity, arsenic, cadmium, lead, histopathology, proteins, lipids, liver

Heavy metal pollution is increasing day by day around the world and toxicity caused due to their non-biodegradable, biological accumulation and carcinogenic nature poses a significant threat to human health (Ahmadijokani et al., 2022). India is facing a major problem of heavy metals in the groundwater and heavy metal toxicity was reported in groundwater of various states of India such as Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal. The main source of Arsenic (As) exposure to 100 million people is through drinking water (Nordstrom et al., 2010). Cadmium (Cd) is a toxic heavy metal and its biological half-life is more than 20 years (Johri et al., 2010). Cd toxicity inhibits the liver metabolic enzyme systems containing sulphhydryl groups and rupture of oxidative phosphorylation in mitochondria, which results in increased lipid peroxidation, hepatic congestion, ischemia, and hypoxia (Habeebu et al., 1998). Lead (Pb) poisoning is the most widely studied occupational and environmental hazards. The uptake of Pb decreases the intelligence level, destroys emotional stability and also causes hearing disabilities in children (Ibrahim et al., 2011). Hepatotoxicity refers to toxicity of the liver resulting from various chemicals and xenobiotics including heavy metals and their metabolites (Navarro et al., 2006). The effect of long-term exposure to a

mixture of heavy metals like As, Cd and Pb at low doses produced hepatotoxic effects in albino rats (Bhattacharjee et al., 2016).

Curcumin has long been recognized for its pharmaceutical properties and efficacy as a food colorant (El-Desoky et al., 2021) and serves as anti-inflammatory and anti-tumorous agent. It ameliorates oxidative stress and inhibits the generation of Reactive Oxygen Species (ROS) both *in vivo* and *in vitro* (Eybl et al., 2006). It was used for the cure of oxidative stress and liver disease caused by Pb (Abdel-Moneim et al., 2015). Ascorbic acid, also known as vitamin C has a therapeutic role in the protection against heavy metals due to its antioxidant properties (Shaukat et al., 2018). The objective of the present study was to determine the changes in histopathology and biochemical components in the liver of male albino rats caused due to combination of heavy metals i.e. As, Cd, Pb at doses higher than the Maximum Permissible Limit of Bureau of Indian Standards (BIS) in drinking water for duration of 60 and 90 days and the possibility of combination of curcumin and ascorbic acid in different doses for protective effect against these changes.

MATERIALS AND METHODS

A total of 84 sexually mature male albino rats weighing 100-150 gm were procured from Disease

Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar after clearing the proposal through the Institutional Animal Ethics Committee (IAEC). The Institutional Animal Ethics Committee (IAEC) vide letter no IAEC/2019/104-123 dated 15/07/2019 approved this experimental protocol. The rats were given 5-fold and 10-fold concentrations of maximum permissible limit of As (0.05 mg/ l), Cd (0.003 mg/ l) and Pb (0.01 mg/ l) as recommended by the Bureau of Indian Standards (BIS) in a mixture through drinking water. The rats were provided with As in the form of sodium arsenite, Cd in the form of cadmium chloride, and Pb in the form of lead acetate. The curcumin and ascorbic acid were given to rats by oral gavage one hour before exposure to heavy metals. The rats were treated for a minimum of 60 days (42 rats- Group Ia to VIIa) and a maximum of 90 days (42 rats- Group Ib –Group VIIb). The grouping and treatment of rats were done as follows with six rats in each group:

Group I a/ Group Ib: 0.25mg/ l of As+ 0.015mg/ l of Cd+0.05mg/ l of Pb (LDHM/ 5-fold)

Group IIa/ Group IIb: 0.50mg / l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb (HDHM/ 10-fold)

Group IIIa/Group IIIb: 0.25mg/ l of As+0.015mg/ l of Cd+0.05mg/ l of Pb + 200 mg of curcumin and 100 mg of ascorbic acid /kg of Body weight (LDHM+ high dose C +A)

Group IVa/Group IVb: 0.25mg/ l of As+0.015mg/ l of Cd+0.05mg/ l of Pb + 100mg of curcumin and 50 mg of ascorbic acid /kg of Body weight (LDHM +low dose C +A)

Group Va/Group Vb: 0.50mg/ l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb +200 mg of curcumin and 100 mg of ascorbic acid /kg of Body weight (HDHM+ high dose C +A)

Group VIa/VIb: 0. 50mg/ l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb + 100 mg of curcumin and 50 mg of ascorbic acid /kg of Body weight (HDHM + low dose C +A)

Group VIIa/Group VIIb: Control

At the end of 60 and 90 days of treatment, the rats were dissected with cervical dislocation, the liver was taken and processed for histological studies by a method of Luna (1968). The photographs of histological slides were obtained under an optical light microscope (Magnus) under 100X and 400X magnifications with the help of Magvision software. The liver tissue was homogenized and the tissue homogenate was centrifuged at 5000 r.p.m. for about 20 minutes and

the supernatant was collected and stored in a deep freezer for biochemical estimations. The different biochemical parameters like proteins (Lowry et al., 1951), lipids (Folch et al., 1957), phospholipids (Ames, 1966), cholesterol (Zlatkins et al., 1968), and fatty acids (Lowry et al., 1976) were analysed quantitatively in homogenates of liver tissues. The comparison for different parameters of between the treated rats and control rats was done using SPSS one way ANOVA. Comparisons were made for treated rats at 60 and 90 days by using Tukey's method.

RESULTS AND DISCUSSION

The liver of control rats at both 60 and 90 days of treatment showed radially arranged hepatic cords with hepatocytes organized into cords separated by sinusoids (Figs. 1A, 2A). The histological structure of the liver showed various alternations in heavy metal treated groups. LDHM was found to cause dilation of the central vein after 60 days (Fig. 1B) and dilation of central vein, degeneration of hepatocytes, and vacuolisation after 90 days (Fig. 2B). HDHM treated group showed an irregular arrangement of hepatic cords after 60 days alongwith dilation of central vein and infiltration of leukocytes in central vein after 90 days (Figs. 1C, 2C). LDHM + low dose curcumin and ascorbic acid treated group showed degeneration of hepatocytes, dilation of central vein and dilation of sinusoidal space after 60 days (Fig. 1D) and infiltration of leukocytes in central vein and necrosis of hepatocytes after 90 days (Fig. 2D). LDHM + high dose curcumin and ascorbic acid showed restored central vein and hepatocytes after 60 days (Fig. 1E) along with reduced sinusoidal space restored hepatic cords after 90 days (Fig. 2E). HDHM+ low dose curcumin and ascorbic acid showed degeneration of hepatocytes in hepatic cords as well as infiltration by the large mass of leucocytic inflammatory cells after 60 days of treatment (Fig. 1F) and irregular arrangement of hepatocytes and necrosis after 90 days of treatment (Fig. 2F). HDHM + high dose curcumin and ascorbic acid showed infiltration of leukocytes in central vein after 60 days of treatment (Fig. 1G) and irregular arrangement of hepatocytes and necrosis after 90 days of treatment (Fig. 2G). The histological damage was observed in the liver of rats treated with a mixture of low and high doses of heavy metals and this damage was more prominent in 90 days as compared to 60 days. These histological changes were restored in rats treated with a mixture of heavy metals and a combination of curcumin and ascorbic acid in a dose and time-dependent manner. Histopathological changes occurred in liver due to

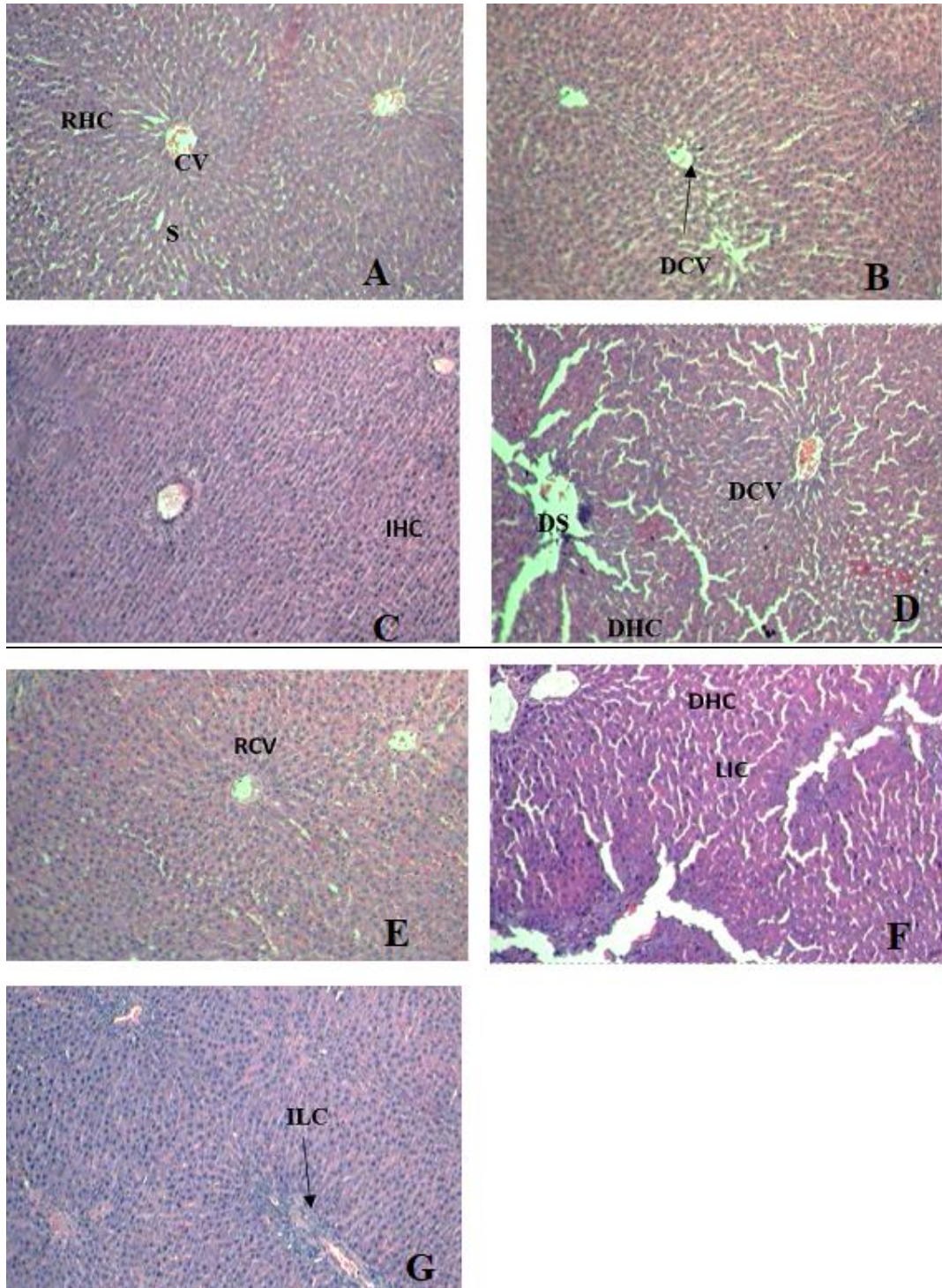


Fig. 1. T.S. of Liver (X100) of rats after 60 days of treatment (A) Control showing Hepatocytes (HC) in radially arranged hepatic cords (RHC) separated by sinusoids(S) (B) LDHM showing dilation of central vein (DCV) after 60 days of treatment. (C) HDHM showing irregular arrangement of hepatic cords (IHC) after 60 days of treatment. (D) LDHM+ low dose C+A showing dilation of central vein (DCV), degeneration of hepatocytes (DHC) and dilation of sinusoid spaces(DS) (E) LDHM+ high dose C+A showing restored central vein (RCV) (F) HDHM+ low dose C+A showing degeneration of hepatocytes (DHC) in hepatic cords and infiltration by large mass of leucocytic inflammatory cells(LIC) (G) HDHM+ high dose C+A showing infiltration of leukocytes in central vein (ILC).

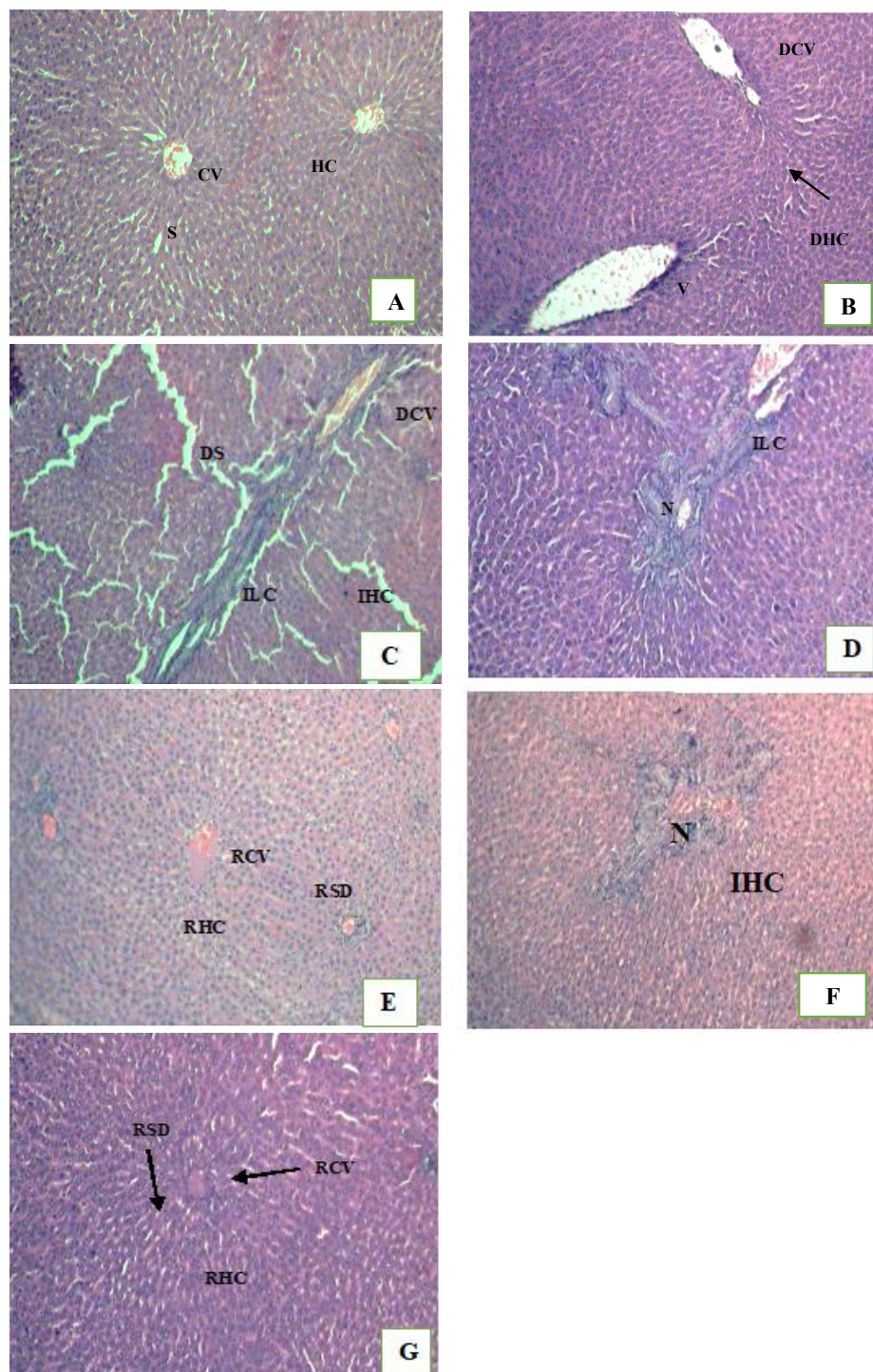


Fig. 2. T.S. of Liver (X100) of rats after 90 days of treatment (A) control rat showing Hepatocytes (HC) in radially arranged hepatic cords (RHC) separated by sinusoids(S) (B) LDHM showing dilation of central vein (DCV), degeneration of hepatocytes (DHC) and vacuolization(V) (C) HDHM showing infiltration of leukocytes in central vein (ILC) and necrosis of hepatocytes(N) (D) LDHM+ low dose C+A showing restored central vein (RCV), reduced sinusoidal space(RS) and restored hepatic cords (RHC) (E) LDHM+ high dose C+A showing irregular arrangement of hepatic cords (IHC), dilation of central vein (DCV) and infiltration of leukocytes in central vein (ILC) (F) HDHM+ low dose C+A showing irregular arrangement of hepatocytes (IHC) and necrosis(N) (G) HDHM+ high dose C+A showing restored central vein (RCV), restored hepatocytes (RHC) in hepatic cords and reduced the dilation of sinusoids (RSD)

As toxicity i.e. moderate sinusoidal dilation, focal necrosis, and these abnormalities in rat liver were cured by co-treatment with curcumin (Mathews et al., 2012). Pb toxicity caused the necrosis of hepatocytes and leucocytic infiltration of hepatic cells in liver of rats (Mehana et al., 2012). The chronic low dose exposure to a mixture of heavy metals i.e. As, Cd, and Pb caused histopathological changes in rat liver i.e. vacuolation in some hepatocytes, fatty degeneration in other hepatocytes, and congestion within central veins and some sinusoids (Bhattacharjee et al., 2016). Liu et al. (2023) also reported histopathological and cytotoxicity caused by different heavy metals (cadmium (Cd), arsenic (As), lead (Pb), and nickel (Ni) contributed to the detoxification ability of curcumin against heavy metals. The content of protein (60 days) and lipid (60 and 90 days) in liver of rats was observed to be significantly low in LDHM, HDHM, HDHM+ high dose C+A and HDHM + low dose C+A as compared to control rats (Table 1). After 90 days of treatment, the protein content in liver of rats was observed to be significantly less in LDHM, HDHM, LDHM + low dose C+A, HDHM+ high dose C +A, and HDHM + low dose C+A as compared to control rats. The non-significant variation in protein and lipid content of liver of LDHM + high dose C+A and LDHM + low dose C+A as compared to control rats indicated the amelioration of hepatotoxicity caused due to low dose of heavy metals at 60 days. However, at 90 days only LDHM + high dose C+A caused amelioration of heavy metals toxicity in protein content. The significantly less content of protein in all the treated groups and that of lipid in LDHM+ low dose C+A, LDHM + high dose C+A treated rats was observed in 90 days as compared to 60 days of treatment. The combination of heavy metals (As, Cd and Pb) leads to overproduction of free radicals which caused damage to protein and lipids by causing oxidation of proteins and peroxidation of membrane lipids (Martemucci et al., 2022). The free radical scavenging properties of antioxidants like curcumin and ascorbic acid prevent the overproduction of free radicals thus protecting the proteins and lipids (Sadighara et al., 2023). The mixture of As, Cd and Pb significantly lowered levels of protein and albumin in liver as compared to control rats (Bhattacharjee et al., 2016). Pb-treated rats showed the suppression of total protein content in liver and curcumin retorted it to normal level as compared to control (Abdelhamid et al., 2020). The liver plays a central role in lipid metabolism, serving as the center for lipoprotein uptake, formation and export to circulation. Alterations in hepatic lipid

Table 1. Biochemical contents in liver of rats treated with heavy metals, curcumin and ascorbic acid

Treatment	Protein (µg/g)		Lipids (mg/g)		Cholesterol (mg/g)		Phospholipids (µg/ml)		Fatty acid (mg/g)	
	60 Days	90 Days	60 Days	90 Days	60 Days	90 Days	60 Days	90 Days	60 Days	90 Days
LDHM	103.05± 2.89 ^a	57.19± 1.01 ^b	18.73± 0.68 ^a	23.43± 0.84 ^a	6.75± 0.10 ^b	18.36± 1.86 ^a	106.41± 5.50 ^{ab}	151.16± 1.82 ^a	4.01± 0.05 ^a	5.01± 0.30 ^a
HDHM	96.96± 2.52 ^a	55.73± 1.57 ^b	19.61± 0.52 ^a	13.23± 1.94 ^a	7.85± 0.70 ^b	24.45± 0.72 ^a	154.15± 4.83 ^b	171.07± 2.82 ^a	5.68± 0.21 ^a	6.18± 0.26 ^a
LDHM + high dose C+A	139.57± 6.31 ^a	142.26± 2.99 ^b	26.93± 1.31 ^b	13.42± 1.43 ^a	5.54± 0.23 ^b	11.19± 0.62 ^a	87.88± 2.27 ^a	91.91± 3.44 ^a	2.31± 0.16 ^a	2.74± 0.14 ^a
LDHM + low dose C+A	119.60± 3.03 ^a	136.46± 1.41 ^b	21.12± 1.83 ^b	13.30± 0.83 ^a	5.02± 0.25 ^a	8.64± 0.18 ^a	93.78± 3.82 ^a	96.61± 2.68 ^a	3.03± 0.33 ^a	3.19± 0.19 ^a
HDHM + high dose C+A	127.66± 4.90 ^a	74.34± 2.38 ^b	19.67± 2.19 ^a	13.29± 1.06 ^a	4.72± 0.53 ^b	13.66± 0.17 ^a	137.05± 2.51 ^b	102.24± 4.1 ^a	3.48± 0.52 ^a	3.97± 0.04 ^a
HDHM + low dose C+A	113.72± 3.17 ^a	64.65± 3.85 ^b	13.30± 0.56 ^a	19.62± 0.87 ^a	5.38± 0.31 ^b	14.08± 0.54 ^a	141.08± 3.66 ^b	193.81± 2.85 ^a	3.62± 0.22 ^a	4.40± 0.38 ^a
Control	143.82± 1.40 ^a	148.61± 2.69 ^a	30.67± 2.19 ^a	32.97± 1.39 ^a	3.81± 0.79 ^a	3.49± 0.57 ^a	39.95± 1.28 ^a	38.02± 1.23 ^a	1.54± 0.10 ^a	1.59± 0.09 ^a

Values Mean ± SE (n=3 from pooled samples of 6 rats); *Significant at p≤0.05 as compared to control; Values with different superscripts (a,b) in a column represents significant difference for 60 and 90 days (p≤0.05)

metabolism could contribute to the development of chronic liver disease (Waghe et al., 2017). The total lipids were elevated in As treated rats as compared to control rats and after the treatment of curcumin with As showed the normal level of total lipids as that of control rats (Mann et al., 2005).

After 60 days of treatment, cholesterol in liver of rats was observed to be significantly high in LDHM, HDHM as compared to control rats. The non-significant variation in cholesterol of liver of LDHM + high dose C+A, LDHM + low dose C+A, HDHM + low dose C+A, HDHM+ high dose C +A treated rats as compared to control rats indicated the ameliorative potential of curcumin and ascorbic acid for hepatotoxicity caused due to low dose of heavy metals at 60 days. However, at 90 days no amelioration of heavy metals toxicity was observed. Cholesterol level in liver was significantly increased in LDHM, HDHM, LDHM + high dose C+A, HDHM + low dose C+A, HDHM + high dose C+A in 90 days of treatment as compared to 60 days of treatment. One study reported high cholesterol levels in liver of male Wistar albino rats treated with heavy metal mixture (20mg/kg Pb, 1.61mg/kg Cd, 0.40mg/kg HgCl₂) (Anyanwu et al., 2020). The cholesterol level was high in male rats treated with a sub-acute heavy metal mixture of Pb (60mg/kg) and Cd(2mg/kg) (Abdulidha et al., 2020). This effect may be due to the increased disturbance of lipid metabolism after the accumulation of heavy metals in the liver. Pb increased the total cholesterol level in liver by inhibiting the activity of hepatic lipoprotein lipase enzyme and the treatment with curcumin + Pb reduced the total cholesterol level in liver tissue to reach the normal range of control rats (Abdelhamid et al., 2020). Buhari et al. (2020) reported that an increase in total cholesterol was observed in Pb, Hg and Cd toxicity in blood of US population. Significant increase in total cholesterol, triglycerides and LDH concentrations upon treatment with buprofezin (BPFN) dose and were restored to normal concentrations after treatment with Vitamin C and curcumin supplementation (Sadia and Qureshi, 2022). After 60 days and 90 days of treatment, the content of phospholipids was found to be significantly increased in liver of all the treated rats and fatty acids content was found to be significantly high in liver of LDHM, HDHM, LDHM +low dose C+A, HDHM + high dose C+A, HDHM + low dose C+A treated rats as compared to control rats. There was no amelioration by curcumin and ascorbic acid in the phospholipid content of liver after 60 and 90 days of heavy metal treatment whereas non- significant variation observed in LDHM

+ high dose C+A as compared to control indicated the amelioration by curcumin and ascorbic acid of fatty acid content in hepatotoxicity for 60 and 90 days of treatment. Phospholipids significantly increased in liver of LDHM, HDHM, HDHM+ high dose C+A an HDHM +low dose C+A treated rats in 90 days as compared to 60 days of treatment. However, there was non-significant variation in the fatty acid content of liver in 90 days as compared to 60 days of treatment A significant increase in phospholipids is in accordance with various earlier toxicity studies caused by different heavy metals, 100 ppm of Cd exposure to rats significantly increased the phospholipid content in liver as compared to control rats (Afolabi et al., 2012). The triglycerides could be taken by the cells in the form of free fatty acids there was an increase in the level of free fatty acids in the liver due to liver problems (Arain et al., 2017). The free fatty acid content was increased with As treatment in drinking water in male albino rats as compared to control rats (Afolabi et al., 2015). The triglycerides/ free fatty acids could be managed by curcumin or ascorbic acid supplementation (Abdel-Moneim et al., 2015).

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AUTHOR CONTRIBUTION STATEMENT

NK designed research. RK conducted experiments. RK, NK and PS wrote the manuscript. NK and PS read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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BIOLOGY OF *APHIS GOSSYPYII* GLOVER ON COTTON AND BRINJAL FOR MASS REARING

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ABSTRACT

The aptness of two hosts viz., cotton and brinjal for mass rearing of *Aphis gossypii* Glover under laboratory conditions was investigated using the age-stage and two-sex lifetable method. The results showed that *A. gossypii* could complete its entire lifecycle on both hosts. The total nymphal duration (7.93 ± 0.33 days), reproductive period (6.95 ± 0.60 days), adult longevity (8.11 ± 0.68 days) and fecundity (24.66 ± 2.34 days) were significantly higher on brinjal than on cotton. The differences in pre-reproductive and post-reproductive periods on both hosts were not statistically significant. The mean reproductive period was longer on brinjal (6.95 ± 0.56 days) than that on cotton (5.06 ± 0.47 days); consequently, the fecundity was also significantly high in brinjal (24.66 ± 2.34 nymphs/ female) compared to cotton (14.11 ± 1.31 nymphs/ female). The difference in an intrinsic rate of increase, infinite rate of increase and doubling time were non-significant. In contrast, the net reproductive rate, gross reproduction rate, and mean generation time on brinjal were significantly higher. Lifetable parameters such as age-stage-specific survival rates, fecundity, life expectancy and reproductive value of *A. gossypii* were higher on brinjal compared to cotton. The results of this study indicate that brinjal is a better host than cotton for the mass rearing of *A. gossypii*.

Key words: *Aphis gossypii*, lifetable, cotton, brinjal, mass rearing, fecundity, reproductive period, intrinsic rate of increase, infinite rate of increase, doubling time, mean generation time

The cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) is a cosmopolitan, polyphagous species found in tropical, subtropical and temperate climates (Kersting et al., 1999). It has a wide host range, perhaps 900 species from 116 plant families, including Cucurbitaceae, Malvaceae, Solanaceae, Rutaceae, and Asteraceae, among others (Blackman and Eastop, 2000). *A. gossypii* causes direct and indirect damage; they reduce crop yield and quality through sap feeding, disease transmission, and the excretion of honeydew. It transmits several plant viruses, including Potato Virus Y (PVY), Cucumber Mosaic Virus (CMV), Tobacco Etch Virus (TEV), Zucchini Yellow Mosaic Virus (ZYMV), and Papaya Ringspot Virus-Type W (PRV-W) that cause economically significant damage to crops (Pinto et al., 2008). Thus, it is critical to control this pest and keep its number below the economic threshold level (ETL). In many agricultural locations, the cotton aphid has reportedly evolved a high resistance to several regularly used insecticides, including organophosphates, carbamates, pyrethroids, and neonicotinoids (Wang et al., 2007). Various studies are being conducted around the world to find effective chemical/botanical

pesticides and biological control agents for managing this notorious pest. There is a need for mass rearing of *A. gossypii* for finding an alternative effective control measure for testing xenobiotic and transgenics for managing this notorious pest.

Insect rearing and multiplication techniques are crucial for biological research on insect pests and the development of management strategies. Humans have learned the art of rearing insects under laboratory conditions. A great deal about the biology of insects was known by introducing insects from the field into the lab and maintaining big colony sizes on natural or artificially developed diets. Insect mass rearing allows researchers to conduct experiments without the seasonal limitations that typically restrict the insect's life history. Mass rearing of insects in protective and controlled laboratory conditions prevents biotic and abiotic stresses, producing nominally healthy insects more likely to perform accurately in the bioassays. It is essential to provide insects with a nutritionally complete diet and a less expensive, quickly produced or procured diet to minimize the cost of multiplication

(Huynh et al., 2021). Rearing on a natural host is always advantageous as it provides complete nutrition. In this study, two natural hosts of *A. gossypii* were tested for their suitability for aphid mass production.

MATERIALS AND METHODS

The culture of *A. gossypii* was obtained from cotton fields of ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. Cotton (RCH- BG II)/ brinjal (Bhagyamathi) leaves were excised from plants, washed with tap water and air dried. The leaf petioles were dipped in a small glass vial (3 cm dia; 5 cm height) filled with fresh water. The leaf was affirmed into the bottle with a cotton plug and parafilm, and the whole set-up was placed into a cylindrical plastic container (15cm in height x 10cm in diameter). Individual aphid-rearing chambers were set up by a single cotton/ brinjal leaf. The gravid females were released on fresh cotton/ brinjal leaves, i.e., five females per leaf for producing nymphs. After releasing gravid females, the container was covered with a single layer of muslin cloth to prevent aphid migration. The aphid culture was maintained in a BOD incubator on cotton/ brinjal leaves at $18 \pm 2^\circ\text{C}$, 16:8 hr, light: dark photoperiod, and $70 \pm 10\%$ RH. The females were inspected after 24 hr for fecundity to have a cohort of the first nymphal stage with the same age (< 24 hr); all the aphids except one new born nymph/leaf were removed using a camel's hair brush (45 nymphs on cotton and 45 on brinjal). Fresh leaves were provided after every six days in cotton and four days in brinjal. These nymphs were observed every 24 hr till their death; the duration of each instar, pre-reproductive, reproductive and post-reproductive periods, adult longevity, total life cycle and fecundity were recorded and analyzed.

According to the age-stage, two-sex lifetable principle (Chi et al., 1985) and method (Chi et al., 2006), twelve parameters; Age-stage-specific survival rates (S_{xj}), Age-specific maternity ($l_x * m_x$), Age-specific survival rate (l_x), Age-stage-specific fecundity (f_x), Age-stage-specific life expectancy (ex_j), Finite rate of increase (λ) and Age-stage-specific reproductive value (V_{xj}), Intrinsic rate of increase (r), Net reproductive rate (R_0) and Mean generation time (T) were calculated, and the age-stage, two-sex lifetables of *A. gossypii* on two hosts were established. Each parameter was calculated using the TWOSEX-MSChart 2020 software (<http://140.120.197.173/Ecology/prod02.htm>); the standard errors were estimated using bootstrapping with 100,000 repetitions. The figures were plotted

using Sigmaplot v12.5 software (Systat Software, San Jose, CA, USA), and the statistical significance of the observed differences was assessed using TWOSEX-MSChart software. $P < 0.05$ was considered statistically significant when evaluating differences between groups using paired bootstrapping. The differences in biological parameters of cotton aphids on two hosts were analyzed by t-test using WASP version 2.0 (Web Agri Stat Package., ICAR-CCARI, India).

RESULTS AND DISCUSSION

The duration of each developmental stage of *A. gossypii* on selected hosts are presented in Table 1. Duration of 1st, 2nd and 3rd nymphal instars and adult longevity were significantly longer on brinjal as compared to cotton. The mean reproductive period (6.95 ± 0.60 days) and mean fecundity (24.66 ± 2.34 nymphs/ female) were higher on brinjal than on cotton. The lifecycle duration was 16.04 ± 0.97 days on brinjal, whereas in cotton it was 12.27 ± 0.75 days. The fecundity was significantly higher on brinjal than on cotton, which could be attributed to a more extended reproductive period and adult longevity on brinjal. In the present study, mean fecundity of *A. gossypii* on cotton was 14.11 ± 1.31 nymphs/ female, which is in line with the findings of Razmjou et al. (2006) reported as 15.30 nymphs/ female and Nimbalkar et al. (2010) reported 17.32 nymphs/ female as mean fecundity of *A. gossypii* on cotton. Higher fecundity on brinjal suggests that brinjal provides better nutrients and less resistance to aphids. Saha et al. (2016) also reported that developmental time and fecundity of *A. gossypii* were significantly higher when fed on the brinjal plant compared to the cotton plant, which is consistent with our findings. The development of insects generally depends on the quality of the diet in the first few instars, which was different among the host (Barros et al., 2010). The longer nymphal duration on brinjal than cotton might have helped aphids take up more nutrients in the nymph stage which helped robust ovary development and fecundity during the adult stage on brinjal.

The intrinsic rate of increase (r) and finite rate of increase (λ) of the *A. gossypii* population were > 0 and > 1 , respectively, for both cotton and brinjal, indicating that aphids could survive on both the hosts (Table 1). The differences in r , λ and doubling time (DT) were non-significant in the two hosts. The net reproductive rate (R_0), gross reproduction rate (GRR), and mean generation time (T) were significantly higher on brinjal as compared to cotton. The “ r ” represents

Table 1. Biological and population parameters of *A. gossypii* (mean± SE) on brinjal and cotton under laboratory conditions

Biological parameters						
Stage/ duration	Cotton (days)	Brinjal (days)	t value			
1 st instar duration	1.62± 0.07	2.29± 0.09	-5.43**			
2 nd instar duration	1.64± 0.13	2.04± 0.12	-2.33*			
3 rd instar duration	1.53± 0.12	1.98± 0.16	-2.15*			
4 th instar duration	1.38± 0.15	1.62± 0.16	-1.11			
Total nymphal duration	6.18± 0.26	7.93± 0.33	-4.13**			
Pre-reproductive period	0.47± 0.07	0.40± 0.07	0.63			
Reproductive period	5.06± 0.47	6.95± 0.60	-2.34*			
Post-reproductive period	0.55± 0.09	0.51± 0.09	0.36			
Adult longevity	6.09± 0.56	8.11± 0.68	-2.29*			
Total life cycle	12.27± 0.75	16.04± 0.97	-3.07*			
Fecundity	14.11± 1.31	24.66± 2.34	-3.93**			
Population parameters						
Host	Intrinsic rate of increase (r/ day)	Finite rate of increase (λ / day)	Net reproductive rate (R_0)	Gross reproduction rate (GRR)	Mean generation time (T/ day)	Doubling time (DT)
Brinjal	0.25± 0.0023	1.28± 0.0029	24.67± 0.0047 ^a	36.37± 0.034 ^a	12.97± 0.01 ^a	2.81± 0.0074
Cotton	0.26± 0.0029	1.28± 0.0024	14.16± 0.0020 ^b	21.61± 0.048 ^b	10.79± 0.91 ^b	2.82± 0.0080
	NS	NS	0.0001	0.003	0.021	NS

*Samples significantly different ($p=0.05$); **Samples significantly different at $p=0.01$; t- value greater than +2 or less than -2 is acceptable; Means followed by different letters in the same column are significantly different by using a paired bootstrap test based on the CI of difference. Standard errors estimated by using 100000 bootstrap resampling.

the growth potential of insect populations; a larger value reflects more rapid development (Xie et al., 2021). The differences of r and λ were non-significant on the two hosts, whereas the value of R_0 and GRR were higher for *A. gossypii* fed on brinjal than cotton. Based on these parameters, brinjal was found to be more conducive to the growth and development of *A. gossypii* than cotton under laboratory conditions. The seasonal nature of aphids, their migration, and parasitoids' attack pose the biggest challenges for the mass-rearing of aphids under field conditions. All these hurdles were solved by rearing aphids under controlled conditions in the laboratory. *A. gossypii* reared on *Hibiscus syriacus* under laboratory conditions had a greater survival rate, fecundity, and longevity than those reared under field conditions. The lifetable parameters, such as intrinsic rate of increase (r), net reproductive rate (R_0), and finite rate of increase (λ), were higher under laboratory conditions compared to the field conditions (Hosseini et al., 2015).

The age-stage specific survival rate (S_{xj}) of *A. gossypii* on cotton (A) and brinjal (B) are shown in Fig. 1. The survival rate differed and overlapped across the developmental stages on both the hosts.

The S_{xj} was highest among first instar nymphs on both hosts. The S_{xj} of third and fourth instar nymphs was higher on brinjal (0.8666 and 0.6444, respectively) as compared to cotton (0.6590 and 0.5454, respectively). The aphids could survive up to 18 days maximum on cotton, whereas they survived up to 22 days on brinjal. The age-specific survival rate (l_x), female age-specific fecundity (f_x), and age-specific maternity ($l_x m_x$) of *A. gossypii* on cotton and brinjal are depicted in Fig. 2. The l_x showed a downward trend as age increased, death of the last adult occurred on 18th day on cotton whereas it was on 20th day on brinjal. The other two parameters, f_x and $l_x m_x$ reached maximum values at 10 day on cotton (3.81 nymphs and 2.681 nymphs/ female/ day, respectively) and at 11 days on brinjal (5.09 nymphs and 3.84 nymphs/ female/ day, respectively). The higher values of f_x and $l_x m_x$ on brinjal indicates that brinjal is more suitable for the development and reproduction of *A. gossypii* (Saha et al., 2016).

The e_{xj} values for all the ages and stages were higher on brinjal as compared to cotton, indicating that aphids developed slowly on brinjal. The age-specific life expectancy was also higher on brinjal throughout the aphid life cycle (Fig. 3a). The variation in l_x , f_x and $l_x m_x$

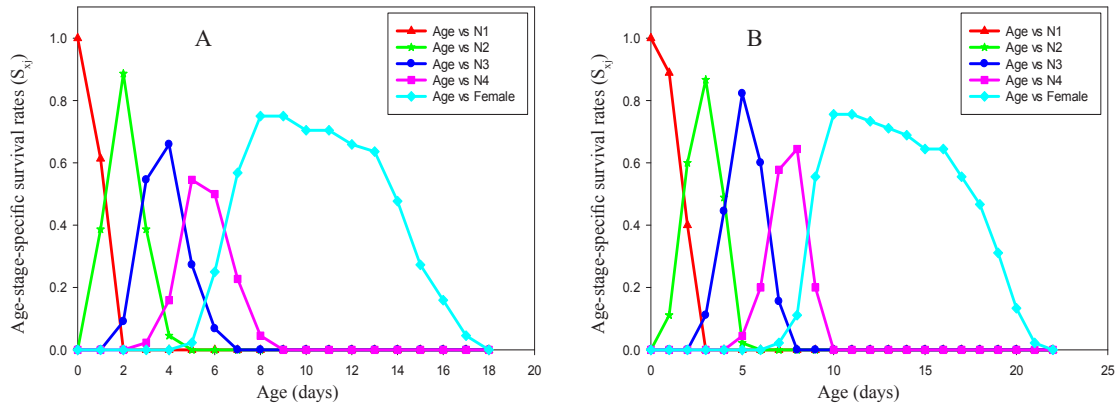


Fig. 1. Age-stage-specific survival rate (S_{xj}) of *A. gossypii* on cotton (A) and brinjal (B)

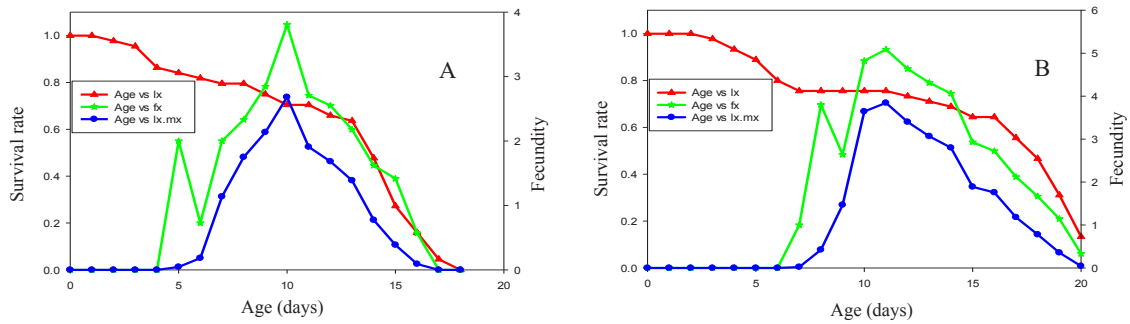


Fig. 2. Population survival rate and fecundity of *A. gossypii* on cotton (A) and brinjal (B). The age-specific survival rate (l_x), female age-specific fecundity (f_x), and age-specific maternity ($l_x m_x$).

on two hosts could be due to nutrient content of the host as all other experimental conditions were uniform for both hosts. The differences in nutrient content in host plants greatly influence the life cycle of herbivorous insects and affect the changing trend of their populations (Obopile and Ositile, 2010, Polat, 2018).

The reproductive value (v_{xj}) of *A. gossypii* feeding cotton (A) and brinjal (B) at age zero ($v_{0,1}$) was 1.27 and 1.28, respectively, which were both close to λ . The peak value of the v_{xj} showed an upward trend with advancing age and developmental stage, with the

highest value at 8 days on cotton (9.47 nymphs/ day) and 10 days on brinjal (16.59 mean nymphs/ day). The highest v_{xj} was in female adults reared on brinjal. The clear distinction between age-specific reproductive values on cotton and brinjal are depicted in (Fig. 3b). Previous studies on *A. gossypii* by Alizadeh (2016) and Satar (1999) also found that the development and reproductive value of *A. gossypii* were affected by host plants. The aphids were successfully cultured on leaves of cotton and brinjal; this helps in space utilization and maintaining culture in BOD throughout the year (Li and Akimoto, 2018). Though the mean generation time is

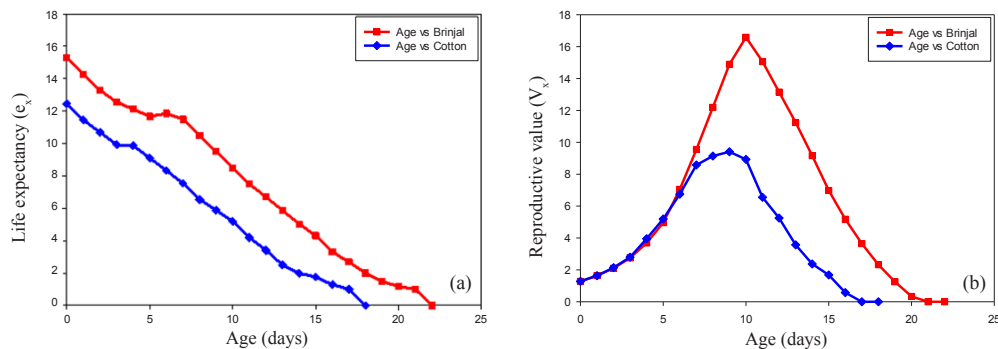


Fig. 3. Comparative age-specific life expectancy (a) and age-specific reproductive value (b) of *A. gossypii* on cotton and brinjal

slightly longer on brinjal, it could still be a better host for aphid mass production as the higher reproductive rate, approximately twice as on cotton, compensates for the increased time.

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AUTHOR CONTRIBUTION STATEMENT

GSU contributed to experimentation, data collection and original draft writing. KMC analyzed the data. VKK contributed to conceptualization, planning, supervision and manuscript editing. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF BEE ATTRACTANTS AND MODE OF POLLINATION ON THE YIELD PARAMETERS OF MUSTARD USING *APIS MELLIFERA* L COLONIES

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ABSTRACT

Among the different bee attractants evaluated, the effectiveness of the treatment bee-Q @ 1.25 % was found to be superior in attracting more bees followed by, jaggery solution @ 10%. The lowest number of bees were attracted in the plots sprayed with coconut water @10%, except open pollination without any treatment spray. The highest no. of siliquae/ plant, siliquae length, no. of seeds/ siliquae, no. of seeds/ plant, seed yield/ plant and 1000 seeds weight were observed in the plot treated with bee-Q @ 1.25 % spray with 209.17 siliquae/ plant, 4.41 cm, 15.70 seeds/ siliquae, 3198.63 seeds/ plant, 13.78g/ plant and 3.35g, respectively. However, the lowest no. of siliquae/ plant, siliquae length, no. of seeds/ siliquae, no. of seeds/ plant, seed yield/ plant and 1000 seeds weight were observed in the plot treated with coconut water @ 10 % spray with 167.33 siliquae/ plant, 3.71 cm, 13.04 seeds/ siliquae, 2176.47 seeds/ plant, 10.32g/ plant and 3.10g, respectively. The highly significant results were obtained in the plots with open pollination with treatments spray which was followed by the plots with open pollination without treatment sprays, plots caged with *Apis mellifera* L. colonies and the least significant results were obtained in the plot caged without insect pollinators.

Key words: *Apis mellifera*, bee attractants, pollination, open pollination, yield parameters, sugar solution, coconut water, jaggery extract, onion extract, sugarcane juice, coriander extract, bee-Q, pomegranate juice

Brassica juncea (L.) Czern & Coss., also known as Indian mustard, belongs to Brassicaceae or Cruciferae family is generally pollinated by insects and is the most important edible oilseed. It is the world's second-largest oilseed crop after groundnut (Anonymous, 2021) with its distribution in China, Canada, India, Australia, France, Germany, etc. Honey bees are considered one of the most effective, cheapest and eco-friendly input methods for triggering crop yield both qualitatively and quantitatively. Mustard is an often-cross-pollinated and self-pollinated crop; adequate pollination is vital for the significant increase in seed production. It not only results in increasing the yield but also improves the quality. It ensures uniform maturity and early harvest of the crop (Anil, 2015). The quantitative seed production of the mustard crop is the ultimate expression of the symbiotic relationship it had with diverse floral visitors as a consequence of the pollination and fertilization process. Seed weight is a direct indicator of seed health and vigour, and it is used to assess the economic characteristics (quantitative and qualitative) characteristics of the plant. The low average yield of mustard could also be attributed to insect pests,

disease damage, poor soil fertility or water stress, also there is evidence that insufficient pollination can also significantly minimize the crop yield (Free, 1999). However, there has been little research in India on the usage of bee attractants. (Patil et al., 2010). Local bee attractants, such as pomegranate juice, sugar solution, honey solution, sugarcane juice, jaggery solution, and onion solution, are commonly utilised in India to boost crop output. Mahadik et al. (2019) studied various indigenous food attractants and revealed that honey solution at 5% attracted the maximum number of bees followed by jaggery solution at 10% and molasses at 10%. The yield of rapeseed and canola can be increased by several % through pollination by insects (Manning and Wallis, 2005; Sabbahi et al., 2005; 2006). Insect pollination increased the number of pods, seeds/ pod, seed weights/ plant, and seed germination (Atmowidi et al., 2007). Goswami and Khan (2014), revealed that open-pollinated plots had the highest % pod set (83.42%) and bee-pollinated plots (75.41%), and caged-pollinated plots (62.80%). Therefore, the present investigation aimed to study the effect of bee attractants to attract *Apis mellifera* L. and also to infer the effect

of bee pollination on the yield-attributing parameters of mustard for seed production.

MATERIALS AND METHODS

The present investigations were carried out during 2021-22 in the experimental field of mustard crop and the apiary at RVSKVV- Krishi Vigyan Kendra, Morena (26°30'10.3"N & 78°0'5.87"E), Madhya Pradesh, India. The healthy seeds of mustard variety RVM- I were sown on 24/ 10/ 2021 at 5 cm depth at a row distance of 45 cm and plant to the plant of 15 cm apart with the plot size of 6.0 m x 3.0 m by following recommended package of agronomical practices. Various pests and diseases were controlled, but no pesticides were employed during the flowering time. To study the effect of different bee attractants to attract *Apis mellifera* L. in the mustard crop, an experiment was laid out in randomized block design with nine treatments replicated thrice. The treatment details are: T1: Open pollination with sugar solution @ 10%, T2: Open pollination with coconut water @ 10%, T3: Open pollination with jaggery @ 10%, T4: Open pollination with coriander @ 1.25%, T5: Open pollination with onion solution @ 10%, T6: Open pollination with sugarcane juice @ 10%, T7: Open pollination with bee-Q @ 1.25%, T8: Open pollination with pomegranate juice @ 10% and T9: Open pollination without treatment (Control). All of the treatments were applied three times at a 10-day interval at the 10% flowering stage of the crop. A one-meter square area was randomly selected and marked in each plot, and the number of *A. mellifera* bees visiting the flowers in that area every five minutes was recorded. During the flowering season, observations were obtained at five separate time slots: 0800, 1000, 1200, 1400, and 1600 hours, and the observed data was averaged daily. These observations were made one day before the spray and one, three, and five days after the spray. The data was square root transformed and statistical analysis was performed in MS Excel.

For the impact of bee pollination on the quality and quantity parameters of mustard, an experiment was laid out in Randomized Block Design with eleven treatments replicated thrice. The treatment details are T1-T9 (as mentioned above), T10: Caged with bees or bee pollination, and T11: Caged without bees or insect pollination. Plots with treatments T1 to T9 were not caged and were always exposed/open to pollination by all agencies. The treatments from T1 to T8 were sprayed three times during the flowering season starting from

the 10% flowering stage at 10 days intervals. The plots with treatment T10 were caged before the beginning of flowers throughout the night to eliminate natural pollination. At the start of flowering, the crop was caged and sprayed with chlorpyrifos 20EC to kill all insects inside the cage and eliminate their contribution to pollination. Following the pesticide waiting time, the *A. mellifera* L. bee colony of determined strength at 10% flowering was placed in fine 16-mesh nylon mosquito net cages of 4x3x2 m. The bee colony was removed when all the floral bloom was exhausted. The plots with treatment T11 were caged before the beginning of flowers throughout the night to eliminate natural pollination. For the observations on qualitative and quantitative parameters, five plants (avoiding edges) were selected randomly for six different characters: number of siliquae/ plants, siliqua length (cm) (/ 10 siliquae/ plant), number of seeds/ siliquae (/ 10 siliquae/ plant), the total number of seeds/ plants, seed yield/ plant (g), 1000 seeds weight (g), and required transformation was applied followed by statistical analysis performed in MS Excel.

RESULTS AND DISCUSSION

The data presented in Table 1 indicates the number of bees attracted/ m²/ 5 min due to different bee attractants on the day before spray and at 1, 3 and 5 days after the first, second and third sprays. According to the data analysis, the number of bees attracted/ m²/ 5 min varied significantly with different bee attractants at 1, 3 and 5 days after the first spray. On a mean basis, bee-Q @ 1.25% was determined to be the most successful and superior treatment in attracting more bees, i.e., 34.67 bees/ m²/ 5 min and the least successful treatment was open pollination without treatment attracting 21.89 bees/ m²/ 5 min. Further in the second spray, the number of bees attracted / m²/ 5 min varied significantly with different bee attractants on the day before the spray and at 1, 3 and 5 days after the spray. On a mean basis, bee-Q @ 1.25% was determined to be the most effective treatment in attracting more bees, i.e., 37.22 bees/ m²/ 5 min and the least successful treatment was open pollination without treatment attracting 24.33 bees/ m²/ 5 min. In the third spray, the number of bees attracted / m²/ 5 min varied significantly with different bee attractants on the day before the spray and at 1, 3 and 5 days after the spray. On a mean basis, bee-Q @ 1.25% was determined to be the most effective treatment in attracting more bees, i.e., 37.89 bees/ m²/ 5 min and the least successful treatment was open pollination without treatment attracting 26.22 bees/ m²/ 5 min.

Table 1. Effect of bee attractants on *A. mellifera* in mustard (rabi 2021-22)

Treatments	Number of bee visits/ m ² / 5 min														
	1 st spray					2 nd spray					3 rd Spray				
	1 DBS	1 DAS	3 DAS	5 DAS	Mean	1 DBS	1 DAS	3 DAS	5 DAS	Mean	1 DBS	1 DAS	3 DAS	5 DAS	Mean
T1: Open pollination with sugar solution @ 10%	20.33 (4.56)*	35.67 (6.01)	27.67 (5.31)	25.00 (5.05)	29.44 (5.47)	22.00 (4.74)	37.67 (6.18)	34.33 (5.9)	27.67 (5.31)	33.22 (5.81)	23.00 (4.85)	42.33 (6.54)	34.33 (5.9)	29.67 (5.49)	35.44 (6.00)
T2: Open pollination with coconut water @ 10%	20.00 (4.53)	26.33 (5.18)	21.33 (4.67)	22.33 (4.78)	23.33 (4.88)	20.33 (4.56)	33.67 (5.85)	28.00 (5.34)	23.67 (4.92)	28.44 (5.38)	21.67 (4.71)	36.00 (6.04)	30.33 (5.55)	25.33 (5.08)	30.56 (5.57)
T3: Open pollination with jaggery @ 10%	20.00 (4.53)	39.00 (6.28)	31.67 (5.67)	27.33 (5.28)	32.67 (5.76)	22.67 (4.81)	41.67 (6.49)	36.67 (6.1)	29.67 (5.49)	36.00 (6.04)	24.67 (5.02)	44.33 (6.7)	36.67 (6.1)	30.33 (5.55)	37.11 (6.13)
T4: Open pollination with coriander @ 1.25%	19.67 (4.49)	29.33 (5.46)	23.00 (4.85)	23.00 (4.85)	25.11 (5.06)	20.67 (4.6)	34.33 (5.9)	29.67 (5.49)	24.67 (5.02)	29.56 (5.48)	22.33 (4.78)	37.67 (6.18)	31.00 (5.61)	26.33 (5.18)	31.67 (5.67)
T5: Open pollination with onion solution @ 10%	20.33 (4.56)	31.00 (5.61)	24.33 (4.98)	23.67 (4.92)	26.33 (5.18)	21.33 (4.67)	35.00 (5.96)	32.00 (5.7)	25.33 (5.08)	30.78 (5.59)	22.33 (4.78)	39.67 (6.34)	32.00 (5.7)	27.67 (5.31)	33.11 (5.80)
T6: Open pollination with sugarcane juice @ 10%	20.67 (4.6)	36.33 (6.07)	28.33 (5.37)	26.33 (5.18)	30.33 (5.55)	22.33 (4.78)	39.67 (6.34)	35.00 (5.96)	28.33 (5.37)	34.33 (5.90)	23.67 (4.92)	43.67 (6.65)	35.00 (5.96)	31.33 (5.64)	36.33 (6.07)
T7: Open pollination with bee-Q @ 1.25 %	20.67 (4.6)	40.33 (6.39)	35.33 (5.99)	28.33 (5.37)	34.67 (5.93)	23.33 (4.88)	43.67 (6.65)	37.67 (6.18)	30.33 (5.55)	37.22 (6.14)	25.00 (5.05)	44.67 (6.72)	37.67 (6.18)	33.33 (5.64)	37.89 (6.20)
T8: Open pollination with pomegranate juice @ 10%	19.67 (4.49)	32.67 (5.76)	26.00 (5.15)	24.33 (4.98)	27.67 (5.31)	21.67 (4.71)	36.00 (6.04)	32.67 (5.76)	26.33 (5.18)	31.67 (5.67)	22.67 (4.81)	41.67 (6.49)	32.67 (5.76)	28.33 (5.37)	34.22 (5.89)
T9: Open pollination without treatment (control)	19.67 (4.49)	23.33 (4.88)	21.67 (4.71)	20.67 (4.60)	21.89 (4.73)	20.00 (4.53)	27.67 (5.31)	24.00 (5.95)	21.33 (4.67)	24.33 (4.98)	21.33 (4.67)	29.33 (5.46)	26.33 (5.18)	23.00 (4.85)	26.22 (5.17)
C.D. p=0.05	NS	0.086	0.090	0.111	0.052	0.098	0.102	0.133	0.106	0.067	0.103	0.100	0.101	0.121	0.071
SEm ±	0.039	0.028	0.029	0.036	0.017	0.032	0.034	0.044	0.035	0.022	0.034	0.033	0.034	0.040	0.024

*Figures in parentheses square root transformed values; NS- Non significant; DBS – Day before spray; DAS – Day after spray

The present findings are in line with the findings of Manjunath (2003) who reported that spraying of bee-Q and fruit boost significantly enhanced visitation by *A. dorsata*, *A. cerana*, *A. mellifera* and other pollinators. However, attractants lost their efficacy after 5 days of spraying with sunflower. Manchare et al. (2020) stated that the bee attractants were sprayed twice, first at 10% blooming and again at 50% flowering. According to the results of bee foraging activity, the intensity of *A. dorsata* increased one day after spraying and decreased seven days later. Spraying bee attractants, such as honey solution 10%, jaggery solution 10%, and molasses 10%, attracted the greatest number of *A. dorsata* up to the fifth day after the first spray and the seventh day after the second spray. The results in the present investigation are partially in line with the findings of Venkataramgowda et al. (2013) who studied on use of bee-Q and fruit Boost as bee attractants in the pollination of niger and were observed for two weeks and estimation of seed yield was determined. Bee-Q at 12.5 gm/ lit and fruit boost at 0.75 ml/ lit was found to dramatically increase the number of bee foragers in niger plots when compared to control plots. Jayaramappa et al. (2011) reported that the studies on bee-Q @ 10, 12.5 and 15 gm/ lit, fruit boost @ 0.50, 0.75 and 1 ml/ lit, cinnamon leaf extract @ 5%, tuberos floral scented water, 10% sugar solution on ridge gourd, which is open pollinated observed that spraying of fruit boost @ 0.50 ml/ lit and bee-Q @ 12.5 gm/ lit enhanced foraging activities of Indian bees and yield by increasing yield parameters like the number of fruits per plant to 19.00 and 17.00 fruits when compared to 10.66 fruits per plant in the open-pollinated plot. The number of fruits was 21.83 and 20.83 fruits per plot when compared to 15.68 fruits per plot in open-pollinated plots. More et al. (2020) reported that there was a significant difference between bee visitation in different treatments and sprays. The bee visits on one, three and five days after the second spray of bee attractants were 13.57, 15.89 and 15.89 bees/ m²/ 5 min, respectively and for the third spray, it was 14.11, 16.25 and 14.82 bees/ m²/ 5 min, respectively. Thus, from the present study, it can be concluded that bee-Q @ 1.25% is the best attractant among the tested treatments in attracting a higher number of bees/ m²/ 5 min which was followed by jaggery @ 10%. The next best treatments were sugarcane juice @ 10%, sugar solution @ 10%, pomegranate juice @ 10%, onion solution @ 10%, coriander @ 1.25%, coconut water @ 10% and the least effective treatment was open pollination without attractants application.

The data recorded on the effect of modes of bee

pollination on quantity and quality parameters are presented in Table 2. Data indicated that all the bee attractants sprayed were significantly effective on seed yield and yield attributing characters. The plot treated with bee-Q @ 1.25% under open pollination conditions was found superior over all other treatments under open pollination conditions in producing the highest mean number of siliquae (209.17 siliquae/plant), highest mean siliquae length (4.55 cm/ plant), highest mean number of seeds/ siliquae (15.70 seeds/ siliquae), highest mean number of seeds/ plant (3198.63 seeds/ plant), highest mean seed yield (13.78g/ plant) and highest mean 1000 seeds weight (3.35g). However, the least no of siliquae (150.50 siliquae/ plant), lowest siliquae length (3.21 cm/plant), least number of seeds/ siliquae (12.24 seeds/ siliquae), least number of seeds/ plants (1749.58 seeds/ plant), lowest seed yield (8.79g/ plant) and lowest 1000 seeds weight (2.96g) were recorded from the crop caged without insect pollination. These results indicated that the application of attractants in open pollination conditions has a significant effect in increasing the yield of mustard crop. The present findings on the yield attributing parameters are completely in line with the findings of Mahadik et al. (2019) reported that the treatment honey solution 5% recorded 105.07 pods/plant, 16.40 number of seeds/ pod, 5.69 wrinkled seed/pod, fruit weight 0.35 kg/ 5 plants, net yield 19.3 q/ha and found superior among all other treatments. Followed by jaggery solution 10% showing results of 103.8 number of pods/plants, 15.40 number of seeds/pods, 6.05 wrinkled seed/pod, 3.46, fruit weight 0.27 kg/ 5 plants, and net yield of 18.00 q/ha. The lowest results showed by treatments with open pollination and pollination without insects. Similarly, Subedi and Subedi (2019) reported that a significant difference was observed in the number of pods, number of seeds /pod and weight of 100 dry seeds in control and open pollination. Also, Patidar et al. (2017) studied the yield of mustard through honey bee pollinators with three pollination treatments The comparative data about modes of pollination in mustard crops revealed that the highest values of mean no. of siliqua/plant (186.44), no. of seeds/siliqua (13.82) and seed yield (20.54 q/ ha) were obtained from plants kept open to all pollinators (OP) followed by plants caged with bee hive (BP) and it was recorded lowest in plants caged pollinator exclusion (PE). Nagpal et al. (2017) conducted a field experiment on the effect of different modes of pollination on yield parameters of Indian mustard showed that the maximum number of pods/plant, pod length, pod setting (%), number of

Table 2. Effect of bee pollination on quality and quantity parameters of the mustard crop during Rabi 2021-22

Treatments	No. of siliquae / plant	Siliquae length (cm)	No. of seeds/ siliquae	Total no. of seeds/ plant	Seed yield/ plant (g)	1000 seeds weight (g)
T1: Open pollination with sugar solution @ 10%	190.50	4.21	14.19	2695.28	12.37	3.23
T2: Open pollination with coconut water @ 10%	167.33	3.71	13.04	2176.47	10.32	3.10
T3: Open pollination with jaggery @ 10%	204.33	4.41	15.48	3158.62	13.40	3.30
T4: Open pollination with coriander @ 1.25%	173.17	3.80	13.41	2323.02	10.97	3.14
T5: Open pollination with onion solution @ 10%	177.67	3.94	13.74	2431.03	11.50	3.19
T6: Open pollination with sugarcane juice @ 10%	195.00	4.30	14.47	2822.59	12.88	3.27
T7: Open pollination with bee-Q @ 1.25 %	209.17	4.55	15.70	3198.63	13.78	3.35
T8: Open pollination with pomegranate juice @ 10%	183.67	4.07	14.04	2576.46	11.99	3.19
T9: Open pollination without treatment (Control)	162.50	3.61	12.84	2086.24	9.76	3.04
T9: Caged with bees or Bee Pollination (BP)	157.33	3.50	12.61	1988.08	9.15	2.98
T10: Caged without bees or Without Insect Pollination (WIP)	150.50	3.21	12.24	1749.58	8.79	2.96
CD p=0.05	3.622	0.052	0.134	4.890	0.074	0.065
SEm±	1.227	0.017	0.045	1.657	0.025	0.022

seeds/pod, thousand seed weight, seed yield/ plot, seed germination (%), seed vigour and oil content (508.72 pods/plant, 5.69 cm, 86.32%, 15.66 seeds/pod, 6.87 g, 17.63 q/ha, 89.20%, 628.12 and 39.42%, respectively) were in open pollination followed by that in bee pollination (404.56 pods/ plant, 4.92 cm, 78.33%, 14.26 seeds/ pod, 6.39 g, 15.57 q/ ha, 85.20%, 542.54 and 38.36%, respectively) and pollinators exclusion (287.56 pods/ plant, 3.89 cm, 65.87%, 12.24 seeds/ pod, 5.30 g, 13.01 q/ha, 78.40%, 385.54 and 37.04%, respectively). Seed yield increased by 35.50 and 19.66 % in open-pollinated and *A. mellifera*-pollinated plots, respectively as compared to pollinators' exclusion. The results of the present experiment are consistent with that of Atmowidi et al. (2007), Kumari et al. (2013), Bhowmik et al. (2014), Kamel et al. (2015) and Hossain et al. (2017). Thus, the findings of the present study could have important implications for crop production and pollinator conservation efforts. However, further research is needed to investigate the specific mechanisms by which bee pollination enhances

crop yields and to optimize the use of bee colonies for pollination in mustard and other crops.

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AUTHOR CONTRIBUTION STATEMENT

M.B. and A.S.Y. conceived, designed research and conducted experiments. A.S.Y. and N. contributed to analytical tools. M.B. and N. wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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PERSISTENCE OF BETA-CYFLUTHRIN AND IMIDACLOPRID RESIDUES IN CASHEW

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ABSTRACT

The QuEChERS analytical method in combination with GC and LC-MS/MS was validated to quantify the insecticide residues in immature kernel, mature kernel, cashew apple and in the field soil. The method performance was satisfactory with the LOQ, recovery and RSD values. Residue persistence of betacyfluthrin and imidacloprid on cashew was carried out after foliar spray of the combination formulation, betacyfluthrin 90 g ℓ^{-1} + imidacloprid 210 g ℓ^{-1} OD (Solomon) at recommended (2.5 ml 10 ℓ^{-1}) and double the recommended (5 ml 10 ℓ^{-1}) doses. The residues of betacyfluthrin and imidacloprid were at below LOQ of 0.05 mg kg^{-1} in all the samples at recommended and double the recommended dose. Soil sample collected at the time of harvest did not show betacyfluthrin and imidacloprid residues at their detection limit of 0.05 mg kg^{-1} . The analytical method standardized for the extraction of betacyfluthrin and imidacloprid residues in cashew matrices offers advantages such as simplicity and also, less time consuming.

Key words: Cashew, combination insecticide, Solomon, QuEChERS, method validation, dissipation, harvest time residues, immature kernels, mature kernels, cashew apple, soil

Cashew (*Anacardium occidentale* L.) is one of the most important plantation crop. Though cashew nuts/kernels are the main consumption part, cashew apples rich in iron, vitamins and minerals are processed into jam, jelly, juice and soft drinks. Cashew is reported to be infested by >180 insect pests. The most serious are tea mosquito bug (*Helopeltis antonii* Signoret), the stem and root borer (*Plocaederus ferrugineus* L., *P. obesus*, *Batocera rufomaculata* De Geer) and thrips (*Scirtothrips dorsalis* Hood.). These attack cashew both spatially and temporally resulting in 11-55% loss in yield (Saroj, 2015; Zote et al., 2018). Timely management measures are essential prevent their damage. Insecticides such as lambdacyhalothrin, profenophos, acetamiprid, triazophos, imidacloprid, endosulfan, monocrotophos, cypermethrin and carbaryl were widely used by farmers against cashew pests (Ramesh Kumar et al., 2015; Saroj, 2015).

Combination formulations of insecticides with different modes of action and broad spectrum activity are considered more effective (Casida et al., 2013). Betacyfluthrin (cyano (4-fluoro-3-phenoxyphenyl) methyl 3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropane carboxylate), a synthetic pyrethroid in the combination having contact activity is recommended for lepidopteran pests. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-

2-ylideneamine), a neonicotinoid with systemic action is widely recommended against sucking pests. The combination product of beta cyfluthrin (9%) + imidacloprid (21%) was introduced in India by Bayer Crop Science Ltd. as Solomon 300 OD and recommended against sucking pests and borers in chilli (Giraddi et al., 2017) and brinjal (Bhargava et al., 2003). Betacyfluthrin 90% + imidacloprid 210% OD (Solomon 300 OD) 1.5 ml for 10 l was found effective in management of tea mosquito bug and thrips in cashew (Zote et al., 2018). Application of insecticides lead to pesticide residues in food commodities which in turn results in health hazards. Information is available on persistence and dissipation behavior of residues of betacyfluthrin and imidacloprid on cashew and hence the present study.

MATERIALS AND METHODS

The material of betacyfluthrin (93% purity), imidacloprid (98.6% purity) its commercial formulation Solomon 300 OD were obtained from Bayer Crop Science Limited, India. Analytical-grade sodium chloride ($\geq 99\%$ purity), anhydrous magnesium sulphate ($\geq 99.5\%$ purity), trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate (Sigma Aldrich) were obtained from Merck, Mumbai, India. The magnesium sulphate was activated at 600°C for a minimum of 4 hr in a muffle furnace before use and

stored in an airtight desiccator. Primary secondary amine (PSA, 40 μm), florisil and graphitized carbon black (GCB) were obtained from Agilent Technologies, Palo Alto, CA, USA. The standard stock solutions (400 $\mu\text{g ml}^{-1}$) were prepared by weighing 10 ± 0.1 mg of certified reference material (CRM) using calibrated analytical balance. The standards of betacyfluthrin and imidacloprid were weighed into a calibrated Class A volumetric flask (25 mL) and dissolved with HPLC grade hexane and acetonitrile respectively for making 25 ml volume. The secondary stock solution (40 $\mu\text{g ml}^{-1}$) was prepared from the stock solution in 25 ml volume by transferring 2.5 ml. The prepared solutions were stored at -20°C until use. All reagents and solvents used were of HPLC grade. The calibration curves were established by injecting standard solutions of both beta cyfluthrin and imidacloprid prepared in hexane and acetonitrile respectively in the range of 0.01-1.0 $\mu\text{g ml}^{-1}$ (0.01, 0.05, 0.1, 0.5 and 1.0 $\mu\text{g ml}^{-1}$). Standard concentration at five levels (0.01, 0.05, 0.1, 0.5 and 1.0 mg l^{-1}) were prepared by adding the betacyfluthrin and imidacloprid with blank extracts of corresponding cashew matrices and soil separately. The untreated immature kernels, mature kernels, cashew apple and soil were fortified with betacyfluthrin and imidacloprid residues at 0.05, 0.25 and 0.50 mg kg^{-1} and extracted as per the procedure explained below with six replications each. Recovery percentage was calculated using the Microsoft Excel 2007 software.

The study on persistence of betacyfluthrin 90 g l^{-1} + imidacloprid 210 g l^{-1} OD (Solomon 300 OD) in cashew was carried out in Elayamuthur, Udumalpet (10.476 N, 77.293 E) during February-May, 2019. The field experiment was conducted in a randomized block design (RBD) with three treatments and replicated thrice. The cashew orchard (variety VRI-3) was maintained as per the recommended agronomic practices in 60 m^2 per plot with spacing of 3 x 2 m. Foliar application of commercial formulation of betacyfluthrin 90 g l^{-1} + Imidacloprid 210 g l^{-1} (Solomon OD) at recommended and double the recommended doses and water spray constituted the untreated control. First foliar application was initiated at fruit setting stage and second at 15 days interval using a high volume knapsack sprayer using 500 l ha^{-1} of water as spray fluid. Samples of immature kernels were collected at 0 (within 2 hr of last application), 3, 7, 14, 21 and 35 days after second spraying. Mature kernel, cashew apple and soil samples were collected at the time of harvest. The field soil samples were collected from the depth of 0-15 cm in the experimental field at random in each replication.

Samples of immature and mature kernel (500 g/ replication) cashew apple (1 kg/ replication) were collected and homogenized in a high volume homogenizer (Robot Coupe, Germany) for 2 min. Immature and mature kernels were processed after the removal of outer shell to represent the edible part. Soil samples (1 kg/ replication) were shade dried by spreading on a polythene sheet on a hard surface and powdered using a wooden mallet, sieved through 2 mm sieve and stored in polythene bag for further analysis. The extraction and cleanup procedure for betacyfluthrin in cashew matrices and soil was done based on the method developed by Silva et al. (2014) with few modifications in sample weight taken for extraction and sorbents used in cleanup step. For extraction a representative sample of 2 g (immature and mature kernel, cashew apple and soil) was weighed into 50 ml polypropylene centrifuge tube, 10 ml of acetonitrile was added and mixed using a vortex mixer for one min. After that, 4.0 g of anhydrous magnesium sulphate (MgSO_4), 1.0 g of sodium chloride (NaCl), 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate salts were added and mixed well in a vortex mixer for 1 min. for proper mixing followed by centrifugation (centrifuge ROTA 4 V/FM) at 6,000 rpm for 10 minutes. To 4.0 ml of supernatant, each 100 mg primary secondary amine (PSA), florisil and 600 mg of anhydrous MgSO_4 were added for removing the co extracts. After centrifugation for 10 min at 3,000 rpm, two ml of supernatant evaporated to near dryness and the residue was dissolved in one ml hexane and filtered into a 2.0 ml vial using 0.22 μm nylon filter for GC-ECD (Shimadzu, GC-2010) analysis.

For extraction and cleanup for imidacloprid residues in cashew matrices and soil QuEChERS (Quick, Easy, Cheap, Effective, Robust and Safe) method was followed with slight modifications (Anastassiades et al., 2003). Homogenized samples of 5.0 g immature kernel, 5.0 g of mature kernel and 10.0g of cashew apple were weighed into a 50 ml polypropylene centrifuge tube and to this 10.0 ml of acetonitrile was added and vortexed for 1 min. To each tube 4.0 g of anhydrous MgSO_4 , 1.0 g of sodium chloride salts were added, vortexed for one minute and centrifuged at 6,000 rpm for 10 min. From the upper supernatant, 6.0 ml extract was transferred into 15 ml small centrifuge prefilled with 100 mg PSA, 25 mg of graphitized carbon black (GCB) and 600 mg of anhydrous MgSO_4 . The tubes were shaken well and centrifuged at 3,000 rpm for 10 min. Finally, one ml of supernatant was directly filtered in to 2.0 ml vial using 0.2 μm nylon membrane filter for LC-MS/MS analysis.

For soil the same procedure was followed with the addition of 10 ml of distilled water before extraction and 10 mg GCB in the cleanup step and analysed in LC-MS/MS. Analysis of the betacyfluthrin residues was carried out in a gas chromatograph (Shimadzu GC 2010, Japan) equipped with electron capture detector (ECD). The extracts were injected and separation was performed in GC equipped with DB-5 (30 m x 0.25 mm x 0.25 μ m) column. An injection volume of 1 μ l was made with the injector temperature set at 280°C in split (1:5) mode and pressure at 74.2 kPa. The flow of carrier gas (He) was set at 0.80 ml min⁻¹ with a linear velocity of 38.1 cm sec⁻¹. The column temperature was set as 150°C, hold for 2 min; increased @ 4°C min⁻¹ to 200°C, hold for 7 min.; increased @ 2°C min⁻¹ to 230°C, hold for 0 min; increased 3.5 °C min⁻¹ to 280°C, hold for 11 min. The injection volume was 1 μ l. The injection port and detector temperature were set at 280 and 300 °C.

Liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC-MS/MS, Waters Alliance LC and Acquity TQD) with an electrospray ionization (ESI) interface in positive mode was used for imidacloprid residue analysis using Xterra analytical column C18, 5 μ m (4.8 x 250 mm) (Waters, Milford, MA, USA). The column temperature was set at 30°C. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) (70:30 ratio), run at a flow rate of 0.5 mL min⁻¹. The injection volume was 20 μ L and imidacloprid residues were eluted in an isocratic mode within 15 min. Optimum MS/MS, parameters set included voltage at 3.5 kV; ion source temperature at 150°C; desolvation temperature at 500°C; flow rates of cone gas and desolvation gas at 50 and 1100 l h⁻¹. To identify the parent and daughter ions, working standard solutions of imidacloprid at 0.5 μ g ml⁻¹ in acetonitrile containing 0.1% (v/v) of formic acid were directly infused into the mass spectrometer and chromatograms were recorded in the full scan mode.

RESULTS AND DISCUSSION

The method has been validated in terms of linearity, accuracy and precision in spiked samples. The results of linearity studies showed good linearity with coefficients of determination (R^2) > 0.99 for both betacyfluthrin and imidacloprid for matrix-matched standard calibration curves of cashew matrices with five points calibration ranging from 0.01 to 1.0 mg l⁻¹. Method accuracy was estimated by recovery experiment at three concentration levels and the results are summarized in Table 1. Currently the QuEChERS method is the

most popular analytical method in the field of pesticide residue analysis in food commodities. Hence we tried QuEChERS method with few modifications for recovery studies. In the present method the samples of untreated cashew immature kernels, mature kernels, cashew apple and soil were fortified with betacyfluthrin and imidacloprid at 0.05, 0.25 and 0.50 mg kg⁻¹ and extracted with six replications in each concentration. The mean per cent recoveries were in the acceptable range of 70 to 120% and < 20 % RSD for all the matrices studied (Fig. 1). Thus, the performance of the method adopted in this study was considered satisfactory for both β -cyfluthrin and imidacloprid (SANTE, 2019).

Since cashew is a complex matrix containing high levels of phenolics, sugar and organic acids, there is every possibility of matrix interference during instrument analysis. In order to improve the recovery rate, different buffering salts and sorbents were employed in extraction and cleanup step. In the present method, sample weight of 2 g was taken for all the matrices except for cashew apple (10 g). Florisil and GCB were tried in the d-SPE cleanup. Florisil was used as a sorbent and GCB to remove pigments along with PSA sorbent (readily adsorbs organic acids in the matrix) in order to accomplish better clean-up and to improve the recovery rate. Buffering salts such as trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate were added to maintain the pH during the extraction and to achieve good recovery of pesticides.

As far as cashew is concerned, a very few published literatures are available regarding the methods for pesticide residue analysis. Earlier, Silva et al. (2014) developed a multiresidue method for simultaneous determination of 20 multiclass pesticides using liquid chromatography coupled to mass spectrometry using electrospray ionization source (LC-ESI-MS/MS). In this method, 10 g of homogenized sample was extracted with 10 ml acetonitrile and PSA was the cleanup sorbent. Recoveries obtained were of 78-110% with precisions of 3.3-5.5%, at all three levels 5, 50, 100 ng g⁻¹ of spiked imidacloprid. Dorea and Lancas (1999) reported a multiresidue extraction method based on matrix solid-phase dispersion (MSPD) and capillary gas chromatography-electron capture detection (GC-ECD) for quantification of diazinon, malathion, parathion, methidathion and pyrazophos and one synthetic pyrethroid permethrin in Brazilian cashew nuts and obtained an acceptable average recovery of 101.4% at 0.1 to 0.6 ppm levels in cashew nut.

The standardized method was successfully applied for studying residue and dissipation pattern of beta-cyfluthrin and imidacloprid residues after application of combination insecticide Solomon (Betacyfluthrin 90 g l⁻¹ + imidacloprid 210 g l⁻¹ OD). In treated plots also, beta-cyfluthrin and imidacloprid residues were at below LOQ (0.05 mg kg⁻¹) in the immature kernels collected in all sampling intervals viz., 0 (within 2 hr after last spray), 3, 7, 14, 21 and 35 days after second spraying. Mature kernel, cashew apple and soil collected at the time of harvest (68 days after last spray) also showed no residues at detectable level in the recommended and double the recommended doses, respectively. Hence, consumption of cashew apple and nuts does not pose risk to consumers.

Field experiments on residue and persistence pattern of quinalphos (Geetha and Regupathy, 1999), monocrotophos, endosulfan and lamdacyhalothrin (Suresh Kumar et al., 2004) in cashew nuts revealed that the residues below detectable limits irrespective of the doses tested. The initial deposits (1 hr after treatment) of monocrotophos, endosulfan and lamda-cyhalothrin was 36.16, 67.79, 31.39 mg kg⁻¹, respectively. After shelling, the concentrations of residues were reduced to 2.32, 2.63 and 0.90 mg kg⁻¹, respectively. The insecticide residues reached below detectable limit (BDL) in the nuts after roasting and removal of testa layer. Similarly monitoring studies revealed that cashew nut samples collected from market were free from pesticide residues and found safe for consumption. This was likely due to presence of two protective coverings in the form of

the outer shell and inside testa layer. Furthermore, the residues reached below detectable levels after shelling, peeling and roasting processes (Ramesh Kumar et al., 2004). Residue and persistence studies were conducted earlier using a combination formulation of betacyfluthrin and imidacloprid in crops such as okra, chilli, brinjal, mango, chickpea, tea etc. Difference in residue persistence and dissipation pattern was observed among fresh as well as processed commodities. Study on persistence of betacyfluthrin and imidacloprid in mango indicated that the residues dissipated with the half-lives of 2.4 and 2.6 days and 3.06 and 4.16 days, respectively. Mature mango fruits at harvest were free from residues of both insecticides. A safe preharvest interval of 8 days is recommended for consumption of mango fruits (Mohapatra et al., 2011). In okra, half-lives were 0.91 and 0.68 days and 0.85 and 0.96 days for betacyfluthrin and imidacloprid at single and double the doses, respectively (Sahoo et al., 2012). The dissipation of betacyfluthrin 8.91% + imidacloprid 19.81% OD in chilli was studied and found 4.20 and 3.80 days as the half lives of betacyfluthrin and imidacloprid. Betacyfluthrin reached LOQ on 15th day after spraying while, imidacloprid reached LOQ on 7 days after spraying (Anju Viswanathan and Ambily Paul, 2019).

In the present study, an analytical method was developed and validated using GC-ECD for betacyfluthrin and LC-MS/MS for imidacloprid residues in cashew. The proposed method is simple, less time-consuming and has high cleanup ability and exhibited better recovery rate of target pesticides.

Table 1. Recovery of betacyfluthrin and imidacloprid (cashew matrices and soil) using GC and LC-MS/MS

Selected analytes	Fortification (mg kg ⁻¹)	Immature kernel		Mature kernel		Cashew apple		Soil	
		*Mean Recovery± SD (%)	RSD (%)	*Mean Recovery ± SD (%)	RSD (%)	*Mean Recovery± SD (%)	RSD (%)	*Mean Recovery± SD (%)	RSD (%)
Beta-cyfluthrin	0.05	88.26± 6.59	7.46	103.99± 2.51	2.42	96.16± 4.75	4.94	111.88 ± 5.48	4.89
	0.25	105.09± 1.89	1.80	84.43± 2.82	3.34	100.10± 6.72	6.71	104.97± 1.33	1.27
	0.50	102.24± 4.14	4.05	82.36± 3.83	4.65	111.19± 3.06	2.75	100.23± 0.44	0.43
Imidacloprid	0.05	96.70± 7.95	8.22	109.72± 4.02	3.67	98.95± 3.04	3.07	102.12± 4.38	4.29
	0.25	101.69± 4.97	4.89	99.50± 2.20	2.21	110.29± 6.17	5.59	99.19± 5.71	5.76
	0.50	103.73± 2.29	2.20	108.20± 6.14	5.67	91.72± 2.40	2.62	96.87± 5.11	5.27

*Mean of 6 replicate analyses; SD- Standard Deviation; RSD- Relative Standard Deviation

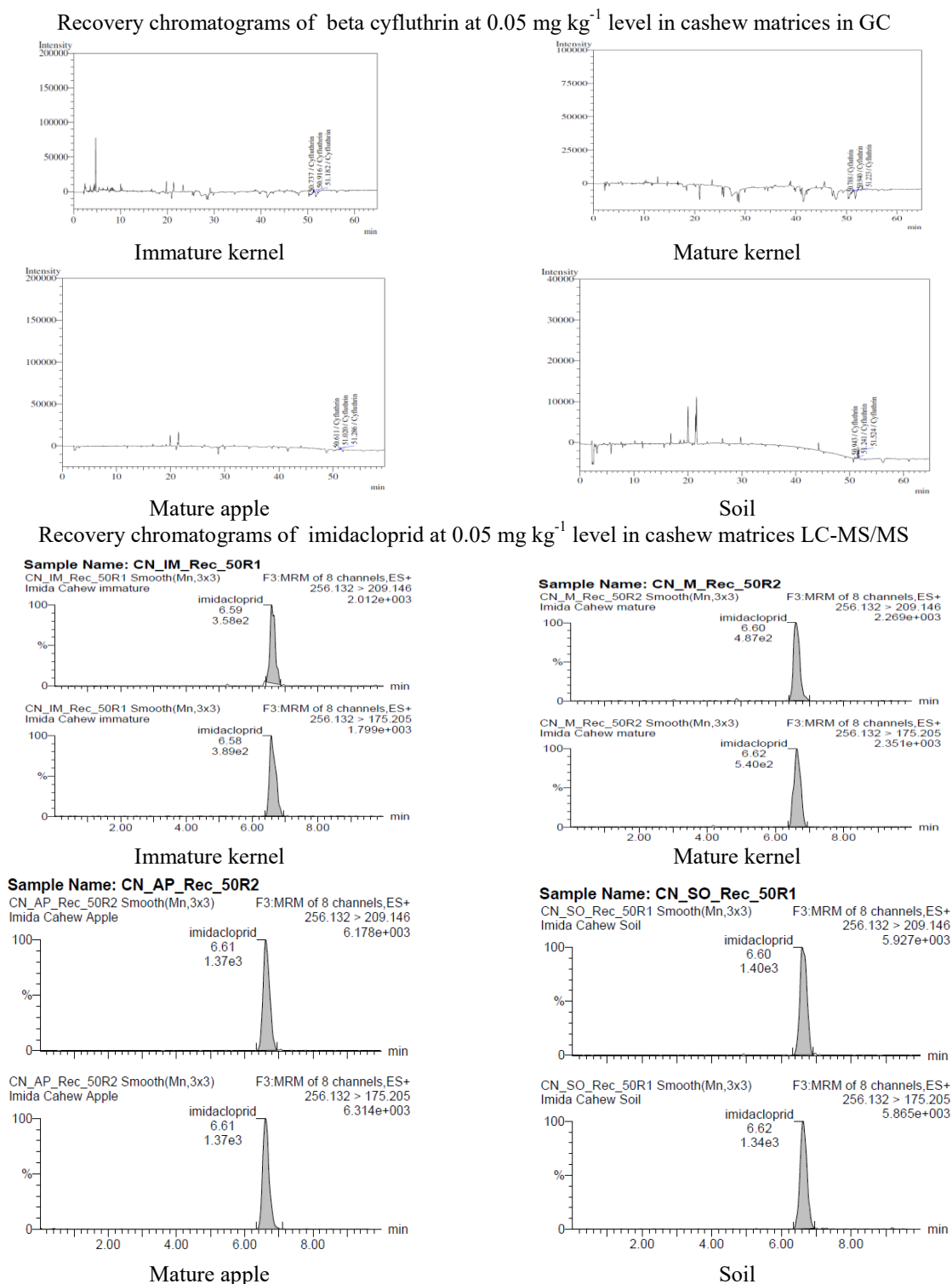


Fig. 1. Chromatograms of recovery of betacyfluthrin and imidacloprid (cashew matrices analysed in GC and LC-MS/MS)

Hence, the validated method was successfully used for studying persistence and dissipation of betacyfluthrin and imidacloprid in cashew crop. The residues of betacyfluthrin and imidacloprid were at below limit of quantification (0.05 mg kg^{-1}) on the day of spray itself in the immature kernels collected after 2 hr of application, mature kernels, cashew apple and soil collected at the time of harvest. Hence, application of Solomon (betacyfluthrin 90 g l^{-1} + imidacloprid 210 g l^{-1} OD) for cashew pest management can be concluded as safe from consumption point of view. The proposed method can be used for monitoring of betacyfluthrin and imidacloprid residues from market cashew nut samples.

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AUTHOR CONTRIBUTION STATEMENT

KB conceived and designed research. KB, VM, AS and PT conducted field experiment. CS and PK conducted lab experiment, NT and PK analyzed data. CS and KB wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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LARVICIDAL POTENTIAL AND RETENTION EFFICACY PERIOD OF CITRONELLA LEAF OIL AGAINST *Aedes aegypti* L.

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ABSTRACT

The mosquito vector *Aedes aegypti* L., plays an important role in transmission of diseases including dengue, chikungunya and zika fever. In this study, citronella leaf oil extracted by hydro-distillation technique has been evaluated for its efficacy against this vector. Citronella leaf oil (100, 125, 150, 175 and 200 ppm) when tested, 150 ppm was found to be the effective larvicidal concentration against 4th instar larvae. LC₅₀ and LC₉₀ toxicity values were observed to be 107.10 and 141.59 ppm, respectively. GC-MS analysis indicated the presence of β -citronellal, geraniol, elemol and β -citronellol as the major components, which might be causing the larval mortality. Retention efficacy of the oil was found to be up to 84 hr in terms of larval killing and during this retention period, a significant delay in development (L4 to adult) was also observed. No effect of storage was observed on its larvicidal efficacy even after six months.

Key words: *Aedes aegypti* L., *Cymbopogon*, development period, essential oils, larvicidal potential, mosquito control, retention efficacy, β -citronellal, geraniol, elemol and β -citronellol

Mosquitoes belonging to the genera *Aedes*, *Anopheles* and *Culex* are common carriers of various diseases such as chikungunya, dengue fever, filariasis, malaria, yellow fever and Japanese encephalitis. According to the National Vector Control Programme, India recorded 123,106 cases of dengue and 82,671 cases of chikungunya in 2021 (NVBDCP, 2021). These are important vectors transmitting parasites and diseases (WHO, 2019). *Aedes aegypti* is a freshwater mosquito that prefers to deposit its eggs in man-made enclosures and its larvae are frequently found in small temporary water collections including desert coolers, containers and earthen pots lying in the peridomestic areas (Kaur et al., 2016). Controlling the mosquito vector is the most effective way to reduce the associated life-threatening diseases, as there is no treatment or vaccine available (Phillips, 2008). Usually insecticides like carbamates, organochlorines, pyrethroids and organophosphates are used, which raise severe concerns on the environment, non-target species and human health (Sathiyathan and Umarajan, 2019). There is an urgent need to develop environmentally safe and more effective alternatives. Plant-based products such as various extracts and extracted essential oils (EOs) serve this purpose (Piplani et al., 2019). The genus *Cymbopogon* has about 140 different fragrance grass species and oils extracted from citronella, lemon and eucalyptus have been approved as ecofriendly insecticides by the Environmental

Protection Agency (EPA) due to their low toxicity and equivalent efficacy (Katz et al., 2008). Citronella oil repels mosquitoes and citronella leaf extracts function as gastrointestinal venom and respiratory poisons thus, prompting larvae to die. Keeping these in mind, the present study evaluated the larvicidal potential and retention efficacy of oil extracted from citronella leaves against *Ae. aegypti*.

MATERIALS AND METHODS

Water samples were taken from various small fresh water collections like desert coolers, roadside ditches, earthen pots and plastic containers etc lying in peridomestic areas of urban regions of Ludhiana district of Punjab (India) from June to September, 2021. From these collected water samples, *Ae. aegypti* larvae were identified and separated using standard keys (Becker et al., 2010). Fresh and young citronella leaves were harvested from the herbal garden of Punjab Agricultural University from August to November 2020 and April to August 2021. The collected leaves were brought to laboratory, cleaned, shade dried for 2-3 days and 250 g of leaves were placed in a 5l capacity round bottom flask fitted in Clevenger apparatus for hydro-distillation oil extraction. The flask was half-filled with water and heated for roughly 4-5 hr on a heating mantle. The temperature was initially set at 100°C for 30 min until boiling occurred and then maintained at 60°C. Oil

vapours generated during the heating process were condensed with cold water steam and collected in the recuperating channel. The oil was carefully collected in a clean glass vial and kept in the refrigerator at 4°C. The oil content/yield (%) of extracted citronella leaf oil was calculated by the formula given below:

$$\text{Oil content (\%)} = \frac{\text{Volume of oil collected after distillation (ml)}}{\text{Weight of withered citronella leaves taken initially (g)}} \times 100$$

The composition of freshly extracted citronella leaf oil was determined using Gas Chromatography-Mass Spectrometry analysis from Central University of Punjab, Bathinda, using the GCMS-QP2010 Ultra programme, with helium as carrier gas flowing at a rate of 1ml/min, split ratio 10:0, injection temperature 250°C and oven temperature programming from 40 to 250°C. A capillary column Rtx-5MS was used in the GC (30 mtr) and 1 µl of oil diluted in 1 ml methanol was injected. For detecting the larvicidal potential of extracted citronella leaf oil, preliminary testing was carried out against 4th instar *Ae. aegypti* larvae by randomly selecting its higher and lower concentrations. Then on this basis, five different concentrations of citronella leaf oil @100, 125, 150, 175 and 200 ppm were prepared by mixing its required amount in 1 ml of DMSO (dimethyl sulphoxide- a non-polar and non-toxic emulsifying agent) so as to make total volume 250 ml by the addition of dechlorinated water in plastic beakers. Dechlorinated water was prepared by storing tap water in opened buckets for 24 hr. Twenty 4th instar *Ae. aegypti* larvae were taken from water samples and treated with five concentrations of citronella oil. A control set (having 250 ml dechlorinated water) and a vehicle-control set (having 1 ml DMSO and 249 ml dechlorinated water) with twenty 4th instar larvae in each beaker were also run simultaneously. All of the experimental sets (treated, control and vehicle-control) were performed in triplicate in the plastic beakers with 250 ml capacity and these beakers were properly covered with muslin cloth. Dog biscuits and crushed yeast in 3:1 ratio (2 mg/ 100 g) were given to all larvae, and larval mortality was recorded after 3, 6, 9, 12, 24, 36 and 48 hr. Larvae not responding or moving after being agitated with a brush were considered dead and were counted in each set. The minimum concentration of the oil that resulted in highest larval mortality within lesser time duration was considered as the effective concentration and that concentration was used for further experimental purpose. After 24 hr of

post-exposure, LC₅₀ and LC₉₀ values were calculated using the log concentration-mortality regression by log probit technique (Finney, 1971) with POLO software (Robertson et al., 1980).

Retention efficacy period was determined by exposing the freshly added twenty *Ae. aegypti* 4th instar larvae in the left over tested solution of the effective concentration after removing all the dead larvae from the beakers of dose-response bioassay (performed earlier). A dechlorinated water control set and a vehicle-control set, were also run in triplicate with their respective treatment sets. After 3, 6, 9, 12, 24, 36 and 48 hr, dead larvae (if any) were counted and replaced with the same number of fresh larvae so as to have total number of larvae as twenty (which were taken initially in the experimental set up). The survived larvae were subsequently monitored to evaluate the retention efficacy on the development duration from 4th larval instar to adult emergence. A control set along with a vehicle-control set was also run simultaneously in triplicate. When larvae got transformed into pupae, the muslin cloth was removed from the beakers and then these beakers were placed in rearing cages. Emerged mosquitoes were fed on sugary juice of deseeded water-soak raisins kept in sterilized petri plate (already kept inside the cages). A moist cotton swab was placed on top of each cage to provide water. In treatment, control and vehicle-control sets, the time taken for each transformation (i.e. from 4th larval instar to pupa and pupa to adult) was recorded, along with recording of adult emergence. To know the effect of storage on the larvicidal efficacy of EO, extracted oil was stored in clean vials wrapped with aluminum foil at 4°C for its usage after 2, 4 and 6 months. Already determined effective larvicidal concentration of oil against *Ae. aegypti* was tested again for freshly prepared and stored oil (2, 4 and 6 months old). Data obtained was statistically analyzed by comparing the larval mortality (during larvicidal bioassay) and developmental duration (during retention efficacy experiments) by one way ANOVA (Duncan multiple range test). The larvicidal potential data of stored citronella oil treated sets was statistically analyzed similarly.

RESULTS AND DISCUSSION

Citronella leaf oil extracted in Clevenger apparatus by hydro-distillation technique was transparent in colour having pleasant floral smell and found to be insoluble in water but soluble in organic solvents and showed an average yield of 1.05± 0.43% (v/w). Cassel

and Vargas (2006) have also reported approximately 1% oil content from fresh citronella leaves following hydro-distillation method of extraction. The GC-MS analysis revealed 57 peaks in the extracted oil, indicating the presence of 57 compounds and out of these the most prevalent were β -citronellal (27.48%) followed by Geraniol (24.10%) > elemol (13.42%) > β -citronellol (9.06%) > geranyl acetate (4.07%) > viridiflorol (3.82%) > 2,6-dimethyl 2,6 octadiene (2.67%) > limonene (1.98%) > N,N-dimethylacetamide (DMA) (1.69%) > 2,4disopropyl methyl (1.59%) as given in Table 1. Exposure of 4th instar *Ae. aegypti* larvae to 100 ppm oil resulted in $20.00 \pm 5.00\%$ larval mortality after 3 hr, this

increased with exposure time and after 48 hr to $56.66 \pm 7.63\%$. No more mortality was observed after 48 hr and the larvae that remained got converted into the pupae stage. With increase in concentration to 125 and 150 ppm, similar mortality trend was found. The exposure of larvae to 175 ppm oil resulted in 100% mortality within 48 hr, but when larvae were exposed to 200 ppm, they died completely within 6 hr (Table 2). Citronella leaf oil @ 150 ppm was found to be statistically effective larvicidal concentration, since it resulted in maximum mortality (100%) of *Ae. aegypti* with the minimal dose of oil (150 ppm) and within lesser duration of treatment (36 hr).

Table 1. Major compounds reported in extracted fresh citronella leaf oil analyzed by GC-MS

Sl. No.	Peak No	Retention time (min)	Area (%)	Molecular formula	Name of compound IUPAC name (Common name)	Structure
1	10	19.839	27.48	C ₁₀ H ₁₈ O	3,7-dimethyloct-6-enal (β -citronellal)	
2	17	23.708	24.10	C ₁₀ H ₁₈ O	3,7-dimethylocta-trans-2,6-dien-1-ol (Geraniol)	
3	37	33.729	13.42	C ₁₅ H ₂₆ O	Cyclohexanemethanol, 4-ethenyl- α,α ,4-trimethyl-3-(1-methylethenyl) (Elemol)	
4	15	22.647	9.06	C ₁₀ H ₂₀ O	3,7-Dimethyloct-6-en-1-ol (β -citronellol)	
5	25	27.947	4.07	C ₁₂ H ₂₀ O ₂	3,7-dimethyl-2,6-octadiene-1-ol acetate (Geranyl acetate)	
6	38	34.539	3.82	C ₁₅ H ₂₆ O	1,1,4,7-Tetramethyl decahydro-1H-cyclopropanE-azulen-4-ol (Viridiflorol)	
7	22	26.945	2.67	C ₁₀ H ₁₈	2,6 dimethyl 2,6 octadiene	
8	3	14.628	1.98	C ₁₀ H ₁₆	1-methyl-4-(1-methylethenyl) cyclohexene (Limonene)	
9	1	8.132	1.69	C ₄ H ₉ NO	N,N-dimethylacetamide (DMA)	
10	27	28.376	1.59	C ₁₅ H ₂₄	2,4disopropyl methyl-1-vinyl cyclohexane (Elemene)	

Table 2. Effect of concentrations of citronella leaf oil on 4th instar larvae of *Aedes aegypti*

Concentration (ppm)	% mortality (Mean± S.D) (n=20)							Range of mortality (within hours)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
100	20.00±5.00 ^b (3-5)	25.00± 5.00 ^b (4-6)	28.33±7.63 ^b (4-7)	30.00±5.00 ^b (5-7)	35.00±5.00 ^b (6-8)	45.00± 5.00 ^b (8-10)	56.66± 7.63 ^b (10-13)	3-48
125	36.66±2.88 ^c (6-8)	56.66± 7.63 ^c (10-13)	65.00±5.00 ^c (12-14)	73.33±5.77 ^c (14-16)	81.66±7.63 ^c (15-18)	85.00± 5.00 ^c (16-18)	90.00± 8.66 ^c (17-20)	3-48
150	65.00±5.00 ^d (12-14)	73.33± 7.63 ^d (13-16)	81.66±5.77 ^d (15-17)	88.33±5.77 ^d (17-19)	91.66±2.88 ^d (18-19)	96.66± 2.88 ^d (19-20)	100.00±0.00 ^d (20)	3-48
175	80.00±5.00 ^e (15-17)	90.00± 5.00 ^e (17-19)	93.33±2.88 ^e (18-19)	95.00±5.00 ^d (18-20)	98.33±2.88 ^e (19-20)	100.00±0.00 ^d (20)	—	3-36
200	91.66±5.77 ^f (17-19)	100.00±0.00 ^f (20)	—	—	—	—	—	3-6
0 (Control)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0
0 (Vehicle-control)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0.00± 0.00 ^a (0)	0

Toxicity values			
Toxicity Value (ppm)	Fiducial limits		χ^2
	Lower limit (ppm)	Upper limit (ppm)	
LC ₅₀ =107.10	100.50	112.41	1.95
LC ₉₀ =141.59	134.44	152.09	1.95

n represents number 4th instar larvae taken; Figures in parentheses range in no. of dead larvae from the start of experiment till that period; Figures followed with different superscripts indicate significant difference (p<0.05, DMRT)

GC-MS analysis of the prepared citronella leaf oil showed the presence of β -citronellal (27.48%) in maximum amount along with other major components like geraniol, elemol and β -citronellol (Table 1). These compounds could be responsible for the larval mortality of *Ae. aegypti* (Katiyar et al., 2011). LC₅₀ and LC₉₀ toxicity values for 4th instar larvae were based on the record of deaths up to 24 hr after being exposed. Manimaram et al., (2012) observed LC₅₀ for larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* as 47.61, 91.23 and 47.21 ppm, respectively; with oil of *C. citratus*, good LC₉₀ at 69 ppm on third and fourth *Ae. aegypti* larvae was observed (Cavalcanti et al., 2004).

When *Ae. aegypti* 4th instar larvae were exposed to the leftover effective concentration @ 150 ppm, 100% mortality was observed up to 6 hr; and thereafter showed a declining trend and no larval mortality was observed after 48 hr. Larval mortality was statistically significant after 9, 12, 24 and 36 hr revealing that prepared oil showed the retention efficacy up to a period of 36 hr. However no larval mortality was observed in the control and vehicle-control sets (Table 3). Thus, the total retention efficacy was 84 hr (48 hr spent during larvicidal bioassay + 36 hr spent during the retention efficacy experiment). Knowing the retention efficacy

period is critical for vector control since it reveals the minimal interval between treatments required to sustain the pesticide's persistence against the targeted vector (Roseli et al., 2007). In field studies, neemarin, a commercial neem extract product, was shown to substantially lower the frequency of *An. stephensi* and *Cx. quinquefasciatus* larvae at recommended doses of 1 and 2 l/ha with a 7-day estimated residual impact. Amer and Mehlhorn (2006) assessed the durability of the larvicidal action of 13 essential oils and observed 100% mortality in three among these (*C. camphora*, *Thymus serpyllum* and *C. limon*) and up to three weeks, these oils were completely effective. Exposure of *Ae. aegypti* 4th instar larvae to leftover effective concentration (150 ppm) resulted in a substantial delaying of development of L4 to pupa and a non-significant delay in pupae to adult development, taking 4.66± 0.28 and 1.83± 0.28 days, respectively. However, it took significantly longer duration in the treated set i.e. 6.49± 0.56 days (Table 3). Citronella leaf oil, on the other hand, had no effect on adult emergence. Sutiningsih et al. (2017) also showed that biolarvicide derived from *Bruceine* spp. leaves slow down the development of *Ae. aegypti* larvae. EO derived from *Pseudocalymma alliaceum* had larvicidal action, while aqueous, ethanol and methanol extracts were found to hinder normal growth and development of *Cx.*

Table 3. Larvicidal retention efficacy of effective concentration (150 ppm) against *Aedes aegypti*

Experimental sets	% mortality (Means S.D) (n=20)							Retention activity time (hours)
	3hr	6 hr	9 hr	12 hr	24 hr	36 hr	48 hr	
Treated citronella leaf oil (150 ppm)	100.00± 0.00 ^b	100.00± 0.00 ^b	98.33± 2.88 ^b	88.33± 5.77 ^b	73.33± 5.77 ^b	58.33± 7.63 ^b	0.00± 0.00 ^a	36
Control	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0
Vehicle-control	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0.00± 0.00 ^a	0

Figures followed with different subscripts indicate significant difference (p<0.05, DMRT) on development and emergence

Experimental set	Duration of developmental period in days (Mean± S.D)			Emergence (%)	
	L4-Pupa	Pupa-Adult	L4-Adult	L4-Pupa	Pupa-Adult
Treated (150 ppm)	4.66± 0.28 ^b (4.5-5)	1.83± 0.28 ^b (1.5-2)	6.49± 0.56 ^b (6-7)	100.00± 0.00 ^a	100.00± 0.00 ^a
Control	3.33± 0.57 ^a (3-4)	1.00± 0.28 ^a (1-1.5)	4.33± 0.85 ^a (4-5.5)	100.00± 0.00 ^a	100.00± 0.00 ^a
Vehicle-Control	3.66± 0.28 ^a (3.5-4)	1.33± 0.28 ^a (1-1.5)	4.99± 0.56 ^a (4.5-5.5)	100.00± 0.00 ^a	100.00± 0.00 ^a

Values are Mean± S.D; Figures in parentheses represents range in days; Figures in subscripts indicate significant difference (p<0.05, DMRT)

Table 4. Effect of storage on larvicidal potential of citronella leaf oil (150 ppm) against *Aedes aegypti*

Storage of oil (months)	% mortality upto (Mean± S.D) (n=20)							Range of mortality (within hours)
	3 hr	6 hr	9 hr	12 hr	24 hr	36 hr	48 hr	
0 (fresh)	61.66± 7.63 ^a (11-14)	68.33± 7.63 ^a (12-15)	73.33± 5.77 ^a (14-17)	86.66± 2.88 (17-18)	93.33± 2.88 ^a (18-19)	98.33± 2.88 ^a (19-20)	100.00± 0.00 ^a (20)	3-48
2	60.00± 5.00 ^a (11-13)	68.33± 5.77 ^a (13-15)	71.66± 2.88 ^a (14-15)	85.00± 5.00 ^a (16-18)	90.00± 5.00 ^a (17-19)	95.00± 5.00 ^a (19-20)	100.00± 0.00 ^a (20)	3-48
4	63.33± 7.63 ^a (11-14)	66.66± 7.63 ^a (12-15)	70.00± 5.00 ^a (13-15)	83.33± 7.63 ^a (15-18)	88.00± 7.63 ^a (16-19)	93.33± 5.77 ^a (18-20)	100.00± 0.00 ^a (20)	3-48
6	65.00± 5.00 ^a (13-14)	66.66± 7.63 ^a (12-15)	70.00± 5.00 ^a (13-15)	78.33± 7.63 ^a (14-17)	90.00± 5.00 ^a (17-19)	91.66± 7.63 ^a (17-20)	100.00± 0.00 ^a (20)	3-48

n represents number of 4th instar larvae taken; Figures in subscripts indicate significant difference (p<0.05, DMRT)

quinquefasciatus larvae thus, extending and delaying larval and pupal duration (Granados-Echegoyen et al., 2014). Plant based EOs have growth regulating effects on a number of insects, slowing or stopping their growth at various phases of development (Regnault-Roger 2013). When 4th instar *Ae. aegypti* larvae were exposed to the effective concentration (150 ppm) of citronella leaf oil stored for two, four and six months, it resulted in 100% larval mortality up to 48 hr, which was statistically non-significant with respect to that of freshly extracted oil (Table 4). Thus, storage has no effect on the larvicidal

efficiency if the oil is stored properly. The oils of *E. globulus* and *A. vera* when preserved in dark bottles, resulted in no significant variations in their larvicidal efficacy after storage (Misharina et al., 2003). Dua et al., (2009) also investigated the effect of storing neem oil at room temperature for 18 months and observed no significant variations in LC₅₀ and LC₉₀ values before and after storage. Santos et al., (2014) tested fresh and stored *Croton rhamnifolioides* essential oil against *Ae. aegypti* and found that the larvicidal efficacy remained unaltered even after three years.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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STRUCTURE AND COMPOSITION OF BIRD ASSEMBLAGE IN KESHOPUR WETLAND OF PUNJAB, INDIA

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ABSTRACT

Survey on avian diversity at Keshopur wetland, district Gurdaspur, Punjab, India revealed the presence of 121 species of birds belonging to 19 orders and 47 families. The maximum number (38) of species in the area belonged to order Passeriformes. 27 winter migratory, 3 summer migratory and 91 resident species of birds were observed. One vulnerable (*Aythya ferina*) and six near threatened species were observed (*Aythya nyroca*, *Anhinga melanogaster*, *Mycteria leucocephala*, *Sterna aurantia*, *Psittacula eupatria*, *Threskiornis melanocephalus*) as per IUCN red list. A particular pattern of arrival and departure of migratory birds was observed. The winter migrants started to appear in October when the temperature started decreasing. The abundance of birds varied significantly during different seasons. Major variation was found in abundance of few species at three different sites of the same wetland. This study revealed that Keshopur Chhamb Community Reserve acts as a refuge site for many waterbirds including wader, waterfowl and many migratory and threatened species.

Key words: Avian diversity, community reserve, ecosystem, migratory birds, Passeriformes, Ramsar site, waterbirds, wetland, vulnerable, near threatened, arrival, departure pattern, season variation

Birds belong to a group of warm blooded vertebrates characterized by feathers, toothless beaked jaws, the hard shelled egg laying, high metabolic rate, a heart with four chambers and a strong yet light weight skeleton. About 10,000 species of birds are present on Earth in different ecosystems. Wetlands are areas of land that are permanently or temporarily covered with water. A large number of wetlands such as swamps, marshes, peatlands etc are present in India. Wetlands are considered to be most distinctive and high yielding ecosystems (Rajasekar et al., 2008) which can be characterized as a transitional world between aquatic and terrestrial ecosystems as they exhibit characters of both ecosystems. A wide diversity of aves depends upon wetlands during their migrant and procreation phase (Luo et al., 2019). Water birds and wetlands are inseparable components as they support an affluent arrangement of water bird communities. About 10% of the bird species globally rely entirely on wetlands, while approximately the same number again utilizing them at some phase in their life span (Gardner et al., 2016). This indicates that globally 20% (approximate) of the avian species utilize wetlands directly or indirectly for foraging, resting, breeding and overwintering (Rannestad et al., 2015). The population of birds dependent on wetlands is going through drastic decrease globally. These noticeable decreases are particularly due to immense loss of wetlands and conversion to land (Saunders et al., 2019).

In a survey conducted by Wildlife Institute of India, it was found that wetlands are dissipating every year @ 2 to 3% (Bal and Dua, 2010). In wetlands, the diversity and abundance of bird species is directly associated with the developing vegetation and compounding. The aquatic birds are fairly receptive to the variations in wetlands (Odewumi et al., 2017). Their population size is directly affected by the food availability (Jagruiti and Geeta, 2017). With the changes in wetlands, the aquatic birdlife is entirely affected which is an indication for us to understand whether the region is environmentally sound or getting contaminated (Odewumi et al., 2017).

In Punjab, six wetlands are of international significance and Keshopur wetland is one such important wetland which has recently been declared as Ramsar site on 26 September, 2019. This wetland was the first declared community reserve of India (Mehta, 2014). The economy of rural areas surrounding it is intensely affected and the contribution from the local community has been recognized as a key factor for its protection. The reserve has many fresh water marshes (natural wetlands) extending to an area of 850 acres and is the main site for migratory birds during the winter season. Due to its conversion into productive agricultural land and fish farms in the past by drainage department of the government, the wetland area has been reduced to its present size which was once spread to many thousand acres. The ecosystem is now on the edge of extinction

and is highly threatened. The disturbance caused by humans directly or indirectly in wetland bird habitats led to decrease in strength of various populations of wetland birds. It is necessary to understand the causes for the decrease in the populations of various water birds and to find the effects of interference of humans. The inestimable information can be obtained on the standing and fitness of wetland by monitoring the birds of wetland (Moilinga and Hassan, 2019). Only by knowing the structure of any region, the significance of local scenery for the conservation of birds can be understood (Harisha, 2016). Wetlands in India cope with enormous anthropogenic pressures as elsewhere, due to which the structure of bird community is strongly influenced (Reginald et al., 2007). Anthropogenic actions are known to cause disruptions to aquatic birds in their natural surroundings including recreation (Aikins et al., 2018; Anderson et al., 2019). Even though these sites are adequately transformed by human actions, still providing suitable environment (Bal and Dua, 2010) and food (Jangral and Vashishat, 2022) to many bird species therefore, the present study was planned to study community structure of birds at Keshopur wetland, a Ramsar site.

MATERIALS AND METHODS

The Keshopur wetland is a freshwater ecosystem and (32°05' 16.3" N, 75°24' 24.2" E) and 245 masl having an area of approximately 344 ha adjacent to the town of Gurdaspur, District Gurdaspur, Punjab. This region was announced as community reserve under Section 36 C of Wildlife protection Act 1972 ensuing a Punjab Government Notification Number 34/13/2007/Ft-V/6133 dated June 25th 2007. The wetland comprised of fresh water marshes owned by Panchayats of five villages categorized into two parts. Miani (162 ha), Dalla (62 ha), Keshopur (55 ha), Matwa (20 ha) form the significant one conterminous block and Magarmudian (45 ha) is a separate patch. The wetland consists of diverse amount of vegetation. The study area was divided into three sites. Site I consisted of trees, shrubs, herbs, grasses, aquatic plants and climbers. It was located near the road and surrounded by agricultural fields. Site II was mainly consisted of small vegetation including herbs, shrubs, climbers, grasses, aquatic plants and some trees. Fish ponds were present at the site which was surrounded by agricultural fields. Site III which was a separate patch consisted of large number of aquatic plants and bamboo trees, herbs, shrubs and grasses. Watchtower was present at all the three sites to see the birds from a distance.

Point count method was used to study and record the diversity of birds at different sites of Keshopur Chhamb Community Reserve. In point count method, all the viewable birds were counted by choosing an appropriate vantage point. Approximately 10-15 minutes 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). At different sites, observations of birds were recorded weekly for one and a half hour between 6:00-9:00 a.m. in the morning and 4:00-7:00 p.m. in the evening using binoculars (Bushnell 13-3450-C, 10×50). Regular observations were taken at weekly intervals throughout the study but they were pooled together into a single monthly observation. Status of species was classified into resident (R), winter migrant (WM) and summer migrant (SM).

The data of four point counts recorded in one month was pooled together. The community features such as Species richness, diversity, evenness and abundance were calculated to determine the bird's community at selected sites. Species richness describes the total number of species of birds in a given area. Relative abundance of birds (%) was calculated using the formula: $n_i/N \times 100$. In this equation, n_i represents the number of *i*th species and N represents the total number of birds seen. Species diversity was calculated using Shannon-Weiner index as explained by (Spellerberg and Fedor, 2003). Species evenness also called as equitability and written as E was determined by the equation: $J = H/H'_{\max}$, Where, H is the observed species diversity and $H'_{\max}$ is the log of total number of species richness. The value of E ranges from 0-1. Annual abundance of avifauna was tabulated and analyzed using two way analysis of variance. CPCS1 software was used to compare the number of species at each selected site. SPSS1 software (Kruskal-Wallis test) was used to compare the seasonal variation between the three sites.

RESULTS AND DISCUSSION

Total 121 species of birds belonging to 19 orders and 47 families were observed (Table 1). The maximum number of species i.e. 38 were belonging to order Passeriformes. Praveen et al. (2016) observed that Passeriformes forms the most predominant group in India with about 54% composition. Anatidae was the most abundant family belonging to order Anseriformes having 11 species. However, Muscicapidae family has

the highest number of birds in India as per the study of Manakadan and Pittie (2001). Similar results were recorded by Mukhopadhyay and Mazumdar (2017) in Bongaon, West Bengal and by Harisha and Hosetti (2017) at Dyamannana Lake, Karnataka. Rawat and Rao (2020) noticed Anatidae as least abundant family in Sheopur city of Madhya Pradesh. Total 107 species of birds were found at Site I where Eurasian coot, common moorhen and northern shoveler were recorded to be the predominant ones having annual abundance 14.10, 13.36 and 10.93%, respectively. 103 species of birds were found at Site II in which common moorhen and Eurasian coot were recorded as the abundant; 113 species were found at Site III where Eurasian coot, common moorhen and northern pintail were the predominant.

As per IUCN red list (IUCN 2020), out of total 121 species, one vulnerable species (*Aythya ferina*) and six near threatened species were observed (i.e. *Aythya nyroca*, *Anhinga melanogaster*, *Mycteria leucocephala*, *Sterna aurantia*, *Psittacula eupatria*, *Threskiornis melanocephalus*). Suryakant (2017) also reported *Mycteria leucocephala* and *Threskiornis melanocephalus* as near threatened species at Urban Wetlands of Kolhapur, Maharashtra. The endangered species can be conserved by studying and conserving their habitat. Most conservation plans for endangered species build on the conservation of habitats (Maleki et al., 2019). In present study, 27 winter migratory, 3 summer migratory and 91 resident birds were observed. This contributed to total composition as 22% of winter migrant, 2.5% of summer migrants and 75.5% of resident

Table 1. Abundance and status of birds (Keshopur wetland)

S. No.	Species	Scientific name	Annual abundance		
			Site I	Site II	Site III
	Order-Accipitriformes, Family-Accipitridae				
1.	Black kite	<i>Milvus migrans</i> (Boddaert, 1783)	0.47	0.42	0.56
2.	Lesser spotted eagle	<i>Clanga pomarina</i> (Brehm, 1831)	0.35	0.27	0.34
3.	Oriental honey buzzard	<i>Pernis ptilorhynchus</i> (Temminck, 1821)	0.05	0.05	0.06
4.	Western marsh harrier	<i>Circus aeruginosus</i> (Linnaeus, 1758)	0.02	0.03	0.02
	Order-Anseriformes, Family-Anatidae				
5.	Bar-headed goose	<i>Anser indicus</i> (Latham, 1790)	1.18	-	0.68
6.	Common pochard	<i>Aythya ferina</i> (Linnaeus, 1758)	1.27	0.88	1.35
7.	Eurasian wigeon	<i>Mareca penelope</i> (Linnaeus, 1758)	7.02	5.57	7.92
8.	Ferruginous duck	<i>Aythya nyroca</i> (Güldenstädt, 1770)	1.03	-	0.91
9.	Gadwall	<i>Anas strepera</i> (Linnaeus, 1758)	7.54	5.95	8.01
10.	Greylag goose	<i>Anser anser</i> (Linnaeus, 1758)	1.55	1.15	1.59
11.	Indian spot billed duck	<i>Anas poecilorhyncha</i> (Forster, 1781)	3.73	0.19	3.35
12.	Lesser whistling duck	<i>Dendrocygna javanica</i> (Horsfield, 1821)	0.48	0.22	0.52
13.	Mallard	<i>Anas platyrhynchos</i> (Linnaeus, 1758)	5.68	5.56	6.15
14.	Northern pintail	<i>Anas acuta</i> (Linnaeus, 1758)	10.06	7.88	10.40
15.	Northern shoveler	<i>Spatula clypeata</i> (Linnaeus, 1758)	10.93	11.36	-
	Order-Apodiformes, Family-Apodidae				
16.	Little swift	<i>Apus affinis</i> (Gray, 1830)	0.60	0.62	0.49
	Order- Bucerotiformes, Family-Bucerotidae				
17.	Indian grey hornbill	<i>Ocyrceros birostris</i> (Hume, 1873)	0.07	0.02	0.02
	Order-Bucerotiformes, Family-Upupidae				
18.	Common hoopoe	<i>Upupa epops</i> (Linnaeus, 1758)	0.40	0.28	0.34
	Order-Ciconiiformes, Family-Ciconiidae				
19.	Asian openbill	<i>Anastomus oscitans</i> (Boddaert, 1783)	0.06	0.18	0.04
20.	Painted stork	<i>Mycteria leucocephala</i> (Pennant, 1769)	0.03	0.02	0.01

(Table 1 contd.)

Order-Charadriiformes, Family-Charadriidae					
21.	Little ringed plover	<i>Charadrius dubius</i> (Scopoli, 1786)	-	-	0.38
22.	Pacific golden plover	<i>Pluvialis fulva</i> (Gmelin, 1789)	-	-	0.08
23.	Red-wattled lapwing	<i>Vanellus indicus</i> (Boddaert, 1783)	1.13	0.82	1.51
24.	White tailed lapwing	<i>Vanellus leucurus</i> (Lichtenstein, 1823)	-	0.01	0.32
25.	Yellow-wattled lapwing	<i>Vanellus malabaricus</i> (Boddaert, 1783)	0.03	-	0.16
Order-Charadriiformes, Family-Jacaniidae					
26.	Pheasant tailed jacana	<i>Hydrophasianus chirurgus</i> (Scopoli, 1786)	0.17	0.22	-
Order-Charadriiformes, Family-Laridae					
27.	Brown headed gull	<i>Chroicocephalus brunnicephalus</i> (Jerdon, 1840)	-	-	0.28
28.	Black headed gull	<i>Chroicocephalus ridibundus</i> (Linnaeus, 1766)	-	-	0.29
29.	River tern	<i>Sterna aurantia</i> (Gray, 1831)	-	-	0.35
Order-Charadriiformes, Family-Recurvirostridae					
30.	Black winged stilt	<i>Himantopus himantopus</i> (Linnaeus, 1758)	-	-	0.96
Order-Charadriiformes, Family-Scolopacidae					
31.	Common greenshank	<i>Tringa nebularia</i> (Gunnerus, 1767)	-	-	0.55
32.	Common sandpiper	<i>Actitis hypoleucos</i> (Linnaeus, 1758)	-	-	0.52
33.	Marsh sandpiper	<i>Tringa stagnatilis</i> (Bechstein, 1803)	-	-	0.44
34.	Spotted redshank	<i>Tringa erythropus</i> (Pallas, 1764)	-	-	0.50
Order- Columbiformes, Family-Columbidae					
35.	Eurasian collared dove	<i>Streptopelia decaocto</i> (Fridvaldszky, 1838)	0.52	0.44	0.58
36.	Laughing dove	<i>Spilopelia senegalensis</i> (Linnaeus, 1766)	0.17	0.11	0.17
37.	Oriental turtle dove	<i>Streptopelia orientalis</i> (Latham, 1790)	0.06	-	0.02
38.	Red collared dove	<i>Streptopelia tranquebarica</i> (Hermann, 1804)	0.09	0.06	0.13
39.	Rock pigeon	<i>Columba livia</i> (Gmelin, 1789)	0.46	0.54	0.65
40.	Spotted dove	<i>Spilopelia chinensis</i> (Scopoli, 1786)	0.21	0.15	0.16
41.	Yellow-footed green pigeon	<i>Treron phoenicoptera</i> (Latham, 1790)	0.13	0.08	0.09
Order-Coraciiformes, Family-Alcedinidae					
42.	Lesser pied kingfisher	<i>Ceryle rudis</i> (Linnaeus, 1758)	0.01	0.12	0.04
43.	White-throated kingfisher	<i>Halcyon smyrnensis</i> (Linnaeus, 1758)	0.05	0.72	0.17
Order-Coraciiformes, Family-Coraciidae					
44.	Indian roller	<i>Coracias benghalensis</i> (Linnaeus, 1758)	0.15	0.07	0.11
Order-Coraciiformes, Family-Meropidae					
45.	Green bee eater	<i>Merops orientalis</i> (Latham, 1801)	0.05	0.05	0.08
46.	Blue tailed bee eater	<i>Merops philippinus</i> (Linnaeus, 1766)	0.02	-	0.03
Order-Cuculiformes, Family-Cuculidae					
47.	Asian koel	<i>Eudynamis scolopaceus</i> (Linnaeus, 1758)	0.11	0.13	0.15
48.	Greater coucal	<i>Centropus sinensis</i> (Stephens, 1815)	0.50	0.27	0.58
Order-Falconiformes, Family-Falconidae					
49.	Peregrine falcon	<i>Falco peregrines</i> (Tunstall, 1771)	0.07	0.05	0.06
Order-Galliformes, Family-Phasianidae					
50.	Black francolin	<i>Francolinus francolinus</i> (Linnaeus, 1766)	0.07	0.04	0.12

(Table 1 contd.)

Order-Gruiformes, Family-Rallidae					
51.	White breasted waterhen	<i>Amaurornis phoenicurus</i> (Pennant, 1769)	0.36	0.22	1.22
52.	Purple swamphen	<i>Porphyrio porphyrio</i> (Linnaeus, 1758)	1.47	2.93	1.22
53.	Common moorhen	<i>Gallinula chloropus</i> (Linnaeus, 1758)	13.36	16.45	13.83
54.	Eurasian coot	<i>Fulica atra</i> (Linnaeus, 1758)	14.10	15.40	15.38
Order-Passeriformes, Family-Alaudidae					
55.	Ashy-crowned sparrow lark	<i>Eremopterix griseus</i> (Scopoli, 1786)	0.22	0.08	0.41
56.	Crested lark	<i>Galerida cristata</i> (Linnaeus, 1758)	0.11	-	0.20
Order-Passeriformes, Family-Campephagidae					
57.	White-bellied minivet	<i>Pericrocotus erythropygius</i> (Jerdon, 1840)	0.06	0.07	0.13
Order- Passeriformes, Family-Cisticolodae					
58.	Yellow-bellied prinia	<i>Prinia flaviventris</i> (Delessert, 1840)	0.07	0.06	0.14
Order-Passeriformes, Family-Corvidae					
59.	Common raven	<i>Corvus corax</i> (Linnaeus, 1758)	0.48	0.34	0.57
60.	House crow	<i>Corvus splendens</i> (Vieillot, 1817)	0.91	0.84	1.10
61.	Indian jungle crow	<i>Corvus macrorhynchos</i> (Wagler, 1827)	0.58	0.46	0.68
62.	Indian treepie	<i>Dendrocitta vagabunda</i> (Latham, 1790)	0.16	0.03	0.10
Order-Passeriformes, Family-Dicruridae					
63.	Ashy drongo	<i>Dicrurus leucophaeus</i>	0.09	0.06	0.09
64.	Black drongo	<i>Dicrurus macrocercus</i> (Vieillot, 1817)	0.17	0.14	0.25
Order-Passeriformes, Family-Estrildidae					
65.	Scaly breasted munia	<i>Lonchura punctulata</i> (Linnaeus, 1758)	0.22	0.13	0.24
66.	Tricolour munia	<i>Lonchura Malacca</i> (Linnaeus, 1766)	0.03	0.01	0.02
Order- Passeriformes, Family-Laniidae					
67.	Long tailed shrike	<i>Lanius schach</i> (Linnaeus, 1758)	-	-	0.58
Order-Passeriformes, Family-Monarchidae					
68.	Asian paradise flycatcher	<i>Terpsiphone paradise</i> (Linnaeus, 1758)	0.05	0.05	0.03
Order-Passeriformes, Family-Motacillidae					
69.	Western yellow wagtail	<i>Motacilla flava</i> (Linnaeus, 1758)	0.02	0.03	0.29
70.	Grey wagtail	<i>Motacilla cinerea</i> (Tunstall, 1771)	0.02	0.04	0.19
71.	Citrine wagtail	<i>Motacilla citreola</i> (Pallas, 1776)	0.02	0.02	0.12
72.	White browed wagtail	<i>Motacilla maderaspatensis</i> (Gmelin, 1789)	0.07	0.07	0.17
73.	Paddy field pipit	<i>Anthus rufulus</i> (Vieillot, 1818)	0.13	0.14	0.24
74.	Long billed pipit	<i>Anthus similis</i> (Jerdon, 1840)	0.04	0.06	0.09
Order-Passeriformes, Family-Muscicapidae					
75.	Oriental magpie robin	<i>Copsychus saularis</i> (Linnaeus, 1758)	0.12	0.05	0.12
76.	Indian black robin	<i>Copsychus fulicatus</i> (Linnaeus, 1766)	0.14	0.15	0.24
77.	Bluethroat	<i>Luscinia svecica</i> (Linnaeus, 1758)	0.03	0.03	0.07
Order-Passeriformes, Family-Nectariniidae					
78.	Purple sunbird	<i>Cinnyris asiaticus</i> (Latham, 1790)	0.08	0.04	0.06
Order-Passeriformes, Family-Passeridae					
79.	House sparrow	<i>Passer domesticus</i> (Linnaeus, 1758)	0.25	0.30	0.36

(Table 1 contd.)

Order-Passeriformes, Family-Ploceidae					
80.	Baya weaver	<i>Ploceus philippinus</i> (Linnaeus, 1766)	0.09	0.06	0.19
81.	Streaked weaver	<i>Ploceus manyar</i> (Horsfield, 1821)	0.13	0.10	0.22
82.	Black breasted weaver	<i>Ploceus benghalensis</i> (Linnaeus, 1758)	0.10	0.07	0.17
Order-Passeriformes, Family-Pycnonotidae					
83.	Red vented bulbul	<i>Pycnonotus cafer</i> (Linnaeus, 1766)	0.39	0.32	0.40
84.	White eared bulbul	<i>Pycnonotus leucotis</i> (Gould, 1836)	0.14	0.06	0.10
Order-Passeriformes, Family-Rhipiduridae					
85.	White throated fantail	<i>Rhipidura albicollis</i> (Vieillot, 1818)	0.02	0.01	0.02
Order-Passeriformes, Family-Sturnidae					
86.	Asian pied starling	<i>Gracupica contra</i> (Linnaeus, 1758)	0.12	0.06	0.15
87.	Bank myna	<i>Acridotheres ginginianus</i> (Latham, 1790)	0.36	0.29	0.54
88.	Common myna	<i>Acridotheres tristis</i> (Linnaeus, 1766)	0.73	0.80	0.88
Order-Passeriformes, Family-Sylviidae					
89.	Common tailor bird	<i>Orthotomus sutorius</i> (Pennant, 1769)	0.06	0.05	0.12
Order-Passeriformes, Family-Timaliidae					
90.	Common babbler	<i>Argya caudata</i> (Dumont, 1823)	0.19	0.12	0.20
91.	Jungle babbler	<i>Argya striata</i> (Lesson, 1831)	0.54	0.51	0.60
Order-Passeriformes, Family-Zosteropidae					
92.	Oriental white eye	<i>Zosterops palpebrosus</i> (Temminck, 1824)	0.06	0.01	0.05
Order-Pelecaniformes, Family-Ardeidae					
93.	Black crowned night heron	<i>Nycticorax nycticorax</i> (Linnaeus, 1758)	-	0.09	-
94.	Cattle egret	<i>Bulbulcus ibis</i> (Linnaeus, 1758)	0.49	0.93	0.35
95.	Eurasian bittern	<i>Botaurus stellaris</i> (Linnaeus, 1758)	-	0.02	0.13
96.	Great egret	<i>Ardea alba</i> (Linnaeus, 1758)	0.34	0.55	0.29
97.	Grey heron	<i>Ardea cinerea</i> (Linnaeus, 1758)	0.12	1.10	0.02
98.	Indian pond heron	<i>Ardeola grayii</i> (Skyes, 1832)	0.47	0.37	0.34
99.	Intermediate egret	<i>Ardea intermedia</i> (Wagler, 1827)	0.19	0.48	0.16
100.	Little egret	<i>Egretta garzetta</i> (Linnaeus, 1766)	0.06	0.11	0.08
101.	Purple heron	<i>Ardea purpurea</i> (Linnaeus, 1766)	0.08	0.61	0.04
102.	Yellow bittern	<i>Ixobrychus sinensis</i> (Gmelin, 1789)	0.30	0.24	0.43
Order-Pelecaniformes, Family-Threskiornithidae					
103.	Glossy ibis	<i>Plegadis falcinellus</i> (Linnaeus, 1766)	0.09	0.91	0.97
104.	Indian black ibis	<i>Pseudibis papillosa</i> (Temminck, 1824)	0.16	0.50	0.49
105.	Black headed ibis	<i>Threskiornis melanocephalus</i> (Latham, 1790)	0.04	0.09	0.03
Order-Piciformes, Family-Capitonidae					
106.	Blue throated barbet	<i>Psilopogon asiaticus</i> (Latham, 1790)	0.04	0.03	0.07
107.	Brown headed barbet	<i>Psilopogon zeylanicus</i> (Gmelin, 1788)	0.03	0.04	0.06
108.	Coppersmith barbet	<i>Psilopogon haemacephalus</i> (Muller, 1776)	0.06	0.05	0.08
Order-Piciformes, Family-Picidae					
109.	Black rumped flameback	<i>Dinopium benghalense</i> (Linnaeus, 1758)	0.07	0.05	0.03

(Table 1 contd.)

(Table 1 contd.)

Order-Podicipediformes, Family-Podicipedidae					
110.	Little grebe	<i>Tachybaptus ruficollis</i> (Pallas, 1764)	1.18	-	0.67
111.	Black necked grebe	<i>Podiceps nigricollis</i> (Brehm, 1831)	0.91	0.26	0.10
Order-Psittaciformes, Family-Psittaculidae					
112.	Slaty headed parakeet	<i>Psittacula himalayana</i> (Lesson, 1832)	0.17	0.11	0.15
113.	Plum headed parakeet	<i>Psittacula cyanocephala</i> (Linnaeus, 1766)	0.13	0.24	0.16
114.	Rose ringed parakeet	<i>Psittacula krameri</i> (Scopoli, 1769)	0.81	0.68	0.77
115.	Alexandrine parakeet	<i>Psittacula eupatria</i> (Linnaeus, 1766)	0.29	0.15	0.16
Order-Suliformes, Family-Anhingidae					
116.	Oriental darter	<i>Anhinga melanogaster</i> (Pennant, 1769)	0.43	0.20	0.23
Order-Suliformes, Family-Phalacrocoracidae					
117.	Indian cormorant	<i>Phalacrocorax fuscicollis</i> (Stephens, 1826)	0.17	1.54	-
118.	Great cormorant	<i>Phalacrocorax carbo</i> (Linnaeus, 1758)	0.33	1.98	-
119.	Little cormorant	<i>Microcarbo niger</i> (Vieillot, 1818)	0.13	3.44	-
Order-Strigiformes, Family-Strigidae					
120.	Indian eagle owl	<i>Bubo bengalensis</i> (Franklin, 1831)	0.01	0.01	-
121.	Spotted owl	<i>Athene brama</i> (Temminck, 1821)	0.01	0.01	-

bird species. However, Mehta (2014) reported 39% of winter migrant and 55% of resident bird species while rest were indigenous species (2013). There was no seasonal variation seen in the resident bird species as they were observed throughout the year, but there was a particular pattern of arrival and departure of migratory birds. The winter migrants started to appear in October when the temperature starts decreasing, elevating the diversity in winter season. Major variation was found in abundance of few species at the three sites of the same wetland. Most of the migratory species recorded were winter visitors only as the maximum abundance was recorded during January. Maximum species richness was in January and minimum in June at all the sites. Highest species diversity was found in during May and lowest during October. Species evenness was maximum during May - July and lowest in October (Table 2). The seasonal variations in environmental factors within the same habitat affect the community structure of birds throughout the year (Xu et al., 2022). Several studies have shown that species richness and abundance of water birds inflates with the emerging vegetation cover in wetlands, especially during breeding periods when water birds are less mobile and more vulnerable to disturbance.

It was found that the Keshopur wetland having great vegetation diversity, is a major habitat site for waterfowl population especially during winters when winter migratory birds reside there. However, it appears that vegetation development affects the composition of the waterfowl breeding population at any wetland (Kristin et al., 1996). Apart from providing food for

herbivorous waterbirds such as seeds, leaves, tubers, and rhizomes, vegetation is a crucial habitat element and significantly influences waterbird habitat usage. The Keshopur wetland provides a great diversity of vegetation for fauna. Emerging plants often provide protection and decrease human interference, which occurs very often at roosting and breeding sites in artificial wetlands (Hattori and Mae, 2001). Dense vegetation often supports invertebrate habitat and food requirements, and increases the viability of eggs or diapausing invertebrates, ultimately increasing their abundance, biomass, and diversity which increases food for water birds (Anderson and Smith, 2000).

The species were not uniformly distributed at the three sites under study area as the majority of winter migratory birds were observed in flocks preferring the ponds away from the road. It was because of more dense aquatic vegetation which protects the birds from severe climatic conditions and predators. Brandolin and Blendinger (2015) also showed in their study that more vegetated ponds provide better shelter to avifauna for their survival. The eurasian coot was most abundant species found at this site. An important pond variable for habitat selection by coots was emerging vegetation, probably because it contributed in protection against aerial predators. As the northern shoveler was one of the most abundant species at the Keshopur a freshwater wetland, the best supporting evidence is from the study of Tietje and Teer, (2015) who observed that freshwater wetlands are of higher quality than saltwater wetlands for wintering shovelers. The flocks of Cormorants were

Table 2. Community structure of birds at three sites (Keshopur wetland)

Month	Site I			Site II			Site III		
	Richness	Diversity	Evenness	Richness	Diversity	Evenness	Richness	Diversity	Evenness
Jun	43.00	3.34	0.89	40.00	3.29	0.89	41.00	3.29	0.89
Jul	59.00	3.45	0.85	55.00	3.59	0.89	60.00	3.72	0.91
Sep	78.00	3.42	0.78	78.00	3.40	0.78	69.00	3.42	0.81
Oct	85.00	2.34	0.53	86.00	2.29	0.51	84.00	2.36	0.53
Nov	89.00	2.79	0.62	81.00	2.62	0.59	95.00	2.97	0.65
Dec	90.00	2.87	0.64	87.00	2.77	0.62	91.00	3.07	0.68
Jan	96.00	2.99	0.65	95.00	3.01	0.66	101.00	3.15	0.68
Feb	91.00	2.94	0.65	90.00	2.98	0.66	99.00	3.18	0.69
Mar	87.00	3.00	0.67	90.00	3.10	0.69	97.00	3.18	0.69
Apr	74.00	3.40	0.79	70.00	3.07	0.72	77.00	3.52	0.81
May	84.00	3.87	0.87	73.00	3.67	0.85	80.00	3.92	0.89

The data for the August was not taken due to rainfall and excessive water logging.

very frequently seen in one pond only near the poplar trees residing on the vegetation protruding from the water. They were also seen utilizing poplar trees for perching in groups and may be for nesting sites also. Previous studies concluded that the higher the nest tree height, the higher the success rate for the breeding (Park et al., 2011). Therefore, the great cormorants may have migrated to areas with higher nesting trees (Lee et al., 2019). Ardeids prefer places where wide areas of wetlands or long banks are located. The number of pheasant tailed jacana was noticed significantly more in lotus vegetation. Pheasant-tailed jacana is always found in reservoirs where the coverage of aquatic vegetation with wide floating leaves is comparatively high. The muddy reservoir beds deliver better foraging grounds for most migratory waders, such as plovers and sandpipers. Such small migratory wading birds are efficiently adapted for feeding on small insects, mollusks, worms, etc. (Henkanaththegedara and Amarasinghe, 2015). Black-winged stilts use a wide range of shallow water wetlands, both for breeding and forage (Pigniczki et al., 2019). They were mostly seen in muddy areas near ponds and shallow water ponds. Northern pintail were found more abundant at this site as compared to the other two sites. Yamaguchi et al. (2012) observed in their study that Northern pintails migratory stop-over sites contained more freshwater wetlands, freshwater lakes and rivers, and other agricultural lands. Shorebirds favoured small mudflats and large bulrush areas over environments with a limited area of high vegetation (Zhenming et al., 2006).

Birds use wetlands as a source of feeding, drinking water, roosting, breeding and social interactions. The richness of avifauna found in present study at Keshopur Chhamb Community Reserve is the magnificent indicator of ecological health. The current condition

of its conservation has shown that Keshopur wetland reduced to about 300 acres of the thousands of acres of land at one point has been restored to about 850 acres by the efforts of forest officials. Present studies conclusively suggest that the habitation of various resident and migratory birds recorded in our study shows that Keshopur wetland is an important habitat for wild birds, which could be used as feeding, breeding, stopover and wintering site by the birds. It acts as a refuge site for many waterbirds including wader, waterfowl and many migratory and threatened species.

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AUTHOR CONTRIBUTION STATEMENT

NV conceptualized the research work and designed the experiments. SJ executed field/lab experiments and collected data. SJ and NV analyzed and interpreted the data. Both authors prepared and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF *Bt* AND NON-*Bt* COTTON ON BIOLOGY OF *THRIPS TABACI* AND ITS MANAGEMENT USING INSECTICIDES

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ABSTRACT

The development of *Thrips tabaci* was studied on transgenic BG I, BG II, non-*Bt*, and *desi* cotton cultivars. The oviposition period was minimum on *desi* cotton, FDK 124 (11.67 days) as compared to BG II (RCH 650) and non-*Bt* cotton (LH 2076). Female longevity was minimum on FDK 124 (17.50 days) followed by RCH 650 (23.17 days) and LH 2076 (21.83 days). Maximum female longevity was recorded on BGI cotton cultivar RCH 308 (26.83 days). Nymphal, pupal period, and total development period were minimum on FDK 124 (8.17, 2.33, and 34.00 days) as compared to *Bt* and non-*Bt* cotton cultivar, LH2076. Based on the higher oviposition period, female longevity, fecundity, and nymphal period, *Bt* cotton hybrids were found to be more favourable for thrips development in comparison to non-*Bt* cotton cultivars and *desi* cotton. The new generation insecticide, spinetoram 11.7SC @ 425g/ha was most effective and also found to be safe to the predators.

Key words: *Thrips tabaci*, *Bt* cotton, biology, insecticides, spinetoran, safety, predators

Cotton (*Gossypium* spp) is the most important commercial crop in India. The major bottleneck in cotton cultivation is the damages caused by bollworm complex and sucking pests. *Bt* cotton was widely adapted in cotton-growing countries globally for the management of the bollworm complex, but it does not provide resistance against sucking pests. As a result of this, nontarget pest insects, with piercing-sucking mouthparts, such as leafhopper, mealybug, thrips, and whiteflies, survive better and emerged as serious pests on *Bt* cotton (Xu et al., 2008; Vennila, 2008). *Thrips tabaci* Lindeman is one such pest of *Bt* cotton causing economic damage in the North-Western zone of India. Earlier, it was known to cause damage during the seedling stage during pre-*Bt* era. However, in 2017, its severe incidence was recorded during vegetative and reproductive stages on various *Bt* cotton hybrids and now it has attained the status of major pest (unpublished data). For its management, chemicals like profenophos 50EC and diafenthiuron 50 WP were used (Anonymous, 2018). It had already attained the status of a regular cotton insect pest in the Punjab province of Pakistan, probably due to overuse of insecticides (Ali et al., 1993). The pertinent literature on the development of *T. tabaci* on *Bt* cotton is not available. A field study on population dynamics showed that *Bt* genotypes were more susceptible than non-*Bt* genotypes (Akram et al., 2013; Jeyakumar et al., 2008). Thrips have a wide host range, reportedly feeding on over 300 plants, under 25 families (Lewis, 1997). No systematic and detailed

study on the comparative development of *T. tabaci* on transgenic *Bt*, non-*Bt* and *desi* cotton has been carried out. The present study was undertaken to find out the development of thrips on transgenic *Bt* and non-*Bt* cotton cultivars and their management on *Bt* cotton.

MATERIALS AND METHODS

The seeds transgenic *Bt* cotton cultivars (*G. hirsutum*) like MRC 6301 (BG I), RCH 308 (BG I), Ankur 3028 (BG II), RCH 650 (BG II), and non *Bt* cotton cultivars like LH 2076 and *desi* cotton cultivar (*G. arboreum*), FDK 124 were sown at weekly intervals in the earthen pots placed in the screen house. Various stages of *T. tabaci* i.e. nymphs and adults were collected from the unsprayed cotton field and released on these potted plants. The adults thus obtained, after confirmation of desired species of thrips (from the documented specimen) were used for raising the culture of *T. tabaci*. The various biological parameters of *T. tabaci* were studied by confining the adults and immature stages in a leaf cage made of a lightweight petri dish measuring 4.8 cm dia. Six circular holes (0.75 cm dia) were made on the sides and one hole (one cm diameter) was made in the centre. One end of the hair clip was placed on cardboard, while the other end was placed above the petri dish, sandwiching the leaf between the leaf cage and cardboard.

The observations on development of *T. tabaci* on transgenic BG I, BG II, non-*Bt* and *desi* cotton cultivars

were carried out under screen house 31.43 ± 0.36 °C RH of $69.67 \pm 3.09\%$. From the maintained culture, five freshly emerged females of *T. tabaci* were taken and released into leaf cages fixed on 40-day-old potted plants of selected cotton genotypes. There were six replications. To record the preoviposition, oviposition, and post oviposition period, the females were transferred daily to a new leaf cage made on the plants of various cultivars until their death. The preoviposition period was considered from the day of emergence of the female to the day when it started laying eggs. The number of days for which egg-laying continued was taken as the oviposition period. The post-oviposition period was observed as the number of days between the last days of oviposition until the death of female. The leaf area was observed daily for the emergence of first instar nymphs. The number of nymphs that emerged from one female represented the fecundity-cum-viability. The nymphs from each female were counted and removed daily at the time of observation. The time between the release of the female and the appearance of the nymphs was considered as the incubation period. Nymphs were observed twice a day to note the change of instars. This process was done till the prepupal stage. The interval between two moultings was taken as the duration of nymphal instars and the period between the time of the release of a freshly hatched nymph and change into the prepupal stage was taken as the total nymphal period. Prepupae were observed twice a day and when the second stage shows sluggish movement, it was considered the beginning of the prepupal stage. The prepupa was somewhat whiteyellowish and that of pupa was dark brownish. The full-length pupa had the antennae folded back over the head and well-developed wing pads. Both the stages showed slight movement when they were disturbed. The female longevity was taken as the period from the adult emergence till death.

Twelve insecticides were evaluated against thrips on *Bt* cotton hybrid, RCH 773. The experiment was repeated four times at two locations (Mansa and Abohar) which are the main cotton growing regions of Punjab, India. There were three replications with treatment being, insect growth regulator (buprofezin 25SC); neonicotinoids (clothianidin 50WDG, imidacloprid 17.8SL, thiamethoxam 25WG and thiacloprid 21.7SC); organophosphate (fipronil 5SC and profenophos 50EC) pyridine carboxamide (dinotefuran 20SG and flonicamid 50WG); synthetic pyrethroids (cypermethrin 25EC) and spinosyn (spinetoram 11.7SC); thiourea derivatives (diafenthiuron 50WP). These were sprayed with a manually operated knapsack sprayer (375e/ ha).

The thrips (nymphs and adults) were recorded from three leaves plant in the upper crop canopy before spray and three, seven, and ten days after spray. The effect on the occurrence of predator plant was also recorded. The seed cotton yield was recorded on a whole plot basis. The means and standard error of biological parameters were computed. The data were statistically analyzed with ANOVA. The means were separated by critical difference (LSD) at $p=0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

There was significant difference in the preoviposition, oviposition, and postoviposition period of *T. tabaci* were recorded on various transgenic BG I, BG II, non-*Bt*, and *desi* cotton cultivars. The preoviposition period was minimum (2.50 days) on BG II cotton cultivar, RCH 650 followed by BG I cotton cultivar MRC 6301 (3.33 days); and oviposition period was minimum on *desi* cotton cultivar, FDK 124 (11.67 days). The post-oviposition period non-significant.

There was a significant difference in female longevity on different cotton cultivars minimum being on the *desi* cotton cultivar, FDK 124 (17.50 days). The maximum female longevity was recorded on RCH 308 (26.83 days) (Fig. 1). Likewise, there was significant difference in fecundity-cum-viability with minimum being on *desi* cotton cultivar, FDK 124 (5.83 days) and maximum on RCH 308 (19.17 days) (Fig. 2). The incubation period varied significant by (Fig. 2), with minimum being on Ankur 3028 (3.17 days) and RCH 308 (3.17 days) and maximum on *desi* cotton cultivar FDK 124 (4.67 days). The difference in the first instar nymphal duration was non-significant (Fig. 2); the second instar nymphal period was minimum on *desi* cotton cultivar FDK 124 (4.33 days) while the total nymphal duration was minimum on *desi* cotton cultivar FDK 124 (8.17 days) and maximum on Ankur 3028 (10.83 days). There was a non-significant difference in the prepupal period on different cotton cultivars (Fig. 2) while there was a significant difference in the pupal period it was significantly lower in FDK 124 (2.33 days) and maximum with Ankur 3028 (3.67 days). Total development period from egg to death of female was on found to be minimum on *desi* cotton cultivar FDK 124 (34.00 days) and maximum with BG I hybrid RCH 308 (40.00 days).

The pooled data of the four field experiments conducted during 2017 and 2018 revealed that the incidence of thrips (nymphs and adults) did not differ significantly in the treatments before spray; and it was

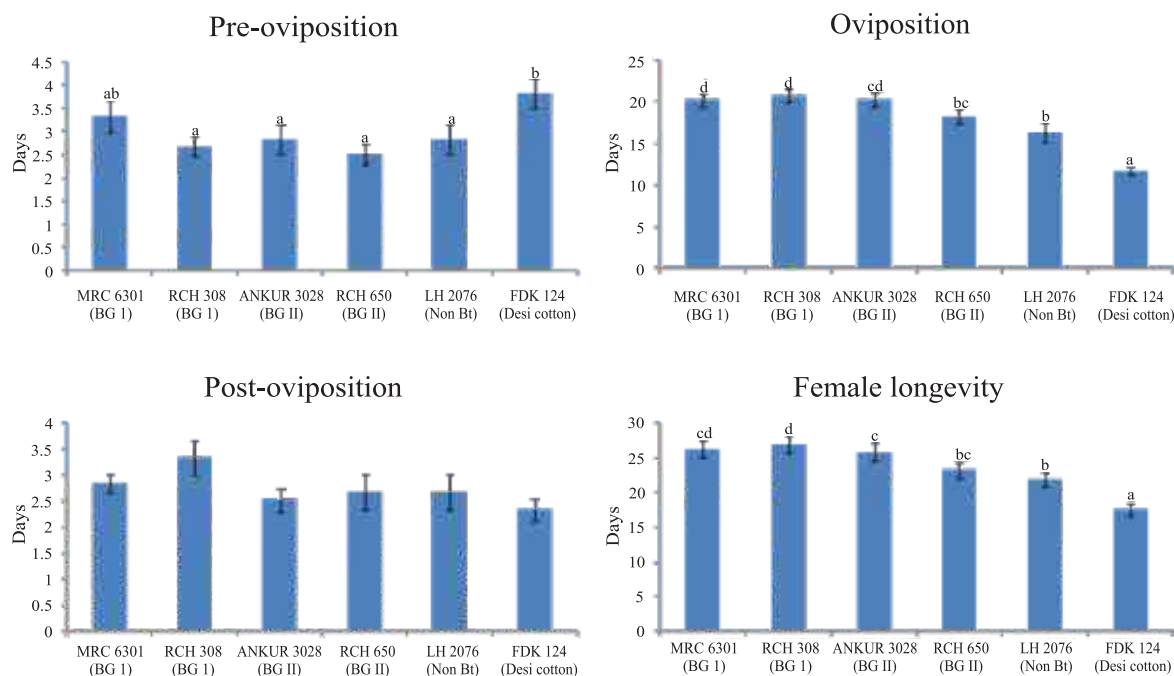


Fig. 1. Effect of various cotton cultivars on oviposition and female longevity of *Thrips tabaci*

adult was significantly less with (spinetoram) 11.7SC i.e. 8.75, 10.42, and 10.16 three leaves followed by standard check profenophos 50EC (11.60, 18.89 and 35.92) and fipronil 5 SC (19.82, 24.45 and 35.51) after 3, 7 and 10 days of application, respectively (Table 1). Seed cotton yield of *Bt* cotton was significantly higher in spinetoram (26.34 q/ha) followed by buprofezin 25SC (24.91 q/ha) (Table 1). The reduction in the population of natural enemies including coccinellids beetle, chrysopa, and spiders was recorded almost in all the treatments except control after three days of spray. After seven and ten days of spray, their occurrence higher in spinetoram 11.7SC being at par with diafenthiuron 50WP (Table 2).

During 2017, an outbreak of thrips *T. tabaci* was recorded in Punjab and Haryana and for its sustainable IPM, host plant resistance plays a vital role. It is imperative to understand its development on transgenic BG I, BG II, non-*Bt* and *desi* cotton cultivars. The present study revealed that its oviposition period, fecundity and female longevity were minimum on *desi* cotton cultivars and maximum on transgenic BG I, BG II, and non-*Bt* cotton cultivars. The maximum female longevity was on BG I cotton cultivar. These results revealed that female of thrips not only oviposit more on *Bt* cotton but also survive longer. Transgenic plants might possess certain biophysical and biochemical characteristics which led to more survival and

oviposition in comparison to non-*Bt* cotton cultivars. A field study on population dynamics showed that *Bt* genotypes found more susceptible for thrips than non-*Bt* genotypes (Akram et al., 2013; Jeyakumar et al., 2008). Salas et al. (1993) and Arrieche et al. (2006) reported the oviposition period of 19.50 days 34.5°C and 65% RH on onion. Present results corroborate the findings of Patel et al. (2013) who reported the oviposition period of 22.98 days on onion. The present study revealed that female longevity was minimum on *desi* cotton cultivar, FDK 124 whereas it was maximum on transgenic BG I cotton Lall and Singh (1968) also reported that female longevity was of 20.1 and 20.2 days on onion at 23.4 °C and 30.8 °C; Patel et al. (2013) reported this as 27.97 days on onion. Salas et al. (1993) and Guzmán et al. (1996) reported adult longevity of 21.5 at 32 °C. Arrieche et al. (2006) reported it as 11.5 days on onion.

Nymphal period was maximum on BGII cotton cultivar, Ankur 3028 and minimum on *desi* cotton cultivar. The present findings agree with those of Fekrat et al. (2009) and Changela (1993), and in contrast with that of Lall and Singh (1968) on onion. The pupal duration was significantly lower on *desi* cotton cultivar whereas it was higher on BG II cotton cultivar. Patel et al. (2013) reported a pupal period of 3.56 ± 0.50 days. Lall and Singh (1968) also recorded pupal period of 2.4 days and Salas et al. (1993) recorded the prepupal and pupal periods of 1.17 and 2.4 days, respectively.

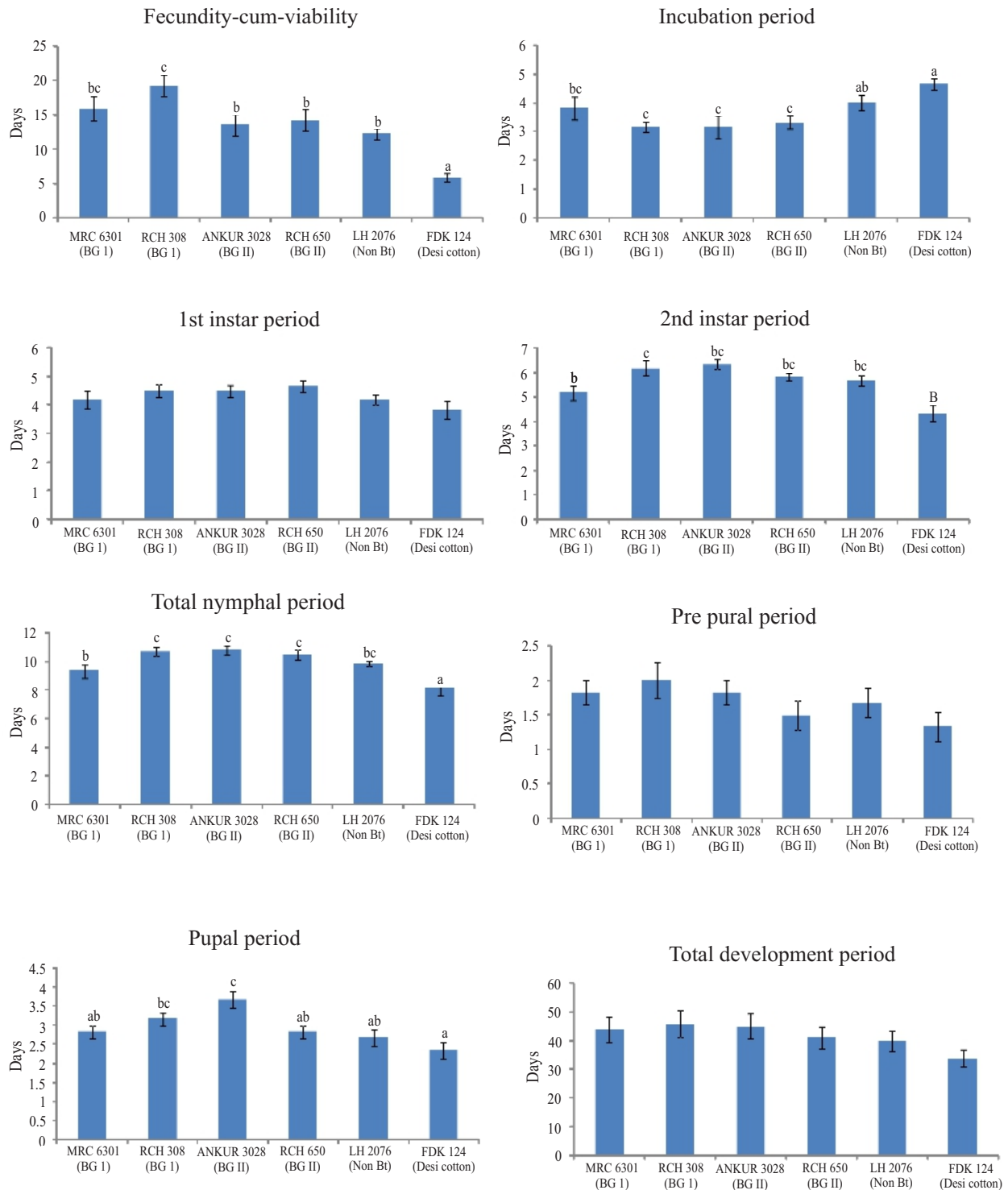


Fig. 2. Effect of various cotton cultivars on fecundity and development period of various stages of *Thrips tabaci*

Similarly, the total development period was minimum on *desi* cotton cultivar and maximum on transgenic BG I cultivar. These results corroborate with those of Patel et al. (2013) on onion; in contrast Salas (1994) reported it as 12.03 days from egg to adult. Patel et al. (2013) observed incubation period of 4.52 days on

onions, and 4.00 to 4.33 days was reported by Salas (1994). Lall and Singh (1968) recorded an egg period of 9.8 days on onion.

Spinetoram 11.7SC was observed to be the most effective insecticide. Among the convention insecticides

Table 1. Efficacy of insecticides against *T. tabaci*

Treatment	Dose (ml/ g / ha)	Population of thrips/ 3 leaves				Seed cotton yield (q/ ha)
		Before spray	3 DAS	7 DAS	10 DAS	
Buprofezin 25SC	1000	59.79	37.49 (6.17)	39.82 (6.36)	47.63 (6.93)	24.91
Clothianidine 50WDG	50	61.02	57.92 (7.63)	64.81 (8.07)	68.62 (8.31)	23.33
Cypermethrin 25EC	120	63.39	44.81 (6.72)	34.38 (5.95)	38.05 (6.25)	23.33
Diafenthiuron 50WP	500	62.67	20.98 (4.66)	27.17 (5.24)	35.43 (5.91)	23.46
Spinetoram 11.7SC	420	65.30	8.75 (3.11)	10.42 (3.36)	10.56 (3.36)	26.34
Dinotefuran 20SG	150	66.59	70.08 (8.38)	74.16 (8.63)	78.95 (8.90)	23.28
Fipronil 5SC	2000	64.79	19.82 (4.55)	24.45 (4.98)	35.51 (6.01)	22.64
Flonicamid 50WG	200	59.07	54.79 (7.44)	56.14 (7.55)	61.04 (7.86)	24.06
Imidacloprid 17.8SL	100	63.80	43.54 (6.60)	45.74 (6.80)	57.28 (7.61)	23.19
Profenofos 50EC	1250	60.34	11.60 (3.55)	18.89 (4.39)	35.92 (6.03)	23.88
Thiamethoxam 25WG	100	66.41	65.69 (7.93)	50.96 (7.18)	54.23 (7.42)	23.84
Thiacloprid 21.7SC	125	59.37	56.35 (7.54)	58.09 (7.68)	60.95 (7.87)	23.92
Control	--	63.96	66.77 (8.20)	78.72 (8.89)	82.14 (9.10)	19.95
LSD (p= 0.05)		NS	(0.82)	(0.52)	(0.60)	2.08

Mean of three replications; Figures in parenthesis square root transformation; DAS: days after spray Data subjected to ANOVA and means separated (LSD, p= 0.05)

Table 2. Safe insecticides to predators in cotton

Treatment	Dose (ml/ g/ ha)	Population of predators/ plant			
		Before spray	3DAS	7DAS	10DAS
Buprofezin 25SC	1000	3.96	3.19	3.08	3.66
Clothianidine 50WDG	50	3.66	2.53	3.23	3.99
Cypermethrin 25EC	120	4.35	2.33	3.03	3.82
Diafenthiuron 50WP	500	4.04	3.23	3.44	3.79
Spinetoram 11.7SC	420	5.03	3.29	3.48	4.11
Dinotefuran 20SG	150	4.16	2.51	3.23	3.79
Fipronil 5SC	2000	4.17	1.90	2.87	3.16
Flonicamid 50WG	200	3.93	2.35	3.01	3.78
Imidacloprid 17.8SL	100	4.65	2.04	2.96	3.41
Profenofos 50EC	1250	4.33	1.93	2.75	3.25
Thiamethoxam 25WG	100	4.48	2.71	2.79	3.37
Thiacloprid 21.7SC	125	3.80	2.42	3.14	3.76
Control	--	4.95	5.50	5.54	5.82
LSD (p=0.05)		NS	1.21	0.75	0.97

Mean of three replications; Predators include coccinellids, chrysopa and spiders

synthetic pyrethroids cause resurgence of whitefly on *Bt* cotton (Abdullah et al., 2006; Virk et al., 2004). The predators natural enemies decreased with insecticide treatments but spinetoram 11.7 SC proved to be more safe to the predators.

Thus, the present study on the development of *T. tabaci* on transgenic BG I, BG II and non-*Bt* cotton cultivars revealed that the oviposition period, female longevity and nymphal period was minimum on *desi* cotton and maximum female longevity and nymphal period was on *Bt* cotton cultivar. Therefore, transgenic

BG II and BG I cultivars are more favourable. Also, pupal duration was significantly lower on *desi* cotton cultivar whereas it was higher on BG II cotton cultivar Ankur 3028. Thus, *T. tabaci* took minimum time to develop on *desi* cotton cultivar. Among the insecticides evaluated on *Bt* cotton spinetoram 11.7SC @ 425 g/ ha was effective in reducing thrips and safe to predators.

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AUTHOR CONTRIBUTION STATEMENT

VK: Conceptualized, planned the work and corrected manuscript, AK: conducted the experiment, data analysis, VJ: data analysis, writing of manuscript

CONFLICT OF INTEREST

No conflict of interest.

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SEASONAL INCIDENCE OF INSECT PESTS AND PREDATORY FAUNA IN BLACK GRAM

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ABSTRACT

The succession and seasonal incidence of insect pests and predatory fauna in black gram were studied in a field experiment at the Agronomy farm, Anand Agricultural University, Anand during kharif 2019 and 2020. The results revealed nine species of insects (7 pests and 2 predators) from seedling to pod maturity stage. Besides, the peak incidence of whitefly, jassid, flower thrips, tobacco caterpillar, Bihar hairy caterpillar, and spiders were observed during 37th standard meteorological week (SMW); with spotted pod borer and coccinellids, maximum incidence was observed during 38th SMW, and the green stink bug during 35th SMW. Correlation coefficients revealed that tobacco caterpillar had a significant positive correlation with minimum temperature, morning RH and evening vapour pressure; and the incidence of spotted pod borer showed a significant negative correlation with bright sunshine hours and rainfall.

Key words: *Vigna mungo*, whitefly, flower thrips, jassid, tobacco caterpillar, Bihar hairy caterpillar, predators, weather factors, incidence, correlation coefficients

Black gram *Vigna mungo* is an important short duration pulse crop and India is the largest producer (Anonymous, 2020). Its productivity is limited by insect pests (Jat et al. 2017), with losses due to sucking pests, defoliators and pod borers ranging from 25.9-67.8% (Justin et al., 2015). Among the sucking pests, the whitefly, a potential vector of mung bean yellow mosaic virus causes a substantial loss of 30-70% (Duraimurugan and Tyagi, 2014). It is necessary to study the population dynamics of these pests along with their predators as has been done earlier (Prasad et al. 2005; Kumar and Singh, 2016; Mohapatra et al. 2018; Yadav et al. 2020). This study analyses the incidence of pests of black gram under middle Gujarat climatic conditions.

MATERIALS AND METHODS

The field experiment was conducted for two consecutive kharif seasons (2019 and 2020) at the Agronomy Farm, B A College of Agriculture, Anand Agricultural University, Anand. Variety T-9 was selected and sown in an area of 200 m² with a spacing of 45 cm row to row and 10 cm plant to plant. For observations on insect pests and predators, the plot was divided into six sectors, with 10 plants/ sector selected randomly. Observations were recorded at weekly intervals starting from one week after germination till crop maturity. All recommended agronomical practices

were followed to raise the crop. Observations on the incidence of whitefly *Bemisia tabaci* (Gennadius) and jassid *Empoasca kerri* Singh-Pruthi were recorded from three leaves (upper, middle and lower)/ plant; thrips *Megalurothrips usitatus* Bagnall were counted from 10 flowers/ plant at the flowering stage; green stinkbugs *Nezara viridula* (L) were counted from 10 plants; for tobacco caterpillar *Spodoptera litura* (F) and Bihar hairy caterpillar *Spilarctia obliqua* (Wlk.) larvae/ plant were counted from one week after germination and continued till the removal of crop; and for spotted pod borer *Maruca vitrata* Geyer, it was similar to the above two larvae but was recorded from the initiation of flowering and continued till harvest. Similarly, the predators viz., spiders and coccinellids (grub and adult) i.e., *Coccinella transversalis* F were counted from germination till harvest. Weekly weather data was obtained from the Department of Meteorology, Anand Agricultural University, Anand. The correlation coefficients between the incidence of insect pests/ predators and weather factors were calculated.

RESULTS AND DISCUSSION

Table 1 provides the details of incidence of insect pests and their predators in black gram. These reveal the occurrence of *B. tabaci*, *E. kerri*, *S. litura* and *S. obliqua* at the seedling stage i.e., at 15 days after sowing (DAS); and these continued up to pod maturity; 25

DAS, *N. viridula* was observed from vegetative to pod formation stage, and it was followed by *M. usitatus*, first observed during flowering stage at 35 DAS and remained in the field up to pod formation (60 DAS); *M. vitrata* appeared with flowering stage and remained till pod maturity (80 DAS). The incidence of all these revealed that these can be categorized as major except for *N. viridula*. These observations corroborate with those of Duraimurugan and Tyagi (2014) and Yadav et al. (2015). The incidence of *B. tabaci* commenced from 31st standard meteorological week (SMW) and persisted till 41st SMW, with peak (8.05 whitefly/ leaf) being during 37th SMW. Sneha et al. (2016), Singh et al. (2017) and Duraimurugan and Tyagi (2014) observed its peak incidence during 37th SMW. Correlation coefficient of its incidence with weather factors revealed that evapotranspiration ($r = -0.587^*$) showed significant negative correlation while, bright sunshine ($r = -0.371$), rainfall ($r = -0.221$), wind speed ($r = -0.174$) and maximum temperature ($r = -0.365$) showed negative correlation; minimum temperature ($r = 0.559$), morning RH ($r = 0.538$), evening RH ($r = 0.478$), morning VP ($r = 0.333$) and evening VP ($r = 0.565$) showed positive correlation. These results are corroborated by Singh and Kumar (2011), Patidar (2015) and Yadav et al. (2015).

The activity of *E. kerri* commenced from 32nd SMW and continued till 41st SMW, with a peak population of 7.76 jassids/ leaf during 37th SMW. Sneha et al. (2016) and Sarode et al. (2003) also observed similar incidence. Correlation coefficients of its incidence with weather factors revealed that evapotranspiration ($r = -0.492$), rainfall ($r = -0.334$), wind speed ($r = -0.287$) and maximum temperature ($r = -0.222$) showed negative relationship; however, bright sun shine ($r = 0.247$), minimum temperature ($r = 0.510$), morning RH ($r = 0.445$), evening RH ($r = 0.359$), morning VP ($r = 0.264$) and evening VP ($r = 0.479$) showed a positive one. These results agree with those of Kundu et al. (2021), and are contrary with those of Mohapatra et al. (2018). Thrips incidence was observed from 35th SMW to 40th SMW, with a peak of (9.31 thrips/ flower) during 37th SMW. These results are in accordance with Kumar and Singh (2016) and Sujatha and Bharpoda (2017). Correlation coefficients showed that evapotranspiration ($r = -0.435$), bright sun shine ($r = -0.142$), rainfall ($r = -0.494$), wind speed ($r = -0.434$) and maximum temperature ($r = -0.059$) showed negative relationship with its incidence; and minimum temperature ($r = 0.437$), morning RH ($r = 0.333$), evening RH ($r = 0.247$), morning VP ($r = 0.216$) and evening VP ($r = 0.400$) showed a positive one. Kumar and Singh (2016) also noticed that morning and evening

RH showed a non-significant positive correlation; while Sujatha and Bharpoda (2017) observed that rainfall and wind speed were negatively associated, and minimum temperature positively correlated. The incidence of *N. viridula* commenced from 32nd SMW and through 40th SMW, with peak being at 35th SMW, agreeing with the results of Sujatha and Bharpoda (2017). Correlation coefficients revealed that evapotranspiration ($r = -0.451$), bright sun shine ($r = -0.322$), rainfall ($r = -0.119$), wind speed ($r = -0.049$) and maximum temperature ($r = -0.411$) showed negative association; while minimum temperature ($r = 0.494$), morning RH ($r = 0.570$), evening RH ($r = 0.462$), morning VP ($r = 0.322$) and evening VP ($r = 0.525$) showed a positive one. Sujatha and Bharpoda (2017) concluded that minimum temperature, morning VP and evening VP had a positive relationship.

The initial incidence of tobacco caterpillar started from 31st SMW and remained till 40th SMW which ranged from 0.77 to 0.66 larvae/ plant (Table 1). The activity considerably increased from 32nd SMW with peak (4.58 larvae/ plant) during 37th SMW. Yadav et al. (2015) and Kumar et al. (2007) reported that its incidence occurred 2 weeks after germination and attained peak during the 6th week after germination. Sneha et al. (2016) also reported a peak incidence during 38th SMW in black gram. The correlation coefficient between incidence and weather factors indicate that evapotranspiration ($r = -0.651^*$) had significant negative association. In contrast, minimum temperature ($r = 0.585^*$), morning RH ($r = 0.619^*$) and evening VP ($r = 0.56^*$) had a significant positive correlation. Kurly and Singh (2021) observed that leaf-eating caterpillar showed a significant positive correlation with minimum temperature and rainfall had a negative and non-significant impact. Mohapatra et al. (2018) reported that the *S. litura* population is positively correlated with temperature and RH.

With *S. obliqua* activity was first noticed from 31st SMW and persisted till 41st SMW and increased from 33rd SMW and attained a peak (11.67 larvae/ plant) in 37th SMW. The present finding is in agreement with that of Sujayanand et al. (2019) on pigeonpea at Uttar Pradesh. The present findings are in agreement with Mohapatra et al. (2018); Patel et al. (2020) and Sujatha and Bharpoda (2017). Correlation coefficients revealed that evapotranspiration ($r = -0.518$), bright sun shine ($r = -0.333$), rainfall ($r = -0.240$), wind speed ($r = -0.211$) and maximum temperature ($r = -0.280$) showed negative correlation; while minimum temperature ($r = 0.512$), morning RH ($r = 0.478$), evening RH ($r = 0.438$),

morning VP ($r=0.300$) and evening VP ($r= 0.525$) showed positive correlation Kurly and Singh (2021) and Berani et al. (2017) observed a positive correlation with minimum temperature and morning and evening RH but a negative correlation with rainfall.

The incidence of spotted pod borer commenced from 35th SMW and sustained up to 41st SMW, with peak population of during 38th SMW. Patel and Borad (2016) and Sneha et al. (2016) reported its peak activity during 37th and 38th SMW.

Correlation coefficients revealed that bright sun shine ($r= -0.695^*$) and rainfall ($r= -0.666^*$) showed a significant negative correlation. Sneha et al. (2016) reported that evening RH and rainfall are negatively associated. Sravani et al. (2015) observed that the pest showed a positive association with both maximum and minimum temperature.

The activity of coccinellids commenced during 33rd SMW and remained up to 40th SMW (Table 1), with a peak observed during 38th SMW. Yadav et al. (2015) and Sujatha and Bharpoda (2017) reported that the peak occurrence was during the 37th SMW. Correlation indicated that evapotranspiration ($r= -0.339$), bright sun shine ($r= -0.236$), rainfall ($r= -0.221$), wind speed ($r= -0.309$) had negative association whereas maximum temperature ($r= -0.507$), minimum temperature ($r= 0.489$), morning RH ($r= 0.454$) and evening RH ($r= 0.299$), morning VP ($r=0.545$) and evening VP ($r= 0.571$) had positive association. These findings are in close proximity with those of Sravani et al. (2015) on RH.

Similarly, the activity of spiders commenced during 33rd SMW with a peak during 37th SMW. Swathi et al. (2018) reported a peak in black gram during the

Table 1. Population dynamics of insect pests and their predators in black gram (kharif, 2019, 2020)

SMW	Incidence of insect pests							No. of predators/ plant	
	Whitefly/ leaf	Jassid/ leaf	Flower thrips/ flower	Green stink bug/ plant	Leaf eating caterpillar larvae/ plant	Bihar hairy caterpillar larvae/ plant	Spotted pod borer larvae/ plant	Coccinellids (grub + adult)	Spider
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31	0.91	1.33	0.00	0.00	0.77	1.02	0.00	0.00	0.00
32	2.72	2.45	0.00	0.13	1.93	2.80	0.00	0.16	0.03
33	3.71	3.11	0.00	0.26	2.57	5.00	0.00	0.23	0.13
34	4.57	3.67	2.69	0.29	3.80	6.10	0.92	0.37	0.21
35	5.69	4.88	6.15	0.51	4.05	9.76	3.14	0.46	0.13
36	7.40	7.59	9.01	0.33	4.58	10.54	4.00	0.52	0.22
37	7.43	7.76	9.31	0.38	4.02	10.91	6.10	0.60	0.21
38	6.29	6.09	7.23	0.27	3.02	8.84	4.28	0.56	0.19
39	4.21	4.37	5.25	0.17	2.60	3.38	4.24	0.26	0.12
40	1.34	2.82	1.31	0.05	0.66	1.26	3.80	0.07	0.04
41	0.00	0.00	0.00	0.00	0.00	0.97	1.03	0.00	0.00

Correlation coefficients

Weather parameters	Whitefly	Jassid	Flower thrips	Green stink bug	Leaf eating caterpillar	Bihar hairy caterpillar	Spotted pod borer	Predators	
								Coccinellids (grub and adult)	Spider
Evapotranspiration, mm (EP)	-0.587*	-0.492	-0.435	-0.451	-0.651*	-0.518	0.264	-0.339	-0.340
Bright Sunshine, h/ day (BSS)	-0.371	0.247	-0.142	-0.322	-0.445	-0.333	-0.695*	-0.236	-0.131
Rainfall, mm (RF)	-0.221	-0.334	-0.494	-0.119	-0.117	-0.240	-0.666*	-0.221	-0.148
Wind Speed, km/ h (WS)	-0.174	-0.287	-0.434	-0.049	-0.002	-0.211	0.274	-0.309	-0.435
Maximum Temperature, °C (MaxT)	-0.365	-0.222	-0.059	-0.411	-0.512	-0.280	0.104	0.507	0.443
Minimum Temperature, °C (MinT)	0.559	0.510	0.437	0.494	0.585*	0.512	0.019	0.489	0.466
Morning Relative Humidity, % (RH1)	0.538	0.445	0.333	0.570	0.619*	0.478	-0.138	0.454	0.461
Evening relative humidity, % (RH2)	0.478	0.359	0.247	0.462	0.549	0.438	-0.113	0.299	0.260
Morning vapour pressure, mm in Hg (VP1)	0.333	0.264	0.216	0.322	0.381	0.300	0.044	0.545	0.483
Evening vapour pressure, mm in Hg (VP2)	0.565	0.479	0.400	0.525	0.596*	0.525	0.123	0.571	0.482

* Significant at $p=0.05$; SMW= Standard Meteorological week

50th SMW. Correlation coefficients indicated that evapotranspiration ($r = -0.340$), bright sun shine ($r = -0.131$), rainfall ($r = -0.148$), wind speed ($r = -0.435$) showed negative correlation whereas maximum temperature ($r = 0.443$), minimum temperature ($r = 0.466$), morning RH ($r = 0.461$) and evening RH ($r = 0.260$), morning VP ($r = 0.483$) and evening VP ($r = 0.482$) had positive association. These findings are in agreement with those of Bharathimeena et al. (2008).

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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MORPHOMETRICS OF INTRASPECIFIC POPULATIONS OF STINGLESS BEE *TETRAGONULA IRIDIPENNIS* SMITH FROM SOUTHERN TAMIL NADU

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ABSTRACT

Stingless bees also referred to as meliponine bees and morphological characteristics of these differ depending on are the species and environmental kinds. The current study explores morphology and morphometrics of *Tetragonula iridipennis* Smith in Tamil Nadu, India. The morphometrics of worker bees were compared over locations. The bees captured in the Kolli hills were significantly larger than those collected elsewhere. The mean value of head length (HL) (1.43 mm), head width (HW) (1.68 mm), antennal length (AL) (1.98 mm), hind leg length (HLL) (3.54 mm) and forewing length (FL) (3.84 mm) were recorded. The results revealed variance of four principal components (PC) reaching 82.4%, as (PC1) 38.58%, (PC2) 20.95%, (PC 3) 19.75% and (PC4) 8.10%. The characters of FL, HW, FW (forewing width), HLL, AL, OOD (ocello ocular distance), HTW (hind tibial width) and HL were the most discriminative in the principal component analysis.

Key words: Stingless bee, *Tetragonula iridipennis*, worker bees, morphometry, head length, hind leg length, forewing length, principal component analysis

Stingless bees are widely known as dammar bees and its native to eight described species in Indian subcontinent with the exception of higher elevations or the drier interior regions. (Rasmussen, 2013). *Tetragonula iridipennis* Smith has been identified as the dominant stingless bee species in Tamil Nadu (Swaminathan, 2000). Previous research on stingless bees has also been dealt with biology and foraging behaviour (Roopa, 2002), (Kuberappa et al., 2005) taxonomy and morphology (George, 1934; Sakagami 1978; Jobiraj and Narendran 2004; Danaraddi et al., 2012), biology and nesting behaviour (Danaraddi et al., 2009). The distribution of *T. iridipennis* has been documented in Kerala by Mohan and Devanesan (1999). One of the most extensively used classification systems for this taxa is morphometric taxonomy. Previously, stingless bees were classified based on their body size, number of hamuli, forewing, hindwing, and cephalic features. Stingless bees can be differentiated from corbiculate Apinae by combination of reduced fore wing venation with the presence of a jugal lobe on the hind wing (Rahman et al., 2013). Studies on the species diversity of *Tetragonula* in various regions of India are scarce. There is a lack of research findings regarding the diversity of stingless bee species in India, especially

in southern districts of Tamil Nadu. In this research, the morphological and morphometric parameters of stingless bees were examined in different topographic regions of Southern Tamil Nadu.

MATERIALS AND METHODS

For the present study, twenty bee specimens were collected from 10 topographic locations viz., Alagar kovil 10.07' N, 78.21'E, Madurai 9.96' N, 78.20' E, Megamalai 9.64'N, 77.40'E, Kadamalaigundu 9.53' N, 77.29' E, Kolli hills 11.24'N, 78.33' E, Erumapatti 11.15'N, 78.29' E, Sirumalai 10.19' N, 77.99'E, Natham 10.50' N, 78.10'E, Kodaikanal 10.29'N, 77.71'E and Batlagundu 10.16'N, 77.75'E. These specimens were preserved as dry and wet in 70% alcohol. The preserved specimens were treated with relaxing fluid (75% alcohol 106 ml, distilled water 98 ml, benzene 14 ml and ethyl acetate 38 ml.) for 2-4 hr. Using a Leica M 205 C stereozoom microscope preserved bees were dissected and fourteen important morphometric measurements were recorded viz. head length (HL), head width (HW), distance between two lateral ocelli (DBO), ocello-ocular distance (OOD), antennal length (AL), hind leg length (HLL), hind tibial length (HTL), hind

tibial width (HTW), hind basitarsus length (HBL), hind basitarsus width (HBW), fore wing length (FL), fore wing width (FW), length of marginal cell (LMC) and number of hamuli in hind wing (NH). Some attributes were represented numerically, while morphometric characters of lengths and widths were measured in mm and a data matrix with 560 measurements was created. The morphological characters were measured as described by Rasmussen (2013). To detect population diversity within *T. iridipennis*, the data was subjected to ANOVA, factor analysis, principal components analysis, and discriminant function analysis using SPSS 21.0 statistical package.

RESULTS AND DISCUSSION

The characters measured in the head region included were head length (HL), head width (HW), distance between two lateral ocelli (DBO), ocello-ocular distance (OOD) and antennal length (AL) (Table 1). The head length (HL) was varied from 1.21 mm (Natham) to 1.46 mm (Kolli hills). Head width (HW) ranged between 1.54 mm (Madurai and Batlagundu) and 1.68 mm in (Kolli hills). These findings agree with those of Danaraddi (2007), who found that the width of the head including eyes in *T. iridipennis* ranged from 1.52 to 1.61 mm. Rasmussen (2013) reported that head length and width were 1.30 and 1.60 mm, respectively in *T. iridipennis*, 1.32 and 1.66 mm in *T. ruficornis*, 1.32 and 1.52 mm in *T. praeterita*, and 1.34 and 1.70 mm in *T. bengalensis*, while they were 1.1 and 1.24 mm in *T. iridipennis* respectively (Rahman et al., 2013). The distance between two lateral ocelli length (DBO) was 0.43 mm at Alagar Kovil location and 0.62 mm at Erumapatti. The ocello-ocular distance (OOD) was 0.20 mm in Alagar kovil, Natham and Batalagundu, while in Kolli hills, it was 0.29 mm. Tej et al. (2017) reported that the mean value of DBO ranged from 0.32 to 0.41 mm of two locations; and Efin et al. (2019) recorded a OOD value 0.28 mm which are comparable. The mean antennal length (AL) of the bees collected in Madurai and Kodaikanal was 1.66 and 1.98 mm in Kolli hills which is in line with the findings of Sharma et al. (2023) which was 1.89 mm.

The characters assessed in the thoracic region were hind leg length (HLL), hind tibial length (HTL), hind tibial width (HTW), hind basitarsus length (HBL), hind basitarsus width (HBW), fore wing length (FL), fore wing width (FW), length of marginal cell (LMC), and number of hamuli in hind wing (NH) (Table 1). The hind leg length (HLL) was greatest in the samples

Table 1. Morphometrics of *T. iridipennis*

Locations/ Characters	Alagarkovil	Madurai	Sirumalai	Natham	Megamalai	Kadamalai gundu	Kodaikanal	Batlagundu	Kolli Hills	Eruma patti	Total mean
HL	1.39± 0.014 ^b	1.29± 0.014 ^d	1.22± 0.005 ^e	1.21± 0.010 ^e	1.31± 0.006 ^{cd}	1.30± 0.005 ^{cd}	1.39± 0.014 ^b	1.31± 0.019 ^{cd}	1.46± 0.014 ^a	1.33± 0.010 ^c	1.32± 0.075
HW	1.59± 0.018 ^d	1.54 ± 0.01 ^e	1.60± 0.005 ^{cd}	1.63± 0.019 ^b	1.58± 0.008 ^d	1.58± 0.009 ^d	1.59± 0.013 ^d	1.54± 0.017 ^e	1.68± 0.009 ^a	1.62± 0.012 ^{bc}	1.59± 0.041
DBO	0.43± 0.014 ^f	0.51± 0.01 ^e	0.61± 0.019 ^{ab}	0.61± 0.019 ^{ab}	0.56± 0.000 ^d	0.51± 0.010 ^e	0.59± 0.000 ^{bc}	0.57± 0.009 ^{cd}	0.60± 0.008 ^{ab}	0.62± 0.005 ^a	0.56± 0.059
OOD	0.20± 0.00 ^d	0.22± 0.008 ^e	0.25± 0.000 ^b	0.20± 0.005 ^d	0.23± 0.005 ^c	0.25± 0.000 ^b	0.23± 0.005 ^c	0.20± 0.000 ^d	0.29± 0.008 ^a	0.23± 0.008 ^c	0.23± 0.028
AL	1.66± 0.046 ^d	1.73± 0.009 ^e	1.75± 0.000 ^c	1.64± 0.000 ^d	1.84± 0.008 ^b	1.76± 0.006 ^c	1.66± 0.014 ^d	1.67± 0.010 ^d	1.98± 0.005 ^a	1.73± 0.009 ^c	1.74± 0.102
HLL	3.50b± 0.041 ^e	3.23± 0.005 ^e	3.40± 0.005 ^d	3.50± 0.006 ^c	3.53± 0.008 ^b	3.40± 0.009 ^d	3.43± 0.013 ^d	3.42± 0.028 ^d	3.54± 0.009 ^a	3.52± 0.009 ^{ab}	3.44± 0.092
HTL	1.41± 0.027 ^b	1.27± 0.008 ^e	1.28± 0.014 ^e	1.46± 0.006 ^c	1.41± 0.005 ^b	1.32± 0.009 ^d	1.41± 0.014 ^b	1.35± 0.000 ^e	1.36± 0.009 ^e	1.46± 0.012 ^a	1.37± 0.067
HTW	0.50± 0.014 ^{bc}	0.39± 0.000 ^e	0.52± 0.000 ^a	0.49± 0.005 ^{bc}	0.52± 0.014 ^a	0.45± 0.005 ^d	0.50± 0.008 ^{bc}	0.49± 0.005 ^e	0.51± 0.008 ^{ab}	0.46± 0.008 ^d	0.48± 0.039
HBL	0.52± 0.000 ^a	0.50± 0.005 ^b	0.52± 0.000 ^a	0.49± 0.005 ^b	0.50± 0.008 ^b	0.53± 0.000 ^a	0.52± 0.014 ^a	0.45± 0.005 ^e	0.47± 0.005 ^c	0.49± 0.005 ^b	0.50± 0.025
HBW	0.23± 0.000 ^d	0.25± 0.000 ^{bc}	0.25± 0.008 ^{bc}	0.25± 0.000 ^{bc}	0.28± 0.005 ^a	0.26± 0.008 ^b	0.23± 0.008 ^d	0.24± 0.005 ^{cd}	0.26± 0.008 ^b	0.28± 0.005 ^a	0.25± 0.017
FL	3.45± 0.087 ^{bc}	3.30± 0.027 ^d	3.45± 0.035 ^{bc}	3.40± 0.008 ^c	3.51± 0.048 ^b	3.38± 0.013 ^c	3.45± 0.017 ^{bc}	3.30± 0.008 ^d	3.84± 0.013 ^a	3.46± 0.005 ^{bc}	3.46± 0.147
FW	1.51± 0.000 ^b	1.33± 0.027 ^f	1.41± 0.036 ^{cd}	1.40± 0.014 ^{de}	1.41± 0.017 ^{cd}	1.36± 0.014 ^{ef}	1.51± 0.018 ^b	1.32± 0.027 ^f	1.56± 0.009 ^a	1.45± 0.005 ^c	1.43± 0.080
LMC	1.23± 0.027 ^{bcd}	1.18± 0.009 ^{de}	1.22± 0.009 ^{cd}	1.21± 0.027 ^{de}	1.25± 0.014 ^{ab}	1.20± 0.009 ^{de}	1.23± 0.008 ^{bcd}	1.19± 0.009 ^{de}	1.29± 0.009 ^a	1.23± 0.014 ^{bcd}	1.22± 0.032
NH	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000	5± 0.000

Values expressed as mean (mm) ± standard deviation; Different superscript letters in same line denote significant differences ($p < 0.05$).

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CONFLICT OF INTEREST

No conflict of interest.

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FORAGING DURATION AND INTENSITY OF *APIS MELLIFERA* L.

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ABSTRACT

An experiment was conducted in the apiary of Uttar Banga Krishi Viswavidyalaya, Pundibari during 2019. For study, six strong *Apis mellifera* L. colonies of similar strength were used. A positive and significant correlation of temperature (maximum and minimum) and relative humidity (minimum) with the foraging duration was found. During the cooler months both foraging duration and foraging intensity was minimum and increased later on. Foraging activity was highly affected by rainfall. The mean foraging duration of foragers throughout the year was 11.85 ± 1.79 hrs/ day. The 25th standard week was recorded with maximum foraging duration, viz. 14.22 hrs. Foraging intensity was low during initial hours but increased in later hours. Foraging intensity was very less in initial weeks and very high in 10th to 25th standard weeks at all the three intervals.

Key words: Honey bee, *Apis mellifera* L., foraging activity, duration, intensity, exit, entry, standard week, weather parameters, correlation, terai region, West Bengal

Pollination service is the basic pillar of crop production that can ensure our food security with different wild and hive bees as preeminent mason (Fikadu, 2019; Reilly et al., 2020; Khalifa et al., 2021). Among them the European honey bee, *Apis mellifera* L. provide highly valued pollination services (Calderone, 2012) and as a single species it is the most frequent pollinator for different crops worldwide (Garibaldi et al., 2013). It is their foraging activity which decides the strength and survival of a colony. Foragers start foraging at early morning and continue foraging up to evening hours. Their foraging duration is influenced by various factors prevailing inside and outside of the hive (Abou-shaara, 2014). External factors influencing the foraging activity includes ambient weather parameters viz. rainfall, temperature, relative humidity, sunshine hours etc. At the time of foraging, forager bees faces a wide range of ambient weather parameters having marked effect on their foraging efficiency and alter the foraging time (Kovac and Stabentheiner, 2011). From this perspective the current study was carried out with a view to observe the foraging activity of *A. mellifera* L. round the year along with the impact of weather parameters on their foraging duration under terai region of West Bengal.

MATERIALS AND METHODS

Six strong *A. mellifera* bee hives of almost similar strength (8-9 frames/hive) were maintained in the apiary unit of Uttar Banga Krishi Viswavidyalaya (26°19' N,

89°23' E, 43 masl), Pundibari, Cooch Behar under the terai agro-ecological region of West Bengal during 2019. The apiary was surrounded by several cultivated and natural vegetations that assure availability of floral resources throughout the year. However, during extreme dearth period sugar syrup was provided. This region is characterized by typical perhumid climate with high rainfall (annual average of 3000mm), high relative humidity (65-95%) and average temperatures varied from 24°C–33.2°C. About 80% of total rainfall received from the South-West monsoon during June–September. Average duration of sunshine hours is more than 8 hours. Short spell of winter occur during December–February. The meteorological data were collected from Meteorological Unit located at the Instructional Farm of Viswavidyalaya.

The foraging duration in terms of time between first exit and last entry was recorded according to Singh (2008) and after the last entry half an hour time was given for confirmation. Number of foragers going out and coming in the hive at 5 minute time period was recorded at 09:00 AM, 12:00 noon and 03:00 PM. Both pollen and nectar gatherers were considered. Time was recorded using a stop watch and bees were counted using a hand-tallycounter. Data were recorded thrice/week considering each bee hive as separate replication. The foraging duration was correlated with environmental parameters by correlation coefficient analysis using Microsoft Excel software.

RESULTS AND DISCUSSION

Foraging duration and its relation with weather parameters: Mean foraging duration of *A. mellifera* round the year was 11.85 ± 1.79 hours/day. During study earliest exit and last entry were recorded at 04:29 AM and 06:53 PM respectively. During initial standard weeks (1st to 8th indicating January-February) foraging duration was little less as compared to rest of the standard weeks which may be due to foggy weather and movement was affected thereby. Maximum last entry and higher foraging duration were recorded from 16th to 37th standard week i.e. April-September when day length was comparatively more. In 15th, 24th, 28th and 39th standard weeks, late first exit and less foraging duration were noted due to incidence of heavy rain (Fig. 1). It was also observed that higher foraging duration associated with floral availability for collecting abundant amount of pollen and nectar which was also supported by ambient weather. Singh (2008) recorded 1st exit of Himalayan honey bees at 06.14 ± 0.004 AM and last entry at 05.28 ± 0.011 PM with average foraging duration of 9.48 ± 0.15 hrs. Singh et al. (2018) reported first exit of *A. mellifera* at 9:30 AM and last entry at 04:15 PM. The correlation analysis of foraging duration revealed a significant positive correlation with maximum and minimum temperature (0.700 and 0.739 respectively) and minimum relative humidity (0.433), whereas it showed positive non-significant correlation with maximum relative humidity, rainfall and sunshine hours. Earlier, Puskadija et al. (2007) noticed positive correlation between average and maximum daily temperature and visits, whereas

high humidity, precipitation, stronger wind and low temperature had negative impact. However, Hemalatha et al. (2018) found a negative correlation of maximum temperature with the foraging activity of different honey bee species in Madurai.

Foraging intensity: During initial weeks (1st to 8th) foraging intensity was recorded very less at 09:00 AM (Table 1). But afterwards that significantly increased by many folds during 12.00 noon and 03.00 PM. Increase in later phase may be due to sunny weather. Higher intensity was recorded in all three intervals during 10th to 25th standard weeks when the weather is warm and shiny with availability of different floral resources. Higher activity of *A. mellifera* during midday hours also reported in previous works (Painkra et al., 2021; Nath et al., 2023). In later weeks, the movement declined from average to minimum in all three intervals that may be due to unavailability of flowering plants as well as fluctuation of environmental factors. During 18th, 28th and 39th standard weeks, external activity of worker bees become almost negligible (Table 1) due to continuous rains and huge losses were detected due to attack by ants. In some cases the number of bees exit when enter some time little less as they may be accidentally died or may forget the way back to hive which may in later interval find the way back to hive with its counterparts who exits from the hive and again get back to hive. Aryal et al. (2016) found during 45th (2012) to 8th (2013) standard weeks less number of foragers came out of hive in four time intervals and also enter into hive in a similar way. The findings obtained from our study are somewhat similar and to some extend contradicting

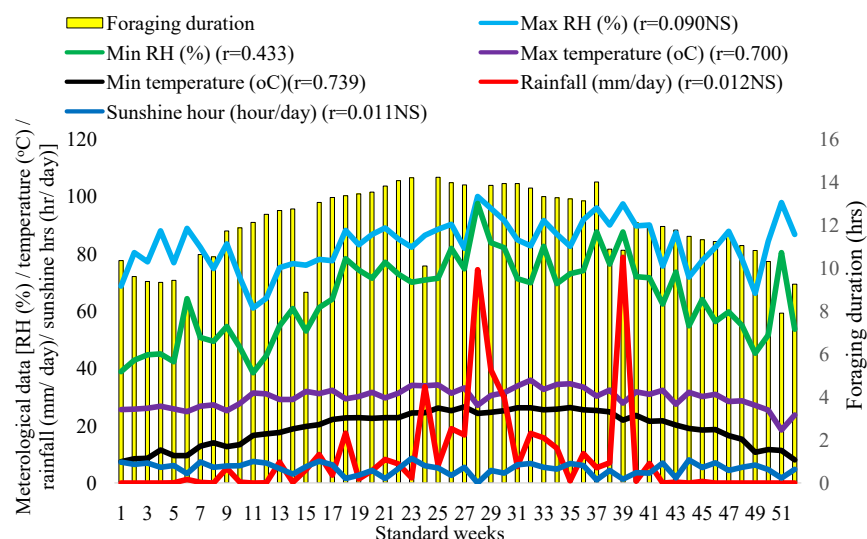


Fig. 1. Foraging duration of *A. mellifera* foragers and its relation with weather parameters (r=Correlation coefficient, NS=Non-significant, p=0.05)

Table 1. Foragers going out and coming in the *A. mellifera* hive (5 min)

Std. week	9:00 hrs		12:00 hrs		15:00 hrs	
	Exit (No.± SD)	Entry (No.± SD)	Exit (No.± SD)	Entry (No.± SD)	Exit (No.± SD)	Entry (No.± SD)
1	33.33± 07.63	28.66± 07.63	153.33± 42.19	155.33± 41.02	98.00± 23.25	104.00± 16.00
2	34.33± 07.23	30.33± 05.85	248.00± 04.58	223.00± 31.48	134.00± 20.07	72.33± 13.27
3	31.33± 07.51	28.00± 11.11	265.00± 39.94	262.67± 56.76	121.33± 30.08	106.33± 23.07
4	29.67± 07.57	25.33± 07.37	307.33± 02.51	309.00± 18.33	137.33± 38.55	115.00± 11.26
5	13.33± 04.93	15.33± 02.51	309.00± 12.49	294.33± 16.50	141.67± 34.03	84.67± 09.29
6	05.00± 01.00	8.33± 01.52	149.00± 04.00	152.33± 03.51	101.00± 32.04	109.33± 48.05
7	20.67± 04.04	24.00± 08.66	290.67± 29.50	304.00± 09.53	169.33± 01.52	161.33± 20.00
8	26.33± 12.85	29.00± 14.79	285.00± 26.51	279.00± 06.08	150.00± 22.33	175.33± 33.94
9	74.67± 14.84	76.33± 27.30	251.00± 37.51	239.00± 63.49	207.33± 40.64	120.00± 31.56
10	108.33± 08.08	102.67± 26.68	191.00± 01.73	178.00± 58.96	152.00± 37.51	153.67± 34.81
11	220.33± 04.93	228.00± 06.92	203.00± 05.00	202.00± 08.18	147.67± 32.39	141.33± 32.51
12	232.00± 18.08	264.66± 18.77	189.67± 71.12	186.00± 71.33	144.67± 19.73	132.00± 16.37
13	211.33± 18.90	250.66± 01.15	175.00± 24.33	288.00± 20.42	111.00± 15.39	138.00± 34.59
14	238.67± 19.55	225.00± 18.33	133.00± 07.00	150.33± 41.19	160.67± 51.05	133.00± 23.38
15	113.33± 31.56	115.66± 35.21	137.67± 24.54	136.33± 25.69	154.00± 11.78	156.00± 19.97
16	173.66± 41.78	170.00± 66.84	150.67± 29.09	185.33± 21.59	102.67± 08.50	120.33± 08.73
17	149.67± 17.93	133.33± 43.37	124.33± 12.85	147.00± 20.42	117.67± 31.89	115.67± 16.62
18	10.33± 01.53	11.33± 02.08	09.67± 03.51	136.67± 00.57	19.00± 02.64	21.67± 04.72
19	108.67± 18.58	106.33± 09.01	88.67± 43.61	110.67± 52.53	111.67± 15.30	94.67± 18.87
20	135.67± 21.22	117.66± 42.52	95.33± 25.73	110.00± 28.16	129.00± 12.16	125.00± 19.28
21	107.00± 20.22	110.66± 27.50	85.67± 12.67	108.67± 09.81	117.00± 12.16	129.67± 12.50
22	132.67± 31.09	136.33± 26.63	126.67± 21.59	125.67± 12.58	122.00± 15.87	120.00± 09.53
23	228.67± 30.62	201.00± 63.37	164.67± 23.79	166.00± 47.65	94.33± 04.50	106.00± 07.21
24	66.33± 08.62	73.00± 05.68	67.33± 24.50	59.33± 22.47	53.67± 26.10	62.00± 21.00
25	134.33± 25.43	137.66± 27.59	102.33± 30.07	87.00± 05.29	89.00± 20.22	87.00± 18.52
26	96.33± 24.78	98.33± 20.79	55.00± 06.24	67.67± 04.04	54.00± 05.56	65.33± 10.21
27	89.67± 42.19	82.67± 38.68	96.67± 01.52	95.00± 02.64	66.67± 24.66	62.33± 27.79
28	11.00± 07.21	09.00± 07.00	00.33± 00.57	00.33± 00.57	04.00± 01.73	04.66± 02.08
29	29.33± 04.61	28.33± 03.05	22.33± 03.21	23.33± 02.51	19.67± 06.02	17.33± 02.30
30	47.67± 13.61	48.33± 13.05	31.67± 06.11	28.67± 05.03	32.33± 12.85	36.33± 12.74
31	71.67± 39.80	69.66± 38.47	47.67± 12.67	46.33± 14.01	40.00± 07.81	38.33± 12.58
32	70.33± 23.71	63.33± 15.30	10.00± 07.54	18.00± 10.14	21.33± 6.80	26.00± 08.00
33	92.33± 09.45	82.67± 13.31	28.00± 13.74	28.33± 10.40	36.67± 18.92	41.67± 22.50
34	83.67± 20.84	72.33± 25.32	34.67± 10.59	26.00± 08.18	65.33± 23.86	62.33± 10.40
35	79.67± 34.12	91.00± 44.19	6.33± 05.68	01.00± 01.73	56.67± 34.81	60.33± 32.92
36	95.67± 22.54	89.00± 31.95	53.67± 21.58	29.33± 01.52	40.00± 17.05	47.00± 22.53
37	64.33± 18.61	57.66± 42.52	10.33± 09.07	12.33± 05.85	50.00± 29.51	58.33± 21.38
38	34.33± 03.51	34.00± 10.44	22.00± 02.64	27.67± 01.52	22.33± 11.93	14.67± 19.59
39	01.00± 01.00	01.33± 00.57	00.33± 00.57	00.33± 00.57	9.00± 06.08	04.33± 03.21
40	85.33± 16.50	79.00± 10.53	102.00± 11.26	97.00± 12.12	57.67± 09.29	60.67± 11.01
41	22.00± 20.67	25.00± 22.27	19.33± 10.50	25.33± 03.78	20.00± 10.39	19.00± 09.53
42	14.33± 04.61	16.67± 04.93	59.33± 12.89	64.67± 23.50	53.00± 13.07	46.33± 06.80
43	14.67± 03.51	15.33± 03.21	12.00± 05.00	17.00± 08.18	29.67± 06.50	26.67± 06.50
44	39.67± 17.21	36.00± 10.81	20.33± 1.15	26.67± 06.11	52.33± 21.12	52.33± 16.25
45	27.67± 16.50	28.00± 17.08	45.67± 17.03	48.67± 25.96	23.67± 13.20	24.33± 14.46
46	06.33± 02.08	07.66± 02.51	43.67± 20.52	64.33± 15.01	13.33± 06.11	16.67± 08.50
47	20.00± 11.53	16.00± 07.21	62.00± 24.55	59.33± 17.24	10.00± 01.73	17.00± 10.44
48	18.67± 10.78	21.00± 15.52	97.00± 41.79	117.33± 37.55	30.33± 14.01	35.66± 11.67
49	30.00± 01.00	34.00± 07.00	151.67± 24.44	120.33± 28.29	88.67± 19.34	87.67± 22.30
50	22.67± 09.07	30.20± 20.22	60.33± 07.37	77.00± 19.05	20.33± 05.13	25.33± 01.53
51	15.33± 04.04	15.33± 04.50	17.33± 39.31	169.33± 42.25	52.33± 17.21	56.67± 21.22
52	24.00± 13.11	27.66± 13.01	251.00± 56.67	233.00± 06.08	13.67± 05.50	17.00± 08.88

with the above mentioned literatures due to variation in prevailing environmental condition, topography and position of colony with the food sources. Keeping these results in view, it is advisable not to apply insecticides during mid-hours when the honey bees are more active.

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AUTHOR CONTRIBUTION STATEMENT

R N and N L conceived and designed experiments. R N and P P performed experiments. S S and N L analyzed data. S S and N L wrote the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF INSECTICIDES AGAINST COTTON PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS)

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ABSTRACT

A field study assessed the efficacy of insecticides against pink bollworm of cotton. Spinosad 45SC treated cotton had the lowest infestation at 23.95% in the first spray, while cypermethrin 25EC had a 26.06% infestation in the second spray. Emamectin benzoate 5SG and spinosad 45SC were the most potent insecticides for locule damage reduction. Emamectin benzoate 5SG had 70.54% mortality in the first spray, while spinosad 45SC had 76.2% mortality in the second spray. Emamectin benzoate 5SG, spinosad 45SC, and cypermethrin 25EC were the most effective insecticides overall.

Key words: *Pectinophora gossypiella*, cotton, spinosad, emamectin, benzoate, cypermethrin efficacy, insecticides, management, field, boll infestation, locule damage, mortality, control.

In India, 160 species of insect pests have been reported to attack cotton crop right from the time of germination to final harvesting of cotton, of which sucking pest and bollworm complex consisting of three notorious bollworms: Pink bollworm (*Pectinophora gossypiella*), Spotted bollworm (*Earias vitella*) and American bollworm (*Helicoverpa armigera*) are considered to be of great menace (Kranthi et al., 2005). Cotton growers in India depend heavily on synthetic pesticides to combat sucking pests. However, due to the continuous and indiscriminate use of synthetic insecticides, there is resistance and hence efficacy has become less reliable (Kranthi et al., 2005). Pink bollworm (PBW) was first reported in 1843 by W.W. Saunders in India. Pink bollworm has more extended developmental period during winter and a shorter one during the summer seasons, with an average life cycle of 32 to 35 days (Naik et al., 2020). In recent years, pink bollworm has been a significant menace to the production of cotton in the past decades leading to large losses in cotton industry up to 34% raw cotton in Punjab (Ghure et al., 2008). With the number of infestations increasing yearly and proposal to ban commonly used insecticides, there is a need for cost-effective alternatives that are more tolerable for the environment and human safety standards. Therefore, this study

was conducted to provide cost-effective alternatives of “to-be-banned insecticides” by comparing their performance with insecticides that are still available in the market for effective management of PBW in cotton in the upcoming years (Naik et al., 2021).

MATERIALS AND METHODS

The research was conducted in the field of the Central Institute of Cotton Research in Nagpur, at a specific location (Field C-5) with specific coordinates and elevation (21°02'21.7"N, 79°03'40.7"E, 321 masl). The experiment was conducted in 2021-2022 and used a randomized block design with 9 insecticidal treatments and three replications. Suraj variety (Non-Bt) was selected. The sowing date was August 2, 2021, and N:P:K (50:25:25) Half of N, full P2O5 and full K2O applied as basal dose and half of N applied 30 days after sowing. Weeding was carried out 4 times, first at 15 days after sowing, then at 40, 60 and 90 days after sowing. A battery-operated sprayer, measuring tape, and insecticidal solutions mixed at the field recommended dose were used. A pre-treatment count was done one day before the treatment, and each treatment was sprayed at the recommended dose. The spraying was done twice, and after a waiting period of three days, observations were made to estimate the ovicidal properties of the

treatments. Observations were made by selecting ten plants per replication for each treatment, and ten bolls were picked from each selected plant. The bolls were dissected, and recordings were made on various parameters, such as boll damage, number of larvae, boll infestation, number of locule, number of damaged locules, mines on the epicarp, exit holes, and number of larvae. These observations were done five times after each spray, starting from one day before spraying (pre-treatment count) and three, six, nine, and fifteen days after spraying.

Using preliminary observations, calculations were made to obtain values such as boll infestation %, locule damage %, and corrected % using Henderson and Tilton's equation (1955). Statistical analysis was conducted using the mean values of pink bollworms boll infestation %, locule damage %, and corrected mortality % for each observation day and the overall value of all observation days. Analysis of variance (ANOVA) was used to compare the mean values, and Duncan Multiple Range Test (DMRT) was used to compare the treatment means at $P=0.05$ using the SPSS program, version 25. The values were compared to estimate the overall ovicidal effectivity of each insecticide formulation in the field.

RESULTS AND DISCUSSION

The data analysis showed that the group of insecticides including spinosad 45SC, emamectin benzoate 5SG, cypermethrin 25EC, profenofos 50EC, thiodicarb 75EC, and fenvalerate 20EC had the lowest % boll infestation after the first spray, with an overall mean reduction value ranging from 23.95% to 25.98%. These treatments were found to be highly significant in comparison with the control group at 49.71%. The insecticides quinalphos 25EC and chlorpyrifos 20EC also showed significant reduction in % boll infestation at 27.39% and 33.57%, respectively. Neem oil 0.3% had the least effectiveness among the treatments with an overall % boll infestation of 37.30%. The results of the second spray also showed that the group of insecticides including cypermethrin 25EC, emamectin benzoate 5SG, spinosad 45SC, profenofos 50EC, fenvalerate 20 EC, thiodicarb 75 WP, and quinalphos 25EC had the lowest % boll infestation, with an overall mean reduction value ranging from 26.06% to 29.40%. Chlorpyrifos and neem oil were found to be less effective with an overall % boll infestation of 37.97% and 37.49%, respectively. All the treatments were found to be significant in comparison with the control group at

53.48%. The studies by Ghure et al. (2008) and Gopala et al. (2000) also reported spinosad 45SC and profenofos 20 EC to be effective against pink bollworm infestation. However, the lower overall numbers reported in the previous studies may be attributed to the studies being conducted in a different location and at a different time when the infestation was not as severe. The findings in the present study are consistent with those of Ghanim et al. (2017), who reported that spinosad was effective in reducing bollworm infestation in cotton. Their study found that spinosad 45SC had a per cent boll infestation of 30.20%, which is higher than the result observed in the present study after the first spray. However, their study was conducted under different environmental conditions and may have used a different concentration or formulation of spinosad. In another study by Sarfraz et al. (2019) they found that thiodicarb and cypermethrin were effective in controlling bollworm infestation in cotton. Their study found that thiodicarb had a per cent boll infestation of 19.78%, which is lower than the result observed in the present study after the first spray. However, their study only evaluated the efficacy of thiodicarb and cypermethrin on American bollworm not pink bollworm.

The values for the data on locule damage after the first are emamectin benzoate 5SG, spinosad 45SC, cypermethrin 25EC, profenofos 50EC, fenvalerate 20 EC, thiodicarb 75 WP, and quinalphos 25EC were the most effective insecticides in controlling bollworm infestation, with an overall mean value of 18.97%, 19.36%, 19.96%, 20.72%, 21.39%, and 22.07%, respectively, after the first spray. These insecticides were significantly more effective than chlorpyrifos 20EC and neem oil 0.3%, which had a locule damage of 27.93% and 29.66%, respectively. All treatments were significantly more effective than the control, which had a boll infestation of 40.88%. The results of the present study were supported by previous research conducted by Gopala et al. (2000), which found that spinosad 45SC and profenofos 20EC were more effective than neem oil 0.3% in controlling bollworm infestation. However, the results of the present study were slightly different from those of Mahalakshmi et al. (2021), which found that spinosad 45SC and emamectin benzoate 5SG were less effective than quinalphos 20EC, thiodicarb 75WP, profenofos 20EC, and cypermethrin 25EC in controlling locule damage. The results of the second spray showed that spinosad 45SC, cypermethrin 25EC, emamectin benzoate 5SG, profenofos 50EC, thiodicarb 75WP, fenvalerate 20EC, and quinalphos 25EC were the most

Table 1. Effect of insecticides against boll infestation, locule damage and mortality caused by *P. gossypiella*

Treatments Concentrations (gm. or ml/ l)	Boll infestation		Locule damage		Mortality	
	1 st spray (mean $\pm$ SE)*	2 st spray (mean $\pm$ SE)	1 st spray (mean $\pm$ SE)	2 st spray (mean $\pm$ SE)	1 st spray (mean $\pm$ SE)	2 st spray (mean $\pm$ SE)
Profenophos 50EC (2.0)	25.06 $\pm$ 1.67 ^a (30.01 $\pm$ 1.10)**	27.91 $\pm$ 2.07 ^a (31.12 $\pm$ 0.79)	20.72 $\pm$ 1.68 ^a (27.04 $\pm$ 1.18)	21.17 $\pm$ 0.98 ^{ab} (27.38 $\pm$ 0.69)	66.43 $\pm$ 3.00 ^a (54.63 $\pm$ 1.84)	69.21 $\pm$ 4.02 ^{ab} (56.39 $\pm$ 2.53)
Chlorpyriphos 20EC (2.5)	33.57 $\pm$ 2.60 ^{bc} (35.37 $\pm$ 1.58)	37.97 $\pm$ 0.87 ^{ab} (38.39 $\pm$ 0.49)	27.93 $\pm$ 2.70 ^b (31.85 $\pm$ 1.72)	30.61 $\pm$ 0.80 ^c (33.59 $\pm$ 0.50)	57.47 $\pm$ 6.86 ^{ab} (49.36 $\pm$.97)	60.28 $\pm$ 7.33 ^b (51.03 $\pm$ 4.28)
Thiodicarb 75 WP (2.0)	25.73 $\pm$ 0.88 ^a (30.47 $\pm$ 0.57)	28.60 $\pm$ 0.90 ^a (31.83 $\pm$ 0.98)	21.39 $\pm$ 0.72 ^a (27.54 $\pm$ 0.51)	21.98 $\pm$ 1.26 ^{ab} (27.94 $\pm$ 0.87)	61.67 $\pm$ 8.40 ^{ab} (51.97 $\pm$ 5.03)	64.11 $\pm$ 3.48 ^b (53.24 $\pm$ 2.09)
Spinosad 45SC (0.3)	23.95 $\pm$ 1.68 ^a (29.27 $\pm$ 1.12)	26.84 $\pm$ 0.55 ^a (29.88 $\pm$ 1.49)	19.36 $\pm$ 1.15 ^a (26.08 $\pm$ 0.83)	19.63 $\pm$ 1.80 ^c (26.25 $\pm$ 1.32)	67.66 $\pm$ 3.24 ^a (55.40 $\pm$.00)	76.25 $\pm$ 1.32 ^a (60.85 $\pm$ 0.90)
Neem oil 0.3% (5.0)	37.30 $\pm$ 1.70 ^c (37.63 $\pm$ 1.01)	37.49 $\pm$ 0.90 ^b (38.24 $\pm$ 0.80)	29.66 $\pm$ 1.18 ^b (32.99 $\pm$ 0.74)	30.45 $\pm$ 1.10 ^{ab} (33.49 $\pm$ 0.69)	47.47 $\pm$ 12.83 ^b (43.24 $\pm$ 7.70)	44.32 $\pm$ 8.65 ^c (41.58 $\pm$ 5.13)
Emamectin benzoate 5SG (0.4)	24.07 $\pm$ 2.16 ^a (29.35 $\pm$ 1.43)	26.07 $\pm$ 1.93 ^a (30.82 $\pm$ 0.83)	18.97 $\pm$ 2.17 ^a (25.75 $\pm$ 1.58)	20.80 $\pm$ 1.03 ^{ab} (27.12 $\pm$ 0.73)	68.17 $\pm$ 6.29 ^a (55.85 $\pm$ 3.87)	71.30 $\pm$ 5.27 ^{ab} (57.83 $\pm$ 3.48)
Cypermethrin 25EC (1.0)	24.43 $\pm$ 0.84 ^a (29.61 $\pm$ 0.56)	26.06 $\pm$ 1.36 ^a (30.92 $\pm$ 0.73)	19.96 $\pm$ 0.63 ^a (26.53 $\pm$ 0.45)	20.65 $\pm$ 0.92 ^{ab} (27.02 $\pm$ 0.65)	66.92 $\pm$ 6.18 ^a (55.11 $\pm$ 3.90)	69.97 $\pm$ 7.24 ^{ab} (57.14 $\pm$ 4.69)
Quinalphos 25EC (2.0)	27.39 $\pm$ 0.59 ^{ab} (31.55 $\pm$ 0.37)	29.40 $\pm$ 1.36 ^a (33.05 $\pm$ 0.84)	22.07 $\pm$ 0.89 ^a (28.01 $\pm$ 0.62)	23.34 $\pm$ 1.07 ^{ab} (28.88 $\pm$ 0.72)	57.92 $\pm$ 7.72 ^{ab} (49.66 $\pm$ 4.52)	59.29 $\pm$ 8.73 ^b (50.58 $\pm$ 5.24)
Fenvalerate 20 EC (1.0)	25.98 $\pm$ 1.00 ^a (31.63 $\pm$ 0.65)	28.43 $\pm$ 1.68 ^a (31.91 $\pm$ 0.83)	21.30 $\pm$ 0.87 ^a (27.48 $\pm$ 0.62)	22.21 $\pm$ 1.06 ^{ab} (28.10 $\pm$ 0.73)	61.57 $\pm$ 3.56 ^{ab} (51.73 $\pm$ 2.12)	62.27 $\pm$ 4.94 ^b (52.18 $\pm$ 2.93)
Control (water spray)	49.71 $\pm$ 1.40 ^d (44.83 $\pm$ 0.80)	53.48 $\pm$ 2.29 ^c (48.06 $\pm$ 0.66)	40.88 $\pm$ 1.14 ^c (39.14 $\pm$ 0.67)	43.53 $\pm$ 1.38 ^d (41.28 $\pm$ 0.80)	0.00 $\pm$ 0.00 ^c (0.00 $\pm$ 0.00)	0.00 $\pm$ 0.00 ^d (0.00 $\pm$ 0.00)
F stat	Sig	Sig	Sig	Sig	Sig	Sig
SEm $\pm$	1.00	0.86	0.53	0.76	2.73	3.00
CD (p= 0.05)	2.95	2.56	1.5	2.26	8.12	8.89

*Values represent means of 3rd day, 6th day, 9th day and 15th day observations; **Figures in parenthesis are sin transformed values.

effective insecticides in controlling locule damage, with an overall mean value of 19.63%, 20.65%, 20.80%, 21.17%, 21.98%, 22.21%, and 23.34%, respectively. Chlorpyriphos 20EC and neem oil 0.3% were significantly less effective, with a boll infestation % of 30.45% and 30.61%, respectively. All treatments were significantly more effective than the control, which had a boll infestation of 43.53%. The results of the present study were supported by previous research conducted by Gopala et al. (2000), which found that spinosad 45SC and profenofos 20 EC were more effective than neem oil 0.3% in controlling bollworm infestation.

The mortality rates of pink bollworm after the first spray were highest for emamectin benzoate 5SG, spinosad 45SC, cypermethrin 25EC, profenofos 50EC, thiodicarb 75WP, and fenvalerate 20 EC, at 68.17%, 67.66%, 66.92%, 66.43%, 61.67%, and 61.57%, respectively. The next batch of insecticides that showed good mortality rates were quinalphos 25EC and chlorpyriphos 20EC, at 57.92% and 57.47%, respectively, followed by neem oil 0.3% at 47.76%. In comparison, the mortality rates after the second

spray were even higher for some insecticides, with spinosad 45SC showing the highest mortality rate at 76.25%, followed closely by emamectin benzoate 5SG, cypermethrin 25EC, and profenofos 50EC, at 71.30%, 69.97%, and 69.21%, respectively.

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AUTHOR CONTRIBUTION STATEMENT

Malsawmtluanga Hnialum, V Chinna Babu Naik and N V Lavhe conceived and designed research, Malsawmtluanga Hnialum conducted experiments, V Chinna Babu Naik and N V Lavhe analysed the data. Malsawmtluanga Hnialum wrote the manuscript and Banka Kanda Kishore Reddy reviewed and corrected it.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECTS OF ANTS ON THE FRUIT SETTING OF *ARTABOTRYS ODORATISSIMUS* R. BR.

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ABSTRACT

Floral volatile compounds exhibit multiple functions such as attracting pollinators, source of food for pollinator and act as defense against herbivore. Thus floral scents may function as allomones to prevent enemies as well as being synomones to attract pollinating mutualist. Flowers are morphological and embryological marvels and the sites of sexual reproduction. The yellow coloured flowers of *Artabotrys odoratissimus* R. Br. are very fragrant. The floral volatile of this plant attracts weaver ants. It plays a crucial role in plant- ant interaction. The present study aims to explore the effect of ants on the reproductive success of *A. odoratissimus*. Ants are detrimental to *A. odoratissimus* and disturb the pollinator visits which resulted in reduced fruit set. The nest constructed leaves experience premature death, thus weaver ants show antagonistic effect in the selected species.

Key words: Ant- plant relationship, *Artabotrys odoratissimus*, floral nectaries, foraging, fruit set, leaves, antagonism, nesting, weaver ants, volatile compounds, attraction

MATERIALS AND METHODS

In all the ecosystems in the universe from desert to ocean species are interact with each other. All living organisms in a community have important relationship with other living organisms. These interactions may be positive or negative. Ants are one of the most interactive organisms in the world. In interspecific interaction, ants may communicate about the location of food with her sisters, they built and care colonies and young ones (Chomicki and Renner, 2017). In general weaver ants (*Oecophylla smaragdina*) exhibit symbiotic relationship with plant leaf (Bronstein et al., 2006; Rico-Gray and Oliveira, 2007), Leaves provide hollow space specialized for housing ants and provide food for ants (Heil, 2008; Stadler and Dixon, 2008). In return, ants protect the plants from herbivores (Heil, 2008; Heil and McKey, 2003). Ant - plant mutualistic relationship is common in tropics. Ants act as a major biocontrol agent on host plants such as *Mangifera indica*, *Citrus* sp., *Anacardium occid* et al., le and *Swietenia mahagoni*, (Peng et al., 1999; Van Mele et al., 2007; Lim and Kitton, 2003; Van Mela and Cue, 2000). The greater quantity of food resources, the angiosperms increase the opportunity for the interaction of ant – plant. The ants use large number of host species for their shelter (Holldobler, 1983) *Artabotrys odoratissimus* R. Br. is a plant belongs to the family Annonaceae also show myrmecophylic relationship with the Weaver ants, The present study discuss about the ant – plant relationship between *Artabotrys odoratissimus* and Weaver ants.

Artabotrys odoratissimus is a large woody garden straggler. The inflorescence is axillary, solitary, flowers green colour in young and yellow colour when ripe. It produces strong smell resembling ripened jack fruit. The flowering start during the rainy season or long summer is followed by a heavy rain also initiate flowering. The social insect weaver ants (genus *Oecophylla*) are belongs to the family Formicidae. *Oecophylla* are obligate arboreal insect. The study was conducted in the plant located at Rani Anna Government College for women, Tirunelveli, Tamil Nadu. Ant and *Artabotrys* were found associated in large colonies. The research was conducted in the plant located at Rani Anna Government College for women, Tirunelveli, Tamil Nadu. Ants and *Artabotrys* were found naturally associated in large colonies. Field work was completed between September 2021 and March 2022. Each day, plants were examined to check for ant relationships with the flowers. During the fieldwork, ant nesting and nectar-gathering behaviours are observed. The average length and width of the nests were used to calculate the nest size. In the field, behavioural observations of nest construction, foraging, and nest protection were made. Four times a day, nest-building behaviour was recorded. Real random leaf sample was used to conduct the measurements. The presence or absence of eggs and pupal cocoons were noted.

RESULTS AND DISCUSSION

Interactions between ants and plants may be facultative or obligate, antagonistic or mutualistic (Rico-Gray and Oliveira, 2007). Floral volatiles play a significant role in attracting the weaver ants.

Nest-building behaviour: Adult weaver ants have reddish colour body and eyes are large. They have 10-segmented antennae with 2-segmented clubs. Adult ants known for their unique nest building behaviour where workers construct nests by weaving together leaves using larval silk. In *A. odoratissimus*, the present study recorded two types nest builded by the Weaver ants. i) Weaver ants bend the green fresh matured leaves (5-8) together and with whitish mucilaginous substances paste along edges, which held the leaves strongly together. The apex of the leaf glued with the petiole of the same leaf. This nest looks like a pouch hanging on the branches. The size varies in height 10 -13 cm and breadth 8 -10 cm. ii) sometimes, the nest construction is done horizontally (elliptical in shape) instead of pouch. 8 -10 leaves are joined vertically without any bend, margin of leaves are glued with the help of white paper like substance. The length varies from 15-18 cm and breadth 5-7 cm. Occasionally, single leaf is folded to form a nest. Hundreds of ants are actively involved in the construction of nests. During the nest building many ants stand on one another, holding down a leaf with another leaf margin, while other ants are employed to fasten the glue. Gluing is done simultaneously in both ends of the leaves. The ants are pulling the nesting leaves on the edges with their mandibles. With help of potential forces a few ants have successfully bent a leaf onto itself. The workers plan between leaves in a systematic coordinated fashion to bind the leaves together. The size of the nest depends on the size of the group. The whitish mucilaginous glue present in the dried nest is soft and appears as membranous sheet. The entrance of the nest is small and oval. Once nest is constructed, workers retrieve the larvae in such a way that causes them to excrete silk, larvae can produce more silk and pupate without a cocoon. The time required to construct a nest is significantly less the 24 hr. Weaver ant's nests are strong and even heavy wind flow cannot collapse the nest. The nest is impermeable to rain water. New nests were consistently being constructed by workers in large colonies.

The leaves used for the woven the nest experience a premature fall down. The nested leaves dried quickly, this may be due to the exposure of abaxial side to

the direct sunlight. The dried nested leaves remain attached to the plant, even after getting detached from petiole, since they are weaved together during fresh condition. The young or mature flower is more susceptible to the weaver ants. Group of ants enter into the thalamus region of the flower and suck the floral scent continuously for 5-7 days, after that the flower dried up. The fruit formation is absent. Rarely fruit formation may occur and the ants may construct the nest over these fruits. These fruits also resulted in premature fall down.

In *A. odoratissimus*, often the nest constructed by pulling the leaves occur next to the inflorescence axis. Fragrant flowers with well developed nectarines are an adaptation for weaver ants. In many plants ant acts as potential pollinators (Peakall et al., 1991; Schoonhoven et al., 2005), but in *A. odoratissimus* weaver ants produce detrimet all effect. The fruit setting is completely absent or highly reduced due to this interaction. This may be due a poor physical fit to connect androecium and gynoecium of flowers, ants have smooth hairless cuticles which are weakly suitable for pollen adhesion. Furthermore, some ants have metapleural glands that secrete anti-microbial agents, required for nest hygiene (Fernández-Marin et al., 2006), but this secretions damaging to pollen longevity and fertility (Galen and Butchart, 2003). Sometimes plants effective pollinators disturbed by the ant colonies. Many findings suggested that, antagonistic ants may harmful to flower visitors or pollinators (Altshuler, 1999; Galen, 1999; Tsuji et al., 2004; Gaume et al., 2005; Nees, 2006; Junker et al., 2007). Ants may be a nectar thieves. So reduce the addictiveness of flowers to the suitable pollinators. Whenever the nectar is removed from a flower, the nectar pollination is reduced by frequency or duration of pollinator to visit the flower (Galen and Geib, 2007). This resulted in poor fruit setting. Galen and Butchart (2003) reported that, some ants are considered to be typical nectar thieves or even robbers, which can decrease a plant's reproductive success by damaging the anthers and pistils or chasing away other potential pollinators (Gaume et al., 2005).

Many studies are reported on beneficial role of ants in plant reproduction. Very limited studies focused on significance of ant pollination. The ant's role in pollination is underestimated. In *A. odoratissimus* ant relationship is detrimental and reduces fruit setting. The ant infected plants are not produces significant numbers of viable seeds. The chemical constituent in the floral scent acting as attractants of ants awaits further study.

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AUTHOR CONTRIBUTION STATEMENT

Study conception and design: Dr. A. Saravana Ganthi
Data collection: Vellankanni K and Suresh Kumar T
Analysis and interpretation of results: Vellankanni K and Suresh Kumar T
Draft manuscript preparation: Dr. A. Saravana Ganthi. All authors reviewed the results and approved the final version of the manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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FIRST HOST REPORT OF EARHEAD WORM, *AUTOBA SILICULA* (SWINHÖE, 1897) ON MAIZE

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ABSTRACT

Earhead worm *Autoba silicula* (Swinhoe, 1897) (Lepidoptera: Erebidæ) is recorded for the first time in India on maize. Larvae were found feeding on maturing grains and silk, fully fed matured larvae pupates under a dome shaped or elongated galleries made of silk inside the cob. Roving survey conducted during 2019 and 2020 recorded on an average 0.40 and 0.45 larvae per plant, respectively, which is below the economic threshold. In the present study, information on species diagnosis with illustrations, occurrence, nature and symptoms of damage are provided.

Key words: *Autoba silicula*, Erebidæ, Lepidoptera, morphology, taxonomy, male genitalia, host plants, maize, cob silk, new record, distribution, occurrence, New Delhi, India

Maize (*Zea mays* L.) is one of the most important cereal crop in the world. Globally, Maize is known as a queen among cereals because of its highest productivity. In India, maize is one of the most important food crop after rice and wheat, cultivated in an area of 96.33 million hectares with a production of 258.99 million tonnes and productivity of 2.69 tonnes per hectare (Anonymous, 2017). It's a staple food for human being and also a quality feed for livestock. Maize is a basic raw material for thousands of industrial products that includes starch, alcoholic beverages, pharmaceutical, oil, protein, food sweeteners, cosmetic, gum, film, textile, package and paper industries etc. Among the insects infest on maize Stem borers, *Sesamia inferens* (Wlk.), *Chilo partellus* (Swinh.), are important insect pests in Delhi and recently, fall armyworm *Spodoptera frugiperda* (J E Smith) infestation is serious and considered as major pest. In 2019-20, we have observed a different lepidopteran larva not previously reported on maize were found feeding on silk and maturing grains. We have reared the larvae and identified morphologically as *Autoba silicula* (Swinhoe, 1897). *A. silicula* (Lepidoptera: Erebidæ) has been reported as a pest of pearl millet, *Pennisetum typhoides* (Burm.) (Kishore, 1996; Vinita et al., 2006); sorghum, *Sorghum vulgaris* (David and David, 1961; Ayyar, 1965; Fletcher, 1921; Hardas et al. (1979, 1980); Nayar et al. (1982), Taley et al. (1974); mango, *Mangifera indica*

L. (Nielsen et al., 1996; Butani, 1993); finger millet, *Eleusine coracana* (Gaertn.) (David, 1962) as earhead and flower feeding caterpillar. In this paper, *A. silicula* (Swinhoe, 1897) is recorded for the first time on maize from India. An account of occurrence, morphological identification with detailed photographic illustrations and symptoms of damage are also provided.

MATERIALS AND METHODS

In 2019-20, feeding of *A. silicula* was observed in maize fields. Maize cobs showing symptoms or presence of *A. silicula* larvae and pupae were placed in plastic bags and brought to the laboratory for further study. The geographical coordinates were taken and data related to the altitude was gathered. Roving survey was conducted to record the symptoms of damage, occurrence of larvae (*A. silicula*). Field collected larvae were reared on cob silk in plastic petri dishes (60 mm × 15 mm), when the mean atmosphere temperature was 15.4 °C and relative humidity was 61%. Cob silk were then replaced every alternate day, together with the absorbent paper lining on the bottom of the box. Fully fed larvae were kept in room temperature for pupation till the emergence of adults. These were then utilized for taxonomic identification. After morphological identification, the abdomen has been detached from specimen with help of forceps, and then placed in 10 per cent KOH for tissue digestion. After complete digestion of the tissue the

abdomen was transferred to cavity block with distilled water to remove traces of KOH solution from sample. Smooth brushing was done using camel hair brush to remove scale and hairs attached to abdomen. After that, the abdomen was transferred to watch glass containing 20 per cent alcohol and cleaned thoroughly. Then the abdomen is placed into the cavity block with 70 per cent alcohol for final clearing. Genitalia was pulled out of the abdomen carefully under stereo binocular microscope. In case of male genitalia, the aedeagus was carefully pulled with the help of forceps holding the valvae with another pair. In female genitalia the hypodermic syringe was used to remove extra material from corpus bursae by making small slit if necessary. The genitalia was placed in cavity block with absolute ethanol. The valvae was stretched apart carefully with the help of fine needle and forceps. Then the genitalia was placed over the clean microscope slide (wiped with 100 per cent ethanol and dried with tissue paper before use) and pure glycerine (98% glycerol) and air bubbles are removed carefully. Then the cover slip was placed upon the glass slide carefully covering the four sides (Robinson, 1976). Photographs were taken with a Leica DFC 425C digital camera attached to a Leica M205FA stereo zoom automontage microscope. The voucher specimens were deposited in the National Pusa Collection, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India.

RESULTS AND DISCUSSION

In 2019-20, *A. silicula* was first observed on maize crop during reproductive stage at IARI, Pusa campus, New Delhi (Fig. 1A). We have noticed the presence of many larvae and pupae on the host plant. The larvae of *A. silicula* were feeding on relatively mature grain, silk and some larvae and pupae remain hidden under a dome-shaped or elongated gallery formed from silken threads and cob silk (Fig. 1B-C).

Taxonomy

Family: Erebiidae Leach, 1815

Subfamily: Boletobiinae

Autoba silicula (Swinhoe, 1897)

=*Eublemma silicula* Swinhoe, 1897: 167;

=*Autoba silicula saturata* Warren, 1913: 225;

=*Eublemma compsoprepes* Turner, 1945: 157.

Distribution: Andhra Pradesh, Bihar (Rao, 1920), New Delhi (Kishore and Jotwani, 1982; Taley et al., 1974), Maharashtra, Telangana.

Host Range: This insect previously recorded on

pearl millet, *Pennisetum typhoides* (Burm.) (Kishore, 1996; Vinita et al., 2006); sorghum, *Sorghum vulgaris* (David and David, 1961; Ayyar, 1965; Fletcher, 1921; Hardas et al. (1979, 1980); Nayar et al. (1982), Taley et al. (1974); mango, *Mangifera indica* L. (Nielsen et al., 1996), (Gardner, 1941); finger millet, *Eleusine coracana* (Gaertn.) (David, 1962); castor, sunflower, cotton, hibiscus, *Albizia lebbbeck*, *Alcearosea*, *Michelia champaca* (NBAIR, 2022).

Survey and population density: During roving survey, twenty plants at 10 randomly selected spots were selected and a total of 0.40 (2019: Nov-Dec) and 0.45 (2020: Nov-Dec) larvae per plant observed.

Material examined: India: 5♂, 9♀, New Delhi: Pusa campus, IARI, 28°25'12"N, 77°07'12"E, 228.61m, 5.xii.2019, net sweep, Coll. Ramaiah M.

Mature larvae: The larvae were initially greenish coloured with brown sclerotized head and later turn into brown yellow colour having numerous hairs and white dots along the lateral sides (Fig. 1A). The pupa was brown in colour and covered with silken webbing threads, made by fully grown larvae (Fig. 1B).

Adult: Wingspan 8.0-9.5 mm, moth pale brown, costa of forewings fading to white, with some dark markings. Wings with rusty brown transverse line. Forewings have a wave-shaped margin (Fig. 2A-B).

Male genitalia: Uncus long, slender, sclerotized, slightly broadened medially and highly sclerotized, hooked apically; socius atrophied; tegumen uniform width, sclerotized marginally, rounded apically; gnatha and gnathal hooks are not prominent; valva membranous broader at base and slightly tapering to apex, valva with spinose valval lobe; costa narrow, rounded apically and median costa slightly bulged outer; transtilla membranous, narrow at both end and wider at the centre; juxta very prominent and slightly sclerotized; sacculus at base of valva, slightly sclerotized but not touching costal margin; saccus poorly developed; vinculum membranous and well developed; aedeagus long, tubular, sclerotized basally and medially with rounded, membranous apex provided elongated with linear deciduous cornutus (Fig. 2C-E).

Female genitalia: Anal papillae well developed, setosed, sclerotized; anterior apophyses longer than posterior apophyses; lamella ante-vaginalis large, slightly sclerotized, proximal membrane allows ventral movement; lamella post vaginalis slightly

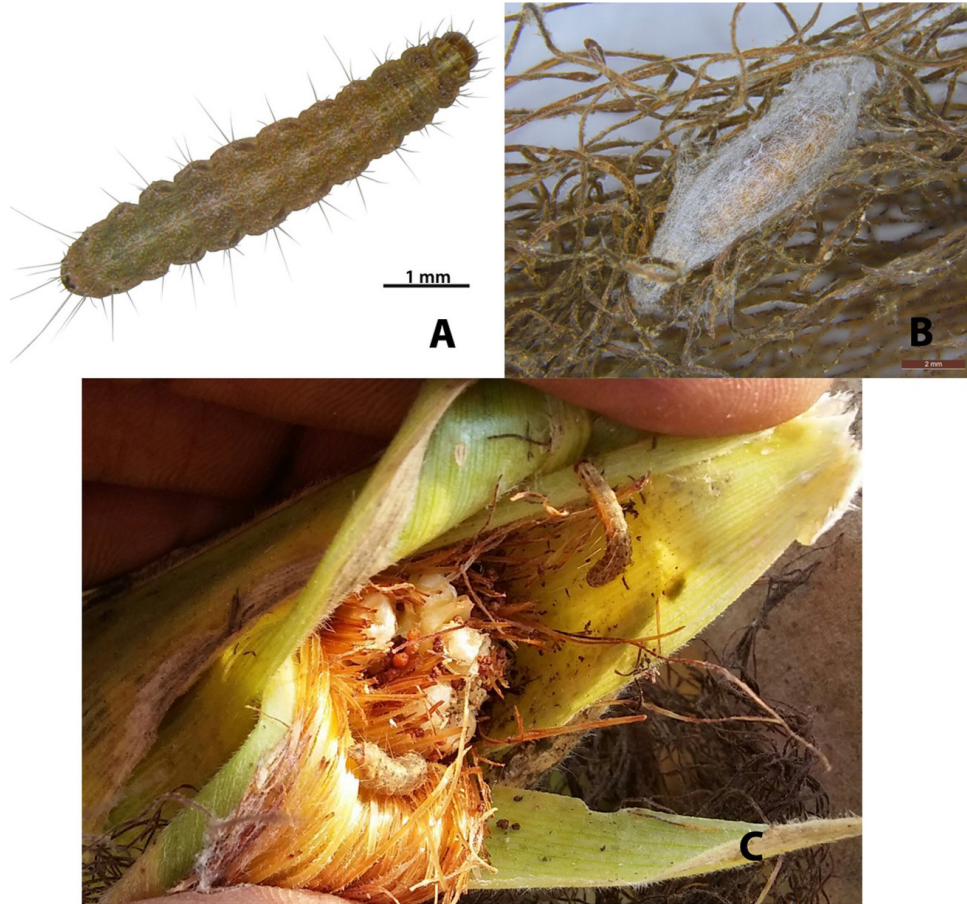


Fig. 1. *Autoba silicula* (Swinhoe, 1897) stages and infestation in the maize field: A: matured larvae, B: pupation inside silk, C: nature and symptoms of damage

sclerotized; ostium bursae small, sclerotized, cup-shaped lobe; ductus bursae long, tubular, curved, uniformly sclerotized. Corpus bursae semioval, signum with pair of different sized sclerotized stripes. Bulla seminalis well developed and membranous (Fig. 2F).

Diagnosis: This species looks similar in forewing pattern to *Eublemma guiera* and *E. gayneri* (Roths.) and tentatively placed next to that species. These three species, however, readily distinguished by their size and differences in color of the forewings.

Discussion: In the present study occurrence of *A. silicula* was recorded on maize in New Delhi. During 2019 and 2020, an average of 0.4 and 0.45 larvae per plant recorded by roving survey, respectively. The insect pest was identified as earhead worm, *Autoba silicula*. The larvae feeding on relatively mature grain, silk and some larvae and pupae remain hidden under a dome-shaped or elongated gallery formed from silken threads and cob silk. *A. silicula* larvae have been observed feeding on several plant species (David and

David, 1961; Ayyar, 1965; Fletcher, 1921; Hardas et al. (1979, 1980); Nayar et al. (1982), Taley et al. (1974); Nielsen et al. (1996); Gardner, 1941; David, 1962). In New Delhi, this species was observed feeding on developing grains of sorghum (Kishore and Jotwani, 1982) as well as serious outbreak (2 to 6 per ear head) was observed on pearl millet by feeding on maturing grains and concealed themselves under a small dome shaped or elongated gallery made of anther and silk (Kishore, 1996; Vinita et al., 2006). In past, this pest was not reported on maize and till date, there is no authentic taxonomy information, detailed description and illustrations are available this species. Our knowledge about the pest status of many maize insect pests and their economic injury level is still incomplete. Information on important taxonomic characters of the larvae and adult is limited. In India, recently invaded fall armyworm, *Spodoptera frugiperda* changed the insect pest's scenario in maize crop, so careful monitoring and timely reporting of changing pest status is necessary to avoid any future outbreak.

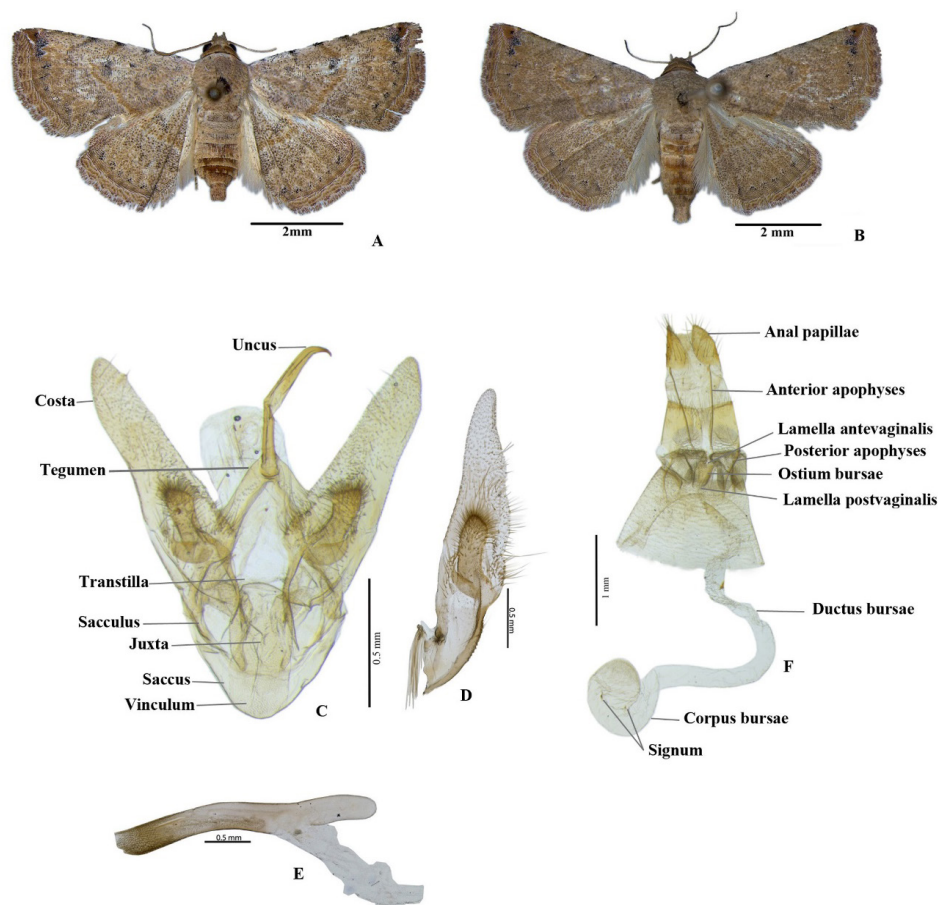


Fig. 2. Habitus and genitalia of *Autoba silicula* (Swinhoe, 1897): A: adult male (dorsal view), B: adult female (dorsal view), C-E: male genitalia, F: female genitalia

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AUTHOR CONTRIBUTION STATEMENT

MR first observed the pest; collected specimens, made biological observations, preparation of the manuscript; SN collected specimens, prepared plate, gathered biological data; NMM identified the insect, made observations; BSN writing up of the manuscript; SPR Planning, guiding the MR and manuscript grammar editing, correction and formatting.

CONFLICT OF INTEREST

No conflict of interest.

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BIOLOGICAL CONTROL OF PINK BOLL WORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) BY *TRICHOGRAMMATOIDEA BACTRAE* NAGARAJA IN COTTON

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ABSTRACT

An experiment was conducted at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore to evaluate the field efficacy of an egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja through inundative releases against *Pectinophora gossypiella* (Saunders) in cotton for two successive field trials during Kharif 2021 and 22. The parasitisation efficacy of *T. bactrae* was tested in the cotton ecosystem and the results revealed that the parasitism on the eggs was found be on par with that observed with NSKE 5% concentration (24.63%), neem formulation 1500 ppm 1% concentration (24.10%), *Pongamia* extract 5% concentration (23.13%), *Jatropha* extract 5% concentration (23.93%) and were found the safest to *T. bactrae*. The emamectin benzoate 5 SG at 190g/ ha (19.49%) and cloranthraniliprole 18.5 SC at 150 mL/ ha (15.26%) were on par with each other. Results suggested that among the insecticides tested emamectin benzoate 5 SG and chloranthraniliprole 18.5 SC were safer to *T. bactrae* and inundative release of *T. bactrae* can achieve sizeable control of *P. gossypiella*, hence it can be integrated with other components for IPM.

Key words: *Gossipium hirsutum*, *Pectinophora gossypiella*, *Trichogrammatoidea bactrae*, biological control, NSKE, neem formulation, *Pongamia*, *Jatropha*, emamectin benzoate, cloranthraniliprole, flubendiamide, spinosad

Cotton (*Gossipium hirsutum* L.) is the most important commercial crop grown in India. Cotton is cultivated in 134.77 lakh hectares in India with a production of 460 kg lint/ ha and ranks first in production with 365.00 lakh bales (ICAR-AICRP, 2019-20). Among cotton bollworms, the *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is a pest of great economic importance in many cotton-growing countries causing yield loss both in terms of quantity as well as quality. It is a worldwide key pest of cotton and its larvae burrow into cotton bolls to feed on the seeds (Sarwar, 2017). The feeding damage allows other insects and fungi to enter the boll and cause additional damage (Shrinivas et al., 2019). Mostly, farmers rely upon chemicals to get rid of these serious pests, but pesticides have not provided a long term solution for their management. A sharp decline (70 to 80%) in the numbers of predatory species occurred in cotton field post chemical applications which was reported by El-Heneidy et al. (1987). As opposed to this approach, biological control is a major component in integrated pest management. It has been considered as a sustainable, economic, environmental-friendly and host specific (Bale et al., 2008). The egg parasitoid, *Trichogrammatoidea bactrae*, was imported into California from Queensland, Australia during 1985 as a potential biological control agent of

PBW (Hutchison et al., 1990, Naranjo et al., 1992). *T. bactrae* is widely distributed in the orient (India, Pakistan, China, Malaysia, Taiwan and Indonesia). It is adapted to terrestrial humid habitats and is known to attack various pests of cotton, sugarcane, fruits and vegetables (Nagaraja, 1978). The development from egg to adult was ranging from 8-9 days. The mean total number of *Corcyra chephalonica* eggs parasitized by fed, mated and virgin parasitoids was 49.0 and 48.7%, respectively (Lim, 1986). Female fecundity and longevity were related directly to temperature (Malik, 2000). Regardless, the optimum temperature for fecundity ranged between 22 and 28°C. Generally the highest numbers of progeny (24.86 and 24.48 adults/ female) were produced at 25 and 28°C (El-Hafez, 1995). In this context, study was conducted to evaluate the efficacy of the egg parasitoid, *T. bactrae* as a biocontrol agent for suppressing the *P. gossypiella* (PBW) population in cotton.

MATERIALS AND METHODS

An experiment was conducted at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore (11.0167N, 76.9350E) to evaluate the field efficacy of an egg parasitoid, *T. bactrae* through inundative releases against *P. gossypiella* in cotton for two successive field

trials during kharif, 2021 and 2022. The parasitoid was released at different application stages of cotton (viz., flowering and boll formation stages). Cotton Bollgard II crop was grown as irrigated crop. The experiment was laid out in randomized complete block design. The egg parasitoid, *T. bactrae* which had been reared on egg cards of the *Corcyra* in the laboratory, was released in the field. The egg card was cut in to small pieces and stapled to the upper side of a cotton leaf with egg side down, in the middle portion of the plant to evaluate parasitoid activity in experimental plot. Egg cards were turned towards the leaves' surface to avoid being eaten by predators (Naranjo et al., 1993). The release of egg parasitoid *T. bactrae* @ 40000/ ha at 15 days interval 3 times from 45 days after sowing (as per recommendation) for the duration of the season (Malik, 2001). The *P. gossypiella* incidence started at flowering stage (45 days) as indicated by the rosette flowers. The occurrence of *P. gossypiella* adults was monitored using Delta traps (Pectino-Lure (SL), Gaiagen Technologies Private Ltd., Bengaluru) @ 12 per ha. The presence and establishment of the parasitoids in the cotton fields was confirmed using egg cards of *P. gossypiella* moth prepared as described above. These cards were left in the field for 24 hours and then brought back to the laboratory. The assessment was repeated every week. In addition, a field infestation of *P. gossypiella* was recorded at weekly intervals. The mean of data were recorded and mean of the two trials data were analysed using SPSS software version 22 with appropriate transformations.

RESULTS AND DISCUSSION

The preliminary studies of the investigation, showed no parasitised eggs in *P. gossypiella* egg cards before the *T. bactrae* release. The results suggested that *T. bactrae* is not present naturally in the field. The eggs recovery was also calculated. The unrecovered eggs were probably be destroyed by predators (Henneberry and Clayton, 1982). The parasitisation efficacy of *Trichogrammatoidea bactrae* was tested in the cotton ecosystem and the results revealed that the parasitism percent of *T. bactrae* on the eggs of *P. gossypiella* was found to be on par at NSKE 5% concentration (24.63%), neem formulation 1500 ppm 1% concentration (24.10%), *Pongamia* extract 5% concentration (23.13%), *Jatropha* extract 5% concentration (23.93%) and emamectin benzoate 5 SG at 190g/ ha (19.49%) and were found the safest to *T. bactrae*. Whereas among the treatments, emamectin benzoate 5 SG at 190g/ ha and chlorantraniliprole 18.5 SC at 150 mL/ ha (15.26%) were on par with each other. Followed by spinosad 45

SC at 220 mL/ ha (11.30%) and flubendiamide 39.5 SC at 125 mL/ ha (10.45%) were on par and were found to be unsafe to the parasitisation of *T. bactrae* (Table 1).

Asha et al. (2019) conducted laboratory studies to evaluate the parasitising efficacy of four *Trichogramma* species against the eggs of *P. gossypiella* and found that the maximum parasitism (>87%) was observed in case of *T. bactrae*. El-Wakeil et al. (2006) stated that no side effects in the adult emergence of *Trichogramma* spp. due to several neem products. Similarly, Sharma and Aggrawal (2019) concluded that botanicals and biopesticides were harmless to slightly harmful with respect to adult emergence of *Trichogramma* sp. which is in agreement with the present results of NSKE, neem formulation, *Jatropha* extract, and *Pongamia* extract on *T. bactrae*. The adult emergence and per cent parasitisation were greatly influenced by insecticides as compared to neem products which were relatively safer to these egg parasitoids (Srinivasan et al., 2001). Perera et al. (2015) revealed that *T. bactrae* was a promising candidate to be promoted as a biocontrol agent of rice leaf folder, over 35-66% parasitism was achieved at laboratory conditions. However, Malik (2001) observed that when *T. bactrae* was released in cotton field, the total parasitism in *P. gossypiella* eggs was 14.72 and 18.53 respectively. Luo et al. (2014) described the same trend in the parasitisation of the inundative release in the field. The present results are in accordance with Malik (2001) and it is suggested that the egg parasitoid, *T. bactrae* proved as an effective biological agent and can be used in IPM of cotton for the management of *P. gossypiella* (El-Hafez and Nada, 2000). Combinations of biological agent *Trichogramma* and chemical control had also been proved to be successful for the management of *P. gossypiella* (Sarwar, 2017).

Emamectin benzoate 5 WG considered to be highly safe to the honey bee/ non-target insects (Husain et al., 2014; Thangavel et al., 2016). Preetha et al. (2009) conducted safety study experiments in the laboratory based on nine insecticides, namely, imidacloprid, thiamethoxam, chlorantraniliprole, clothianidin, pymetrozine, ethofenprox, BPMC, endosulfan and acephate were tested to determine their toxicity against *Trichogramma* sp. by glass vial residue bioassay. Based on the risk quotient assessment, only chlorantraniliprole was found to be safe to *Trichogramma* sp. Spinosad 2.5 SC at the recommended dosage (17.5 a.i./ ha) was found to be extremely toxic to immature and adults of *T. bactrae* (Jalali and Ramani, 2001). Though parasitism rates may seem not enough for the complete

Table 1. Parasitisation efficiency of *Trichogrammatoidea bactrae* after release against pink bollworm in cotton ecosystem (Kharif, 2021-22)

Treatment	Pink bollworm eggs tested in the field (Nos)*	Eggs recovered from the field (Nos)*	Number of parasitised eggs (Nos)*	Parasitism* (%)
T1 - NSKE @ 5%	100	89.33	22.00	24.63 (29.75)a
T2 - Neem formulation 1500 ppm @ 1%	100	83.00	20.00	24.10 (29.40)a
T3 - <i>Pongamia</i> extract @ 5%	100	89.33	20.67	23.13 (28.75)a
T4 - <i>Jatropha</i> extract @ 5%	100	78.00	18.67	23.93 (29.29)a
T5 - Emamectin benzoate 5 SG @ 190 g/ ha	100	78.67	15.33	19.49 (26.20)ab
T6 - Flubendiamide 39.5 SC @ 125 ml/ ha	100	73.33	7.67	10.45 (18.86)c
T7 - Spinosad 45 SC @ 220 ml/ha	100	76.67	8.67	11.30 (19.65)c
T8 - Chlorantraniliprole 18.5 SC @ 150 ml/ ha	100	83.00	12.67	15.26 (23.00)b
T9 - Control	100	77.33	20.33	26.29 (30.85)a
SEd	-	-	-	2.5729
CD (p=0.05)	-	-	-	5.4056

*Mean of three replications and mean of two trials; In a column, means followed by different letters are significantly different (p=0.05) as per Tukey HSD test

management of the *P. gossypiella*, inundative release of *T. bactrae* achieved sizeable control of *P. gossypiella* (Mahalakshmi and Prasad, 2020). To avoid economic damage by *P. gossypiella* in cotton, cotton growers could follow Integrated Pest Management by integrating biological control methods with other management practices viz., cultural methods, pheromone mass trapping methods and chemical control.

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AUTHOR CONTRIBUTION STATEMENT

I Padma-Shree: For collection, analysis and interpretation of data; drafting the manuscript; M Muthuswami: Advisor for the research work and for

drafting the manuscript; K Senguttuvan: Advisor for the research work and for drafting the manuscript; S Rajeswari: Advisor for the research work and providing research facilities; N Manikanda Boopathi: Advisor for the research work and drafting the manuscript structure.

CONFLICT OF INTEREST

No conflict of interests.

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OCCURRENCE OF APHID, *APHIS GOSSYPPI* GLOVER AND COCCINELLIDS ON ISABGOL

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ABSTRACT

Aphid *Aphis gossypii* Glover was found to be the major pest of isabgol in the semi-arid regions of the Rajasthan. The infestation of aphid initiated from 2nd standard meteorological week (SMW) with 6 aphids/plant and fluctuated till 13th SMW. The peak activity of aphids observed on 8th SMW which gradually reached to the level of 56.13 aphids/plant and thereafter gradually decreased up to 7.87 aphids/plant in the fourth week of March when the crop matured. The activities of three coccinellid beetles viz. *Brumoides suturalis* Fabricius, *Cheilomenes sexmaculata* Fabricius and *Coccinella septempunctata* Fabricius were also studied, and their peak population was noticed from 8th to 10th SMW (19th February to 5th March, 2022). The significant positive correlation of aphid population was found with bright sunshine and all three species of coccinellids. *C. sexmaculata* and *C. septempunctata* have the significant positive correlation with bright sunshine, maximum temperature and average temperature while negative non-significant correlation with relative humidity.

Key words: Isabgol, blond psyllium, *Plantago ovata*, aphid, *Aphis gossypii*, ladybird beetles, *Brumoides suturalis*, *Cheilomenes sexmaculata*, *Coccinella septempunctata*, population dynamics

Isabgol (*Plantago ovata* Forst.) commonly known as psyllium, belongs to family Plantaginaceae and is native to Persia (Dhar et al., 2005). The name is originated from two Sanskrit words, 'asp' and 'ghol' referring horse ear as the seed resembles to horse ear, also refers to the boat shaped seeds. India is the largest producer and exporter of Isabgol in the world. Isabgol is an economically important medicinal plant commonly cultivated in different parts of India, Pakistan, Iran and some part of Europe (Singh et al., 2009). In India, it is mainly cultivated in north Gujarat, western Rajasthan and Madhya Pradesh. The isabgol husk is an ayurvedic herb, used in health care for many centuries in South Asia, and is now widely used for its medicinal properties all over the world. Psyllium seed husk provide health benefits for diabetes, constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome symptoms, abdominal pain, obesity, hypercholesterolaemia and lowering blood cholesterol level (Jalanka et al., 2019, Clark et al., 2020, Franco et al., 2020). Furthermore, psyllium polysaccharides are potential natural antioxidant, anti-carcinogenic agent and also have antiulcerogenic property (Patel et al., 2019). It is also added to shakes, juices, yogurt, syrups, soups, bread and even in ice creams to improve the fiber content of the food (Belorio and Gomez, 2022). *Plantago* seeds contains of 6.85% ash, 23.5% crude

fiber, 8.7% protein and 50.65% carbohydrates (Pendse et al., 1976). The yield of isabgol is mainly affected by biotic and abiotic factors. Aphid, *Rhopalosiphum maidis* (Fitch) is the major insect pest (Kumawat, 2008) whereas the other pests viz. field cricket, *Gryllus* sp; whitefly, *Bemisia tabaci* (Genn.); aphid, *Aphis gossypii* (Glover) and termites, *Odontotermes obesus* Rambur and *Microtermes obesi* Holmgren are also reported on isabgol by Jindla et al. (1984), Farooqi and Sreeramu (2001) and Reddy (2009). The aphids are active sap suckers at bud and later causing considerable loss to the crop, and use of man-made insecticides for its control may reduce profit and export potential both. Aphids are reported to be the major pests of isabgol in Punjab and Madhya Pradesh (Upadhyay and Mishra, 1999) causing serious losses to crop. Regular occurrence of aphid on isabgol crop in Rajasthan caused enormous economic yield losses. Very few research works has been done on the management of aphids in Rajasthan. Therefore, to get the scenario of aphid infestation on isabgol, the present study was conducted to know the population dynamics of aphids and its coccinellid predators.

MATERIALS AND METHODS

The experiment was conducted at the Instructional Farm, College of Agriculture, Jodhpur, during *Rabi* season of 2021-22. It is situated at 26°15' North

latitude, 73°01' East longitude with altitude (elevation) of 231 meters above mean sea level in western part of Rajasthan.

The field experiment was carried out on isabgol variety RI- 1. The crop was sown in three replication of 10 m × 10 m plot size at spacing of 30 cm × 5 cm and all the recommended agronomic practices were followed. The crop was left for natural infestation by the pest; no plant protection measures were taken throughout the crop season. The incidence of aphid was recorded on five plants selected randomly from each quadrat to record the aphid population at weekly interval and the aphids were counted on three bristle or three leaves from each tagged plant. The observations on coccinellids and their grubs were counted per plant, and mean observation of five randomly selected plants were recorded. The data of meteorological parameters and insect population were recorded for statistical analysis. The simple correlation was computed between the mean populations of aphid and natural enemies with weather parameters by using the Microsoft Excel.

RESULTS AND DISCUSSION

The aphid population on isabgol during *Rabi* 2021-22 was ranged from 6 to 56.13 aphids/ plant. The aphid infestation was initiated from 2nd standard meteorological week (SMW) i.e. January 8-14, 2022 when the pest started its multiplication from 6 aphids/ plant and fluctuated till 13th SMW (26th March, 2022). The peak activity of aphids was observed on 8th SMW which reached to the level of 56.13 aphids/ plant, when the prevailing sunshine, minimum temperature, maximum temperature, evening relative humidity and morning relative humidity were 9.20 hrs., 17.70°C, 28.90°C, 46.40% and 59.70%, respectively. A negligible rainfall was occurred during the season. Thereafter, the aphid population started to decline gradually with maturity of crop in the ending of March. The correlation between aphid population and weather parameters were non-significant positive with minimum temperature ($r = 0.136$), maximum temperature ($r = 0.395$) and average temperature ($r = 0.267$) while significant positive correlation when the factor was bright sunshine ($r = 0.652$).

These results are in partially agreement with Prajapati et al. (2020) that the aphid infestation was started from 1st week of January (1st standard meteorological week) and gradually increased up to 4th week of February (8th standard meteorological week) with a peak population of 2.40 aphid index. Ghetiya and Butani (1995) and Khinchi and Kumawat (2012) also observed that the population of aphid increased

up to the last week of January, highest population were observed during January and February. The results of correlations are in close accordance with the findings of Prajapati et al. (2020) revealed that aphid population had non-significant and negative correlation with evening relative humidity, wind velocity and rainfall. These results are also collaborate with the findings of Kataria and Kumar (2015) showed that maximum population of aphids were observed in the months of November to February which gradually started declining in March and a positive correlation was found between aphid population and maximum temperature whereas a negative correlation found with minimum temperature, relative humidity and rainfall.

The natural enemies played an important role in minimizing the pest population. In the present study, the population of three striped ladybird beetle, *Brumoides suturalis* Fabricius; six spotted zigzag ladybird beetle, *Cheilomenes sexmaculata* Fabricius and seven spotted ladybird beetle, *Coccinella septempunctata* Fabricius was recorded. The population of *B. suturalis* commenced on crop in the 2nd week of January (five week after sowing) when minimum and maximum temperature remained 10.90°C and 16.10°C, evening relative humidity and morning relative humidity was 69.70 and 88.10 per cent and sunshine was 6 hrs, respectively (Table 1). A very negligible rainfall was occurred during the crop period. After that the population fluctuated till reached to its peak in the 10th standard meteorological week (1.20 grubs and adults/ plant). Thereafter, the population started to decline gradually with maturity of crop in the ending of March. The population of *C. sexmaculata* reached to its peak in the 8th standard meteorological week with 2.33 grubs and adults/plant while *C. septempunctata* reached to maximum (4.27 grubs and adults/plant) in the 9th standard meteorological week. Correlation between aphid and coccinellids were observed significantly positive with *C. septempunctata* ($r = 0.821$), *B. suturalis* ($r = 0.914$) and *C. sexmaculata* ($r = 0.853$).

The positive correlation of *B. suturalis* population was found with bright sunshine ($r = 0.699$), maximum temperature ($r = 0.607$), minimum temperature ($r = 0.387$) and average temperature ($r = 0.503$). The negative correlation was found with rainfall ($r = -0.619$), morning RH ($r = -0.520$), evening RH ($r = -0.426$), average RH ($r = -0.506$) and rainfall ($r = -0.502$). The positive correlation of *C. sexmaculata* population was found with bright sunshine ($r = 0.757$), maximum temperature ($r = 0.759$), average temperature ($r = 0.673$) and minimum temperature ($r = 0.572$). The negative correlation was

Table 1. Population dynamics of *A. gossypii* and its natural enemies with weather parameters on isabgol

SMW#	Date of observation	Temp. (°C)		Avg. temp. (°C)	RH (%)		Rainfall (mm)	Bright sunshine (hrs.)	Mean no. of aphids / plant	Mean No. of natural enemies / plant		
		Min.	Max.		Morning	Evening				<i>Brumoides suturalis</i>	<i>Cheilomenes sexmaculata</i>	<i>Coccinella septempunctata</i>
50	Dec. 10, 2021	13.40	23.00	18.20	80.60	55.90	0.00	8.51	0.00	0.00	0.00	0.00
51	Dec. 17, 2021	11.90	22.90	17.40	72.10	43.30	0.00	7.82	0.00	0.00	0.00	0.00
52	Dec. 24, 2021	13.40	20.60	17.00	82.70	77.60	3.40	4.56	0.00	0.00	0.00	0.00
1	Jan. 01, 2022	12.90	20.20	16.55	82.90	57.00	10.00	5.18	0.00	0.00	0.00	0.00
2	Jan. 08, 2022	10.90	16.10	13.50	88.10	69.70	0.00	6.00	6.00	0.33	0.00	0.00
3	Jan. 15, 2022	12.80	22.20	17.50	78.70	47.80	0.00	6.38	16.07	0.40	0.27	0.67
4	Jan. 22, 2022	11.70	19.80	15.75	84.80	56.10	6.00	7.04	15.93	0.53	0.53	1.87
5	Jan. 29, 2022	11.70	24.20	17.95	80.90	51.90	0.00	8.07	23.27	0.60	0.87	2.47
6	Feb. 05, 2022	14.40	23.80	19.10	78.60	53.40	0.50	8.84	30.13	0.80	1.40	3.07
7	Feb. 12, 2022	16.90	26.60	21.75	75.70	70.50	0.00	9.31	42.07	0.93	1.87	3.53
8	Feb. 19, 2022	17.70	28.90	23.30	59.70	46.40	0.00	9.20	56.13	1.07	2.33	3.67
9	Feb. 26, 2022	18.60	29.40	24.00	70.90	44.40	0.00	9.35	47.67	1.20	2.27	4.13
10	Mar. 05, 2022	21.60	33.60	27.60	53.30	32.70	0.00	9.18	37.20	1.13	2.13	4.27
11	Mar. 12, 2022	26.30	34.00	30.15	66.50	33.60	0.00	6.21	30.07	1.00	1.73	4.07
12	Mar. 19, 2022	28.60	35.10	31.85	77.20	45.20	0.00	8.46	23.07	0.73	1.67	3.67
13	Mar. 26, 2022	30.90	36.30	33.60	47.40	35.90	0.00	9.63	7.87	0.53	1.53	2.27
Correlation coefficient of minimum temperature (°C)												
Correlation coefficient of maximum temperature (°C)												
Correlation coefficient of average temperature (°C)												
Morning RH (%)												
Correlation coefficient of evening RH (%)												
Correlation coefficient of average RH (%)												
Correlation coefficient of rainfall (in mm)												
Correlation coefficient of bright Sunshine (hrs.)												
Correlation coefficient of mean aphids												
									0.136 ^{NS}	0.387 ^{NS}	0.572 ^{NS}	0.526 ^{NS}
									0.395 ^{NS}	0.607*	0.759*	0.732*
									0.267 ^{NS}	0.503 ^{NS}	0.673*	0.636*
									-0.368 ^{NS}	-0.520 ^{NS}	-0.674*	-0.508 ^{NS}
									-0.204 ^{NS}	-0.426 ^{NS}	-0.479 ^{NS}	-0.497 ^{NS}
									-0.307 ^{NS}	-0.506 ^{NS}	-0.619*	-0.539 ^{NS}
									-0.502 ^{NS}	-0.619*	-0.340 ^{NS}	-0.208 ^{NS}
									0.652*	0.699*	0.757*	0.620*
									-	0.914*	0.853*	0.821*

#SMW- Standard Meteorological Week

*Significant at (p= 0.05)

NS – Non significant Correlation coefficient- r values

observed with morning RH ($r = -0.674$), average RH ($r = -0.619$), rainfall ($r = -0.340$) and evening RH ($r = -0.479$). The positive correlation of *C. septempunctata* population was found with bright sunshine ($r = 0.620$), average temperature ($r = 0.636$), maximum temperature ($r = 0.732$) and minimum temperature ($r = 0.526$). Negative correlation was found with morning RH ($r = -0.508$), evening RH ($r = -0.497$), average RH ($r = -0.539$) and rainfall ($r = -0.208$). The results on population of lady bird beetles were supported by the other earlier workers. According to Prajapati et al., 2020 the population of lady bird beetles was increased throughout the crop season and reached at peak (2.56 adults/plant), in second week of March (10th SMW). Kataria and Kumar (2015) found that the maximum temperature shows positive correlation in the case of aphid population and relative humidity shows positive correlation in case of its associated insects such as coccinellids and ant population. Khinchi and Kumawat (2012) also observed that the population of *C. septempunctata* was non-significantly correlated with the meteorological parameters but was positively correlated with the aphid population.

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AUTHOR CONTRIBUTION STATEMENT

RSO: Conducted research, written manuscript, MMK: Conceptualized, planned and guided during the study and corrected manuscript, SP: Planned the work and provided the materials for the study. DR: Provided the technical guidance during the study.

CONFLICT OF INTEREST

No conflict of interest.

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BIORATIONAL MANAGEMENT OF GRAM CATERPILLAR *HELICOVERPA ARMIGERA* (HUBNER) IN CHICKPEA UNDER ORGANIC FARMING

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ABSTRACT

Chickpea is vulnerable to the broad range of insect pests of which the gram caterpillar *Helicoverpa armigera* is a major pest, and farmers merely rely on pesticides to manage this pest. As pesticides result in many environmental hazards, biorational products are given importance. In this study, Brahmastra and Agniastra @ 10, 15 and 20 litres ha⁻¹ and Neemastra @ 250 litres ha⁻¹ were evaluated against *H. armigera* in chickpea in 2019-20 and 2020-21. Incidence of *H. armigera* larvae was significantly less in Agniastra @ 20 litres ha⁻¹ (2.58/ metre plant row) followed by Brahmastra @ 20 litres ha⁻¹ (2.65/ metre plant row) compared to untreated control (3.77/ metre plant row). Agniastra gave maximum pest reduction over control with the least damaged pods. The grain yield was also highest with Agniastra @ 20 litres ha⁻¹ (1011.67 kg ha⁻¹) with economic returns of Rs. 4382.06 ha⁻¹.

Key words: Biorational management, *Helicoverpa armigera*, chickpea, Brahmastra, Agniastra, incidence, pod damage, yield, organic farming

Chickpea (*Cicer arietinum* L.) is an important legume crop (Jukanti et al., 2012; Bakr et al., 2004) and its global production is 13.10 mt on an area of 13.54 million ha with the productivity of 968 kg/ ha (FAOSTAT, 2015). In Punjab it was cultivated over an area of 1.50 thousand ha with production of 1.80 thousand mt (Anonymous, 2021). Reduction in its productivity is due to biotic and abiotic stresses. A number of insect pests and diseases attack chickpea but eleven have been reported to cause economic losses (Rahman et al., 1982). Among these, the gram caterpillar *Helicoverpa armigera* (Hubner) is serious and cause huge yield losses. Its larvae shift from leaves to the developing seeds and pods along with development of larval instars (Reed and Pawar, 1982). It is reported from Palearctic, Oriental, Ethiopian and Australian regions and survives under varied climatic conditions (CAB, 2000). Its larvae feed upon vegetative and reproductive parts, i.e., leaves, flowers and pods of chickpea causing 90% losses (Ahmad et al., 2015). In India, *H. armigera* had been reported on many crops like cotton, pigeonpea, sunflower, corn, chilli, tomato and okra besides chickpea (Wubneh, 2016; Patil et al., 2017).

Chickpea production is affected due to the heavy infestation by *H. armigera* (Chaudhary and Sharma, 1982; Russel et al., 1999; Sarwar et al., 2009; 2011). A broad spectrum use of chemical insecticides for management of *H. armigera* has caused the resistance

development (Kranthi et al., 2002; Yang et al., 2013; Bird, 2018). Indiscriminate use of insecticides is also harmful to the pollinators, natural enemies and human beings (Mesnage and Seralini, 2018). Non-judicious applications of chemical insecticides can be effectively overcome by integrating biocontrol agents under pest management program. Use of biocontrol agents to manage *H. armigera* is effective and environmentally safe (Abid et al., 2020). Some biorational approaches which do not harm the beneficial organisms like parasitoids and predators are most important. This study evaluates some biorational products like Brahmastra, Agniastra and Neemastra for the against *H. armigera* in chickpea under organic conditions.

MATERIALS AND METHODS

The present studies were carried out at the Organic Research Farm, School of Organic Farming, Punjab Agricultural University (PAU), Ludhiana in Punjab. University's recommended chickpea variety PBG5 was raised under organic conditions as per package of practices of PAU, Ludhiana (Anonymous, 2021). Various biorational products viz., Brahmastra, Agniastra @ 10, 15 and 20 litres ha⁻¹, Neemastra @ 250 litres ha⁻¹ and one blanket spray with 100% water were applied during 2019-20 and 2020-21. These were prepared by using or collecting the locally available plant/ animal products (Devrat, 2019). The experiment was laid out in randomized block design with three

Table 1. Efficacy of bio-rational products on larval population of *H. armigera* in chickpea under organic conditions

Treatments	Larval counts (nos. m ⁻¹ plant row)						PROC						% Damaged pods						PROC		Cost of treatment (Rs./ ha)	Net returns (Rs./ ha)
	2019-20		2020-21		Pooled		2019-20		2020-21		Pooled		2019-20		2020-21		Pooled					
	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS				
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean				
Brahamastra @ 10L ha ⁻¹	2.82	3.80	2.20	2.93	2.94	2.93	19.23	21.97	21.43	25.17	21.95	21.95	14.40 (57.60)	12.67 (20.83)	13.54	24.61	24.00	24.31	1000	1314.13		
Brahamastra @ 15L ha ⁻¹	2.55	3.60	2.07	2.98	2.80	2.98	26.92	26.08	26.19	23.89	25.77	25.77	13.27 (53.07)	12.00 (20.25)	12.64	30.54	28.00	29.27	1250	2324.31		
Brahamastra @ 20L ha ⁻¹	2.40	3.47	1.93	2.80	2.65	2.80	30.77	28.82	30.95	28.57	29.78	29.78	11.83 (47.33)	11.67 (19.96)	11.75	38.05	30.00	34.03	1500	3220.81		
Agniastra @ 10L ha ⁻¹	2.73	3.73	2.27	2.88	2.90	2.88	21.15	23.34	19.05	26.45	22.50	22.50	14.08 (14.08)	12.33 (20.53)	13.21	26.27	26.00	26.14	1250	1350.81		
Agniastra @ 15L ha ⁻¹	2.60	3.60	2.00	2.87	2.77	2.87	25.00	26.08	28.57	26.87	26.63	26.63	13.62 (54.47)	12.00 (20.25)	12.81	28.71	28.00	28.36	1625	3095.38		
Agniastra @ 20L ha ⁻¹	2.33	3.40	1.87	2.73	2.58	2.73	32.69	30.18	33.33	30.27	31.62	31.62	11.57 (46.27)	11.00 (19.35)	11.29	39.44	34.00	36.72	2000	4382.06		
Neemastra @ 250L ha ⁻¹	2.67	3.93	2.13	3.00	2.93	3.00	23.08	19.23	23.81	23.47	22.40	22.40	13.43 (53.73)	13.00 (21.12)	13.22	29.67	22.00	25.84	1000	2975.81		
Blanket spray	3.33	4.80	2.67	3.75	3.64	3.75	3.85	1.44	4.76	4.34	3.60	3.60	18.75 (18.75)	15.67 (23.30)	17.21	1.83	6.00	3.92	500	38.31		
Untreated control	3.47	4.87	2.80	3.92	3.77	3.92	-	-	-	-	-	-	19.10 (76.40)	16.66 (24.06)	17.88	-	-	-	-	-		
LSD (p=0.05)	0.67	0.53	0.53	0.36	-	0.36	-	-	-	-	-	-	(3.39)	(1.80)	-	-	-	-	-	-		

L ha⁻¹: Litres per hectare; DAS: Days after spray; Values in parentheses are sine transformed means; PROC: % reduction over control; Price of organic chickpea = Rs. 6875/ q; Labour cost for spraying = Rs 500/ ha /two sprays; Brahamastra @ Rs. 20/ l; Agniastra @ Rs. 30/ l; Neemastra @ Rs. 100/ 100 l; Ingredient price (Rs 300/ 40/ 120/ kg of tobacco, green chilli and garlic, respectively)

replications, and an untreated control. Two sprays were carried out, first at pod initiation and second 15 days thereafter. Observations were recorded on the larval incidence/ m plant row at 7 and 15 days after spray (DAS), using three rows/ replicated plot. The damaged pods were recorded at the time of harvest wherein random samples of 200 pods/ plot were collected and % pod damage worked out. Data on crop yield was recorded from each plot after harvest and computed to per hectare. The experiment was set up in a randomized full block design using the CPCS 1 program (Singh and Cheema, 2001).

RESULTS AND DISCUSSION

All the biorational products tested against *H. armigera* on chickpea were superior over untreated control and blanket spray due to low larval counts, less damaged pods, high PROC and more grain yield. As regards larval counts (larvae m⁻¹ plant row), in 2019-20, at 7 DAS, the Agniastra @ 20 l ha⁻¹ (2.33 larvae/ metre plant row) and Brahamastra @ 20 l ha⁻¹ (2.40 larvae/ metre plant row) had significantly low counts which were at par with Brahamastra and Agniastra @ 10 and 15 l ha⁻¹ (2.55-2.82 larvae m⁻¹ plant row), and Neemastra @ 250 l ha⁻¹ (2.67 larvae m⁻¹ plant row) over untreated control (3.47 larvae m⁻¹ plant row) and blanket spray (3.33 larvae m⁻¹ plant row). A similar trend was there at 7 and 15 DAS in 2020-21. Pooled data also revealed that incidence was significantly low in Agniastra @ 20 l ha⁻¹ (2.58 larvae m⁻¹ plant row) and Brahamastra @ 20 l ha⁻¹ (2.65 larvae m⁻¹ plant row) over their lower dosages (2.77-2.94 larvae m⁻¹ plant row) (Table 1).

A similar trend was observed in 2020-21. In 2019-20, Agniastra @ 20 l ha⁻¹ (32.69 and 30.18%) and Brahamastra @ 20 l ha⁻¹ (30.77 and 28.82%) showed higher PROC over other treatments at 7 and 15 DAS, respectively. A similar trend was reported for 2020-21 (Table 1). As far as damaged pods are concerned Agniastra @ 20 l ha⁻¹ was observed to be superior (11.29 %); Agniastra @ 20 l ha⁻¹ (39.44 and 34.00%) and Brahamastra @ 20 l ha⁻¹ (38.05 and 30.00 %) recorded higher reduction during 2019-20 and 2020-21, respectively. A similar trend was reported for 2020-21 (Table 1); pooled data also revealed similar results. Santhosh et al. (2008) documented 63.33 and 50.00 % of *Spodoptera litura* (F) larval mortality over 31.62 and 29.78 % for *H. armigera* in Agniastra and Brahamastra, respectively. Similarly, 60.00 and 53.33% cumulative mortality in *S. litura* larvae were reported by Krishna Naik (2011). Agniastra @ 20 l ha⁻¹ (976.67

and 1046.67 kg ha⁻¹) gave maximum grain yield in 2019-20 and 2020-21, respectively. Present findings for grain yield in Brahamastra and Agniastra @ 10 l ha⁻¹ (952.50-956.67 kg ha⁻¹) fall in range of 895.84-1082.84 kg ha⁻¹ in chickpea given by Chandra et al. (2014). Higher returns were achieved in Agniastra @ 20 l ha⁻¹ (Rs.4382.06/-) followed by Brahamastra @ 20 l ha⁻¹ (Rs.3220.18/-) (Table 1). Overall, the biorational products viz., Brahamastra, Agniastra and Neemastra were found effective against *H. armigera* on chickpea crop under organic conditions.

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AUTHOR CONTRIBUTION STATEMENT

The author is the sole contributor for planning, technical inputs and conduct of the study.

CONFLICT OF INTEREST

No conflict of interest.

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INSECTICIDAL RESISTANCE IN *HELICOVERPA ARMIGERA* (HUBNER) INFESTING CHICKPEA

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ABSTRACT

A laboratory experiment was conducted at Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, India during 2017-18. Resistance to five test insecticides viz., chlorantraniliprole, cyantraniliprole, emamectin benzoate, indoxacarb and spinosad was investigated against *H. armigera* infesting chickpea from different locations of Western Maharashtra (Ahmednagar, Kolhapur, Nashik, Pune, Sangali, Satara and Solapur). Results revealed that, *H. armigera* infesting chickpea of Solapur recorded 7.00-fold resistance to indoxacarb 15.8% EC which was higher than other field populations *H. armigera*. The resistance ratios varied greatly among the populations and high resistance factor of indoxacarb 15.8% EC against all field populations of *H. armigera* infesting chickpea from all 7 locations revealed that development of decreased susceptibility to low level resistance against this insecticide whereas chlorantraniliprole 18.5% SC, cyantraniliprole 10.26% OD, emamectin benzoate 5% SG and spinosad 45% SC found to be toxic.

Key words: *Helicoverpa armigera*, resistance, chickpea, indoxycarb, chlorantraniliprole, cyantraniliprole, emamectin benzoate, spinosad, Ahmednagar, Kolhapur, Nashik, Pune, Sangali, Satara, Solapur

Helicoverpa armigera (Hubner) is a prominent polyphagous pest damaging chickpea in many global agricultural systems. Almost 30% of all pesticides used worldwide are directed against *H. armigera* (Ahmad, 2007). The unrestrained usage of a limited group of insecticides against successive pest generations of *H. armigera* imposed severe selection pressure ensuing in resistance and leading to field control failures. *H. armigera* is the noctuid species reported enormous cases of insecticide resistance worldwide with evolved resistance against organochlorines, organophosphates, carbamates, pyrethroids (Kranthi et al., 2002 and www.pesticideresistance.org), spinosad (Aheer et al., 2009), emamectin benzoate, indoxacarb (Qayyum et al., 2015) and *Bacillus thuringiensis*-derived toxins (Zhang et al., 2011). The field populations of *H. armigera* also indicated development of resistance to multiple insecticides (Faheem et al., 2013). Insecticide resistance in *H. armigera* is reported due to the combined effects of insensitivity of acetylcholine esterase to insecticides, expression of higher levels of esterases, phosphatases and a specific protein called p-glycoprotein ATPase (Srinivas et al., 2004). Hence the change in susceptibility in insect-pests to different insecticides is need to be detected from time to time which alert growers about changes in resistant populations, development of novel resistance and helping them in taking correct pest control decisions

and improve the sustainable use of insecticides. Hence keeping this in view, the present investigation was planned to detect the levels of insecticidal resistance in the field populations of *H. armigera* infesting chickpea grown in different districts of Western Maharashtra region of Maharashtra during 2017-18.

MATERIALS AND METHODS

Large sized larvae of *H. armigera* were collected from chickpea fields grown in different districts of Western Maharashtra region of Maharashtra separately in clean plastic vials along with sufficient green pods to avoid starvation. Immediately these larvae were carried to the Post Graduate Laboratory, Dept. of Agril. Entomology, College of Agriculture, Latur for further culturing. The collected L1 larvae were individually reared on natural diet (green pods) till pupation in round plastic vials (measuring 4 cm dia and 5 cm height). The pupae were transferred to round clean plastic containers (measuring 16 cm diameter and 16 cm height) covering top with muslin cloth secured firmly with rubber band. The sexes were determined in pupal stages on the basis of distance between genital and anal apertures. It is less in the case of male and more in the case of female (Srivastava and Pande, 1966; Dani et al., 1980). The freshly emerged adults were released into standard oviposition cage (measuring 50 cm x 30 cm) covered

with black muslin cloth. The oviposition cage was placed over the water trough in order to create humidity. The proportion of female and male in the cage was 1:1 in order to get fertilized eggs. Cotton swab dipped into 10 per cent honey solution was provided to serve as food for the adults. A strip of cotton cloth toweling (6 x 17 cm) and/or chickpea pods were hung vertically inside each oviposition cage as oviposition substrate. The eggs laid on the toweling and/or chickpea pods were kept in a transparent plastic box (26 cm x 17 cm x 6 cm). The eggs from each pair were kept separately. After hatching, neonate larvae were transferred separately into plastic vials to avoid cannibalism. Daily the larvae were fed on natural diet. The 2nd instar larvae of 'F1' generation were used for conducting the bioassay studies. The rearing of *H. armigera* population collected from different districts of Western Maharashtra region was carried out separately at ambient room temperature of 28 ± 3° C. The susceptible population of *H. armigera* was developed by rearing five generations of *H. armigera* without selection pressure of any insecticide under laboratory conditions (Tripathy and Singh, 2000).

The insecticides which are commonly used by farmers (viz., chlorantraniliprole, cyantraniliprole, emamectin benzoate, indoxacarb and spinosad) were selected for studying the levels of insecticide resistance in *H. armigera* infesting chickpea. All the insecticides were procured from market and dilutions required were prepared from the formulated product only with distilled water. In *H. armigera* bioassay, each insecticide was used in five concentrations (two lower than recommended, one recommended and two higher than recommended) rendering 20 to 80 per cent mortality in pilot tests. However, care was taken to retain the recommended dosage of each insecticide as one of the concentrations. Newly moulted 2nd instar larvae of *H. armigera* from F₁ laboratory culture were exposed to different insecticides using pod dip method (IRAC Method No. 7) recommended by Insecticide Resistance Action Committee with slight modification. Formulated insecticides were diluted using distilled water as a solvent. Sufficient number of non-infested, untreated and fresh pods was collected from unsprayed chickpea plots. Then these pods were dipped into the test solution for 60 seconds, dried on paper towel and transferred to labelled clean plastic rearing vials. Two treated pods per treatment were maintained in each vial to avoid starvation stress during the test. One newly moulted 2nd instar F1 larva was placed on these dried pods and then the vial was covered with a plastic lid. Ten larvae per treatment per replication were exposed to treated

Pods. Three replicates each of five concentrations and one control (distilled water) were used for each test insecticide at ambient room temperature. Observation on larval mortality was recorded at 48 hrs after exposure period. Larvae regarded as dead when they were not able to move on probing with a blunt probe or brush. The setup of bioassays was maintained separately for every location. The mortality data of each treatment was corrected with respect to control mortality as per Abbott (1925) for *H. armigera* bioassays. The resistance factor (RF) was calculated with formula given by Pate and Bhamare (2016). The resistance ratio values were used to indicate resistance levels or categories as given by Reddy and Bhamare (2016).

RESULTS AND DISCUSSION

The resistance ratios of test insecticides for all 7 locations of Western Maharashtra was found to be in the range of 2.18 to 2.58 fold for chlorantraniliprole 18.5%SC, 2.31 to 2.93 fold for cyantraniliprole 10.26%OD, 1.60 to 1.94 fold for emamectin benzoate 5%SG, 5.23 to 7.00 fold for indoxacarb 15.8%EC and 1.52 to 1.98 fold for spinosad 45%SC. The toxicity of test insecticides was noticed in the order of spinosad 45%SC > emamectin benzoate 5%SG > chlorantraniliprole 18.5%SC > cyantraniliprole 10.26%OD > indoxacarb 15.8%EC.

Spinosad 45%SC, emamectin benzoate 5%SG, chlorantraniliprole 18.5%SC and cyantraniliprole 10.26%OD were more toxic and *H. armigera* populations were found to be susceptible to these insecticides whereas decreased susceptibility to low level of resistance developed for indoxacarb 15.8%EC against *H. armigera*. These results are in conformity with the findings of Karjule et al. (2017) who monitored development of insecticide resistance in *H. armigera* infesting pigeonpea from Marathwada region and exhibited that all the populations indicated susceptibility to chlorantraniliprole 18.5%SC (1.13 to 1.96 fold), cyantraniliprole 10.26% OD (1.74 to 2.10 fold) and emamectin benzoate 5%SG (2.09 to 2.54 fold). From Telangana, Deepa (2015) indicated that *H. armigera* larvae collected from Mahaboobnagar population recorded the resistance factor of 1.3, 2.0 and 2.6 fold to chlorantraniliprole at 24, 48 and 72 hours, respectively and 1.1, 1.7 and 2.5 fold resistance ratio to emamectin benzoate at 24, 48 and 72 hours, respectively. Similarly, Bird (2015) revealed that the resistance ratio for chlorantraniliprole was 2.9 fold. Bird et al. (2017) could not detected resistance in *H. armigera* populations to

Table 1. Insecticidal resistance in field population of *H. armigera* infesting chickpea

Sr. No.	Strain	Chlorantraniliprole 18.5% SC		Cyantraniliprole 10.26% OD		Emamectin benzoate 5% SG		Indoxacarb 15.8% EC		Spinosad 45% SC	
		LC ₅₀ ml/ g/l	RR	LC ₅₀ ml/ g/l	RR	LC ₅₀ ml/ g/l	RR	LC ₅₀ ml/ g/l	RR	LC ₅₀ ml/ g/l	RR
1	Ahmednagar	0.0343	2.28	0.0509	2.57	0.0202	1.60	0.1035	6.72	0.0124	1.82
2	Kolhapur	0.0388	2.58	0.0517	2.61	0.0224	1.77	0.0806	5.23	0.0104	1.52
3	Nashik	0.0354	2.36	0.0582	2.93	0.0217	1.72	0.0959	6.22	0.0119	1.75
4	Pune	0.0375	2.50	0.0570	2.87	0.0245	1.94	0.1078	7.00	0.0122	1.79
5	Sangali	0.0328	2.18	0.0521	2.63	0.0196	1.55	0.0815	5.29	0.0135	1.98
6	Satara	0.0363	2.42	0.0528	2.66	0.0209	1.65	0.0961	6.24	0.0125	1.83
7	Solapur	0.0372	2.48	0.0459	2.31	0.0232	1.84	0.1037	6.73	0.0120	1.76
8	Susceptible	0.0150	-	0.0198	-	0.0126	-	0.0154	-	0.0068	-

emamectin benzoate and also reported low but detectable levels of survival of *H. armigera* at discriminating concentrations of indoxacarb. Gill and Dhawan (2006), Stanley et al. (2009), Khan et al. (2010) and Pan et al. (2017) revealed that *H. armigera* was highly susceptible to spinosad. Wang et al. (2017) showed that the indoxacarb-selected population, Yishui population, Shandong and Handan populations exhibited decreased sensitivity, low-level resistance and moderate-level resistance to indoxacarb 15.8%EC, with the resistance ratios of 4.36, 8.06 and 15.34 fold, respectively. More or less similar trend of results were obtained by Agboyi et al. (2016) revealed that spinosad was more toxic to *P. xylostella* populations than the other insecticides with LC₅₀ and LC₉₀ values less than 1 and 15 µg per ml, respectively. Reddy and Bhamare (2016) exhibited that *Earias vittella* population from different locations of Marathwada region registered variations in susceptibility to chlorantraniliprole 18.5%SC, cyantraniliprole 10.26%OD and emamectin benzoate 5%SG.

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AUTHOR CONTRIBUTION STATEMENT

A S Bade and V K Bhamare conceived and designed research, A S Bade conducted research, analyzed the data and wrote the manuscript which was guided by V K Bhamare.

CONFLICT OF INTEREST

No conflict of interest.

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AGE SPECIFIC AND FERTILITY LIFETABLE OF *Callosobruchus maculatus* (F.) ON CHICKPEA

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ABSTRACT

Pulse beetle (*Callosobruchus maculatus*) (F.) is a pernicious pest of different stored grain pulses and decrease the nutritional and economic values of the pulses, which can cause huge losses in terms of quality and quantity. The present study was conducted for determining the rate of multiplication of *C. maculatus* was conducted at ambient temperature and relative humidity under Palampur conditions on preferred host chickpea, *Cicer arietinum*. Under conditions of abundant space and food supply, population of *C. maculatus* increased with 0.03485 (r_m) and 1.035 (λ) per day. The population multiplied 1.276 times in one week and mean time for doubling the population was 19.88 days. True generation time was 63.07 days. Life expectancy of adult was 12.37 days. The results of the study pave ways to create awareness among researchers and farmers about the nature and extent of damage caused by the *C. maculatus* in chickpea.

Key words: Life table, survivorship, pulse beetle, *Cicer arietinum*, life expectancy, doubling time, gross reproductive rate, net reproductive rate, finite rate of increase, generation time, potential fecundity, doubling time

A variety of insect-pests wreak havoc on stored grains. Among several grain pests, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a prominent pest of stored legume grains, with infestations beginning at pod maturity (Umar and Turaki, 2014). It attacks maturing crops in the field and causes qualitative and quantitative loss in storage. It feeds on variety of hosts which include pigeonpea, pea, cowpea, chickpea, blackgram, horsegram, and other legumes (Srivastava and Subramanian, 2016). The pulse beetle, is an economically important pest of stored chickpeas, which produces losses up to 10-30% in a short period of two months (Philips and Throne, 2010). However, in some case the infestation level may reach upto 100 per cent (Govindan et al., 2020). Infestation begins either on mature pods when they are approaching harvest or when in storage. Adult beetle soon after emergence lays eggs on the surface of the seed kept in storage or on pods in the fields. The larva burrows directly into the seeds and larvae's feeding activity can almost entirely hollow out the seeds, leaving a distinctive emergence hole as the adult emerges (Giga and Smith, 1983). Adults do not consume food and larva within the seeds eats the endosperm, causing the seed to be completely destroyed. In storage, beetle populations grow so quickly that it turns the entire stored grains into flour. It is estimated that all the activities of insect pests like feeding on grain,

their presence in the cereal grains and products, and the expenditure for the strategies used to destroy them have caused a significant economic loss. If we can save these losses, our stockpiles of food grains could grow enormously, thus enabling us to feed millions of hungry people worldwide (Kalpna et al., 2022). In the ecological studies of any insect-pest, a life table constitute to be an essential analytical tool that provides detailed information on population dynamics to generate simple but more informative statistics. It also includes a detailed summary of survival, development, and life expectancy (Ali and Rizvi, 2007). The collection of data on life-table at particular temperatures and humidity gives an important task for pest management in different environmental conditions. Hence, the age and female fecundity life tables of *C. maculatus* were studied on chickpea grains under laboratory conditions.

MATERIALS AND METHODS

The experiment was conducted under laboratory conditions at Entomology Department of CSKHPKV, Palampur (17-22°C, 55-65%RH) during 2018. At the beginning of the experiments, to synchronize the age of eggs, ten pairs of *C. maculatus* were transferred from the stock culture on 100 g of chickpea seeds. After 12 hr, 50 eggs on seeds were collected for further investigation. The collected eggs were transferred into

the container which was covered with the white muslin cloth. The collected eggs were checked daily until the emergence of adults. Duration of adult longevity was also recorded daily until the death of the last female. After the emergence of adults, the females with males were placed into each plastic case containing chickpea. The duration of oviposition and post-oviposition periods as well as longevity, daily fecundity (eggs/ reproduction day), and total fecundity (eggs during reproduction period) were recorded for one generation. The life table was built based on the concepts as studied by Birch (1948), Southwood (1978) and Chakraborty and Mondal (2015). For calculating the age-specific survivorship different parameters viz., age-specific survival (l_x) and mortality (dx) were built. In case of age-specific female fertility life table age specific survival (l_x) and average number of female offsprings (m_x) for each age interval (x) were employed. The other demographic parameters including gross reproductive rate (GRR) or potential fecundity, net reproductive rate (R_0), approximate

generation time (T_c), innate capacity for natural increase (r_c), true intrinsic rate of increase (r_m), true generation time (T), finite rate of natural increase (λ), doubling time (DT), and weekly multiplication rate (WR), were calculated using survivorship and fertility schedules.

RESULTS AND DISCUSSION

The various growth parameters of the beetle were worked out. The results revealed the net reproductive rate (R_0) of 9.01 females/ female on chickpea. While true of generation (T) time was 63.07 days. The approximate rate of increase (r_c) was recorded slightly lower than the actual rate of natural increase (r_m) and those were 0.0347 females/ female per day and 0.0348 females per female per day respectively. The finite rate of increase was 1.03 females/ female per day (Table 1). These results are in accordance with Bidar et al. (2021) who observed finite rate of increase to the tune of 1.155 females/ female/ day on chick pea. Similar studies were undertaken by Chakraborty and Mondal (2015) who

Table 1. Age-specific female fertility lifetable of *C. maculatus* on chickpea

x	l_x	m_x	$l_x m_x$	$x l_x m_x$	$e^{-r_x} l_x m_x$		$e^{-r_x} . l_x m_x$	
					$r=0.036$	$r=0.034$	$r=0.036$	$r=0.034$
0	1.00							
1-58	0.62	Immature stages						
59	0.62	Preoviposition period						
60	0.62	2.85	1.767	106.02	223.4713	251.9632	0.203779	0.229761
61	0.62	2.67	1.6554	100.9794	201.9545	228.159	0.184159	0.208054
62	0.62	1.82	1.1284	69.9608	132.7942	150.3252	0.121093	0.137079
63	0.62	1.65	1.023	64.449	116.1334	131.728	0.1059	0.12012
64	0.62	0.94	0.5828	37.2992	63.82142	72.53642	0.058198	0.066145
65	0.62	1.38	0.8556	55.614	90.38223	102.9299	0.082418	0.09386
66	0.62	0.68	0.4216	27.8256	42.96139	49.0236	0.039176	0.044704
67	0.58	1.28	0.7424	49.7408	72.97617	83.44042	0.066546	0.076088
68	0.58	0.72	0.4176	28.3968	39.59761	45.36626	0.036108	0.041369
69	0.54	0.33	0.1782	12.2958	16.29977	18.71174	0.014863	0.017063
70	0.43	0.42	0.1806	12.642	15.93518	18.32982	0.014531	0.016715
71	0.36	0.15	0.054	3.834	4.596195	5.297467	0.004191	0.004831
72	0.29	0	0	0	0	0	0	0
73	0.18	0	0	0	0	0	0	0
74	0.11	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0
		$\Sigma m_x =$	$\Sigma l_x m_x =$	$\Sigma x l_x m_x =$	$\Sigma =$	$\Sigma =$	$\Sigma =$	$\Sigma =$
		14.89	9.0066	569.0574	1020.923	1157.811	0.930962	1.055787

Fertility life table parameters of *C. maculatus* on chickpea based on above observations are calculated as under:

Gross reproductive rate (GRR) or Potential fecundity= 14.89 females/ female; Net reproductive rate (R_0)= 9.01 females/ female; Approximate generation time (T_c)= 63.18 days; Innate capacity for natural increase (r_c)= 0.0347 females/ female/ day; True intrinsic rate of increase (r_m)= 0.03485 females/ female/ day; True generation time (T)= 63.07 days; Finite rate of natural increase (λ)= 1.035 females/female/day; Weekly multiplication rate (WE)= 1.276 females/female; Doubling time (DT)= 19.88 days

X: pivotal age in days; l_x : survival fraction of females; m_x : female eggs per female

reported r_m value of 0.055 on the greengram. Further, on the basis of (r_m) values, the descending order on different food grains for *Callosobruchus chinensis* (L.) was gram (0.097), pea (0.0928), black gram (0.0892) and soybean (0.085) and the finite rate of increase was 1.25, 1.24, 1.22 and 1.23 females/ female/ day on gram, pea, soybean and black gram, respectively (Borude et al., 2012). Overlap between the stages was observed in terms of reproductive value, life expectancy and survival rate of pulse beetles because of the variation in the development period among the individuals (Singh and Boopathi 2022), which signifies the importance of observing the different biological parameters. Naseri et al. (2022) observed similar trend of different parameters of life table studies of *C. maculatus* on the Sari cultivar of soybean with R_o , r_m and λ with 14.60, 0.061 and 1.06 values, respectively.

The difference in the values of various fertility parameters in respect to the present investigations with some of the previous supporting literature corresponds to the difference in hosts as well the difference in the relative ambient conditions viz. temperature and humidity. It is well documented that for survival and reproduction, this bruchid must rely on materials obtained during the larval stage (Bhoge et al., 2023). Further, the growth parameters of this bruchid is greatly influenced by type of host plant, seed parameters (shape, size, weight, seed surface texture), availability of seeds, strain of the beetle, mating behavior, and generation (Cope and Fox 2003; Kazemi et al., 2009; Ahuchaugu, 2021). Also, in our study, the pre-oviposition period of 1 day was observed and adults started laying eggs after that. This corresponds to the earlier studies which marked no significant pre-ovipositional period for this bruchid and also the adults did not need food or water, and could reproduce immediately after one to two days of emergence (Kazemi et al., 2009; Mohammadi et al., 2020). Life expectancy (ex) of *C. maculatus* was recorded, starting from adult emergence. A gradual decrease in 'ex' was found with the advancement of the age of the insect. On the first day of its emergence as an adult, the expected life was 12.37 days (Fig. 1). These results are in accordance with the findings of Jaiswal et al. (2018), who observed the average lifespan of 10.15 days of pulse beetle. Different workers have reported the life span of adult of pulse beetles from 9.89-15.5 days (Varma and Anandhi, 2010; Sharma et al., 2018; Solanki and Mittal, 2018; Muhammadi et al., 2020); which is more or less similar with the present findings.

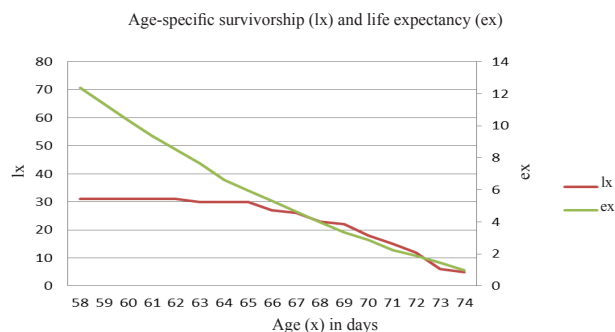


Fig. 1. Age-specific survivorship (lx) and life expectancy (ex) of *C. maculatus* on chickpea

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AUTHOR CONTRIBUTION STATEMENT

US conceived and designed research. US conducted experiments and SS analyzed the data. Both the authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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OCCURRENCE OF FALL ARMYWORM *SPODOPTERA FRUGIPERDA* (J E SMITH) AS EARHEAD CATERPILLAR ON FINGER MILLET

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ABSTRACT

Fall Armyworm, *Spodoptera frugiperda* considered as a polyphagous and potential pest of cereals. Recently it was found infesting on earheads of finger millet at ZARS, V C Farm, Mandya, Karnataka, India. Screening of 230 finger millet genotypes during Kharif-2018 and 2019 revealed that, fist earhead shape genotypes recorded maximum level of incidence i.e. 0.15 to 0.50 larvae/earhead, whereas, compact earhead shape recorded moderate level of incidence 0.06 to 0.37 larvae/earhead and minimum level on semi-compact earhead shape genotypes i.e. 0.01 to 0.20 larvae/earhead. However, droopy/lax and open earhead genotypes were free from infestation completely. During various stages of earhead development, A maximum level of incidence was recorded during doughing stage (0.23 larvae/ earhead), followed by milky stage (0.16 larvae/earhead) and maturity stage (0.07 larvae/earhead). However, at flowering stage, there was no incidence.

Key words: Fall armyworm, *Spodoptera frugiperda*, earhead caterpillar, finger millet, *Eleusine coracana*, invasive pest, compact, semi-compact, open-droopy and lax, maturity stage, dough stage, milky stage

Finger millet belongs to the family “Poaceae” scientifically called as *Eleusine coracana* (L.) Gaertn. The word “*Eleusine*” is derived from a Greek word, means “Goddess of Cereals” (Chalam and Venkateshwaralu, 1965). It is an annual plant, widely grown in Asian and African countries of the world including India. Finger millet is a very hardy crop as well as a great source of nutritive value and is a climate change complaint crop. The crop is being widely cultivated through the year for food, fodder, medicinal and nutritive value in Asian and African countries (Mirza and Marla, 2019) both in rain-fed and irrigated conditions under varied agro climatic conditions. This crop is also found prove to various native and invasive pest attacks. The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is considered as one of the most ruinous pests native to the America. It is an invasive species predominant in several countries viz., Brazil, Argentina and USA (Prowell et al., 2004; Clark et al., 2007) causing economic losses across a wide range of crops viz., maize, cotton, soybean, beans (Pogue 2002; Nagoshi et al., 2007; Bueno et al., 2010), rice, number of weeds and other grasses (Nabity et al., 2011) in large parts of the world. The occurrence of *S. frugiperda* as an invasive pest in Asia was reported for the first time on maize in Karnataka in India (Sharanabasappa et al., 2018;

Ganiger et al., 2018 and Shylesha et al., 2018). It is well known to be a “pest of cereals”, due to its preference to “Gramineae” family and incidence was also noticed on fodder crops viz., maize and sorghum (Keerthi et al., 2021) and other hosts as listed by Deshmukh et al. (2021). Recently *S. frugiperda* has been reported on finger millet (Chikkarugi and Vijaykumar, 2022). In view of this status of its abundance, incidence and host resistance were studied during 2018 and 2019 kharif on 230 finger millet genotypes, and those are given herein.

MATERIALS AND METHODS

To know the abundance of *S. frugiperda*, 230 finger millet genotypes representing droopy (fingers drooping and lax), open (straight fingers), semi-compact (tops of fingers curved), compact (incurved fingers) and fist (very incurved fingers) earhead shapes were examined during the dough stage of the crop by selecting 20 earheads randomly in each genotype of different sets viz., Finger Millet Initial Varietal Trial (FMIVT), Finger Millet Advanced Varietal Trial (FMAVT), National Screening Nursery Finger Millet (NSNFM), State Multi Location Trial (SMLT), National Bureau of Plant Genetic Resources (NBPGR), Kenyan and local land races were obtained from the All India Coordinated Research Project (AICRP) on Small Millets, V. C. Farm, Mandya. The observations on the number of

S. frugiperda caterpillars infesting different types of earheads were recorded and the mean larval population was worked out.

Similarly, to know the pest status, observations were made on *S. frugiperda* caterpillars on 11 selected fist shape earhead genotypes namely., EC886329, EC886337, EC886336, EC886340, EC886328, EC886342, EC886343, Hamsa, GE4449, GPU-67 and Udu mallige (susceptible to earhead caterpillars) during *Kharif* 2018 and 2019 at different stages of the crop, including flowering, milky, dough and maturity. In each genotype, 20 earheads were selected randomly, observations were made on the number of *S. frugiperda* caterpillars and mean larval population was calculated. To interpret the replicated data from each trial (under respective trials) by using the OP Stat software the means were separated by Tukey's HSD (Tukey, 1965) and ANOVA (Gomez and Gomez, 1984; Hosmand, 1988).

RESULTS AND DISCUSSION

The experimental studies revealed that out of 230 genotypes evaluated, droopy or lax (6 genotypes) and the open type earhead (26) genotypes were fully free of *S. frugiperda* infestation. Whereas, semi-compact (110) genotypes recorded minimum level of incidence with an average larval population varied between 0.01

to 0.20 larva/earhead ($\bar{X} = 0.060 \pm 0.033$), followed by compact (68) genotypes which recorded moderate mean larval population ranged between 0.06 to 0.37 larva/earhead ($\bar{X} = 0.166 \pm 0.062$) (Fig. 1). However the fist earhead shape (20) genotypes recorded maximum level of *S. frugiperda* incidence (Fig. 2), ranged between 0.15 to 0.50 larva/earhead ($\bar{X} = 0.233 \pm 0.087$) (Table 1). These findings are in conformity with Chikkarugi et al. (2022) who also found higher mean larval population of earhead caterpillars on fist earhead genotypes and these findings were supported by early workers Fletcher (1921) who revealed that, more compact or tight-fisted varieties are generally more vulnerable to the attacks, as they provide hiding places for the caterpillar within the closed earhead. Similar reports were obtained from Ali et al. (1987) compact and fist types showed a higher proportion in 30.0 and 40.0% level of infestation. Likewise, In finger millet, fist type of earheads had a very high level of infestation both in terms of mean larval number per earhead and % earhead infestation (Hegde, 1989) and reports of Paul et al. (1980) revealed that compact earhead genotypes viz., SPV-122 and SPV-369 exhibit highly susceptible reaction against *H. armigera*. Meanwhile, Balasubramanian et al. (1979) found that open and loose panicles had fewer numbers of earhead bug, caterpillars and webworms than the semi compact and compact types. In Sorghum, Mote and



Fig. 1. Infestation of *S. frugiperda* on compact earhead shape genotypes of finger millet



Fig. 2. Infestation of *S. frugiperda* on fist earhead shape genotypes of finger millet

Table 1. Abundance *S. frugiperda* on genotypes of finger millet during earhead stage

Earhead type	No. of genotypes	Mean larval population/ earhead	
		Mean± SD	Range
Droopy/ Lax type	6	0	0
Open type	26	0	0
Semi-compact type	110	0.060± 0.033	0.01 - 0.20
Compact type	68	0.166± 0.062	0.06 - 0.37
Fist type	20	0.233± 0.087	0.15 - 0.50

*Observations from a set of 230 finger millet genotypes under FMIVT, FMAVT, NSNFM, SMLT, NBPGR, Kenyan and local

Pokharkar (1981) reported that, compact earheads viz., CS-3541, SPV-102 genotypes were more susceptible to attack of *S. elongella* and *E. subnotata* and Li et al. (2021) was also of some opinion against fall armyworm.

Eleven fist earhead type genotypes viz., EC886329, EC886337, EC886336, EC886340, EC886328, EC886342, EC886343, Hamsa, GPU-67, GE4449 and Udurumallige were chosen for screening against the occurrence of *S. frugiperda* caterpillars at different stages of earhead development viz., flowering, milky, dough and matured stage during kharif 2018 and 2019. The mean larval population ranged between 0.07 to 0.23 larva/earhead. There was no incidence noticed at flowering stage. The number of larvae per earhead noticed during milky, dough and maturity stages were 0.16, 0.23 and 0.07 larva/earhead respectively (Table 2). With this, it can be presumed that *S. frugiperda* might cause severe injury during doughing stage of the crop. These results were in conformity with findings of Mital et al. (1980), Ramadan et al. (2004) and Raveendra et al. (2018) which revealed that population of the sorghum earhead caterpillar, *C. gnidiella*, was highest during the doughing stage of the crop and narrowed

Table 2. *Spodoptera frugiperda* incidence at earhead growth stages and pest status

Earhead growth stages	Occurrence of species	Mean No. of larva/ earhead	Pest status
Flowering	✕	0.00	** (Occasionally serious)
Milky	✓	0.16	
Dough	✓	0.23	
Maturity	✓	0.07	

*Minor (< 0.20 larvae/ earhead); **occasionally serious (0.20 – 0.50 larvae/ earhead); ***Major (> 0.50 larvae/ earhead)

down with grain maturity and hardening. The results of Hosam and Gepaly (2019), noticed a complex of lepidopteran larvae of *E. gayneri*, *C. gnidiella*, and *P. simplex* were maximum on dough earhead stage of sorghum. These studies were supported by past findings of Hegde (1989) in finger millet where he found the infestation of *C. angustipennella* increased to 51% at doughing stage. Chikkarugi et al. (2020) and Sharma et al. (2022) reported earhead caterpillars incidence was noticed maximum at doughing stage of the earhead in finger millet. Occurrence of *S. frugiperda* has been confined to the fist and compact earhead shaped genotypes. Thus, the results of this study infer that, the farmers may endorse to go for cultivation of open or semi compact type of earhead finger millet genotypes to combat the menace of earhead caterpillar *S. frugiperda*.

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AUTHORS CONTRIBUTION STATEMENT

NMC, LV conceived and designed the research work plan. HRR and BS along with NMC conducted field the experiments, SN and MSK contributed in data analysis and preparation of manuscript. All authors approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF SELECTED RICE LANDRACES ON THE FECUNDITY AND SURVIVAL OF RICE LEAF FOLDER *CNAPHALOCROCIS MEDINALIS* (GUENEE)

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ABSTRACT

The rice leaf folder, *Cnaphalocrocis medinalis* (Guenée) is a significant pest causing outbreaks. This study focuses on the impact of selected rice landraces on the survivability of *C. medinalis*, aiming to identify potential sources for IPM and future breeding programs. The research encompassed 35 landraces, categorized as resistant, moderately resistant and susceptible based on net house screenings along with standard resistant check (TKM6) and susceptible check (TN1). The study evaluated fecundity, larval emergence, survival rates, and adult emergence on these genotypes. Results showed that resistant and moderately resistant genotypes significantly reduced the pest's fecundity and survival across various lifestages compared to susceptible ones. The findings indicated the possible existence of antibiosis factors in resistant genotypes.

Key words: *Cnaphalocrocis medinalis*, rice, landraces, antibiosis, survivability, fecundity, larval emergence, pupal survival, adult emergence, host plant resistance, IPM

The rice leaf folder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), is a serious pest of rice causing outbreaks (Dale, 1994). Its significance has escalated with the adoption of high-yielding varieties and the associated changes in agricultural practices, leading to decreased crop yields. Effective IPM strategies involve identifying sources of resistance and understanding the mechanisms that induce resistance against such pests. Plant resistance to insect infestation can be categorized into three factors: antibiosis, antixenosis, and tolerance (Painter, 1951). Factors like fecundity and survival of insect pests may be significantly affected by the quality of the host plant (Awmack and Leather, 2002). The host plant's morphological, physical, physiological, and chemical attributes interact with the pest, influencing its ability to seek out suitable hosts and affecting how effectively it can locate and exploit those hosts (Muller, 1983). Interaction between host plants and pests provides a foundation for developing IPM strategies. Resistant varieties provide a sustainable approach, but donors of resistance remain largely unexplored. It is necessary to identify such sources and utilization of rice landraces, which were traditionally cultivated by farmers. With this objective in mind, the present study evaluates the impact of selected landraces on the survivability of *C. medinalis*.

MATERIALS AND METHODS

The material comprised 35 distinct landraces obtained from various regions of Odisha, along with a standard resistant check, TKM6 and a susceptible check, TN1. These consists of resistant, moderately resistant and susceptible genotypes which were rated on the basis of adjusted damage rating (ADAR) obtained from nethouse screening during kharif, 2021 and rabi, 2021-22. The ADAR between 1-30% (rating 1-3) were considered as resistant, between 31-50% (rating 5) as moderately resistant and 50% (rating 7-9) as susceptible. The ADAR % was computed using the observations on the damaged area in mm² of the test entry and the susceptible check. The % plant damage calculated was considered for conversion into damage scores following the standard evaluation system (SES) for rice (IRRI, 2013).

Cnaphalocrocis medinalis was reared following the protocol outlined by Waldbauer and Marciano (1979) at 25± 5° C, and 60± 10% RH. Larvae were collected from the field and were released onto the rearing chamber containing 30-day-old potted TN1 plants. Upon pupation, the pupae were collected and placed in petridishes lined with filter paper, then transferred to adult emergence cages. The newly emerged adult moths

were immediately utilized for further experimental studies. The fecundity study was carried out within an ant-proof oviposition cages. Each cage contained two resistant, one moderately resistant, one susceptible category of Odisha landrace rice genotypes and the standard resistant and susceptible checks. Ten pairs of adult moths were collected from the adult emergence cage and introduced into the oviposition cage. To provide nourishment for moths, a cotton ball soaked in a 20% honey was placed inside the chamber. The moths were allowed to lay eggs for a period of three days. Subsequently, the eggs laid on each genotype were counted. This experiment was replicated three times. The survivability was observed inside an insect-proof nethouse at ICAR-NRRI, Cuttack. After oviposition, transparent mylar cages were used to cover the potted plants containing eggs. The first instar larval emergence was observed with observations repeated thrice. After the larval stage, the count was taken for the surviving 5th instar larvae, followed by the prepupae and pupae. Once pupation occurred, the pupae were transferred to an adult emergence chamber, and the number of emerging adults was recorded. The data were analyzed in a completely randomized block design, with data presented as mean values along with standard errors (mean $\pm$ SE). The data were subjected to ANOVA, and means were compared using Tukey's honestly significant difference (HSD) test ($p=0.05$) using IBM SPSS version 22.

RESULTS AND DISCUSSION

The genotypes selected consisted of 19 resistant, 10 moderately resistant, and 6 susceptible (Table 1). The fecundity of *C. medinalis* revealed significant variations (66.67 $\pm$ 1.20 to 175.33 $\pm$ 4.37; in resistant ones it was 66.67 $\pm$ 1.20 to 73.33 $\pm$ 2.33; with Kalakusuma exhibiting the least; in the moderately resistant ones it ranged from 88.00 $\pm$ 1.16 to 100.33 $\pm$ 0.88; with the least being in Agnisar (88.00 $\pm$ 1.16); while among the susceptible ones it varied from 144.33 $\pm$ 0.88 to 178.00 $\pm$ 5.20; with maximum being with Nimei (178.00 $\pm$ 5.20), followed by N. umerchudi (175.33 $\pm$ 4.37) and Safari (168.00 $\pm$ 2.08). During 2023, also similar trend was observed. In the survivability study, the number of first instar larvae emerging from eggs ranged from 51.00 $\pm$ 1.53 to 164.00 $\pm$ 8.08 with notably, resistant landraces like Bhatta (51.00 $\pm$ 1.53), Basudha (52.00 $\pm$ 1.16) and Bayabhandha (55.33 $\pm$ 1.20) showing the least values; while the susceptible ones like Nimei (164.00 $\pm$ 8.08), N. umerchudi (163.00 $\pm$ 5.29) and Safari (156.67 $\pm$ 1.67) showing maximum values. During 2023 too

similar trend was observed. Survival of fifth instar larvae, ranged from 34.33 $\pm$ 1.20 to 136.33 $\pm$ 8.09, with resistant ones, particularly Basudha (34.33 $\pm$ 1.20), Bhatta (34.33 $\pm$ 2.33) and Bayabhandha (37.67 $\pm$ 2.03) showing the least survival rates; in the susceptible N. umerchudi (136.33 $\pm$ 8.09), Nimei (134.33 $\pm$ 9.39) and Safari (130.67 $\pm$ 3.53), these values were maximum, with similar trend seen in 2023.

Number of larvae entering the prepupal stage ranged between 30.33 $\pm$ 1.20 and 131.33 $\pm$ 9.26 with resistant Basudha (30.33 $\pm$ 1.20), Bhatta (30.67 $\pm$ 2.60) and Kalakusuma (29.67 $\pm$ 2.67) showing least values and the susceptible N.umerchudi (131.33 $\pm$ 9.26), Nimei (127.00 $\pm$ 8.89) and Safari (123.00 $\pm$ 2.89) showing more; this trend was seen in 2023 too. Likewise pupation ranged from 27.33 $\pm$ 1.20 to 126.33 $\pm$ 7.51 with the least values being in the resistant Basudha (27.33 $\pm$ 1.20), Bhatta (28.00 $\pm$ 2.89) and Kalakusuma (29.67 $\pm$ 2.67); the susceptible ones such as N.umerchudi (126.33 $\pm$ 7.51), Nimei (123.67 $\pm$ 8.97), and Safari (115.67 $\pm$ 1.67) showed highest pupal numbers; in 2023 also similar trend was observed. During 2022, the total number of adults that emerged ranged from 19.00 $\pm$ 1.00 to 109.33 $\pm$ 2.03; least values were in the resistant ones like Basudha (19.00 $\pm$ 1.00), Bhatta (19.67 $\pm$ 0.33) and Kalakusuma (20.67 $\pm$ 2.19); and maximum with susceptible types- Nimei (109.33 $\pm$ 2.03), N.umerchudi (108.33 $\pm$ 5.46), and Safari (105.67 $\pm$ 5.04); with similar trend observed during 2023.

The results indicate that when reared on resistant and moderately resistant landraces, *C. medinalis* showed less fecundity, as well as lower rates of larval emergence and survival across various life stages, including larvae, pupae, and adults, compared to those reared on susceptible landraces. Analyzing the impact of damage (ADAR) on the survivability, it was observed that resistant and moderately resistant genotypes with lower ADAR exerted the most pronounced effects on fecundity ($r=0.958$, $R^2=0.919$, $p<0.005$), adult emergence ($r=0.861$, $R^2=0.742$, $p<0.005$), larval emergence ($r=0.852$, $R^2=0.726$ and larval survival ($r=0.846$, $R^2=0.716$, $p<0.005$) in contrast to the effects on prepupal ($r=0.682$, $R^2=0.465$, $p<0.005$) and pupal survival ($r=0.603$, $R^2=0.364$, $p<0.005$). Thus, a positive trend was noted in fecundity and survivability of larvae, pupae and adults with increase in the susceptibility (Table 1).

This trend can be attributed to the conducive nature of susceptible genotypes for the pest, whereas it faced

Table 1. Effect of rice landraces on survivability of lifestages of *C. medinalis*

S. No.	Genotypes	Score	No. of eggs laid		No. of 1 st instar larvae emerged		No. of 5 th instar larvae survived		No. of pre-pupa formed		No. of pupa formed		Total no. of Adults emerged	
			2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
1	Benababar(R)	3	71.00±1.16 ⁱ	77.00±3.79 ^{efghij}	58.67±1.86 ⁱ	55.67±2.19 ^{ghi}	40.67±1.86 ^{hij}	43.67±3.84 ^{deghij}	36.67±1.86 ^{ikt}	36.33±1.76 ^{ghi}	34.33±1.67 ^{jkl}	30.00±2.08 ^d	24.33±0.88 ^g	26.00±2.52 ^f
2	Kalajeera (B)(R)	3	72.33±1.20 ^j	65.33±3.53 ^{ij}	57.00±0.10 ⁱ	52.33±2.33 ⁱ	40.67±1.67 ^{hij}	41.00±3.51 ^{ghij}	36.00±1.00 ^{ikt}	33.00±1.16 ⁱ	32.67±0.67 ^{kl}	28.00±1.00 ^d	23.33±0.33 ^g	23.0±0.58 ^f
3	Basudha(R)	3	68.33±0.67 ^j	60.00±4.62 ^j	52.00±1.16 ⁱ	48.00±3.22 ⁱ	34.33±1.20 ^j	33.67±4.81 ^j	30.33±1.20 ^j	28.00±1.16 ⁱ	27.33±1.20 ^j	23.33±1.20 ^d	19.00±1.00 ^g	20.67±2.33 ^f
4	BayaBhanda(R)	3	70.00±0.58 ^j	68.00±1.64 ^{hij}	55.33±1.20 ^j	49.33±1.86 ⁱ	37.67±2.03 ^j	36.67±5.46 ^{hij}	34.00±2.89 ^{kl}	31.67±1.45 ⁱ	31.67±3.48 ^{kl}	26.67±1.76 ^d	23.00±3.22 ^g	23.67±3.84 ^f
5	Bhalunki(R)	3	71.33±0.88 ^j	72.00±2.62 ^{ghij}	58.67±0.88 ⁱ	54.33±2.40 ^{hi}	40.00±1.00 ^{ij}	42.33±3.28 ^{efghij}	36.00±1.00 ^{ikt}	34.67±0.88 ⁱ	33.33±1.33 ^{kl}	28.00±1.00 ^d	23.00±1.16 ^g	26.00±3.22 ^f
6	Bhatta(R)	3	68.33±1.20 ^j	67.33±2.40 ^{hij}	51.00±1.53 ⁱ	45.33±2.19 ^j	34.33±2.33 ^j	35.33±1.45 ^{ij}	30.67±2.60 ^j	28.67±0.67 ⁱ	28.00±2.89 ^j	20.67±1.45 ^d	19.67±0.33 ^g	20.00±0.58 ^f
7	Manepuri (R)	3	72.00±2.08 ^j	78.00±3.42 ^{efghij}	61.00±1.53 ^{ghi}	56.33±1.45 ^{ghi}	42.67±2.03 ^{hij}	44.67±4.84 ^{deghij}	38.33±2.60 ^{ikt}	37.00±1.53 ^{ghi}	36.00±3.22 ^{ikt}	30.33±3.38 ^d	26.00±2.65 ^{efg}	25.67±2.33 ^f
8	Mahasuri(R)	3	71.33±1.20 ^j	76.00±2.65 ^{efghij}	60.00±1.53 ^{hi}	57.00±2.52 ^{ghi}	41.67±2.33 ^{hij}	46.00±2.65 ^{deghij}	37.67±2.73 ^{ikt}	38.33±2.60 ^{ghi}	35.00±3.00 ^{kl}	28.33±1.33 ^d	25.67±2.73 ^{efg}	26.00±2.52 ^f
9	Jangalijata(R)	3	69.67±0.88 ^j	74.00±4.02 ^{efghij}	57.67±1.20 ^j	53.67±1.86 ⁱ	44.67±2.03 ^{hij}	42.33±1.45 ^{efghij}	42.33±2.73 ^{efghij}	34.33±1.20 ^j	40.67±2.96 ^{efghij}	28.33±0.88 ^d	28.67±1.86 ^{efg}	25.00±0.58 ^f
10	Pahadiabanki(R)	3	71.67±1.45 ^j	75.00±4.12 ^{efghij}	59.00±1.53 ⁱ	56.00±3.22 ^{ghi}	42.67±1.76 ^{hij}	44.33±3.84 ^{deghij}	39.33±2.03 ^{hij}	36.67±1.76 ^{ghi}	37.00±2.31 ^{hij}	29.33±1.33 ^d	26.00±1.16 ^{efg}	25.33±0.67 ^f
11	Kalakusuma(R)	3	66.67±1.20 ^j	64.33±2.33 ^{ij}	56.33±2.60 ^j	52.33±1.76 ^j	37.33±2.40 ^j	38.67±6.01 ^{ghij}	33.00±2.52 ^{kl}	31.67±1.20 ^j	29.67±2.67 ^j	24.33±1.20 ^d	20.67±2.19 ^g	21.33±2.85 ^f
12	Kaliasaru(R)	3	71.33±1.86 ^j	76.00±5.51 ^{efghij}	59.67±2.91 ⁱ	58.33±3.53 ^{efghi}	41.67±2.33 ^{hij}	45.00±4.04 ^{deghij}	37.53±2.27 ^{ikt}	36.67±2.03 ^{ghi}	34.67±1.86 ^{ikt}	30.00±2.08 ^d	24.00±0.58 ^g	26.00±2.52 ^f
13	Kanhav(R)	3	71.67±0.67 ^j	74.00±3.12 ^{efghij}	60.67±1.20 ^{hi}	57.00±3.06 ^{ghi}	42.67±0.88 ^{hij}	42.33±5.18 ^{efghij}	38.67±1.45 ^{hij}	35.00±0.58 ⁱ	35.67±2.03 ^{ikt}	29.67±0.88 ^d	25.67±2.03 ^{efg}	26.67±2.33 ^f
14	Kansapurimajhi jhuli(R)	3	73.33±2.03 ^j	73.67±4.66 ^{efghij}	62.00±2.08 ^{ghi}	58.00±1.53 ^{efghi}	43.67±3.38 ^{efghij}	48.33±3.28 ^{deghij}	39.67±3.84 ^{efghij}	39.67±1.45 ^{efghi}	37.67±4.33 ^{efghij}	31.33±3.84 ^d	24.67±2.91 ^{efg}	24.33±2.60 ^f
15	Menaka(R)	3	73.33±2.33 ^j	72.00±4.62 ^{efghij}	60.67±1.45 ^{hi}	54.67±1.67 ^{hi}	42.33±1.20 ^{hij}	42.33±5.18 ^{efghij}	38.33±1.76 ^{ikt}	34.67±0.88 ⁱ	36.00±2.08 ^{ikt}	30.00±1.16 ^d	25.67±1.45 ^{efg}	25.33±1.20 ^f
16	Mogra(R)	3	71.00±1.73 ^j	77.00±2.62 ^{efghij}	58.33±1.76 ⁱ	54.00±3.79 ^{hi}	39.33±1.20 ^{ij}	39.33±4.81 ^{ghij}	35.00±1.16 ^{kl}	32.67±0.33 ⁱ	32.00±1.16 ^{kl}	28.67±0.88 ^d	22.00±1.53 ^g	25.67±4.41 ^f
17	Nagara(R)	3	72.67±1.45 ^j	75.67±4.33 ^{efghij}	60.00±2.52 ^{hi}	56.67±4.41 ^{ghi}	41.00±0.58 ^{hij}	43.67±2.03 ^{deghij}	37.67±1.67 ^{ikt}	37.00±1.53 ^{ghi}	35.00±2.52 ^{ikt}	28.67±1.20 ^d	25.67±2.73 ^{efg}	25.67±0.88 ^f
18	Padmakesari(R)	3	70.67±1.20 ^j	76.00±3.82 ^{efghij}	58.67±0.88 ⁱ	54.33±2.33 ^{hi}	41.00±1.16 ^{hij}	43.67±1.86 ^{deghij}	38.00±2.08 ^{ikt}	35.67±2.33 ^{hi}	35.33±2.40 ^{ikt}	29.33±1.45 ^d	25.67±2.19 ^{efg}	26.00±2.08 ^f
19	Radhajugal(R)	3	71.33±0.88 ^j	70.00±1.28 ^{ghij}	57.67±1.76 ⁱ	53.00±1.53 ⁱ	40.33±1.33 ^{ij}	40.67±4.37 ^{ghij}	36.67±2.19 ^{ikt}	33.00±1.53 ⁱ	34.00±3.06 ^{ikt}	27.67±1.76 ^d	24.00±0.58 ^g	24.33±0.88 ^f

(contd.)

(contd. Table 1)

20	Agnisar(MR)	5	88.00± 1.16 ^a	85.67± 1.45 ^{defghi}	76.00± 2.00 ^{gh}	73.33± 2.40 ^{defg}	58.00± 2.65 ^{efgh}	63.00± 2.31 ^{defg}	54.00± 3.06 ^{efghij}	52.00± 1.16 ^{defgh}	51.33± 2.85 ^{efghij}	46.33± 1.20 ^e	41.00± 1.53 ^{cd}	42.00± 1.73 ^e
21	Ankul(MR)	5	92.67± 1.76 ^{ghi}	89.00± 0.58 ^{defgh}	82.37± 1.84 ^f	78.67± 3.84 ^d	63.67± 1.20 ^e	65.67± 2.40 ^{def}	60.67± 1.67 ^{ef}	61.67± 1.45 ^d	58.33± 1.33 ^{ef}	56.00± 1.53 ^e	45.00± 1.00 ^e	52.00± 2.52 ^e
22	Chinamali-k(MR)	5	92.00± 2.08 ^{ghi}	91.67± 3.38 ^{defg}	78.33± 1.86 ^f	75.33± 3.53 ^{def}	61.00± 2.31 ^{ef}	63.33± 3.84 ^{defg}	57.80± 2.72 ^{efg}	56.00± 1.53 ^{de}	55.67± 3.18 ^{efg}	50.33± 2.91 ^e	42.00± 2.89 ^{cd}	46.33± 2.33 ^e
23	Maguramanji(MR)	5	96.67± 1.33 ^{ghi}	85.00± 1.73 ^{defghi}	82.67± 0.88 ^f	79.00± 2.52 ^d	65.00± 2.08 ^e	68.33± 2.33 ^d	61.67± 2.73 ^e	62.33± 2.60 ^d	59.33± 2.40 ^e	54.00± 0.58 ^e	45.33± 2.03 ^e	49.67± 3.48 ^e
24	Maharaji(MR)	5	96.33± 2.33 ^{ghi}	94.67± 1.45 ^{def}	82.00± 1.73 ^f	77.33± 4.33 ^d	61.00± 2.65 ^{ef}	63.33± 3.53 ^{defg}	57.00± 3.22 ^{efgh}	57.00± 3.22 ^d	55.00± 3.79 ^{efgh}	50.00± 1.53 ^e	43.67± 0.88 ^e	46.67± 2.67 ^e
25	Mahipal-B(MR)	5	89.67± 1.20 ^{ghi}	93.33± 1.76 ^{def}	77.00± 0.58 ^{fg}	72.33± 2.03 ^{defgh}	55.67± 0.88 ^{efghi}	57.33± 3.67 ^{defghij}	51.00± 1.00 ^{efghijk}	52.67± 2.33 ^{defg}	48.33± 1.20 ^{efghijk}	47.67± 2.33 ^e	37.00± 2.08 ^{def}	44.00± 2.65 ^e
26	Majhaliyhuli(MR)	5	94.33± 1.86 ^{ghi}	85.33± 2.19 ^{defghi}	81.00± 2.89 ^f	76.33± 2.40 ^{de}	58.67± 1.45 ^{efg}	60.33± 5.67 ^{defghi}	54.00± 1.53 ^{efghij}	53.67± 1.20 ^{ef}	51.33± 1.86 ^{efghij}	48.00± 2.65 ^e	38.67± 2.33 ^{de}	43.33± 0.88 ^e
27	Mayurkantha-k (MR)	5	93.33± 1.33 ^{ghi}	97.00± 2.00 ^{le}	81.67± 2.60 ^f	79.33± 3.38 ^d	62.67± 3.18 ^e	68.00± 2.08 ^{de}	59.33± 3.76 ^{ef}	58.67± 3.84 ^d	57.00± 3.79 ^{ef}	51.00± 1.73 ^e	45.67± 2.19 ^e	47.33± 1.76 ^e
28	Motahalka(MR)	5	100.33± 0.88 ^e	106.67± 0.33 ^d	87.33± 0.88 ^f	82.67± 2.60 ^d	62.33± 1.20 ^e	62.33± 7.67 ^{defgh}	56.67± 2.33 ^{efgh}	58.00± 3.22 ^d	53.67± 3.33 ^{efgh}	51.33± 0.67 ^e	41.00± 4.00 ^{cd}	46.33± 2.40 ^e
29	Nadalghanta(MR)	5	97.33± 1.86 ^{gh}	91.67± 1.67 ^{defg}	86.67± 1.67 ^f	79.00± 3.00 ^d	64.33± 2.33 ^e	64.00± 4.00 ^{efg}	60.67± 2.67 ^{ef}	61.00± 2.52 ^d	59.00± 3.00 ^e	54.67± 2.73 ^e	46.67± 1.67 ^e	50.67± 0.67 ^e
30	Kathidhan(S)	7	155.67± 3.48 ^{de}	148.00± 4.58 ^{abc}	143.00± 4.58 ^{abc}	139.33± 3.53 ^b	113.33± 3.48 ^{bc}	120.67± 5.46 ^{bc}	108.00± 2.65 ^{bc}	108.00± 2.65 ^{bc}	103.67± 3.48 ^{bcd}	99.00± 1.53 ^b	91.33± 2.85 ^b	93.33± 2.73 ^{bcd}
31	Nimei(S)	9	178.00± 5.20 ^a	169.00± 8.08 ^a	164.00± 8.08 ^a	161.33± 5.37 ^a	134.33± 9.39 ^a	148.00± 7.00 ^a	127.00± 8.89 ^a	126.00± 9.17 ^a	123.67± 8.97 ^a	116.00± 1.16 ^a	109.33± 2.03 ^a	110.00± 2.08 ^a
32	Ganjamratnachudi (S)	7	144.33± 0.88 ^f	133.33± 0.88 ^e	128.33± 0.88 ^e	120.87± 3.58 ^e	103.33± 0.67 ^d	105.67± 4.33 ^c	98.33± 1.20 ^d	101.00± 1.53 ^e	95.33± 1.20 ^d	91.33± 0.33 ^b	89.33± 4.70 ^b	88.33± 0.88 ^d
33	Numerehudi(S)	9	175.33± 4.37 ^{ab}	168.00± 5.29 ^a	163.00± 5.29 ^{ab}	162.23± 4.40 ^a	136.33± 8.09 ^a	146.33± 4.33 ^{ab}	131.33± 9.26 ^a	128.67± 9.82 ^a	126.33± 7.51 ^a	120.00± 3.61 ^a	108.33± 5.46 ^a	113.00± 3.46 ^a
34	Safari(S)	9	168.00± 2.08 ^{bc}	161.67± 1.67 ^{ab}	156.67± 1.67 ^{ab}	150.37± 2.69 ^{ab}	130.67± 3.53 ^{ab}	131.33± 8.74 ^{abc}	123.00± 2.89 ^{ab}	121.67± 1.86 ^{ab}	115.67± 1.67 ^{ab}	115.33± 4.10 ^a	105.67± 5.04 ^a	107.67± 5.84 ^{ab}
35	Ramkrushna bilasha (S)	7	146.67± 2.91 ^{ef}	139.33± 4.91 ^{bc}	134.33± 4.91 ^{de}	133.07± 5.36 ^{bc}	106.67± 3.18 ^{cd}	111.33± 6.89 ^c	101.0± 2.65 ^{cd}	99.67± 1.86 ^c	97.00± 1.53 ^{cd}	92.33± 2.03 ^b	89.67± 4.63 ^b	91.33± 4.81 ^{cd}
36	TKM6(RC)		71.00± 2.08 ^j	63.67± 3.93 ^j	58.67± 3.93 ^j	53.03± 1.04 ⁱ	41.67± 2.85 ^{hij}	40.33± 4.33 ^{ghij}	37.00± 2.08 ^{hij}	33.00± 1.53 ^j	33.33± 1.45 ^{kl}	28.33± 1.45 ^d	22.67± 0.67 ^g	24.00± 1.16 ^f
37	TNI(SC)		160.33± 4.41 ^{cd}	152.33± 7.22 ^{abc}	147.33± 7.22 ^{bcd}	149.60± 7.43 ^{ab}	122.33± 6.33 ^{abc}	130.33± 6.07 ^{abc}	117.67± 5.84 ^{abc}	113.00± 5.13 ^{abc}	115.00± 5.00 ^{abc}	111.33± 5.78 ^a	101.00± 2.08 ^{ab}	103.33± 1.20 ^{ab}
CD			5.855	9.443	8.005	9.158	8.643	8.889	9.129	8.411	9.085	6.058	7.089	7.180
SE(m)			2.074	4.053	2.835	3.243	3.061	4.565	3.233	2.979	3.217	2.146	2.511	2.543
CV			3.810	7.575	6.026	7.243	8.46	8.37	9.813	9.273	10.284	7.573	10.056	9.781
F _{Cal}			280.724	61.41	149.92	118.525	103.46	53.59	88.41	106.53	85.83	199.23	132.49	133.68
r			0.958		0.852		0.846		0.682	0.603			0.861	
R ²			0.919		0.726		0.716		0.465	0.364			0.742	

Data represented as Mean± SE of three replications. In a column, mean value followed by the same letters not significantly different at p=0.05 as per Tukey's HSD test.
R- Resistant, MR- Moderately resistant, S- Susceptible. RC- Susceptible check, r = Correlation coefficient, R²= Coefficient of determination

challenges in growth, survival, and reproduction on resistant genotypes (Abenes and Khan, 1990). The reduction in fecundity and survival of *C. medinalis* on resistant and moderately resistant genotypes suggest potential antibiosis factor. This factor has the capacity to significantly curtail the population buildup of compared to that in susceptible varieties (Khan and Joshi, 1990). Similar findings were reported by Punithavalli et al. (2014), revealing lower numbers and survivability of *C. medinalis* eggs, larvae, and pupae on resistant and wild genotypes. Rajdurai et al. (2021) observed significant differences in adult emergence in resistant transgenic and wild rice compared to susceptible checks (TN1). Rekha et al. (2003) observed substantial reduction in moth emergence on transgenic plants. Larvae that fed on susceptible genotypes exhibited higher survival rates, shorter growth and development periods, and increased pupation rates (Dhakshayani et al., 1993). Resistant landraces such as Bhatta, Basudha, Bayabhandha, Kalakusuma, Kalajeera (I) and TKM6 performed exceptionally well. This might be due to certain biophysical or biochemical factors that curtailed the population buildup. These genotypes are likely to be rich sources of valuable genes for insect pest resistance, and hold promise for IPM.

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AUTHOR CONTRIBUTION STATEMENT

AKN carried out the experiments, analyzed the data, and wrote the manuscript. AS and PG guided the experiments and participated in statistical analysis.

SSD participated in the experiments and revised the manuscript. SDM and TS reviewed the draft. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF SOME INSECTICIDES AND BIOPESTICIDES AGAINST MUSTARD APHID *LIPAPHIS ERYSIMI* KALT.

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ABSTRACT

The mustard aphid *Lipaphis erysimi* Kalt can reduce yields up to 96%. In rabi 2017-18, a field study was conducted at the Crop Research Centre (CRC), Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut (Uttar Pradesh), to assess the efficacy of some insecticides and biopesticides. Thiamethoxam 25WG @ 100 g/ ha was observed to be more effective with least incidence at 3, 7, and 10 days after the first and second sprays. Imidacloprid 17.8SL @ 100 ml/ ha was the second-best treatment. Dimethoate 30EC @ 1000 ml/ ha, fipronil 5SC @ 500 ml/ ha, *Beauveria bassiana* @ 2.0 kg/ ha, and *Metarhizium anisopliae* @ 2.0 kg/ ha were the other treatments found effective.

Key words: *Lipaphis erysimi*, mustard, incidence, insecticides, biopesticides, thiamethoxam, dimethoate, *Beauveria bassiana*, *Metarhizium anisopliae*, yield, economics, cost benefits

Mustard (*Brassica juncea*) is an important oilseed crop (Kumar et al., 2022; Kamil et al., 2017), with production, area, and yield being 72.37 mt, 36.59 million ha, and 1980 kg/ ha, respectively, during 2018-19 (Seerja and Kumar, 2022); for India, these are 6.33 mt, 6.41 million ha, and 6979 kg/ ha, respectively (Taki, 2022), and it is majorly grown in northwest Madhya Pradesh (Gautam et al., 2019). Many biotic and abiotic factors are responsible for its reduced yield (Deka et al., 2017, and various diseases, insect pests, and weeds are responsible. It is affected by more than three dozen insect pests known in India (Sinha et al., 2018). Amongst these the mustard aphid, *Lipaphis erysimi* Kalt. is the key pest (Sahoo, 2012). There are various options available for its management, but insect resurgence and insecticide resistance are the serious problems. Therefore, the present study to evaluate the efficacy of certain insecticides and biopesticides, along with establishing their cost benefits.

MATERIALS AND METHODS

The insecticides were procured from the local market of Modipuram and biopesticides were procured from the Entomology department, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut. The field experiment was conducted with treatments done two times during rabi, 2017-18 at the Crop Research Centre (CRC), Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram,

Meerut (29°40'N, 77°42'E, 237 masl). Seven treatments including control were evaluated viz., T1 (Imidacloprid 17.8%SL @ 100 ml/ ha), T2 (*Beauveria bassiana* @ 2 kg/ ha), T3 (Thiamethoxam 25 WG @ 500-1000 g/ ha), T4 (Fipronil 5 SC @ 500 ml/ ha), T5 (*Metarhizium anisopliae* @ 2 kg/ ha), T6 (Dimethoate 30 EC @ 1000 ml/ ha) and T8 (Check) in natural field plots (plot size of each treatment: 4.2 x 3 m²) with three replications in a randomized block design. Observations were made on 10 randomly selected plants on the top 10 cm twig/ plot; one day before (pretreatment), and at 3, 7, and 10 days after the first and second spray, and 10 days after the first spray was considered as pretreatment observation for the second spray. The cost benefit ratio was determined for each treatment and incremental cost benefit ratio (ICBR) worked out. Data were subjected to ANOVA after necessary transformation (Panse and Sukhatme, 1978) with OPSTAT software.

RESULTS AND DISCUSSION

The data on the efficacy of insecticides and biopesticides reveal that thiamethoxam 25WG (77.00, 34.66 and 91.66 aphids/ 10 plants at 3, 7 and 10 days after the first spray, respectively; and 37.33, 21.33 and 17.00 aphids/ 10 plants at 3, 7 and 10 days after the second spray) is the most superior (Table 1). These findings derive support from Kumar et al. (2013) with thiamethoxam 25% WDG @ 100 g/ ha. Ghule and Bagde (2016) observed that 0.003% thiamethoxam as

Table 1. Efficacy of insecticides and biopesticides against *L. erysimi*

S. No.	Treatment	Dose	DBS	First spray				Second spray			Yield (q/ha)	Increase in yield over control (q/ha)	Value of increase yield (Rs/ha)	Cost of treatment (Rs/ha)	Net profit (Rs/ha)	Cost benefit ratio
				3 rd DAT	7 th DAT	10 th DAT	10 th DAT	3 rd DAT	7 th DAT	10 th DAT						
1	Imidacloprid 17.8 SL @	100 ml	281.00 (16.78)	77.66 (8.86)	35.66 (6.04)	93.33 (9.71)	93.33 (9.71)	38.00 (6.24)	22.33 (4.82)	17.66 (4.31)	16.90	7.10	26625.00	2450	24175.00	1:9.86
2	<i>Beauveria bassiana</i> @	2.0 kg	271.00 (16.48)	144.66 (12.06)	117.33 (10.87)	167.66 (12.98)	167.66 (12.98)	151.33 (12.34)	122.66 (11.12)	124.66 (11.21)	15.50	5.70	21375.00	2300	19075.00	1:8.29
3	Thiamethoxam 25 WG @	100 g	281.00 (16.78)	77.00 (8.83)	34.66 (5.97)	91.66 (9.62)	91.66 (9.62)	37.33 (6.19)	21.33 (4.72)	17.00 (4.24)	15.75	5.95	22312.50	2010	20302.00	1:10.10
4	Fipronil 5 SC @	500 ml	276.66 (16.65)	137.33 (11.67)	99.66 (10.03)	154.66 (12.47)	154.66 (12.47)	93.33 (9.71)	78.00 (8.88)	63.00 (7.99)	14.90	5.10	19215.00	2390	16825.00	1:7.03
5	<i>Metarhizium anisopliae</i> @	2.0 kg	268.00 (16.40)	152.33 (12.38)	124.66 (11.21)	173.66 (13.21)	173.66 (13.21)	155.33 (12.50)	125.66 (11.25)	128.33 (11.37)	11.70	1.90	7125.00	1450	5675.00	1:3.91
6	Dimethoate 30 EC @	1000 ml	271.66 (16.51)	96.33 (9.86)	50.00 (7.14)	107.00 (10.39)	107.00 (10.39)	86.33 (9.34)	72.33 (8.56)	57.66 (7.65)	12.10	2.30	8625.00	1450	7175.00	1:4.94
7	Control		279.33 (16.74)	288.66 (16.95)	297.33 (17.18)	308.67 (17.43)	308.67 (17.43)	312.33 (17.54)	317.33 (17.68)	287.66 (16.93)	9.80	-	-	-	-	-
	SE (M) ±		0.258	0.06	0.09	0.056	0.056	0.09	0.05	0.06						
	CD (p=0.05)		N/S	0.19	0.30	0.17	0.17	0.28	0.18	0.21						

Market price of mustard Rs. 3850/q, Sprayer rent Rs. 50/ day, Labour charge Rs. 200/ day and two labour for one days were used. C: B = Value of saved yield over control (Rs./ha)/ total cost of protection (Rs./ha).

the best. The economics of treatments revealed that highest C: B (1:10.00) was obtained from plots treated with imidacloprid 17.8%SL @ 100 ml/ ha followed by thiamethoxam 25WG @ 100 g/ ha (1: 9.86), while the least (1:3.91) was found in *Beauveria bassiana* @ 2 kg/ ha. Mishra and Yadav (2013) obtained maximum cost: benefit ratio of 1.92 and 1.87 with imidacloprid. Thus, to reduce risk, some newer insecticides have been found more appropriate.

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CONFLICT OF INTEREST

No conflict of interest.

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FIRST RECORD OF THREE SPECIES OF *HELOCHARES* (COLEOPTERA: HYDROPHILIDAE) FROM WESTERN GHATS OF INDIA

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ABSTRACT

Hydrophilidae is one of the families of aquatic beetles which are known as water scavenger beetles. There are about 212 species of aquatic beetles belonging to Hydrophilidae from India. 10 species of genus *Helochares* Mulsant, 1844 had been reported from India out of which two species has been reported from Western Ghats. The present study documents three more species namely *Helochares crenatus* Régimbart, 1903, *Helochares lentus* Sharp, 1890, *Helochares negatus* Hebauer, 1995 from Western Ghats region of Tamil Nadu and Kerala as new records.

Key words: Hydrophilidae, *Helochares*, aquatic beetles, water scavenger beetles, new record, taxonomy, Western Ghats, distribution, Tamil Nadu, Kerala, Species diversity

The Western Ghats are a mountain range running parallel along the western coast of India starting from Gujarat and ending in Tamil Nadu covering the states of Maharashtra, Goa, Karnataka and Kerala. It is well known for its biodiversity and high number of species endemism. About 24.79% species of animals are found exclusively living in this region. The order Coleoptera is hard bodied beetles and holometabolous in nature. India harbours 776 species of aquatic beetles belonging to 137 genera, 17 families under 3 suborders. Western Ghats of India harbours around 154 described species of aquatic coleopterans, out of which, 42 species under 18 genera belongs to the family Hydrophilidae (Chandra et al., 2017; 2020). The genus *Helochares* Mulsant, 1844 is a large genus of the Hydrophilidae comprising 159 described species distributed worldwide (Hansen, 1999; Short and Hebauer, 2006; Short and Fikacek, 2011; Giron et al., 2021). An adult *Helochares* occurs in ponds and at the edge of slow streams, or on surface of wet stones covered with leaves (Yang et al., 2021). The present study reports three species of *Helochares* Mulsant, 1844 as new records from the Western Ghats of India.

MATERIALS AND METHODS

This study is based on some recent collections of 17 examples of genus *Helochares* Mulsant, 1844 by ZSI scientists in different sampling sites of Western Ghats from Kanyakumari District, Tamil Nadu (08.46770°N, 77.33164°E; 08.49090°N, 77.32606°E) and Trivandrum District, Kerala (08.67534°N, 77.08522°E). The specimens are preserved in the

repository of the Zoological Survey of India, Southern Regional centre, Chennai and Zoological Survey of India, Western Ghat Regional Centre, Kozhikode. The material examined was collected by means of light trap near water bodies. The beetles were killed using ethyl alcohol. The specimens were studied in the Western Regional Centre, Zoological Survey of India, Pune. The photographs were taken using Leica stereo zoom microscope (S9i).

RESULTS AND DISCUSSION

A total of 10 species of *Helochares* Mulsant, 1844 have been recorded from India so far (d'Orchymont 1928 and Giron et al., 2021) (Table 1).

Three species of *Helochares* Mulsant, 1844 are reported as new records from Western Ghats of India. These are *H. crenatus* Régimbart, 1903, *H. lentus* Sharp, 1890, *H. negatus* Hebauer, 1995. Identification of specimens are based on Dong and Bian (2021), Devi et al. (2014), Species synonyms are only based on the original citation and the latest citation on Giron and Short (2021). The latest citation is indicated by a colon symbol (:) between species and author's name. The details of new recorded species like material examined, distribution with images of adult are given.

Helochares crenatus Régimbart, 1903 (Fig. 1)

1903. *Helochares* (*Graphelochares*) *crenatus* Régimbart, Ann. Soc. Ent. Fr., 72: 54.

2021. *Helochares crenatus*: Giron and Short, *ZooKeys* 1045: 173

Table 1. Distribution of *Helochaeres* in India

Species	Distribution
<i>Helochaeres altus</i> d Orchymont, 1943	Tamil Nadu (Nilgiri)
<i>Helochaeres anchoralis</i> Sharp, 1890	Andhra Pradesh, Assam, Bihar, Himachal Pradesh, Maharashtra, Manipur, Tripura, Uttar Pradesh and West Bengal
<i>Helochaeres crenatuloides</i> d Orchymont, 1943	Madhya Pradesh, Uttar Pradesh
<i>Helochaeres crenatus</i> Régimbart, 1903	Andhra Pradesh, Bihar, Madhya Pradesh, Pondicherry, Tamil Nadu, Tripura, West Bengal
<i>Helochaeres densus</i> Sharp, 1890	Andaman Island, Madhya Pradesh, Nicobar Is., Tamil Nadu, Uttarakhand, Uttar Pradesh
<i>Helochaeres hiekei</i> Hebauer, 1995	Karnataka
<i>Helochaeres lentus</i> Sharp, 1890	Kerala, Tamil Nadu
<i>Helochaeres minor</i> d Orchymont, 1925	Bihar
<i>Helochaeres negatus</i> Hebauer, 1995	Kerala, Tamil Nadu
<i>Helochaeres pallens</i> (MacLeay, 1825)	Andaman Island, Assam, Bihar, Madhya Pradesh, West Bengal



Figs. 1-3. Dorsal habitus of *Helochaeres* spp.
1. *Helochaeres negatus* Hebauer, 1995; 2. *Helochaeres crenatus* Régimbart, 1903; 3. *Helochaeres lentus* Sharp, 1890

Material examined: 1 ex., Kilaviar, Kulasekaram range, Kanyakumari district, Tamil Nadu, 22.ii.2020, coll. Varadaraju and party.

Distribution: India: Andhra Pradesh, Bihar, Madhya Pradesh, Pondicherry, Tamil Nadu, Tripura and West Bengal. Elsewhere: Cambodia, Indonesia and Philippines.

Helochaeres negatus Hebauer, 1995 (Fig. 2)

1995. *Helochaeres* (*Hydrobaticus*) *negatus* Hebauer, Acta Coleopterologica 11(3): 5

2021. *Helochaeres negatus*: Giron and Short, ZooKeys 1045: 188

Material examined: 13 ex., Kuttar, Kulasekaram Range, Kanyakumari district, Tamil Nadu, 21.ii.2020, coll. Varadaraju and party; 2 ex., Vithura, Peppara WLS, Trivandrum district, Kerala, 4.xii.2021, coll. P. Girish Kumar and party.

Distribution: India: Kerala, Tamil Nadu. Elsewhere: Bangladesh, Nepal.

Helochaeres lentus Sharp, 1890 (Fig. 3)

1890. *Helochaeres lentus* Sharp, Trans. of the Ent. Soc. of Lond., 352

2021. *Helochaeres lentus*: Giron and Short, ZooKeys 1045: 180

Material examined: 1 ex., Vithura, Peppara WLS, Trivandrum district, Kerala, 4.xii.2021, coll. P. Girish Kumar and party.

Distribution: India: Andhra Pradesh, Kerala. Elsewhere: Bangladesh, Cambodia, China, Indonesia, Laos, Malaysia, Nepal, Sri Lanka, Thailand and Vietnam.

Two species of *Helochaeres* were earlier recorded from Western Ghat i.e., *H. altus* d Orchymont, 1943 and *H. hiekei* Hebauer, 1995 (Hansen, 1999). The present study reports three more species from one of the 36 global biodiversity hotspots. Devi et al. (2014) mentioned *Helochaeres atropiceus* Régimbart, 1903 as a new record to Manipur as well as India. However, Giron and Short (2021) transferred the species to the genus *Peltochaeres* Régimbart, 1907 based on the redescription and biological notes on *Peltochaeres atropiceus* by Jia and Tang (2018). Therefore, this species is not included in the list of Indian *Helochaeres* (Table 1).

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AUTHOR CONTRIBUTION STATEMENT

Both the authors have equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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A CHECKLIST OF TERMITE FAUNA (ISOPTERA) OF KURUKSHETRA, HARYANA

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ABSTRACT

This study is aimed to know the termite community of the Kurukshetra through sampling by the random hand-picking method, and identified with the keys given by Roonwal and Chhotani, (1989) and Chhotani (1997). A total of 11 species belonging to two families (Termitidae and Rhinotermitidae), two subfamilies (Macrotermitinae and Coptotermitinae) and three genera (*Odontotermes*, *Microtermes* and *Coptotermes*) were observed. Out of these two species are new records from Kurukshetra i.e. *Coptotermes kishori* and *Odontotermes profeae*. Termitidae is the most diverse of the two families, and the study reveals the presence of total of 19 species under five genera, four subfamilies and two families.

Key words: Termite, Termitidae, Rhinotermitidae, Macrotermitinae, *Coptotermes* *Odontotermes*, Kurukshetra Campus, species, genera, subfamilies, checklist

The insect order Isoptera comprises of termites, which make up 10% of all animal biomass (Krishna et al., 2013; Effowe et al., 2021). Termites are significant as pests of forestry, housing, and agriculture. Sen Sharma et al. (1975) estimated that these destroy around 33% of the timber produced in India. These are interestingly referred to as “eusocial cockroaches” in the order Blattodea Brunner von Wattenwyl, 1882, and are frequently referred to as “white ants” (Klambhampati and Eggleton, 2000). From all across the world, 3106 species (both alive and extinct) have been recorded (Matsui et al., 2009; Krishna et al., 2013; Effowe et al., 2021). In “Fauna of India and adjacent countries Isoptera (Termites),” Roonwal and Chhotani (1989) and Chhotani (1997) provided the most significant contributions to termites in the Indian region. From the Indian subcontinent, they identified 337 species in seven families and 59 genera (Bangladesh, Bhutan, Myanmar, India, Nepal, Pakistan and Sri Lanka) (Paul et al., 2018). However, it is now anticipated that the ultimate tally would exceed 350 species across the Indian subcontinent (<https://termiteexpert.in/page.php?pageid=22>). In India, approximately 300 species belonging to 52 genera and six families have been documented which make up 9.7% of the world’s termites (Krishna et al., 2013; Rajmohana et al., 2019; Gupta et al., 2021). From Kurukshetra, only 17 species were reported under two families and five genera denoting 5.7% of the Indian termites and 46% of the Haryana termites i.e. 38 (Paul et al., 2018; Vidyashree et al., 2018; Poonia, 2019; Bhanupriya et al., 2022).

This study aims to identify the diversity of the termite fauna of Kurukshetra, Haryana.

MATERIALS AND METHODS

Survey was carried out at the Kurukshetra University (29° 57 N, 76° 48 E, 250 masl) and the samples were collected with random hand selection method (Kakkar et al., 2015; Gupta and Kakkar, 2015; Bhanupriya et al., 2022). Termites were picked up from living trees, tree bark, leaf litter, standing or fallen wooden logs, mounds and the ground. Collected samples were kept in a mixture of glycerol and 70% ethanol, and brought to laboratory for identification using keys (Roonwal and Chhotani, 1989; Chhotani, 1997; Krishna et al., 2013). It was based on a variety of diagnostic features of the soldier caste, including head length, head width, head shape, mandible length, mandible plus head length, body length, body width, and body colour, as well as the tibial spur, tarsal segments, and antennal segments (Scheffrahn and Su, 1994; Wang et al., 2009; Kakkar et al., 2017). The compound microscope was used to evaluate these with measurements, and images were documented, with mean and SD (Mahapatro et al., 2018).

RESULTS AND DISCUSSION

The present study revealed more termites from trees and a total of eleven species were identified under two families (Termitidae and Rhinotermitidae), and subfamilies each (Macrotermitinae and Coptotermitinae). These were under three genera

(*Microtermes*, *Odontotermes* and *Coptotermes*). Of these *Coptotermes kishori* and *O. profeae* were reported first-time from the Kurukshetra campus. Maximum number of species was obtained from a single genus *Odontotermes* i.e. seven (7) of the subfamily Macrotermitinae (Termitidae). *Coptotermes* and *Microtermes* were observed with two species each belonging to subfamilies Macrotermitinae and Coptotermitinae. Subfamily Macrotermitinae (Family: Termitidae) is represented by two genera i.e. *Microtermes* and *Odontotermes* whereas subfamily Coptotermitinae of family Rhinotermitidae is denoted by a single genus *Coptotermes* only. Termitidae was the most dominant family as compared to others (Aiman Hanis et al., 2014; Kakkar et al., 2015; Ranjith and Kalleshwaraswamy, 2021; Bhanupriya et al., 2022); in the present study Termitidae was also found highly diverse with nine species; *O. feae*, *O. assmuthi* and *O. obesus* were the widely distributed in all potential habitats. These observations corroborate with those of Kakkar et al. (2015); Gupta and Kakkar (2015) that *O. feae*, *O. gurdaspurensis*, *O. obesus* and *M. obesi* are the widely scattered species in this study area.

Termite diversity is quite abundant in Kurukshetra University, and eleven species had been reported (Gupta and Kakkar, 2015), which increased to 17 species under five genera, four subfamilies, and two families (Kakkar et al., 2015). In the present study, two species were reported as new records from this area, making it to a total of 19 species (Table 1). Depending on the

Table 1. Diversity of termite fauna from Kurukshetra

Family and Subfamily	Genus	Species
Termitidae	<i>Microcerotermes</i>	<i>M. beelsoni</i>
Amitermitinae	<i>Microtermes</i>	<i>M. imphalensis</i>
Macrotermitinae		<i>M. mycophagus</i>
		<i>M. obesi</i>
	<i>Odontotermes</i>	<i>O. anamallensis</i> ;
		<i>O. assmuthi</i> ;
		<i>O. bhagwatii</i> ;
		<i>O. brunneus</i> ;
		<i>O. feae</i> ;
		<i>O. feaeoides</i> ;
		<i>O. guptai</i> ;
		<i>O. gurdaspurensis</i> ;
		<i>O. microdentatus</i> ;
		<i>O. obesus</i> ;
		<i>O. parvidens</i> ;
		<i>O. profeae</i>
Rhinotermitidae	<i>Coptotermes</i>	<i>C. heimi</i>
Coptotermitinae		<i>C. kishori</i>
Heterotermitinae	<i>Heterotermes</i>	<i>H. gertrudae</i>

season and habitat, termites may be present or absent due to food preferences and environmental factors (Basu et al., 1996; Gupta and Kakkar, 2015; Vidyashree et al., 2018). *Odontotermes* was the only genus that was widely diverse and available all over the year except February (Gupta and Kakkar, 2015). For example, *O. feae* was collected throughout the year. Since termites and moisture content have a positive correlation, the highest number of termites was found during periods of low relative humidity, rainfall levels, and some wet days (rainy days) (Gathorne-Hardy et al., 2001; Shanbhang and Sundararaj, 2011; Sattar et al., 2013; Ahmed et al., 2018). In terms of species composition, biomass, species richness, and density, the termite community is significantly altered by habitat alterations (Basu et al., 1996).

AUTHOR CONTRIBUTION STATEMENT

The concept and layout of this manuscript were jointly designed by all authors. BP completed the initial manuscript preparation, data analysis, and literature reviews. All authors made revisions, edited the final draft, and approved it.

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CONFLICT OF INTEREST

No conflict of interest.

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POLLINATOR ACTIVITIES IN *MOMORDICA CYMBALARIA* CUCURBITACEAE

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ABSTRACT

Momordica cymbalaria is an underutilized, non-cultivated cucurbit often seen as a weed. A study to record diversity of pollinators in *M. cymbalaria* was conducted during September to November, 2021 in Virudhunagar (Tamil Nadu). A total of 26 species (18 hymenopterans, 5 dipterans, 2 coleopterans and 1 hemipteran) were found as pollinators. Hymenopterans were the predominant (69.23%; 38.47% solitary bees and 15.38% each of social bees and ants). Dominating pollinators species was *Lassioglossum* sp. >*Dorymyrmex* sp. >*Technomyrmex albipes* >*Trigona iridipennis* > *Halictus* sp. >*Apis florea*. The mean population of *Lassioglossum* was maximum (6.24 bees/ m²/ 5 min) during 1000 to 1200 hr. Other solitary and social bees were also foraging maximum during 1000 to 1200 hr. Ants were found frequenting flowers from 0900 to 1800 hr. Species richness was maximum during 1000 to 1200 hr (17 species) and minimum during 1600 to 1800 hr (6 species). Species diversity and evenness not noticed throughout the hours of observation indicated unevenness. Thus, *M. cymbalaria* was mainly pollinated by solitary bees like *Lassioglossum* sp. and to a little extent by *T. iridipennis*.

Key words: *Momordica cymbalaria*, Athalakai, pollinators, foraging activity, hymenopterans, solitary bees, *Lassioglossum* sp., *Trigona iridipennis*, *Apis florea*, ants, honey bees, diversity indices, non-cultivated cucurbits

Momordica cymbalaria Hook (Fenzl.) (Cucurbitaceae) is an under-utilized, non-cultivated, season-bound vegetable harvested from wild sources. It is vernacularly called Athalakai (Tamil), Kasarakae or Kasarakaya or Kasarakai (Telugu), Karchikai (Kannada) and Kadavanchi (Marathi) (Rekha, 2015). Fruits are mainly used for culinary purposes (Parwathi and Kumar, 2002). The plant possesses antioxidant (Prashanth et al., 2013), antidiabetic and hypolipidemic (Rao et al., 1999; Koneri et al., 2006) antihelminthic, antimicrobial (Ramnath and Kumar, 2012) and wound healing properties (Kolluru et al., 2016). Tubers and leaf decoction are widely used in traditional medicine preparations. Fruits have abortifacient properties. Cucurbits being cross pollinated, pollination plays a role in determining the yield (Motzke et al., 2015). The yield of a non-cultivated monoecious cucurbit climber like *M. cymbalaria* varies widely due to pollination gap. Although attention has been given on the pharmacological aspects, its pollination biology remains to be explored. The present study analyses the diversity of pollinators and their pollination biology in *M. cymbalaria*.

MATERIALS AND METHODS

The study was carried out in Erichanatham village, Sivakasi block, Virudhunagar District in Tamil Nadu

(9°63'25" - 33" N, 77°81'82" - 84" E). Periodical field survey was conducted at weekly intervals during September to November, 2021. The experimental plots were kept free from chemical sprays during the flowering period. The diversity of floral visitors/ pollinators were recorded in five randomly selected 1m² area during flowering period during 0600 - 0800 hr, 0800 - 1000 hr, 1000 - 1200 hr, 1200 - 1400 hr, 1400 - 1600 hr and 1600 - 1800 hr for five min with three replications. The data were later averaged time-wise and group-wise to infer the pollinator fauna as well as the dominance of a particular group. Visual observations on pollen and nectar feeding insects were made and their role in pollination determined. Further, based on their foraging mode pollinators were grouped as side or top workers. Pollinators using their proboscis into the flower were considered as nectar collector while, pollinators carrying pollen load on their hind legs were determined as pollen collectors (Balachandra et al., 2014). Relative abundance, species richness and other diversity indices were computed (Davila et al., 2012). The mean values were square root transformed and compared (LSD, p= 0.05) with AGRES to assess the foraging activity (abundance) of a floral visitor. Pollinator efficiency was calculated based on the relative abundance and foraging activity of an insect species at peak pollinating hours.

Table 1. Foraging activity of pollinators of *M. cymbalaria* at different hours of the day and diversity indices

Pollinators	Foraging Mode*	Feeds on [#]	No. of individuals/5 min/m ² * at indicated hours						Mean
			0600 – 0800	0800 – 1000	1000 – 1200	1200 – 1400	1400 – 1600	1600 – 1800	
<i>Apis cerana indica</i>	T/S	P&N	0.00	0.4	0.00	0.00	0.00	0.00	0.067
Apidae: Hymenoptera			(0.70)	(0.94) ^d	(0.70) ⁱ	(0.70) ^b	(0.70) ⁱ	(0.70) ^f	
<i>Apis florea</i>	T/S	P&N	0.00	0.2	2.87	1.98	0.00	0.00	0.842
Apidae: Hymenoptera			(0.70)	(0.83) ^{de}	(1.83) ^d	(1.57) ^e	(0.70) ⁱ	(0.70) ^f	
<i>Apis dorsata</i>	T/S	P&N	0.00	0.00	0.02	0.00	0.00	0.00	0
Apidae: Hymenoptera			(0.70)	(0.70) ^e	(0.83) ^{ij}	(0.70) ^b	(0.70) ⁱ	(0.70) ^f	
<i>Trigona iridipennis</i>	T/S	P&N	0.00	3.23	3.86	2.02	0.00	0.00	1.518
Apidae: Hymenoptera			(0.70)	(1.93) ^b	(2.08) ^{bc}	(1.58) ^e	(0.70) ⁱ	(0.70) ^f	
<i>Lasioglossum</i> sp.	T/S	P&N	0.00	5.57	6.24	4.5	1.46	0.48	3.042
Halicitidae: Hymenoptera			(0.70)	(2.46) ^a	(2.59) ^a	(2.23) ^{ab}	(1.40) ^{cd}	(0.98) ^d	
<i>Halictus</i> sp.	T/S	P&N	0.00	3.07	3.22	1.73	0.62	0.20	1.473
Halicitidae: Hymenoptera			(0.70)	(1.88) ^b	(1.93) ^{cd}	(1.49) ^e	(1.05) ^e	(0.83) ^e	
<i>Amegilla zonata</i>	T/S	P&N	0.00	0.95	1.67	0.96	0.20	0.00	0.63
Apidae: Hymenoptera			(0.70)	(1.20) ^c	(1.47) ^e	(1.21) ^f	(0.83) ^{gh}	(0.70) ^f	
<i>Nomia</i> sp.	T/S	P&N	0.00	0.74	1.63	0.98	0.27	0.00	0.603
Halicitidae: Hymenoptera			(0.70)	(1.11) ^c	(1.45) ^e	(1.22) ^f	(0.87) ^{fg}	(0.70) ^f	
<i>Megachile</i> sp.	T/S	P&N	0.00	0.43	1.48	0.86	0.32	0.18	0.545
Megachilidae: Hymenoptera			(0.70)	(0.96) ^d	(1.40) ^e	(1.16) ^f	(0.90) ^{fg}	(0.82) ^e	
<i>Chrysis</i> sp.	T/S	P&N	0.00	0.23	1.45	0.96	0.46	0.00	0.517
Chrysididae: Hymenoptera			(0.70)	(0.85) ^d	(1.39) ^{ef}	(1.20) ^f	(0.97) ^{ef}	(0.70) ^f	
<i>Camponotus pennsylvanicus</i>	T/S	P&N	0.00	0.00	0.98	1.62	1.22	0.00	0.637
Formicidae: Hymenoptera			(0.70)	(0.70) ^e	(1.21) ^{gh}	(1.45) ^e	(1.31) ^d	(0.70) ^f	
<i>Monomorium minimum</i>	T/S	P&N	0.00	0.24	1.08	3.04	3.02	1.67	1.508
Formicidae: Hymenoptera			(0.70)	(0.86) ^d	(1.25) ^{fg}	(1.88) ^d	(1.87) ^b	(1.47) ^c	
<i>Dorymyrmex</i> sp.	T/S	P&N	0.00	0.28	2.85	4.65	3.65	2.85	2.38
Formicidae: Hymenoptera			(0.70)	(0.88) ^d	(1.83) ^d	(2.26) ^a	(2.03) ^a	(1.83) ^a	
<i>Technomyrmex albipes</i>	T/S	P&N	0.00	0.28	3.88	3.97	3.68	2.47	2.38
Formicidae: Hymenoptera			(0.70)	(0.88) ^d	(2.09) ^b	(2.11) ^{bc}	(2.04) ^a	(1.72) ^b	
<i>Licilia</i> sp.	S	N	0.00	0.00	0.60	0.53	0.00	0.00	0.188
Calliphoridae: Diptera			(0.70)	(0.70) ^e	(1.04) ⁱ	(1.01) ^g	(0.70) ⁱ	(0.70) ^f	
<i>Musca</i> sp.	S	N	0.00	0.00	0.63	0.46	0.03	0.00	0.187
Muscidae: Diptera			(0.70)	(0.70) ^e	(1.06) ^{hi}	(0.98) ^g	(0.72) ^{hi}	(0.70) ^f	
<i>Tabanus</i> sp.	S	N	0.00	0.00	0.82	0.36	0.28	0.00	0.243
Tabanidae: Diptera			(0.70)	(0.70) ^e	(1.15) ^{ghi}	(0.92) ^g	(0.88) ^{fg}	(0.70) ^f	
Gnats	S	N	0.00	0.00	2.68	3.46	1.63	0.00	0.295
Cecidomyiidae: Diptera			(0.70)	(0.70) ^e	(1.78) ^d	(1.99) ^{cd}	(1.46) ^c	(0.70) ^f	
S.Ed			-	0.0693	0.0801	0.0730	0.0509	0.0458	
CD (p= 0.05)			-	0.1408	0.1629	0.1485	0.1034	0.0931	
Species richness (S)			0	12	17	16	13	06	
Relative abundance (%)			0	66.67	94.44	88.89	72.22	33.33	
Shannon's diversity index			0	0.43	1.12	1.10	0.91	0.61	
Shannon's evenness index			0	0.40	0.91	0.91	0.82	0.79	
Simpson's diversity index			0	4.60	11.14	10.65	6.57	3.56	
Simpson's evenness index			0	0.38	0.66	0.67	0.51	0.59	

*Each value is a mean of 15 observations; Figures in parentheses square root transformed values; In a column, means followed by same letter(s) on par by LSD (p= 0.05); *T - Top worker; S - Side worker; T/S - Top and side worker; # P- Pollen; N- Nectar

RESULTS AND DISCUSSION

Twenty-six insect species visiting flowers were identified as pollinators. Of all the pollinators, hymenopterans contributed the highest share (18 species; 69.23%) followed by dipterans (5 species; 19.23%), coleopterans (2 species; 7.69%) and a hemipteran (1 species; 3.85%). Solitary bees and wasps were the predominant with 10 species (38.46% share) succeeded by social bees and ants with 4 species each (15.38% share each). Family wise distribution revealed the Apidae as the dominant family (6 species) followed by Formicidae (4 species), and Halictidae (3 species) (Table 1). Species-wise means pooled over hours of observation when compared, solitary bee *Lassioglossum* sp. was found to be the most abundant one followed by ants *Dorymyrmex* sp. and *Technomyrmex albipes*, and social bee *Trigona iridipennis*. This was followed by ant, *Monomorium minimum*, solitary bee *Halictus* sp. and social bee *Apis florea*. The foraging activity started during 0800 - 1000 hr (especially after flowers bloomed at 0930 hr), peaked during 1000 - 1200 hr, and declined thereafter. However, ants' activity were found peaking during 1200 - 1400 hr and their activity continued till 1600 hr- 1800 hr.

The population of *Lassioglossum* increased from 4.5 /m²/ 5 min (0800 - 1000 hr) to a maximum of 6.24 /m²/ 5 min (1000 - 1200 hr) followed by a decline (5.57 /m²/ 5 min; 1200 - 1400 hr). Similar trend was recorded for *T. iridipennis*. All other solitary bees, social bees, and dipterans except gnats were also found foraging maximum during 1000 - 1200 hr. Gnats were found foraging maximum during 1200 - 1400 hr. Ants were found to be the permanent visitors from 0900 - 1800 hr. Thus, the pollinator diversity foraging across hours of observation varied. During 0800 - 1000 hr, solitary bees were the predominant followed by social bees. Ants' activity was found minimal and dipterans were entirely absent. During 1000 - 1200 hr, solitary bees again dominated followed by ants. From 1200 till 1800 hr ants' activity dominated. Ants were the only arthropods frequenting flowers during 1600 - 1800 hr.

Relative abundance (RA) increasing from 0800 - 1000 hr, reached the maximum during 1000 - 1200 hr, and decreased thereafter. During 1600 - 1800 hr least value were observed in accordance with the visual observation of ants alone frequenting more during that time. Similar trend was also noticed in species richness (S) Shannon's diversity indices depicted that both the diversity and abundance of pollinator species were maximum at 1000 - 1400 hr and Shannon's H being

0.91 during 1000 - 1400 hr indicated near evenness of the species during those two hours of observation. However, such diversity and evenness were not noticed during the other hours. Least diversity and evenness (0.43 and 0.40) were recorded during 0800 - 1000 hr followed by 1600 - 1800 hr (0.61 and 0.79). Similar trend was noticed in Simpson's indices.

The results obtained from this study are in accordance with the findings of Subhakar et al. (2013) that *Tetragonula iridipennis*, *Halictus guttuorosus* and *Apis florea* were the major pollinators in bitter gourd. Saeed et al. (2012) also recorded the maximum activity of *Apis dorsata*, *T. iridipennis* and *Eristalinus laetus* in bitter gourd. Yogapriya et al. (2019a, b) also reported hymenopterans as the major flower visitors/ pollinators and *T. iridipennis* as a major pollinator of bitter gourd.

Thus, it is clear that the *M. cymbalaria* was mainly pollinated by solitary bees like *Lassioglossum* sp. and to a little extent by social bee *T. iridipennis*. This highlighted the importance of conservation of solitary bees and wild bee colonies. Further, as the diversity and abundance of pollinators was high during 1000 - 1600 hr, spraying insecticides during late evening hours should alone be attempted in the neighbouring crop ecosystems to conserve them.

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AUTHOR CONTRIBUTION STATEMENT

YA and ST designed the research. YA conducted the experiment and analyzed the data. ST made correction in the monograph.

CONFLICT OF INTEREST

No conflict of interest.

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ASSESSMENT OF IPM MODULES AGAINST INSECT PESTS OF OKRA

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ABSTRACT

Field experiments at two locations were conducted for the validation of IPM modules against sucking and borer pests of okra. In both locations, the proposed IPM module led to the least incidence of sucking pests viz., leafhopper *Amrasca biguttula biguttula* (Ishida), whitefly *Bemisia tabaci* (Gennadius) and aphid *Aphis gossypii* Glover (1.63, 2.41 and 0.79% leaf, respectively). The least shoot and fruit damage caused by *Earias* spp. was in the IPM and BIPM modules (4.67-4.81; 7.65-8.01%, respectively). Thus, IPM module was found to be effective with maximum reduction of both sucking and borer pests, which led to the maximum cost-benefit ratio of 1:8.34.

Key words: Okra, *Amrasca biguttula biguttula*, *Bemisia tabaci*, *Aphis gossypii*, *Earias* spp., IPM, BIPM modules, cost-benefit ratio.

Okra (*Abelmoschus esculentus*) is an important vegetable and it is regarded as a vital nutritional component of the human diet (Anonymous 2019a). Major constraint in okra production are insect pests causing yield loss of 32.06 to 94.00%, mainly due to the sucking pests- leafhopper, *Amrasca biguttula biguttula* (Ishida), whitefly *Bemisia tabaci* (Gennadius) and aphid *Aphis gossypii* Glover; 17.46 to 48.00% loss due to two-spotted mite, *Tetranychus urticae* (Koch) and avoidable yield loss of 36.00- 90.00% by *Earias* spp. are estimated (Sastry and Singh, 1974; Chaudhary and Dadeech, 1989; Singh and Brar, 1994; Misra et al., 2002; Kumaran et al., 2007). To manage these, farmers resort to indiscriminate use of broad-spectrum pesticides resulting in many hazards like resistance to insecticides, secondary pest outbreaks, phytotoxicity, toxicity to beneficial organisms, intoxication of farm personnel and environmental pollution like contamination of groundwater (Birah et al., 2012; Halder et al., 2014); this is in addition to the residue problems in fruits (Anonymous, 2019b). Based on the survey conducted among major okra growing districts of Tamil Nadu, only 13.33% of okra farmers were practicing IPM (Meenambigai, 2017a). The use of newer generation pesticides, along with the economics of IPM is of paramount importance from the farmer's point of view. This study evaluates two IPM modules comprising seed treatment, botanicals, biocontrol agents such as microbial, parasitoids and predators, and novel insecticides along with their cost: benefits.

MATERIALS AND METHODS

The field experiment was carried out in farmer's field at two locations, Narasipuram (10°99'35"N, 76°75'71"E, 488 masl- location I) and Thondamuthur (10°99'47"N, 76°83'28"E, 450 masl- location II), in Coimbatore district of Tamil Nadu, India. Okra seeds (CO 4 hybrid) were sown in a flat bed with a spacing of 45x 30 cm during kharif 2017. The crop was raised by following Tamil Nadu Agriculture University (TNAU) recommended agronomic practices except for plant protection measures. The experiment was laid out in a randomized complete block design (RCBD) with five replicates in a plot size of 100 m². Each replication was separated by a 2 m alley to serve as buffer zone. Three IPM modules were formulated and evaluated along with untreated control. Farmer's routine module (M₃) was formulated based on the preliminary survey on pesticide usage patterns in major okra growing districts of Tamil Nadu (Meenambigai et al., 2017b). Biocontrol agents used were procured from the Department of Agricultural Entomology, TNAU, and pesticides were procured from local shops. IPM module (M₁) comprised of seed treatment with imidacloprid 600FS (48% w/w) @ 5 mL kg⁻¹ seed; sowing of maize as border crop; installation of yellow sticky trap 15 cm above the crop canopy @ 24 traps ha⁻¹ at 30 DAS; release of *Chrysoperla zastrowi sillemi* (Esben-Peterson) eggs @ 5000 ha⁻¹ at 30 DAS; release of *Trichogramma chilonis* Ishii (5 releases at weekly interval) @ 1,00,000 ha⁻¹ from 30

DAS; collection and destruction of affected shoots; installation of pheromone traps (Ervit® lure traps) @ 10 traps ha⁻¹; spraying of neem seed kernel extract (NSKE) 5% at 30 and 50 DAS; imidacloprid 200 SL (17.8% w/w) @ 0.2 ml l⁻¹ at 25 and 35 DAS, dimethoate 30 EC @ 2 ml l⁻¹ at 40 DAS and emamectin benzoate 5 SG @ 0.4 g l⁻¹ at 45 and 55 DAS; Biointensive pest management (BIPM) module (M₂) comprised of all the components from IPM module except seed treatment with imidacloprid and spraying of imidacloprid and dimethoate. Instead, spraying of *Bacillus thuringiensis* var *kurstaki* (18000 IU mg⁻¹) @ 2 ml l⁻¹ at 35 DAS was included. Farmer's routine module (M₃) comprised of spraying of imidacloprid 200 SL (17.8 SL) @ 1 ml l⁻¹ at 30 and 40 DAS, dimethoate 30 EC @ 2 ml l⁻¹ + acephate 75 SP @ 1 g l⁻¹ at 35 and 45 DAS, flubendiamide 480 SC (39.95% w/w) @ 1 ml l⁻¹ at 40 and 50 DAS. In unprotected control module (M₄) no IPM practices were followed.

Observations on *A. biguttula biguttula* (15 DAS), *A. gossypii* and *B. tabaci* (25 DAS) were recorded after the first appearance from the abaxial surface of the leaf at the top, middle and bottom of the canopy at 6 AM. These were monitored at ten days interval from ten randomly selected plants/ replication. The mean incidence of these was calculated/ leaf/ replication for each treatment and the overall mean computed for the entire crop duration. Shoot and fruit damages were recorded in ten randomly selected plants. Shoot damage was recorded from 35 DAS to the end of crop duration and expressed as the overall mean %. Fruit damage was recorded at the time of every harvest and the mean % damage was worked out on a weekly basis (three pickings/ week) and expressed as the overall mean %. The cumulative yield of all the pickings was computed and expressed in kg/ plot and finally computed as q/ ha. The economics in terms of cost-benefit (C: B) analysis was calculated. Data on incidence of pest from the two locations separately, as well as pooled ones, were subjected to ANOVA (Gomez and Gomez, 1984). Pair-wise comparisons were performed by using Least Significant Difference (LSD) test (p=0.05). Statistical analysis of data was carried out in STAR version 2.0.1 software (IRRI, Los Banos, Philippines)

RESULTS AND DISCUSSION

The results revealed that in both the locations, significant differences were observed in the incidence of *A. biguttula biguttula* ($F=98.12$, $df=3, 12$, $p < 0.0001$; $F=83.45$, $df=3, 12$, $p < 0.0001$), *A. gossypii* ($F=171.78$, $df=3, 12$, $p < 0.0001$; $F=42.05$, $df=3, 12$,

$p < 0.0001$), and *B. tabaci* ($F=12.94$, $df=3, 12$, $p < 0.0005$; $F=53.68$, $df=3, 12$, $p < 0.0001$). With pooled data also similar results were obtained. IPM module (M₁) registered the lowest incidence of *A. biguttula biguttula*, *A. gossypii*, and *B. tabaci* (1.63, 2.41 and 0.79/ leaf, respectively). A similar trend of shoot and fruit damage were noticed in both locations (Table 1). Reduction of sucking pests over control was highest (64.57 to 76.68%) in the IPM module, followed by chemical control (51.63 to 68.39%) and BIPM (36.68 to 52.06%). Thus, IPM module (M₁) was significantly superior. Seed dressing with imidacloprid controlled *A. biguttula biguttula*, *A. gossypii*, and *B. tabaci* for up to 40 - 50 days after germination (early growth period) in okra crop (Venkataravanappa et al., 2011; Singh et al., 2014; Verma et al., 2014). Dipankar et al. (2020) reported that the IPM module comprising seed treatment with imidacloprid 600 FS @ 5 ml kg⁻¹ per seed, installation of yellow sticky trap @ 50 ha⁻¹ and spraying of acetamiprid 20 SP @ 0.3 g l⁻¹ was most effective against, *A. biguttula biguttula* and *B. tabaci* in okra. The release of *C. carnea* and the usage of neem products resulted in an effective reduction of sucking pests in okra (Praveen and Dhandapani 2001). Preetha and Nadarajan (2007) observed maximum suppression of sucking pests of okra in the IPM module consisting of seed treatment with imidacloprid, release of *T. chilonis* and *Chrysoperla* eggs, installation of pheromone traps and spraying of *Bacillus thuringiensis*. Patel et al. (2009) and Birah et al. (2010) observed reduced incidence of *A. biguttula biguttula* and *B. tabaci* with a module consisting of maize border crop, seed treatment with imidacloprid and foliar application of neem oil/ NSKE. Highly significant differences in mean shoot damage ($F=111.98$, $df=3, 12$, $p < 0.0001$; $F=95.09$, $df=3, 12$, $p < 0.0001$) and fruit damage ($F=283.86$, $df=3, 12$, $p < 0.0001$; $F=275.28$, $df=3, 12$, $p < 0.0001$) were observed among the treatment modules. With pooled data also similar results were obtained. Both IPM (M₁) and BIPM (M₂) modules were found to be superior with mean % shoot damage being 4.67 and 4.81, respectively, while it was 7.65 and 8.01, respectively with fruit damage. Maximum reduction of shoot and fruit damage was recorded from both IPM (65.07% and 72.59%) and BIPM (64.01% and 71.29%) modules (Table 1). Preetha and Nadarajan (2006) obtained maximum suppression of shoot and fruit borer in the module consisting of release of *T. chilonis* and *Chrysoperla*, installation of pheromone trap and spraying of *B. thuringiensis*. Birah et al. (2012) reported that the biointensive IPM module comprising imidacloprid, maize as barrier crop, clipping off deadhearts and foliar sprays of NSKE was the most

Table 1. Efficacy and cost benefit of IPM modules against major insect pests of okra

Treatment	*Mean <i>A. bigutula</i> <i>bigutula</i> / leaf			*Mean <i>A. gossypii</i> / leaf			*Mean <i>B. tabaci</i> / leaf			*Mean shoot damage (%)			*Mean fruit damage (%)		
	L I	L II	Pooled	L I	L II	Pooled	L I	L II	Pooled	L I	L II	Pooled	L I	L II	Pooled
M ₁ -IPM	1.56 ^d (1.24)	1.70 ^d (1.29)	1.63 ^d (1.27)	2.24 ^d (1.57)	2.57 ^c (1.70)	2.41 ^d (1.64)	0.79 ^c (0.87)	0.79 ^d (0.89)	0.79 ^c (0.88)	3.74 ^c (1.93)	5.60 ^c (2.37)	4.67 ^c (2.16)	7.00 ^c (2.65)	8.29 ^c (2.88)	7.65 ^c (2.77)
M ₂ -BIPM	3.37 ^b (1.84)	3.34 ^b (1.84)	3.35 ^b (1.84)	4.28 ^b (2.05)	4.87 ^b (2.18)	4.58 ^b (2.12)	1.40 ^b (1.17)	1.40 ^b (1.18)	1.40 ^b (1.18)	3.86 ^c (1.96)	5.77 ^c (2.40)	4.81 ^c (2.19)	7.37 ^c (2.65)	8.65 ^c (2.88)	8.01 ^c (2.83)
M ₃ -Farmer's routine	2.16 ^c (1.43)	2.27 ^c (1.52)	2.21 ^c (1.48)	2.92 ^c (1.71)	3.23 ^c (1.84)	3.07 ^c (1.77)	1.06 ^b (1.10)	1.08 ^c (1.04)	1.08 ^b (1.07)	6.40 ^b (2.53)	9.61 ^b (3.10)	8.01 ^b (2.83)	13.93 ^b (3.73)	16.61 ^b (4.08)	15.27 ^b (3.91)
M ₄ -Control	6.95 ^a (2.63)	7.05 ^a (2.65)	7.00 ^a (2.64)	6.83 ^a (2.54)	7.63 ^a (2.69)	7.23 ^a (2.62)	2.27 ^a (1.49)	2.23 ^a (1.49)	2.23 ^a (1.49)	9.88 ^a (3.14)	14.81 ^a (3.85)	13.37 ^a (3.66)	26.04 ^a (5.10)	29.77 ^a (5.46)	27.90 ^a (5.28)
SEd	0.0948	0.1016	0.0695	0.0962	0.1029	0.0704	0.0948	0.1016	0.0695	0.0962	0.1029	0.0704	0.0948	0.1016	0.0695
CD (p=0.05)	0.2067	0.2214	0.1434	0.1594	0.2243	0.1454	0.2067	0.2214	0.1434	0.1594	0.2243	0.1454	0.2067	0.2214	0.1434

Economics/ cost benefits					
Treatment module	Yield of healthy fruits (q ha ⁻¹)	Increase in yield over control (q ha ⁻¹)	Increase in yield over control (%)	Cost of increased yield (Rs ha ⁻¹)	Cost of treatment (Rs ha ⁻¹)
M ₁ -IPM	193.51	102.79	110	123349	13200
M ₂ -BIPM	167.83	77.11	85	92533	12000
M ₃ -Farmer's routine	168.37	77.65	86	93176	10403
M ₄ -Control	90.72				
				Net profit (Rs ha ⁻¹)	Cost: benefit ratio (BCR)
				110149	1:8.34
				80533	1:6.71
				82773	1:7.96

*Overall mean incidence during entire crop duration; L I= location I (N=5); L II = location II (N=5); Values in parentheses are square root transformed values; Means with the same letter in the same column are not significantly different ($p \leq 0.05$, LSD test). Spray volume = 500 l ha⁻¹; Cost of okra was Rs. 1200 q⁻¹

effective module. Similarly release of *T. chilonis* and *C. carnea* registered with 70.30% reduction in boll damage in cotton than modules containing insecticides alone (Brar et al., 2001). Gautam et al. (2013) observed the efficacy of damage (18.8%) in okra treated with NSKE 5%. Neem preparations were found relatively safe against predators and parasitoids such as *C. carnea* and *T. chilonis* compared with pesticides. Conservation of natural enemies could have in turn led to reduction of pests (Rao and Raguraman, 2005). The pest *E. vittella* was effectively suppressed giving highest yield with emamectin benzoate 5 SG (Laichattiwar and Meena, 2014; Dhaker et al., 2017). Javed et al. (2019) observed that IPM and BIPM modules were effective and contributed for least shoot and fruit damage.

The IPM module registered a highest marketable fruit yield (193.51 q ha⁻¹) giving maximum cost-benefit ratio of 1:8.26 followed by the farmer's routine module (1:7.59). Though a nearly similar yield was recorded from the BIPM and the farmer's routine module, the use of a few relatively low-cost conventional pesticides in the farmer's routine module led to a higher C: B ratio as compared to the BIPM module (1:6.71) (Table 1). Mohankumar et al. (2016) observed that IPM approach was effective against insect pests giving maximum cost: benefit ratio of 1: 2.53 to 1: 3.23 in okra. Borkakati et al. (2020) observed maximum yield and cost: benefit ratio (1: 8.46) in the IPM plot, although the population of *A. biguttula biguttula* and *B. tabaci* were recorded minimum in the chemical module (1: 7.98). Thus, in both locations, the proposed IPM module (M₁) was found superior with highest yield and cost-benefit ratio.

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AUTHOR CONTRIBUTION STATEMENT

K Bhuvaneswari conceptualized the research work, designed the experiments, read and approved the manuscript; C Meenambigai executed field experiments, collected, analyzed and interpreted the data, and prepared the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFECT OF BIOPESTICIDES ON THE LARVAL MORTALITY OF *CHRYSOPERLA ZASTROWI SILLEMI* (ESBEN-PETERSON)

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ABSTRACT

The efficacy of biopesticides; *Bacillus thuringiensis*, *Lecanicillium lecani*, *Beauveria bassiana*, Azadirachtin and fipronil (chemical check) on the larval mortality of *Chrysoperla zastrowi sillemi* was carried out during the year 2020-21 by two different methods viz. residue deposit and diet contamination, under laboratory conditions. Result showed that in residue deposit method, the lowest larval mortality after 7 days was observed in *Bacillus thuringiensis* (9.33%) followed by *Metarhizium anisopliae*, *Lecanicillium lecani* and *Beauveria bassiana* (23.0, 24.0 and 26.67%, respectively). In diet contamination method, the lowest larval mortality (10.00%) was observed in *Lecanicillium lecani* followed by *Metarhizium anisopliae*, *Beauveria bassiana* and *Bacillus thuringiensis* (11.33% , 13.67% and 28.33%, respectively). The highest larval mortality in both the methods were observed in the check chemical fipronil (82.67% in residue deposit and 89.33% in diet contamination) followed by Azadirachtin (40.33% in residue and 58.00% in diet contamination). Keeping in view, the results demonstrated from the investigation the microbial pesticides was found to be compatible with *Chrysoperla zastrowi sillemi* and can safely be included in the integrated pest management programme against the pests.

Key words: biopesticides, petridishes, residue deposit method, diet contamination method, chemical check, entomopathogenic fungi, entomopathogenic bacteria, integrated pest management, natural enemies, low toxicity

Green lacewings has a significant role to play in biocontrol of insect pests for having high host searching ability, voracious feeding habit and accountable to mass production. Sixty nine species of Lacewings belonging to 21 genera have been reported from different crop systems in India. Among them *Chrysoperla zastrowi sillemi*, *C. scelestes*, *Mallada boninensis* are efficiently used. In India, *Chrysoperla zastrowi sillemi* (older name *Chrysoperla carnea*) where, *sillemi* was ascertain to be a subspecies of *Chrysoperla zastrowi* and scientific name has been recoined as *Chrysoperla zastrowi sillemi* (Esbén-Peterson). The efficacy of *Chrysoperla* sp was demonstrated as a biological control agent in field crops, orchards and green house crops (Nasreen et al., 2004). On the other hand, a good number of microbial pesticides viz., entomopathogenic fungi, entomopathogenic bacteria and botanicals have great potential in pest management and act as a component in integrated pest management system. Successfulness of these components as an efficient control tactics not only relies on its effectiveness against insect pests, but also on its low toxicity to non target insects (Natural enemies). Taking into account, the importance of both the natural predator and the biopesticides, the present investigation was conducted to determine the

compatibility of the predator, *Chrysoperla zastrowi sillemi* with the microbial pesticides for their inclusion in integrated pest management programme.

MATERIALS AND METHODS

The laboratory experiment study of the efficacy of microbials and botanical pesticides on *Chrysoperla zastrowi sillemi*, was conducted in the Biocontrol laboratory, Department of Entomology, College of Agriculture, OUAT, Bhubaneswar during 2020-21. The experiment was laid out in completely randomized design with 7 treatments (at field recommended doses) each replicated thrice at 27± 2°C and relative humidity of 65%. *C. zastrowi sillemi* adults were procured from the National Bureau of Agriculturally Important Insects (NBAII), Bengaluru, Karnataka, India. Eggs of *Corcyra cephalonica*, the factitious host of *C. zastrowi sillemi* were obtained from the Biocontrol Laboratory, Department of Entomology, OUAT, Bhubaneswar. The adults were reared in controlled condition in the Laboratory in one litre capacity glass jars to maintain the stock culture. For the experiment 7 treatments with 3 replications each are taken into account. From the stock culture, the first instar larvae were separated out

Table 1. *Chrysoperla zastrowi sillemi* larval mortality as influenced by biopesticides by residue deposit method and diet contamination method

Treatments	Concentration	Dose	Mortality of larvae (%)						Decrease in mortality at 7 DAT over chemical check (%)	
			Residue deposit Method			Diet contamination method			Residue deposit Method	Diet contamination method
			3 DAT	5 DAT	7 DAT	3 DAT	5 DAT	7 DAT		
T ₁ <i>Bacillus thuringiensis</i>	1.77x10 ⁹ cfu/ml	2 ml/L	5.00* (12.74)**	8.67* (17.06)**	9.33* (17.76)**	8.67* (17.118**)	20.67* (27.03)**	28.3* (32.15)**	88.71	68.29
T ₂ <i>Lecanicillium lecani</i>	2.13x10 ⁹ spores/ml	5 ml/L	2.67* (9.30)**	11.33* (19.64)**	24.00* (29.31)**	0.67* (4.693)**	3.33* (10.51)**	10.00* (18.41)**	70.97	88.81
T ₃ <i>Beauveria bassiana</i>	2.03x10 ⁹ spores/ml	5 ml/L	3.00* (9.85)**	13.33* (21.39)**	26.67* (31.07)**	1.33* (6.62)**	5.00* (12.87)**	13.67* (21.69)**	67.74	85.03
T ₄ <i>Metarhizium anisopliae</i>	2.27x10 ⁹ spores/ml	5 ml/L	1.67* (7.36)**	10.33* (18.69)**	23.00* (28.64)**	0.67* (4.693)**	3.67* (11.04)**	11.33* (19.66)**	72.18	87.32
T ₅ Azadirachtin	1500 ppm	5 ml/L	23.67* (29.06)**	37.00* (37.44)**	40.33* (39.40)**	38.6* (38.436)**	51.33* (45.74)**	58.00* (49.58)**	51.22	35.07
T ₆ Fipronil (chemical check)	5%SC	2 ml/L	63.33* (52.71)**	79.00* (62.70)**	82.67* (65.39)**	68.00* (55.529)**	81.67* (64.63)**	89.33* (70.90)**	-	-
T ₇ Untreated control	-	-	0.67* (4.56)**	2.67* (9.32)**	5.33* (13.22)**	0.33* (3.292)**	4.33* (12.01)**	6.67* (14.96)**	93.55	92.53
SE(m)±			1.031	0.734	0.726	0.136	0.33	0.329	-	-
CD (0.05)			3.159	2.247	2.223	0.417	1.01	1.007	-	-

DAT-Days after treatment, *Mean of three replications, **Figures in the parenthesis are arc sin values.

and the mortality effect of biopesticides was studied by the Residual Deposit and the Diet Contamination methods. In Residual Deposit method, inner surface of the glass petridishes were sprayed with different treatments separately at maximum recommended doses by means of a pneumatic polysprayer and air dried followed by placing 10 numbers of first instar larvae. In Diet Contamination Method, UV treated *Corcyra* eggs were sprayed with different treatments separately at the maximum field recommended doses and air dried. Larval mortality in both the methods were observed at 3rd, 5th and 7th day after treatment (DAT) and mortality percentage was worked out. Decrease percentage in mortality at 7 DAT over chemical check was determined. Data collected on the relevant observations made during the investigation was subjected to the required statistical analysis analyzed with OPSTAT statistical software with one factor analysis involving arc sine transformation in complete randomized design (CRD).

RESULTS AND DISCUSSION

Mortality of *Chrysoperla zastrowi sillemi* larvae at 3 DAT after treatment of pesticides ranged from 1.67 to 63.33% in Residue Deposit method and 0.67% to 68% in Diet Contamination method (Table 1). In Residue Deposit method, the lowest mortality was observed in *Metarhizium anisopliae* treated larvae and in Diet Contamination method, lowest mortality was observed in *Lecanicillium lecani* (0.67%) and highest mortality was observed in Fipronil (63.33% in residue deposit and 68.00% in diet contamination).

At 5 DAT, mortality percentage ranged from 8.67- 79% in residue deposit method and 3.33- 81.67 per cent in diet contamination method. The larval mortality determined by residue deposit method and diet contamination method (Table 1) revealed that the cumulative mortality at 7 DAT varied from 9.33 to 82.67 percent in residue deposit method and 10.00 to 89.33% in diet contamination method. In residue deposit method, lowest cumulative mortality at the end of 7 DAT was in *Bacillus thuringiensis* accounting 9.33% larval mortality followed by *Metarhizium anisopliae* with 23.00% larval mortality. In diet contamination method, *Lecanicillium lecani* recorded the lowest mortality (10.00%) followed by *Metarhizium anisopliae* (11.33%). Lower mortality due to the treatments clearly depicted that microbial pesticides can be recommended along with the predator, green lacewing in IPM programme. Fipronil followed by Azadirachtin proved to be toxic to *C. zastrowi sillemi*

by recording higher larval mortality. With respect to decrease percentage in mortality on 7 DAT, *Bacillus thuringiensis* showed 88.71% decrease mortality over chemical check clearly indicated that Bt was the safest among the pesticides used in residue deposit method. In case of Diet contamination method, *Lecanicillium lecani* showed 88.81 percent decrease in mortality over chemical check confirming its compatibility with the bioagent *Chrysoperla*.

Working on effect of biopesticides, Thungrabaeb and Thongma (2007) also found low larval mortality in *M. anisopliae* and the present findings were in opine with the findings of above authors. Mortality per cent of Bt in the present investigation corroborate the findings of Morsy (2017). Larval mortality in Azadirachtin in the present finding contradicted the finding of Vinuela et al. (2000) which reported that Azadirachtin had no significant effect on *Chrysoperla* larvae. Imam et al. (2015) reported that *B. bassiana* caused death of larval instar between 9 and 41% which was correspondingly at the same range as that of present findings.

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AUTHOR CONTRIBUTION STATEMENT

The authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EFFICACY OF INSECTICIDES AND SOME ORGANIC PRODUCTS AGAINST BRINJAL SHOOT AND FRUIT BORER *LEUCINODES ORBONALIS* (GUENEE)

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ABSTRACT

Seven organic products and an insecticide- emamectin benzoate 5SG (0.002%) were evaluated against the brinjal shoot and fruit borer *Leucinodes orbonalis* (Guenee) at the research farm, Palampur. The results among the organic products, *Agniastra* (5%), *Brahmastra* (5%) and cow urine (10%) were effective. Emamectin benzoate was superior to the organic products giving maximum yield of fruits and marketable fruits (43.97 and 42.57 q/ ha, respectively).

Key words: Efficacy, organic, prophylactic spray, emamectin benzoate, *Agniastra*, *Brahmastra*, cow urine, fruit yield

Brinjal (*Solanum melongena* L.) is an important vegetable and its native of India (Choudhary, 1970). In India, it is cultivated in 0.73 million ha with an annual production of 12.51 mt and grown in Odisha, Bihar, Karnataka, West Bengal, Andhra Pradesh, Maharashtra and Uttar Pradesh (NHB, 2018). In Himachal Pradesh, it is grown in 1,213 ha with a production of 27,710 mt (Anonymous, 2018). Brinjal is attacked by number of insect and mite pests viz., *Leucinodes orbonalis* (Guenee), *Henosepilachna vigintioctopunctata*, *Euzophera perticella* Ragonot, *Urentius sentis* Distant, *Eublemma olivacea* Walker, *Amrasca biguttula biguttula* Ishida, *Bemisia tabaci* (Gennadius), *Trialeurodes vaporariorum* (Westwood), *Aphis gossypii* Glover, *Myzus persicae* (Sulzer) and *Polyphagotarsonemus latus* (Banks) (Chandel et al., 2016). Of these, *L. orbonalis* is the most serious causing extensive damage both in vegetative and reproductive stages (Banerjee et al., 2009; Panja et al., 2013). Infestation due to leafhopper, whitefly and shoot and fruit borer results in about 70-92% loss in yield (Rosaiah, 2001; Raju et al., 2007). Emamectin benzoate @ 6.25 g a.i./ ha has proved the most effective against jassid, whitefly, aphid and borer (Kalawate and Dethé, 2012). A number of insecticides have been recommended, but their indiscriminate use has posed a serious problem and hazards (Pal, 2004). Use of organic amendments and indigenous plant products can be an alternative. The significant reduction in *L. orbonalis* was observed after spraying of vermiwash with biopesticides (Mishra et al., 2014). Also 7% and 5% diluted *panchagavya* applications were found to be effective (Kumar et al.,

2015). Cow urine (20%) and neem leaf extract (10%) had been found effective (Patel et al., 2017). Organic amendments and plant products are known to leave no toxic residues and are non- phytotoxic. The present study evaluates the efficacy of some organic products.

MATERIALS AND METHODS

Field experiment was conducted during kharif 2017, at the Experimental Farm, Department of Entomology, CSK HPKV, Palampur (32.6° N 76.3° E, 1290 masl). Seedlings of variety Arka Keshav, were transplanted with at 60 x 45 cm spacing in plot size of 3 x 2 m. Randomized block design (RBD) was followed with treatments being *Agniastra* (5%), *Brahmastra* (5%), cow urine (10%), *Darekastra* (10%), *Jeevamrit* (10%), *Panchgavya* (10%) and *Tamralassi* (5%) and an insecticide, emamectin benzoate 5SG @0.002%. Two sets of trials were maintained in set 1, the treatments were applied starting from 20 days after transplanting till harvest and in set 2, treatments were applied after the appearance of *L. orbonalis* moths in the pheromone trap. A total of 12 and 9 sprays of organic products were given in set 1 and 2 whereas, organic products were applied at 10 days interval in both the sets. A total 6 sprays of emamectin benzoate was applied in set 1 and 5 sprays in set 2 at 20 days interval. Observations were recorded on the fruit infestation terms of drooping shoots and number of damaged fruits, % shoot infestation computed to at weekly intervals from five randomly selected plants and fruit infestation (number and weight basis) from each picking. Marketable fruit yield was also recorded. Data were subjected to ANOVA

after transformation through CPCS-1 software as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Result indicated that set 1 (prophylactic sprays) was effective against *L. orbonalis* as compared to set 2 (sprays applied after the appearance of pest) and treatments were significantly superior to the control (Table 1). Among all the treatments, minimum mean % shoot infestation was recorded in emamectin benzoate (2.09%) which was followed by cow urine (8.75%), *Agniastra* (9.66%) and *Brahmastra* (9.91%). Maximum total% mean shoot infestation was recorded in *Jeevamrit* (13.34%). In set 1, shoot infestation was observed minimum (5.42%) in *Agniastra*, followed by *Brahmastra* (6.11%), cow urine (6.11%) and *Jeevamrit* (6.11%). But in set 2, minimum shoot infestation was in cow urine (11.11%) followed by *Brahmastra* (13.70%) and *Agniastra* (13.89). These findings are similar to those of Anil and Sharma (2010) with emamectin benzoate. Jagadeesha (2010) concluded that the fermented plant products were found to be on par with vermicompost + neem cake + recommended dose of fertilizers + *Pongamia glabra* + *Agniastra*. The superiority of emamectin benzoate against *L. orbonalis* was also reported by Islam et al. (2016). Shoot infestation was the lowest (2.5%) in the plots

treated with Tracer 45 SC followed by Proclaim 5 SG (4.06%) being statistically similar (Akter et al., 2017). Based on % reduction in shoot infestation emamectin benzoate @ 0.0025% was found superior (89.56%) by Shah et al. (2012) in Anand, Gujarat.

The data presented in Table 1 reveal that in Set 1, all the treatments differed significantly and no significant differences were observed for number of pickings. Mean minimum fruit infestation (number basis) was observed in emamectin benzoate (3.73%) which was statistically at par with at par with cow urine (4.43%) and *Agniastra* (5.56%) as compared to control (13.46%). In set 2, minimum infestation was in emamectin benzoate (5.37%). Minimum fruit infestation (weight basis) was recorded in emamectin benzoate (3.35%) which was statistically at par with *Agniastra* (4.13%), cow urine (4.21%) and *Brahmastra* (5.20%). In set 2, no significant difference was observed in number of pickings. Among all the treatments, maximum yield of fruits was obtained in emamectin benzoate (51.67 q/ ha) which was at par with *Brahmastra* (45.50 q/ ha) and *Agniastra* (41.67 q/ ha) in Set 1. Whereas in Set 2, maximum yield of fruits was in emamectin benzoate and *Jeevamrit* (36.27 q/ ha) followed by *agniastra* (28.33 q/ ha); while minimum was in cow urine (20.30 q/ ha) followed by *Panchagavya* (22.13 q/ ha). Kalawate and Dethé (2012) observed emamectin

Table 1. Effect of treatments on infestation (on number and weight basis) by *L. orbonalis*

Treatment	Dose (ml/ l)	% Shoot infestation			% Fruit infestation (on number basis)			% Fruit infestation (on weight basis)		
		Set 1	Set 2	Mean	Set 1	Set 2	Mean	Set 1	Set 2	Mean
<i>Agniastra</i> (5%)	50	5.42 (13.35)	13.89 (21.38)	9.66 (19.70)	2.98 (2.00)	8.14 (2.68)	5.56 (2.34)	2.86 (2.00)	5.40 (2.38)	4.13 (2.19)
<i>Brahmastra</i> (5%)	50	6.11 (14.27)	13.70 (21.71)	9.91 (19.03)	4.45 (2.17)	7.08 (2.95)	5.55 (2.56)	4.16 (2.15)	6.24 (2.62)	5.20 (2.39)
Cow urine (10%)	100	6.39 (14.59)	11.11 (20.99)	8.75 (17.37)	3.82 (2.20)	5.03 (2.15)	4.43 (2.18)	3.60 (2.15)	4.81 (2.27)	4.21 (2.21)
<i>Darekastra</i> (10%)	100	6.39 (13.19)	18.15 (24.87)	12.27 (18.00)	8.84 (3.14)	11.86 (3.41)	10.35 (3.28)	7.76 (2.96)	10.01 (3.31)	8.89 (3.14)
<i>Jeevamrit</i> (10%)	100	6.11 (14.27)	20.56 (26.35)	13.34 (20.31)	6.35 (2.77)	12.34 (3.66)	9.35 (3.22)	6.11 (2.64)	11.33 (3.61)	8.72 (3.13)
<i>Panchgavya</i> (10%)	100	6.81 (15.00)	17.59 (24.39)	12.20 (17.79)	11.62 (3.24)	15.91 (3.29)	13.77 (3.27)	10.02 (3.17)	11.29 (3.52)	10.66 (3.35)
<i>Tamralassi</i> (5%)	50	7.78 (16.18)	17.41 (24.44)	12.60 (20.31)	8.29 (2.97)	12.42 (3.61)	10.36 (3.29)	9.05 (3.08)	5.47 (3.54)	7.26 (3.31)
Emamectin benzoate 5SG (0.002%)	0.4	1.39 (6.69)	2.78 (10.95)	2.09 (8.82)	2.09 (1.77)	5.37 (2.46)	3.73 (2.12)	2.00 (1.74)	4.69 (2.44)	3.35 (2.09)
Control	-	6.25 (14.21)	19.07 (22.19)	12.66 (18.20)	8.42 (3.16)	18.09 (4.43)	13.46 (3.80)	8.91 (3.23)	20.66 (4.74)	14.79 (3.99)
CD (p= 0.05)		3.09	3.69	1.93	2.6	1.5	0.35	1.2	2.7	0.37

Figures in parentheses are sine transformed values

benzoate and spinosad are efficient. Similarly, Anwar et al. (2015) reported that after 7th day of spraying lowest % fruit infestation (26.93%) was recorded in emamectin benzoate. Wankhede and Kale (2010) reported that emamectin benzoate 5% SG was the best. Emamectin benzoate, abamectin and spinosad showed better efficacy than IGR-based biopesticide buprofezin (Islam et al., 2016). According to Akter et al., (2017) the lowest % fruit infestation (8.43%) was recorded in the plots treated with Tracer 45 SC followed by Proclaim 5 SG (25.92%) and Bt (35.67%). Suganya et al. (2005) reported that emamectin benzoate and spinosad were equally effective in tomato. Anil and Sharma (2010) also observed minimum (16.58%) fruit infestation in emamectin benzoate followed by endosulfan (20.42%) and agrospray oil T (20.61%). Islam et al. (2016) obtained maximum marketable yield with emamectin benzoate.

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AUTHOR CONTRIBUTION STATEMENT

Shagun Sood conducted the experiment and recorded the data. PC Sharma conceptualized the experiment and supervised the experiment. Nitika Negi analysed the data and assisted in compilation of results.

CONFLICT OF INTEREST

No conflict of interest.

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ASSESSMENT OF IPM PRACTICES AGAINST YELLOW STEM BORER *SCIRPHOPHAGA INCERTULAS* (WALKER)

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ABSTRACT

An on-farm trial on the assessment of IPM practices against rice yellow stem borer *Scirphophaga incertulas* (Walker) in rice was carried out at five locations at randomly selected farmers' fields of KVK adopted villages of *Scirphophaga incertulas* (Walker) Nalgonda District, Telangana during rabi 2015-16, 2016-17 and 2017-18. The IPM practice consisted of seed treatment, nursery management, clipping off leaf tips before transplanting, application of recommended dosages of fertilizers, application of chlorantraniliprole granules @ 10 kg/ ha, the release of biocontrol agent *Trichogramma japonicum* @ 1,25,000/ ha at five releases from 20 DAT at an interval of 20 days, installation of pheromone traps @ 10/ ha, spraying of cartap hydrochloride 50SP @ 2.0 g/ l at the vegetative stage and chlorantraniliprole 18.5SC @ 0.3 ml/ l at the panicle initiation stage. The results on pest incidence like mean deadhearts were significantly lower in IPM demonstration plots (9.9%) than in farmers' practice (17.7%) and the white earheads incidence was *Scirphophaga incertulas* (Walker) (9.0%) *Scirphophaga incertulas* (Walker) in IPM demonstration plots compared to 19.3% in farmers' practice. The grain yield was higher in demonstration plots 6588.3 kg/ ha, with a increase of 12.0% over the farmers' practice of 5913.3 kg/ ha. The gross returns, net returns, and benefit-cost were obtained more in IPM demonstrations as compared to farmers' practices.

Key words: Rice, IPM, Yellow stem borer, Dead hearts, White ear heads, Yield, Gross returns, Net returns, Cost of cultivation, B: C ratio

Rice (*Oryza sativa* L.) is one of the most important cereal crop and a staple food for more than half of the world's population (Liu et al., 2014). In India, more than 100 insect pest species attack rice (Matteson, 2000), and twenty insect species are identified as major pests. These cause yield losses of about 10 -60% (Bhogadhi and Bentur, 2015; Jena et al., 2018). Among these, the yellow stem borer, *Scirphophaga incertulas* (Walker) (Crambidae: Lepidoptera), is the most damaging, causing annual yield losses of 27-34% (Prasad et al., 2007). The stem borer causes deadhearts at the vegetative stage and white earheads at the reproductive phase (Sulagitti et al., 2018); and yield losses vary from 17 to 51% (Muralidharan and Pasalu, 2006; Singh et al., 2018; Singh and Triveni, 2019). Stem borer incidence is recorded in early sown and late sown crop to an extent of 20.0% and 80.0%, respectively (Singh et al., 2018). The deadhearts incidence ranged from 11.2-40.1%, and white earheads from 27.6-71.7% (Krishnaiah and Varma, 2010). The IPM provides farmers with a variety of options for managing pests, diseases, and weeds. The IPM technology demonstrated are location specific and

have cost effectiveness on farmers' fields as compared to conventional farmers' practice. Thus, location-specific IPM models are more adoptive to farmers (Gururaj Katti et al., 2021). There is a need to popularize and disseminate the improved IPM technologies. Therefore, the present study to assess the IPM technologies against yellow stem borer in rice in Nalgonda District, Telangana.

MATERIALS AND METHODS

Field experiments were carried out by the Krishi Vigyan Kendra, Kampasagar at farmers' fields in adopted villages of Duggaepally and Babusaipet villages of Tripuraram (M), Nandhiphad, Bhalunaik Thanda, and Nanya Thanda villages of Miryalaguda Mandal, and Somararam (V) Naeruducharla (M) (NL 16°51'48", EL 79°25'57") of Nalgonda District, Telangana during rabi 2015-16, 2016-17, and 2017-18. The demonstrations were conducted in five locations every year, covering selected adopted villages in the Nalgonda District. An on-farm trial of IPM with rice variety MTU 1010 included cultural, biological,

mechanical and chemical methods. These comprised carbofuran seed treatment @1g/ kg seed, application of carbofuran granules 1 kg/ 5 cents nursery before 5 days of pulling of nursery; cultural practices followed include: clipping off leaf tips before transplanting, application of recommended dosages of fertilizers 120-60-60 NPK/ ha, installation of pheromone traps @10 ha for monitoring collection and destruction of egg masses, application of chlorantraniliprole granules 0.4%G @ 10 kg/ ha at 20 days after transplanting, release of *Trichogramma japonicum* @1,25,000/ ha with five releases at 20 DAT, spraying of cartap hydrochloride 50SP @ 2g/ l at vegetative stage and chlorantraniliprole 18.5SC @ 0.3ml/ l at panicle initiation stage. Whereas in farmers' practices with rice variety MTU 1010, no seed treatment, application of cartap hydrochloride granules @ 1 kg/ 5 cents at 15 days after sowing, indiscriminate use of fertilizers, no pheromone traps, application of carbofuran 3G granules @ 15 kg/ ha at 15-20 days after transplanting, no release of biocontrol agents, spraying of lambda cyhalothrin @ 2ml/ l at vegetative stage, and bifenthrin @ 2ml/ l at panicle initiation stage were practised. The demonstrations were laid out in an area of 0.1 ha and an adjacent 0.1 ha was treated as a farmers' practice.

Observations were recorded on the incidence of deadhearts, white earheads, yield, and economics. Incidence of deadhearts (DH%) was recorded randomly on five hills/ m² from 30 days after transplanting (DAT) to 60 DAT at a 15 days interval, and the white earheads (WEH%) recorded on five randomly selected hills/ m² from 75 DAT to before harvesting of the crop at 15 days interval. The yield data was recorded by the random crop cutting method and increase in yield over the farmers' practices was analyzed as per standard statistical procedures through t-test. The gross returns, net returns, cost of cultivation, and benefit-cost ratio

computed was calculated and computed, as per standard procedures.

RESULTS AND DISCUSSION

The data showed that the % incidence of deadhearts was 10.6, 9.8, and 9.4 in the IPM demonstration plots as compared to farmers' practice 18.6, 16.4, and 18.2% during rabi 2015-16, 2016-17, and 2017-18, respectively (Table 1). The deadhearts were significantly lower in IPM demonstration plots (9.9%). The white earheads were 10.2, 8.8, and 8.0% in IPM demonstration plots during rabi 2015-16, 2016-17 and 2017-18, respectively. In farmers' practice, the pest incidence was higher due to the non-adoption of IPM practices and non-recommended and higher dosages of pesticides. These results clearly indicated that demonstrations through IPM practices had a given positive impact. Similar findings were reported by Ramulamma et al. (2022); Sandeep et al. (2020); Banerjee et al. (2015); Kumar et al. (2018); Sudharani et al. (2021); Nayak et al. (2019) who reported that deadhearts and white earheads were lower in IPM plots.

The grain yield was recorded 6240.0, 7260.0, and 6215.0 kg/ ha in IPM demonstration plots as compared to 5665.0, 6825.0, and 5230.0 kg/ ha during rabi 2015-16, 2016-17, and 2017-18, respectively (Table 2) was observed. Thus, a mean grain yield of 6588.3 kg/ ha in IPM recommended practices. The increase yield over the farmers' practice was about 12.0% in IPM demonstrations. These results coincide with Ramulamma et al. (2022) that adopting IPM practices gave higher yield. Sandeep et al. (2020) assessed that IPM demonstration plots recorded more yield. Banerjee et al. (2015); Kumar et al. (2018) obtained higher yields in demonstration plots as compared to farmers' practice and Kumar et al. (2018). The IPM practices led to higher gross returns, net returns and

Table 1. Incidence of *S. incertulas* in rice (rabi 2015-16 to 2017-18 KVK, Kampasagar, Nalgonda District, Telangana)

Year	Deadhearts (%)		White earheads (%)	
	Demonstrations practice	Farmers practice	Demonstrations practice	Farmers practice
2015-16	10.6 [#]	18.6 [#]	10.2 [#]	19.8 [#]
2016-17	9.8 [#]	16.4 [#]	8.8 [#]	18.2 [#]
2017-18	9.4 [#]	18.2 [#]	8.0 [#]	19.8 [#]
Mean	9.9	17.7	9.0	19.3
t-value	-10.2		-12.2	
p-value	0.002*		0.0001*	

*Significant at p<0.05; [#]Mean of five farmers

Table 2. Yield and economics of rice against yellow stem borer of demonstration vs farmers practice during rabi from 2015-16 to 2017-18 at KVK, Kampasagar, Nalgonda District, Telangana

Year	Yield (kg/ha)		Yield $\pm$ %	Gross returns (Rs./ha)		Cost of cultivation (Rs./ha)		Net returns (Rs./ha)		B:C ratio	
	Demonstration practice	Farmers practice		Demonstration practice	Farmers practice	Demonstration practice	Farmers practice	Demonstration practice	Farmers practice	Demonstration practice	Farmers practice
2015-16	6240.0 [#]	5665.0 [#]	10.2	91355.0 [#]	80897.5 [#]	48354.0 [#]	50790.0 [#]	43001.0 [#]	30107.5 [#]	1.9 [#]	1.6 [#]
2016-17	7260.0 [#]	6825.0 [#]	6.4	118123.0 [#]	107019.5 [#]	64870.0 [#]	67325.0 [#]	64868.2 [#]	67323.4 [#]	1.8 [#]	1.6 [#]
2017-18	6265.0 [#]	5250.0 [#]	19.3	105927.5 [#]	92260.0 [#]	50700.0 [#]	53512.5 [#]	55227.5 [#]	38747.5 [#]	2.1 [#]	1.7 [#]
Average	6588.3	5913.3	12.0	105135.2	93392.3	54641.3	57209.2	54365.6	45392.8	1.9	1.6
t-value	1.16										
p-value	0.15**										

**Non significant at p<0.05; [#]Mean of five farmers

benefit-cost ratio were Rs. 1,05,135.2/ha, Rs. 54,365.6/ha and 1.9 as compared to farmers' practice. The input expenditure is lower in the IPM demonstration plots of Rs. 54,641.3/ha over the farmers' practice Rs. 57,209.2/ha (Table 2). Similar results were observed by Ramulamma et al. (2022). Chakraborty (2012) reported that IPM plots' recorded better net returns and benefit-cost ratio.

From the above findings, it is clearly indicated that the IPM practices comprising seed treatment with carbendazim, nursery management, clipping off leaf tips before transplanting, application of recommended dosages of fertilizers, application of chlorantraniliprole granules, the release of *Trichogramma japonicum*, installation of pheromone traps, and spraying of chlorantraniliprole 18.5SC @0.3 ml/l minimized the rice yellow stem borer incidence, obtained higher grain yield, gross returns, net returns, and benefit-cost ratio in IPM demonstration plots. Hence, the adoption of IPM practices proved to be economical, ecofriendly, and sustainable in the rice production system.

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AUTHOR CONTRIBUTION STATEMENT

All authors read, reviewed, agree and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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EVALUATION OF IPM MODULES AGAINST THE FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J E SMITH) ON MAIZE

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ABSTRACT

Fall armyworm *Spodoptera frugiperda* (J E Smith) has become a devastating pest in maize. Field experiments were carried out to assess the efficacy of various IPM modules against this pest. Amongst these, biointensive module recorded highest number of coccinellids (1.27/ plant) and ants (0.42/ plant), while chemical control module led to highest larval reduction over control (86.04%) and being superior with significantly higher grain yield (53.45 q/ ha). This also gave a 1.86 B:C ratio followed by the IPM module (48.06 q/ ha) with 1.85 B:C ratio.

Key words: *Spodoptera frugiperda*, modules, chemical control, biointensive, IPM, insecticides, coccinellids, ants, maize, yield, benefit cost ratio

Fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is an invasive and a polyphagous pest of many crops causing significant damage to maize (CABI, 2019). More than 350 plant species have been reported as host plants, causing significant damage to economically valuable crops (Montezano et al., 2018). In India, maize is cultivated in an area of 9.86 million ha with a production of 31.51 mt and productivity of 31.95 q/ ha (FAO, 2021). Among the major maize-growing states, Karnataka stands first with an area of 1.68 million hectares and a production of 5.18 million tonnes with a productivity of 30.92 q/ha (FAO, 2021). In 2016, the FAW invaded the African continent, and then spread rapidly to >28 countries of southern and eastern Africa (Goergen et al., 2016). From India it was reported in 2018 (Sharanabasappa et al., 2018), and it has been appearing in severe form, especially during rainy and post-rainy seasons, causing heavy yield losses throughout the country (Deshmukh et al., 2020). FAW has the potential to destroy up to 80 mt of maize worth USD 18 billion/ year, affecting about 600 million people in Africa, Asia-Pacific and the Near East (FAO, 2020). A recent study from India, the pesticide expenditure to produce 100 kg of maize grains has increased from US\$ 0.124 in 2017 to US\$ 1.39 in 2020 (Deshmukh et al., 2021). The increasing problems due to continued usage of pesticides and failure of control strategies to check the pests, necessitates the development of IPM strategies (Pretty and Bharucha, 2015). From India, good number of natural enemies have been reported (Shylesha et al., 2018; Sharanabasappa et al., 2019;

Navik, 2020; Firake and Behere, 2020). It is essential to explore these natural enemies as a component in IPM and the present study attempts this.

MATERIALS AND METHODS

Field experiments were carried out during rabi 2019 and 2020 at the experimental sites located at the Agricultural and Horticultural Research Station (14.2959° N, 75.8323°E), Kathalagere, Davanagere and the farmer's field (14.1435° N, 75.5539° E), Surahonne village of Honnali taluka of Davanagere district of Karnataka, The maize hybrid, CP-818 was sown at a spacing of 60x 30 cm with plot size of 125 m² for each module. The experiments were laid in randomized block design (RBD) with five replications. The sprays were prepared by mixing the required quantity of insecticides or biorationals at required dosages in normal water at 500 l/ ha. The treatments include- biointensive module [Installation of pheromone traps (10/ ha) at the time of sowing, removal of egg masses and neonates (at fortnightly interval from 15- 49 DAS), spraying of sugar solution 10% (two sprays at fortnightly interval, 1st at 15 and 2nd at 30 DAS, spraying of *Metarrhizium rileyi* @ 3 g/ l (two sprays at 21 and 35 DAS)], IPM module [installation of pheromone traps (10/ ha) at the time of sowing, removal of egg masses and neonates (at fortnightly interval from 15 DAS), spraying of *Metarrhizium rileyi* @ 3g/ l (single spray at 15 DAS), spraying of emamectin benzoate @ 0.5g/ l (single spray at 21 DAS)], chemical control

module [(seed treatment with cyantraniliprole 19.8 % + thiamethoxam 19.8 % @6 ml/ kg of seed, spraying of emamectin benzoate 5SG @ 0.5 g/ l (at 15 DAS), spraying of chlorantraniliprole 18.5SC @ 0.4ml/ l (28 DAS)] and control [no plant protection measures were applied, served as an untreated control]. Observations on the fall army worm were made on 20 plants/ replications at weekly intervals from 15 to 50 DAS. The effect of modules was determined by observing the larval incidence and plant damage (Davis and Williams, 1992); and yield along with the cost-benefit ratio. The grain yields obtained were converted into q ha⁻¹ and subjected to one-way ANOVA with SPSS software for each year as well as pooled (p = 0.05).

RESULTS AND DISCUSSION

During 2019-20, larval density of fall army worm was significantly higher in the untreated control (1.19/ plant), and reduction over control was significantly less in the chemical control module (92.0%), followed by the IPM module (67.22%) and biointensive module (52.10%). In 2020-21, the chemical control module led to maximum reduction (79.55%) over control, followed by the IPM module (75.55%) and the biointensive module (61.77%). Pooled data revealed that the reduction over control was maximum in chemical control module (0.24/ plant) followed by IPM module (0.44/ plant). During 2020-21, significantly lowest mean leaf damage was observed with chemical control module (1.44) followed by the IPM module (1.53) and biointensive module (2.75); maximum decrease in the leaf damage was in IPM module (64.48) followed by chemical control (66.58) and biointensive module (36.23). The chemical control module gave significantly higher grain yield (53.45 q/ha; maximum cost of cultivation was in the chemical control module (Rs. 43115); and this gave maximum gross returns (Rs.80175) and net profit (Rs. 37060), with a cost-benefit ratio of 1.86. Also, the maximum yield loss (avoidable) was compensated in the chemical control module (47.91%) with yield increment upto 91.98% (Table 2). The difference in larval load was due to the seed treatment in chemical intensive module; incidence was only 0.10 and 0.28/ plant at 14 and 21 DAS, respectively during 2019-20; in 2020-21, it was 0.13 and 0.24/ plant at 14 and 21 DAS, respectively (Table 1, 2).

The presented results are consistent with those of Babu et al. (2021), who found that seed treatment with thiamethoxam 19.8+ cyantraniliprole 19.8%t @ 4 ml/ kg resulted in a lower incidence. Suganthi et al.

(2022) found that cyantraniliprole+ thiamethoxam 19.8FS seed treatment provided effective protection. Two sprays of *Metarrhizium rileyi* @ 3 g/ l at 21 and 35 DAS in biointensive module and one spray of *M. rileyi* @ 3 g/ l at 15 DAS, IPM module led to less number of larvae/ plant. *M. rileyi* was found to reduce infestation by 58.91-62.87% (Mallapur et al., 2018). Two sprays of sugar solution (10%) at 15 and 30 DAS resulted in increase in ant density in bi intensive module. Canas and O'Neil (1998) reported an increase in ant population in sugar-treated maize. The application of sugar in maize fields enhanced the number of individual natural enemies, linked to lower leaf area damage and whorl infestations. Sugar could play a vital role by preserving the natural enemies (Canas and O'Neil, 1998). Different IPM methods should be utilized in a coordinated manner (Bista et al., 2020). Due to the broad spectrum and high insecticidal activity, new pesticide compounds demonstrate better efficacy in suppressing FAW in maize. Despite the fact that the chemical control module produced the highest yield (53.45 q/ ha), it is recommended to include/ follow the IPM module because its benefit-cost ratio (1.85), that is nearly identical to that of chemical control module.

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AUTHOR CONTRIBUTION STATEMENT

SSD, KCM and KBA planned and designed this experiment. KBA analyzed the data and SSD, KCM and SS assisted in developing the manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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Table 1. Incidence of *S. frugiperda* in maize (rabi 2019-20, 2020-21)

Incidence	Mean number of larvae/ plant* in 2019-20										Mean number of larvae/plant* 2020-21									
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	49 DAS	Overall larval mean	% RoC**			14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	49 DAS	Overall larval mean	% RoC**	Pooled mean	
Modules																				
Bio intensive module	1.08±0.19 (1.25) ^b	0.77±0.17 (1.12) ^{bc}	0.48±0.06 (0.99) ^b	0.40±0.02 (0.95) ^b	0.42±0.09 (0.95) ^b	0.25±0.05 (0.87) ^b	0.57 ^b	52.10 ^c			1.56±0.21 (1.43) ^b	1.62±0.17 (1.45) ^b	0.62±0.25 (1.03) ^b	0.40±0.05 (0.95) ^a	0.69±0.19 (0.08) ^b	0.30±0.06	0.86 ^c	61.77 ^b	0.71	
IPM module	1.20±0.08 (1.30) ^b	0.58±0.20 (1.03) ^{ab}	0.12±0.03 (0.79) ^a	0.12±0.00 (0.79) ^a	0.14±0.06 (0.80) ^a	0.18±0.05 (0.82) ^a	0.39 ^{ab}	67.22 ^b			1.73±0.08 (1.49) ^b	0.25±0.06 (0.86) ^a	0.28±0.07 (0.89) ^a	0.48±0.25 (0.96) ^a	0.10±0.03 (0.77) ^a	0.12±0.03 (0.80) ^a	0.55 ^{ab}	75.55 ^a	0.44	
Chemical control module	0.10±0.04 (0.77) ^a	0.28±0.08 (0.88) ^a	0.31±0.05 (0.90) ^{ab}	0.42±0.04 (0.96) ^b	0.35±0.07 (0.92) ^b	0.16±0.05 (0.81) ^a	0.27 ^a	92.0 ^a			0.13±0.21 (0.79) ^a	0.24±0.05 (0.86) ^a	0.30±0.07 (0.89) ^a	0.25±0.04 (0.86) ^a	0.23±0.03 (0.85) ^a	0.16±0.04 (0.81) ^a	0.46 ^a	79.55 ^a	0.24	
Control	1.16±0.08 (1.28) ^b	1.30±0.30 (1.32) ^c	1.02±0.09 (1.23) ^c	1.08±0.03 (1.26) ^c	1.30±0.11 (1.34) ^b	1.29±0.37 (1.31) ^b	1.19 ^c	-			2.07±0.26 (1.59) ^b	2.93±0.25 (1.85) ^c	2.28±0.26 (1.66) ^b	2.26±0.14 (1.66) ^b	2.65±0.14 (1.77) ^c	1.29±0.35 (1.31) ^b	2.25	-	1.72	
SEM±	0.054	0.051	0.040	0.026	0.050	0.057	-	-			0.051	0.049	0.075	0.064	0.051	0.065	-	-	-	
CD (p=0.05)	0.12	0.22	0.095	0.08	0.12	0.19	-	-			0.16	0.15	0.231	0.20	0.15	0.20	-	-	-	
CV (%)	7.26	14.39	7.09	5.98	8.70	14.34	-	-			7.66	8.76	15.02	13.00	9.93	15.44				
Leaf damage																				
	Mean leaf damage /plant* (1-9 scale) in 2019-20										Mean leaf damage /plant* (1-9 scale) in 2020-21									
Modules	14 DAS**	21 DAS	28 DAS	35 DAS	42 DAS	49 DAS	Mean leaf damage (1-9 scale)	% RoC**			14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	49 DAS	Mean leaf damage (1-9 scale)	% RoC**	Pooled mean	
Bio intensive module	2.28 ^b	2.72 ^c	2.64 ^b	3.21 ^b	2.87 ^b	4.18 ^c	2.98 ^b	33.59			2.31 ^b	2.16 ^b	2.22 ^b	2.86 ^b	3.45 ^b	3.52 ^b	2.75	36.23	2.87	
IPM module	2.19 ^b	2.02 ^b	1.57 ^a	2.18 ^a	2.30 ^a	2.27 ^a	2.09 ^a	53.50			2.12 ^b	1.38 ^a	1.19 ^a	1.60 ^a	1.49 ^a	1.418 ^a	1.53	64.48	1.81	
Chemical control module	1.22 ^a	1.67 ^a	1.85 ^a	2.19 ^a	2.31 ^a	3.29 ^b	2.08 ^a	53.52			1.31 ^a	1.16 ^a	1.22 ^a	1.90 ^a	1.65 ^a	1.42 ^a	1.44	66.58	1.77	
Control	2.26 ^b	3.14 ^d	5.10 ^c	5.22 ^c	5.46 ^c	5.79 ^d	4.49 ^c	-			2.21 ^b	3.48 ^c	4.00 ^c	5.30 ^c	5.54 ^c	5.39 ^c	4.32	-	4.41	
SEM±	0.09	0.07	0.10	0.22	0.08	0.13	-	-			0.18	0.12	0.08	0.10	0.19	0.18	-	-	-	
CD (p=0.05)	0.26	0.22	0.31	0.67	0.24	0.40	-	-			0.54	0.36	0.24	0.30	0.60	0.56	-	-	-	
CV (%)	9.68	6.67	8.12	15.29	5.48	7.49	-	--			19.67	13.00	7.90	7.54	14.34	14.04	-	-	-	

Values in parentheses $\sqrt{x}+0.5$ transformed values; *Mean number of 100 plants; **RoC - Reduction over control. Means sharing similar letters in the same column not significantly different by Tukey's HSD test, $p=0.05$ at $p<0.01$. NS= Non-Significant at $p\geq0.05$

Table 2. Cost economics for the management of *S. frugiperda* (pooled)

Treatments	Yield q/ha	Cost of production (Rs/ha)	Cost of Protection (Rs/ha)	Total cost of cultivation (Rs/ha)	Gross Income (Rs/ha)	Net Income (Rs/ha)	B:C ratio	Avoidable yield loss (%)	Yield increment over control (%)
Bio-intensive module	36.56 ^c	33856	4425	38281	54840	15559	1.43	23.85	31.32
IPM module	48.06 ^b	33856	5050	38906	72090	32184	1.85	42.07	72.62
Chemical control module	53.45 ^a	33856	9259	43115	80175	37060	1.86	47.91	91.98
Control	27.84 ^d	33856	0	33856	41760	7904	1.23	-	-
SEm±	1.15	-	-	-	-	-	-	-	-
CD (p=0.05)	3.54	-	-	-	-	-	-	-	-
CV (%)	6.19	-	-	-	-	-	-	-	-

Note: Cost of maize = Rs 1500/q. Cost of insecticides: Sugar = Rs.32 (1 kg), Rs 1600 (50kg); *Metarrhizium rileyi*, (1500g) = Rs.150; Emamectin benzoate 5 SG (250g) = Rs.550; Cyantraniliprole+Thiamethoxam (Fortanza duo for seed treatment) (80ml) = Rs.1600; Chlorantraniliprole 18.5 SC (200ml) = Rs. 2,475; Pheromone traps = Rs. 150 (No.= 1), Rs.1500 (No.= 10); Cost of labour : a. Men = Rs. 400/day, b. Women = Rs. 300/day; Standard spray volume = 500 lit/ha. Means sharing similar letters in same column not significantly different by Tukey's HSD test at p= 0.05

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EFFICACY OF INSECTICIDES AGAINST RICE WHITE BACKED PLANTHOPPER

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ABSTRACT

Efficacy of some insecticides (pymetrozine 50WG, sulfoxaflor 24SC and imidacloprid 17.8SL) was evaluated against rice white backed plant hopper (WBPH) *Sogatella furcifera* Horvath. Sulfoxaflor 24SC @218.7 g a.i./ ha and pymetrozine 50WG @ 187.5 g a.i./ ha gave significantly results (7.57 and 7.82/ hill, respectively). However, highest grain and straw yield (40.87 and 64.33 q/ ha) was observed with pymetrozine 50WG @ 187.5 g a.i./ ha and sulfoxaflor 24SC @ 218.7 g a.i./ ha (40.41 and 64.22 q/ ha). Regarding incremental cost benefit ratio (ICBR), it was observed that maximum ICBR was obtained with pymetrozine 50 WG @ 187.5 g a.i./ ha (1: 3.55) followed by sulfoxaflor 24SC @ 218.7 g a.i./ ha (1: 2.99). Thus, pymetrozine 50 WG at 187.5 g a.i./ ha and sulfoxaflor 24SC at 218.7 g a.i./ha can be recommended against rice WBPH.

Key words: Rice, *Sogatella furcifera*, insecticides, pymetrozine 50WG, sulfoxaflor 24SC and imidacloprid 17.8SL, yield, cost benefit ratio

Rice is cultivated under 43.78 million ha with productivity of 2705 kg/ ha during 2019-20 (Anonymous, 2020a), and Gujarat has 0.90 million ha with productivity of 2192 kg/ ha (Anonymous, 2020b). About 100 insects were reported as pests, among them 21 insect pests are major pests (Jena et al., 2018; Pathak and Dhaliwal, 1981). Among these the white backed plant hopper (WBPH) *Sogatella furcifera* Horvath (Homoptera: Delphacidae) causes significant damage (Shamim et al., 2009), as it attacks from late vegetative stage to grains hardening stage, causing “hopper burn” symptoms (Dale, 1994). The yield losses vary from 10%- 90% (Elanchezhyan et al., 2020; Pandi et al., 2018; Sujithra and Chander, 2013; Kulshreshtha, 1974). Availability of rice crop throughout the year coupled with susceptible varieties in some pockets along with heavy irrigation, higher doses of nitrogenous fertilizers, indiscriminate use of insecticides, lead to serious damage (Elanchezhyan et al., 2020; Shankar et al., 2018). Farmers normally deploy chemical control against this pest. Kumar et al. (2022), Adhikari (2016), Guruprasad et al. (2016) and Bhanu (2015) revealed that pymetrozine 50WG and sulfoxaflor 24SC are superior against the BPH and WBPH giving higher yield. The introduction of new insecticides that are safe, quickly degradable with better efficacy. So, there is a need to evaluate the new groups with different modes of action of insecticide requires evaluation of their efficacy, and this study evaluates few of these.

MATERIALS AND METHODS

The field experiment was carried out at the Main Rice Research Station (22°47'49''N; 72°34'29''E; 30 masl, Anand Agricultural University, Nawagam, Gujarat during kharif, 2018 and 2019. The experiment was laid out in randomized block design with variety GR 11 in plots of size 2.7x 2.4 having three replications of eight treatments viz., three dosages of pymetrozine 50WG @ (187.5, 150.0 and 112.5 g a.i./ha) and sulfoxaflor 24 SC @ (218.7, 175.0 and 131.25g a.i./ ha) with standard check imidacloprid 17.8SL @ 25.0 g a.i./ ha and untreated control. Twenty-five days old seedlings were transplanted in a spacing of 20x 15 cm with the treatments applied in the form of foliar spray with knapsack sprayer. First spray was applied based on the WBPH incidence and buildup at 50 days after transplanting (DAT); second after 15 days of the first spray. The incidence of WBPH was observed on 5 randomly selected hills/ plot before and after treatments (5, 10 and 14 days after spray). Grain and straw yield were recorded in kg/ plot and then converted into q/ ha. The data on incidence were square root transformed and statistically analyzed (Steel and Torrie, 1980), with means compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results given in Table 1 revealed that WBPH

Table 1. Efficacy of newer insecticides against white backed plant hoppers (WBPH) in rice (*Kharif*, 2018 and 2019)

Sr. No.	Treatments	Dose (g a.i./ha)	No. of WBPH/ hill									
			<i>Kharif</i> , 2018					<i>Kharif</i> , 2019				
			Before spray	1 st spray	2 nd spray	Mean	Before spray	1 st spray	2 nd spray	Mean	Cumulative mean	
1	Pymetrozine 50 WG, 0.037%	187.5	4.59 (20.53)	3.75 ^a (13.57)	2.75 ^a (7.04)	3.25 ^a (10.05)	3.93 (14.93)	2.59 ^a (6.22)	2.45 ^a (5.52)	2.52 ^a (5.87)	2.89a (7.82)	
2	Pymetrozine 50 WG, 0.030%	150.0	4.50 (19.79)	3.86 ^a (14.43)	2.89 ^{ab} (7.85)	3.38 ^{ab} (10.90)	3.93 (14.92)	3.41 ^b (11.14)	3.23 ^b (9.94)	3.32 ^b (10.53)	3.35b (10.72)	
3	Pymetrozine 50 WG, 0.022%	112.5	4.48 (19.60)	3.97 ^{ab} (15.28)	3.16 ^c (9.45)	3.56 ^c (12.20)	3.93 (14.93)	3.85 ^c (14.33)	4.06 ^c (15.95)	3.95 ^c (15.13)	3.76c (13.60)	
4	Sulfoxaflor 24 SC, 0.043%	218.7	4.54 (20.10)	3.73 ^a (13.42)	2.72 ^a (6.89)	3.22 ^a (9.90)	3.89 (14.60)	2.53 ^a (5.90)	2.39 ^a (5.21)	2.46 ^a (5.55)	2.84a (7.57)	
5	Sulfoxaflor 24 SC, 0.036%	175.0	4.49 (19.67)	3.82 ^a (14.07)	2.86 ^{ab} (7.68)	3.34 ^{ab} (10.64)	3.84 (14.25)	3.35 ^b (10.74)	3.28 ^b (10.26)	3.32b (10.50)	3.33b (10.59)	
6	Sulfoxaflor 24 SC, 0.026%	131.3	4.68 (21.40)	3.94 ^{ab} (14.99)	3.01 ^{bc} (8.56)	3.47 ^{bc} (11.56)	3.86 (14.39)	3.80 ^c (13.93)	4.00 ^c (15.51)	3.90 ^c (14.71)	3.69c (13.08)	
7	Imidacloprid 17.8 SL, 0.005%	25.0	4.56 (20.33)	4.13 ^b (16.57)	3.43 ^d (11.28)	3.78 ^d (13.80)	3.70 (13.18)	4.40 ^d (18.85)	4.40 ^d (18.90)	4.40d (18.88)	4.09d (16.23)	
8	Untreated	-	4.52 (19.92)	4.85 ^c (23.04)	4.62 ^c (20.83)	4.74 ^c (21.92)	3.66 (12.89)	5.64 ^e (31.28)	6.23 ^e (38.36)	5.94 ^e (34.73)	5.34e (28.01)	
SE (m)±			0.07	0.076	0.079	0.055	0.12	0.073	0.07	0.051	0.019	
CD (p=0.05)			NS	0.216	0.226	0.154	NS	0.209	0.201	0.143	0.052	
CV (%)			2.68	5.69	7.5	6.48	5.34	5.97	5.63	5.81	6.15	

Figures in parentheses retransform values and those outside $\sqrt{x} + 0.5$ (square root) transformed value; Treatment means with the letter(s) in common not differing significantly by DMRT (p=0.05).

incidence at pretreatment did not vary significantly among the treatments. Plots treated with the sulfoxaflor 24SC @ 218.7 g a.i./ ha and pymetrozine 50WG @ 187.5 g a.i./ ha led to significantly less incidence (9.90 and 10.05/ hill, respectively), and these were at par with each other. During 2nd year, sulfoxaflor 24SC @ 218.7 g a.i./ ha and pymetrozine 50WG @ 187.5 g a.i./ again were found superior, with cumulative mean data confirming that higher dose of sulfoxaflor 24SC @ 218.7 g a.i./ ha and pymetrozine 50 WG @ 187.5 g a.i./ ha are significantly superior (7.57 and 7.82/ hill, respectively). Imidacloprid 17.8SL @ 25.0 g a.i./ ha and untreated control plots recorded more incidence (16.23 and 28.01/ hill, respectively). These observations corroborate with those of earlier workers viz., Kumar et al. (2022), Konchada (2017) and Seni and Naik (2017) on pymetrozine 50WG. Ghosh et al. (2013) and Chander et al. (2012) revealed that sulfoxaflor 14SC was highly effective. Naik et al. (2016) reported that pymetrozine 50 WG @ 0.6 g/ l and imidacloprid + ethiprole @ 0.25 g/ l are effective. Shankar et al. (2018) observed that sulfoxaflor 24SC @ 375 ml/ ha is superior, while Guruprasad et al. (2016) revealed that sulfoxaflor 24SC @ 438.0 ml/ ha was the best.

The results presented in Table 2 revealed that higher grain and straw yield recorded in different treatments was found significantly superior over untreated control. The highest grain and straw yield (40.87 and 64.33 q/ ha, respectively) was recorded from the plots treated with pymetrozine 50 WG @ 187.5 g a.i./ha followed by sulfoxaflor 24 SC @ 218.7 g a.i./ha (40.41 and 64.22 q/ha, respectively). Whereas, lowest grain and straw yield were recorded in plots treated with imidacloprid 17.8 SL @ 25.0 g a.i./ha (31.37 and 46.27 q/ha, respectively) and untreated control plot (28.59 and 43.88 q/ha, respectively). The data presented in Table 2 also showed that highest incremental cost benefit ratio (ICBR) (1: 3.55) was obtained from the plots treated with pymetrozine 50 WG @ 187.5 g a.i./ha followed by sulfoxaflor 24 SC @ 218.7 g a.i./ha (1: 2.99). The present findings are in close conformity with the finding of earlier workers viz., Guruprasad et al. (2016), Konchada (2017) and Shankar et al. (2018) who reported that application of pymetrozine 50 WG and sulfoxaflor 24 SC recorded highest grain yield. The present experiment summarized that the plots treated with pymetrozine 50 WG at 187.5 g a.i./ ha and sulfoxaflor 24 SC at 218.7 g a.i./ha significantly reduced the buildup of white backed plant hoppers population with higher yield and economics in rice crop.

Table 2. Economics of newer insecticides evaluated against white backed plant hoppers (WBPH) in rice

Insecticides	Dose (g a.i./ ha)	Quantity of insecticides required for two sprays (L/kg/ ha)	Cost of insecticides for two sprays (₹)	Total cost of treatments including labour charge (₹/ ha)	Yield (q/ ha)			Net gain over control (q/ ha)			Realization over control (₹/ ha)			Total realization over control (₹/ ha)	ICBR
					Grain	Straw		Grain	Straw		Grain	Straw			
Pymetrozine 50 WG, 0.037%	187.5	0.75	6563	7836	40.87	64.33		12.28	20.45		21488	6339		27827	1: 3.55
Pymetrozine 50 WG, 0.030%	150.0	0.60	5250	6524	35.91	52.08		7.31	8.20		12801	2543		15344	1: 2.35
Pymetrozine 50 WG, 0.022%	112.5	0.45	3938	5211	31.83	50.03		3.24	6.15		5667	1906		7572	1: 1.45
Sulfoxaflor 24 SC, 0.043%	218.7	1.82	7746	9019	40.41	64.22		11.82	20.34		20678	6307		26985	1: 2.99
Sulfoxaflor 24 SC, 0.036%	175.0	1.46	6198	7472	35.67	50.64		7.08	6.76		12387	2097		14484	1: 1.94
Sulfoxaflor 24 SC, 0.026%	131.3	1.09	4648	5922	32.90	47.25		4.31	3.37		7535	1044		8579	1: 1.45
Imidacloprid 17.8 SL, 0.005%	25.0	0.28	1208	2481	31.73	46.27		3.14	2.39		5487	742		6228	1: 2.51
Untreated	-	-	-	-	28.59	43.88		-	-		-	-		-	-

500 litre spray solution required for one spray per ha and two sprays were given during the cropping season; Labour charge @ ₹ 318.40/ day × two labour= ₹ 636.8/ ha/ Spray; Price of rice grain= ₹ 1750/ q and rice straw= ₹ 310/ q; Insecticide cost as per market rate Pymetrozine 50 WG, ₹ 1050/ 120 g, Sulfoxaflor 24 SC, ₹ 850/ 200 ml and Imidacloprid 17.8 SL, ₹ 2150/ 500 ml.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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VALIDATION OF BIOINTENSIVE IPM PRACTICES AGAINST PEST COMPLEX OF ORGANIC BLACK RICE

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ABSTRACT

Field Experiment to validate the Biointensive IPM (BIPM) package on black rice variety “Kola Chaul” revealed that BIPM package consisting seedlings root dip with *Pseudomonas fluorescens* @ 2% solution; application of organic manure MUKTA @ 2 t ha⁻¹; application of *Beauveria bassiana* @ 10¹³ spores ha⁻¹ against sucking pests, use of bird perch (10 ha⁻¹), 6 releases of *Trichogramma japonicum* @ 50,000 ha⁻¹ at 10 days interval starting from 30 days after treatment (DAT) for stem borer and leaf folder infestation along with need based application of botanicals NSKE 5% (5 l/l⁻¹) could suppress the major rice pests and produce higher grain yield as compared to the farmer’s practice (FP). Maximum grain yield of 3124.8 kg ha⁻¹ and 3139.10 kg ha⁻¹ was recorded in BIPM field as compared to 2882.6 kg ha⁻¹ and 2897.20 kg ha⁻¹ in farmers practice plot during 2020-21 and 2021-22, respectively. Thus, BIPM practice was observed to be superior to FP with higher benefit: cost ratio.

Key words: Kola Chaul, *Pseudomonas fluorescens*, *Trichogramma japonicum*, BIPM, farmers’ practice, Assam, grain yield, net benefit, stem borer, deadheart, white earhead, leaf folder, yield, additional yield over chemical control, C:B ratio

India produced 118.40 mt of rice from an area of 43.78 m ha with productivity of 2.7 t ha⁻¹ milled rice during 2019-20 (Anonymous, 2021). As per the estimate of Viraktamath (2013), India has to produce at least 140-150 mt of milled rice by 2030 to maintain the present level of self-sufficiency. However, the biotic pressure of insect pest is also high and >70 pests are associated with rice, out of which 20 species are regular (Bhogadhi and Bentur, 2015). As a result, rice cultivation has become chemocentric leading to the pollution of soil-air-water as well as human health hazard. Hence, biointensive integrated pest management (BIPM) is the need of the hour. Moreover, it is necessary to make innovative changes in plant protection measures and improve the education of farmers who can fully understand both direct and indirect benefits and risk involved in it (Trivedi and Ahuja, 2011). Validation of IPM has reduced use of pesticides up to 20 g a.i.ha⁻¹ as against 2250 g a.i.ha⁻¹ used in the fields with farmers’ practices (Anonymous, 2012). The present attempts validation of BIPM practices against major pests of organic black rice especially lepidopteran pests associated with rice.

MATERIALS AND METHODS

Field experiment was carried out at the Instruction -

cum-Research (ICR) Farm, AAU, Jorhat (26.757874°N; 94.2037°E) during 2020-21 and 2021-22 on variety “Kola Chaul”. Twenty-five days old seedlings were transplanted during last fortnight of July and recommended agronomic practices as per package of practices of AAU, Jorhat were followed. The BIPM package as mentioned below was evaluated in comparison with farmers’ practice (FP) where chlorantraniliprole 18.5SC @20 g.ai ha⁻¹ was applied with an area of 0.5 ha each and data were collected from 10 randomly selected 1 m² quadrats. Four rounds of chemical sprays were imposed at 35, 45, 55 and 65 days after transplanting (DAT) in farmers practice plots. A set of IPM tactics were implemented at BIPM practiced plot in a systematic way, - organic manure Mukta @ 2 t ha⁻¹ was applied at the time of final ploughing. Seedlings root dip treatment of uprooted seedlings with *Pseudomonas fluorescens*@2% solution was carried out before transplanting. To control sucking pests *Beauveria bassiana* @ 10¹³ spores ha⁻¹ was applied just after transplanting and second spray was imposed 15 DAT. Application of bird perch (10 ha⁻¹) during vegetative stage before panicle initiation was helpful for IPM. Egg parasitoid *Trichogramma japonicum* @ 50,000 ha⁻¹ was released at 30 DAT and subsequent release

was conducted at 10 days interval for management of lepidopteran pests especially stem borer *Scirpophaga* sp. and leaf folder *Cnaphalocrocis medinalis*; and need based application of botanicals NSKE 5% (5 ml/l) was imposed during milky stage against gundhi bug *Leptocoris acuta*.

RESULTS AND DISCUSSION

Table 1. Effect of stem borer and leaf folder on yield of black rice

[illegible]

self-pollinated (Tsunoda and Takahashi, 1984), so when pollen grains from anther fall by gravity onto the stigma of rice flower then only pollination takes place and this function may be accelerated by different arthropod fauna present in rice ecosystem. Therefore, the chance of pollination is higher in BIPM plot. Moreover, from the present investigation it had been observed that BIPM practice plot was superior to FP plot with superior benefit: cost ratio of 1.43 and 1.16. Use of ecofriendly pesticides was the reason for higher population of predators in BIPM plot as compared to FP plot was in conformity with Dash et al. (2006).

Thus, it can be interpreted that BIPM package consisting of different tactics viz., seedlings root dip with *Pseudomonas fluorescens* @ 2% solution; application of organic manure Mukta @ 2t ha⁻¹; application of *Beauveria bassiana* @ 10¹³ spores ha⁻¹ against sucking pests, use of bird perch (10 ha⁻¹), 6 releases of *Trichogramma japonicum* @ 50,000 ha⁻¹ at 10 days interval starting from 30 DAT for stem borer and leaf folder infestation along with need based application of botanicals NSKE 5% (5 ml/ l) could suppress the major rice pests and produce higher grain yield as compared to the farmer's practice plot. Therefore, the BIPM package may be recommended for the rice farmers associated with organically cultivated black rice or "*Kola Choul*" which will give maximum yield as compared to chemocentric agriculture and BIPM will help to increase the income of farmers.

Integrated pest management involves integration of IPM viz., cultural, mechanical, biological and finally the chemical control (Dent, 1991), (Pretty et al., 1998; Atanassov et al., 2002). However, least or no use of chemical pesticides encourages conservation of natural enemies (Garg et al., 2002; Saikia et al., 2017; Borkakati et al., 2018; Tandon and Srivastava, 2022). Earlier attempts with BIPM practices showed satisfactory results over chemo-centric agriculture in regards to sugarcane (Saikia et al., 2020) and vegetables like tomato, brinjal etc. (Saikia and Borkakati, 2019; Borkakati et al., 2020)

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AUTHOR CONTRIBUTION STATEMENT

RNBK and DKS: Conceptualized, planned the work; RNBK, JG and MG: Conducted research, written manuscript; RNBK, RPP & SB: corrected manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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BIOLOGY AND MORPHOMETRIC STUDY OF OKRA SHOOT AND FRUIT BORER *EARIAS VITELLA* (F.)

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ABSTRACT

Investigations on biology of shoot and fruit borer (*Earias vitella*) on okra revealed that the freshly laid eggs were spherical, bluish green and finally become brownish. These measured $0.52 \pm 0.02 \times 0.51 \pm 0.02$ mm. The incubation period was 3.35 ± 0.76 days, and larval stage passed through four instars. The first, second, third and fourth instar was 2.16 ± 0.71 , 1.43 ± 0.50 , 2.26 ± 0.73 and 3.30 ± 1.02 days, respectively with the total larval period of 9.13 ± 1.71 days. These were measured 1.77 ± 0.28 , 2.60 ± 0.40 , 6.45 ± 0.56 and 14.15 ± 2.12 mm long respectively and 0.27 ± 0.03 , 0.36 ± 0.08 , 1.24 ± 0.07 and 3.23 ± 0.51 mm, respectively in breadth. The pre-pupa lasted for 1.43 ± 0.50 days. The male and female pupa measured 9.78 ± 0.46 mm and 3.14 ± 0.31 mm, 10.14 ± 0.69 mm and 3.37 ± 0.09 mm, respectively, with duration of 7.90 ± 0.92 and 7.53 ± 0.50 days, respectively. The adult male measured 9.13 ± 0.41 mm in length and 21.02 ± 0.98 mm in breadth with expanded wings. Whereas, female 10.71 ± 1.14 mm in length and 22.30 ± 1.48 mm in breadth with expanded wings. The preoviposition, oviposition and post-oviposition periods were 1.50 ± 0.68 , 6.00 ± 1.33 and 4.30 ± 1.53 days, respectively. The fecundity was 265.00 ± 86.75 eggs/ female. The total lifecycle of male was 30.51 ± 6.00 days, while in female it was 32.60 ± 5.59 days.

Key words: Okra, shoot and fruit borer, biology, fecundity, morphometrics, instars, incubation period, longevity, lifecycle, oviposition,

Okra, *Abelmoschus esculentus* (L.) is one of the most important vegetable (Jan et al., 2021; Nagachandrabose, 2022); (Khanzada and Khanzada, 2018; Kumar and Das, 2021). In India, it is cultivated on 5.1 lakh ha with an annual production of 6094 thousand mt and a productivity 12.00 mt/ ha. Major okra growing states are Gujarat, West Bengal, Bihar, Andhra Pradesh, Assam and Orissa (Anonymous, 2018a). In Gujarat, it is mainly cultivated in Junagadh, Vadodara, Surat, Bhavnagar, Valsad and Anand districts on 75.27 thousand ha with total production of 921.72 thousand MT and a productivity of 12.28 mt (Anonymous, 2018b). Several biotic and abiotic factors are responsible for low yield in the okra crop, and among them, incidence of insect pests is important. Shoot and fruit borer *Earias vitella* and *E. insulana* (Ahmed et al., 2012; Tanni et al., 2019) are the most serious as it. *Earias vitella* being predominant (Kathiriya et al., 2007; Kumar et al., 2014); (Dongarjal and Bhamare, 2016). In India, an estimated loss of 69% is observed (Sharma et al., 2010; Kedar et al., 2014). Keeping in view the economic importance of *E. vitella* in okra, studies on biology and its morphometric were done.

MATERIALS AND METHODS

The study was carried out in the laboratory of Department of Entomology, C P College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during August 2019 to October 2019. Biology was studied on okra variety Gujrat Okra-5 at $26.81 \pm 1.53^{\circ}\text{C}$ and $67.82 \pm 08.13\%$ RH. To raise the initial culture of *E. vitella*, infected fruits were collected from college farm and brought to the laboratory. These were kept in circular galvanized trays and as soon as pupae were formed these were removed to glass jar (20 x 14 cm). The top of glass jar was covered with pieces of muslin cloth with the help of rubber bands and kept in oviposition cage for emergence of adults. On emergence of male and female tender shoots and fruits were provided for oviposition. Egg, larvae, pupa and adult were observed Size of eggs were measured under the microscope with ocular micrometer and size of larvae, pupa and adult was measured with graph paper. The number and duration of larval instar was determined on the basis of exuviae casted off. Incubation period was calculated from laying to hatching and hatching % was calculated. Sex of the adult was identified from the pupa

on the basis of marking on genitalia and anal opening of the pupa. The newly emerged male and female were used to study the preoviposition, oviposition and post-oviposition periods. Number of eggs laid by each female was recorded daily till the death of the female, to observed the fecundity. Longevity of male and female were also calculated. The period from egg laying to the death of adult was considered as a total lifecycle. The data on various biological parameters were subjected to analysis in software SPSS.

RESULTS AND DISCUSSION

It was observed that *E. vitella* did not lay eggs on leaf surface. In laboratory conditions, the eggs were laid singly in a small group of 2 to 3 on surface of the tender shoot and hairy part of fruits. Kathiriya et al. (2007) reported similar observation. The freshly laid eggs were spherical, bluish green which projected upward and gave it crowned appearance. Later, eggs turned into dark green and finally became creamy brown and after emergence of the larvae, the egg shell became transparent with a tiny emergence hole made by the larva. Vennila et al. (2007) and Patel et al. (2010) corroborate. Maximum mortality was observed in egg stage and first larval instars was observed while minimum was in the fourth larval instar (Shah et al., 2014). The length of eggs varied from 0.52 ± 0.02 mm, while the breadth 0.51 ± 0.02 mm. Patel et al. (2010) reported it was $0.52 \pm 0.02 \times 0.51 \pm 0.02$ mm. The incubation period varied from 3.35 ± 0.76 days; it was 2.8 to 3.5 days by Dhillon and Sharma (2004); 3.66 ± 0.75 days by Kathiriya et al. (2007); 3.33 ± 0.48 days by Patel et al. (2010); 2.30 days by Syed et al. (2011) and 2.97 ± 0.30 days by Sahito et al. (2019). The hatching

of eggs varied from $73.27 \pm 3.56\%$, similar with the findings of Dhillon and Sharma (2004), Kathiriya et al. (2007), Patel et al. (2010) and Sahito et al. (2019).

The larva was observed to pass through four larval instars. Kathiriya et al. (2007) and Patel et al. (2010) observed four larval instars on cotton genotypes the larval period was from 8.2 to 9.2 days on buds and 9.2–12.2 days on bolls with the pre-oviposition period of 2.42 days (Dhillon and Sharma, 2004). The freshly hatched larvae were light creamy white, possessed hairs on dorsal and lateral side with black dots and also with dark brown head capsule; and later on it turned black. After feeding it turned into pale brown. The larva had three pairs of thoracic legs on each thoracic segment and five pair of prolegs on third to sixth and tenth abdominal segment. Patel et al. (2010) reported that length and breadth of first instar of *E. vitella* larva was on an average 1.75 ± 0.29 mm and 0.26 ± 0.04 mm. Thus, the present findings are in conformation with those reported in past. The duration of first instar larva varied from 1 to 3 days with a mean of 2.16 ± 0.71 days (Table 1). Shitole and Patel (2010) and Sahito et al. (2019) reported that the average duration of first instar larva of *E. vitella* was 2.51 ± 0.61 days and 2.50 ± 0.22 days, respectively when reared on okra. Thus, the above findings are more or less corroborating with present findings.

Morphometrics of larval instars are given in Table 1. These observations and those on larval period almost corroborate with those of earlier workers (Kathiriya et al., 2007; Patel et al., 2010; Shitole and Patel (2010); Sahito et al., 2019; Sayed et al., 2011).

The fourth instar larva became sluggish, stopped

Table 1. Morphometry and biology of *E. vitella*

Sl.No.	Life stage	Period (Days)			Length (mm)			Breadth (mm)		
		Min.	Max.	Mean $\pm$ S.D.	Min.	Max.	Mean $\pm$ S.D.	Min.	Max.	Mean $\pm$ S.D.
1.	Egg	2	5	3.35 ± 0.76	0.5	0.54	0.52 ± 0.02	0.5	0.55	0.51 ± 0.02
2.	Larva									
	I instar	1	3	2.16 ± 0.71	1.34	2.16	1.77 ± 0.28	0.23	0.32	0.27 ± 0.03
	II instar	1	2	1.43 ± 0.50	1.86	3.1	2.60 ± 0.40	0.24	0.49	0.36 ± 0.08
	III instar	1	3	2.26 ± 0.73	5.6	7.23	6.45 ± 0.56	1.13	1.36	1.24 ± 0.07
	IV instar	2	5	3.30 ± 1.02	10.7	17.04	14.15 ± 2.12	2.45	4.13	3.23 ± 0.51
3.	Pupa									
	Male	7	8	7.53 ± 0.50	9.1	10.8	9.78 ± 0.46	2.82	3.64	3.14 ± 0.31
	Female	7	9	7.90 ± 0.92	9.36	11.13	10.14 ± 0.69	3.24	3.51	3.37 ± 0.09
4.	Adult									
	Male	27	44	32.60 ± 5.59	8.45	9.68	9.13 ± 0.41	19.8	22.7	21.02 ± 0.98
	Female	22	39	30.51 ± 6.00	9.1	12.49	10.71 ± 1.14	20.4	24.56	22.30 ± 1.48

feeding and contracted. The contracted larva has darker brown colour with white and orange spots on the dorsal and lateral side. The duration of pre-pupal stage was 1.43 ± 0.50 days. Dhillon and Sharma (2004) observe pre-pupal period of 19.8 to 23.7 hours and Patel et al. (2010) was 1.4 ± 0.33 days. Sex differentiation in pupa was done on the basis of female and male genitalia. In female, sex organs were present on 8th and 9th segments of abdomen and in male it was present on 9th abdominal segment. Anal slit present on 10th abdominal segments of pupa in both sexes. Kathiriya et al. (2007) reported that the pupa was dirty white to light brown, silken, boat shaped cocoon and pupated inside it. The female pupa measured $10.14 \pm 0.69 \times 3.37 \pm 0.09$ mm, male pupa measured $9.78 \pm 0.46 \times 3.14 \pm 0.31$ mm (Table 1). Patel et al. (2010) observed similar more or less morphometrics. The duration of pupal stage varied from 7.53 ± 0.50 and 7.90 ± 0.92 days in female and male, respectively. The present finding are in close agreement with those of Patel et al. (2010) and Shitole and Patel (2010).

The male and female moths were pale white with green longitudinal wedge-shaped band in the middle of the forewings. Hind wings were pale white and smaller than forewings. Head was smaller than thorax. Antennae were filiform and yellowish grey with creamy white hair. Eyes were black. Abdomen was cylindrical, long with pale hairs. The male was distinguished from the female by the presence of tuft of hair on the last abdominal segment. The present findings on sex differentiation are in close agreement with those of Kathiriya et al. (2007) and Patel et al. (2010). The measurements of adults agree with those of earlier workers (Kathiriya et al., 2007; Patel et al., 2010). The preoviposition period was 1.50 ± 0.68 days. Patel et al. (2010) reported it as 1.45 ± 0.49 days. The oviposition period was 6.00 ± 1.33 days found to agree with Patel et al. (2010).

The post-oviposition period lasted 4.30 ± 1.53 days. Patel et al. (2010) observed it as 4.40 ± 1.68 days. The fecundity of female varied from was 265.50 ± 86.75 eggs/ female (Table 1). Patel et al. (2010) observed 228 ± 8.10 eggs/ female Mishra et al. (2016) gave similar results. The longevity of male was 8.70 ± 2.11 days, while in female it similar more or less morphometrics. The duration of pupal stage varied from was 11.16 ± 2.62 days. Patel et al. (2010) observed that the female survived longer than male similar to the present study. The total lifecycle was 30.51 ± 6.00 days in male and 32.60 ± 5.59 days in female (Table 1). Patel et al. (2010) observed that the total life cycle of female and male was 34.18 ± 4.68 and 29.90 ± 3.62 days, respectively.

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AUTHOR CONTRIBUTION STATEMENT

Ankur has conducted the entire work under the guidance of Ravindra Kumar Patel and Mohan Lal Tatarwal. Tanmaya Kumar Bhoi has analysed the data. Ipsita Samal has given constructive suggestions while writing this manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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DIVERSITY OF MAJOR PREDATORS UNDER DIFFERENT INSECTICIDE REGIMES IN TWO IMPORTANT GROWTH STAGES OF RICE

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ABSTRACT

The effect of twelve different groups of insecticides on diversity of major predators (insects and spiders) during active tillering and bootleaf stage of rice crop was evaluated in West Bengal. The results showed that spinosad, emamectin benzoate, sulfoxaflor and pymetrozine were highly safe while buprofezin, imidacloprid, indoxacarb and chlorantraniliprole were moderately so. The insecticides like lambdacyhalothrin, fipronil and chlorpyrifos were found to be toxic. During active tillering stages the predator diversity was maximum in emamectin benzoate treatment and least in case of lambdacyhalothrin. Similar trend was observed for evenness index in the emamectin benzoate and lambdacyhalothrin. During boot leaf stage, Shannon index was maximum in sulfoxaflor and minimum in lambdacyhalothrin. Overall, insecticidal treatments resulted in decrease in diversity of predators during both stages of rice crop.

Key words: Diversity, insect, spider, Shannon index, evenness index, ecosystem, insecticide, *Ophionea* spp., *Paederus* spp, *Cyrtorhinus lividipennis*, *Lycosa pseudoannulata*, *Oxyopes* spp., *Phidippus indicus*

West Bengal has wide diversity of flora and fauna due to favorable climate and therefore there exists wide varieties of predators of the crop pests in rice ecosystems. Nearly 300 species of insect pests attack rice crop (Gailce Leo Justin and Preetha 2014) of which only 23 species cause notable damage (Pasalu and Katti, 2006). Among them, yellow stem borer (YSB), *Scirpophaga incertulas* (Walk.), brown planthopper (BPH), *Nilaparvata lugens* (Stål) and Asian rice gall midge (GM), *Orseolia oryzae* (Wood-Mason) cause huge crop losses (Seni and Naik 2017). Farmers apply insecticides at indiscriminate rates and frequencies to overcome pest problems, leading to pest resurgence and destruction of natural enemies (Katti et al., 2001, Satpathi et al., 2005). Monocrotophos, chlorpyrifos, triazophos and cartap hydrochloride are commonly used against rice stem borer and leaf folder, while quinalphos and imidacloprid are used against planthoppers (Sarao and Mahal, 2012). Recent studies have shown that acephate 95%SG, rynaxypyr 20%SC, dinotefuran 20%SG, cartap hydrochloride 50%SP, fipronil 5%SC, pymetrozine 50%WDG are few newer insecticides being recommended for use against borer and hopper pests (Seni and Naik, 2017; Adhikari et al., 2019). The natural enemies often control insect pests, especially in places where use of broad spectrum pesticide is avoided. Hill et al. (2017) reported that broad spectrum pesticide application altered natural enemy communities thereby

facilitating secondary pest outbreaks. Wyckhuys (2019) reported that biological pest control is annually worth hundreds of dollars but remains unknown to nearly 70% of farmers globally. Therefore it is imperative to select specific and safe insecticides to conserve the natural enemy fauna. The present study focuses on this in rice crop.

MATERIALS AND METHODS

Field experiments were conducted during (June to November 2020, 2021) at the Regional Research Station, Chakdaha, Bidhan Chandra Krishi Viswavidyalaya (24.50° N, 86.00 to 89.00° 39.75 masl) 9.75 m. Short duration variety, IET 4786 was transplanted on 15th and 18th July during 2020 and 2021, respectively in plots of 5x5 m size each with spacing of 0.20 m (row to row) x 0.15 m (plant to plant) and 1 m intermediate distance between the plots. The experiment was laid out in randomized block design with thirteen treatments replicated thrice. The crop was raised following normal agronomic practices. Twelve insecticides as given in Table 1 were applied at maximum tillering (42 days after transplanting DAT) and boot leaf stage (63 DAS) using knapsac sprayer 600 l/ ha as spray fluid. Observations were recorded on the incidence of major predators on randomly selected 20 hills/ plot at 3 and 14 days after spraying. Data were converted to angular transformed values to perform ANOVA treatment means were

Table 1. Important predators associated with rice crop

Sl. No.	Scientific name	Target pests	Number of predators (Mean± SE, n=20)	
			Tillering	Boot leaf
Insects				
1.	<i>Ophionea ishii ishii</i> (Habu), Coleoptera	SB, LF, H	3.15± 0.177	3.15± 0.135
2.	<i>Ophionea indica</i> (Thunberg), Coleoptera	SB, LF, H	5.14± 0.157	4.10± 0.105
3.	<i>Drypta japonica</i> (Bates), Coleoptera	SB, LF, H	-	2.08± 0.103
4.	<i>Micraspis discolor</i> (F.), Coleoptera	SB, LF, H	5.15± 0.131	3.09± 0.087
5.	<i>Paederus riparius</i> (L.), Coleoptera	SB, LF, H	3.13± 0.105	2.08± 0.078
6.	<i>Paederus fuscipes</i> (Curtis), Coleoptera	SB, LF, H	3.26± 0.189	2.14± 0.117
7.	<i>Cyrtorhinus lividipennis</i> Reuter, Hemiptera	H	1.07± 0.136	8.16± 0.142
8.	<i>Camponotus compressus</i> (F.), Hymenoptera	SB, LF, GB	2.13± 0.125	1.17± 0.082
9.	<i>Solenopsis geminata</i> (F.), Hymenoptera	SB, LF, GB	1.12± 0.187	2.08± 0.078
10.	<i>Andrallus spinidens</i> (F.), Hemiptera	SB, LF	1.13± 0.125	1.20± 0.105
11.	<i>Cheilomenes sexmaculata</i> (F.), Coleoptera	SB, LF	2.07± 0.094	1.23± 0.110
12.	<i>Brumoides suturalis</i> (F.), Coleoptera	SB, LF	3.08± 0.078	1.11± 0.099
Spiders				
13.	<i>Lycosa pseudoannulata</i> (Boes and Strand), Lycosidae	SB, LF, H	4.1± 0.081	4.11± 0.11
14.	<i>Marpisa bengalensis</i> Tikader, Lycosidae	SB, LF, H	2.10± 0.063	3.09± 0.091
15.	<i>Oxyopes javanus</i> Thorell, Oxyopidae	SB, LF, H	4.10± 0.094	4.09± 0.087
16.	<i>Oxyopes lineatipes</i> (C.L. Koch), Oxyopidae	SB, LF, H	3.09± 0.087	2.11± 0.11
17.	<i>Phidippus indicus</i> Tikader, Salticidae	SB, LF, H	3.09± 0.087	3.38± 0.078
18.	<i>Pardosa sumatrana</i> Thorell, Salticidae	SB, LF, H	2.15± 0.108	1.08± 0.079
19.	<i>Thomisus sikkimensis</i> Tikader, Thomisidae	SB, LF, H	1.08± 0.091	-
20.	<i>Zygoballus narmadaensis</i> Tikader, Thomisidae	SB, LF, H	1.06± 0.084	-
21.	<i>Araneus inustus</i> (L. Koch), Araneidae	SB, LF, H	1.13± 0.069	2.17± 0.13
22.	<i>Argiope aemula</i> (Walckenaer) Araenidae	SB, LF, H	1.06± 0.084	2.16± 0.12
23.	<i>Argiope aemula</i> (Walckenaer), Araenidae	SB, LF, H	2.07± 0.082	2.08± 0.091
24.	<i>Neoscona bengalensis</i> Tiader. and Bal., Araneidae	SB, LF, H	2.11± 0.079	2.08± 0.091
25.	<i>Tetragnatha bengalensis</i> Walck, Tetragnathidae	SB, LF, H	3.12± 0.078	3.11± 0.089
26.	<i>Tetragnatha maxillosa</i> Thorell, Tetragnathidae	SB, LF, H	1.08± 0.078	2.14± 0.108

SB=Stem borer, LF=Leaf folder, H=Green leaf hopper, brown planthopper and whitebacked planthopper, GB: Gandhi bug

separated by LSD, at $p=0.05$ (Gomez and Gomez 1984). The predator diversity was worked out by computing diversity (Shannon and Weaver 1949) and evenness index (Pielou, 1966).

RESULTS AND DISCUSSION

The insect predators observed belonged to the orders Coleoptera, Hemiptera, Hymenoptera and Araneae (Table 1). *Ophionea ishii ishii* and *Ophionea indica*, *Drypta japonica*, *Paederus riparius* and *Paederus fuscipes* were the common carabid beetles observed to predate on lepidopteran larvae, hopper pests and gallmidge in rice field. Among the coccinellid beetles *Micraspis discolor*, *Cheilomenes sexmaculata* and *Brumoides suturalis* were predominant. Black ant *Camponotus compressus* and red ant *Solenopsis*

geminata population were prevalent when the field became dry (Fig 1). *Cyrtorhinus lividipennis* and *Andrallus spinidens* consumed both mature and immature stages of hopper and lepidopteran pests, respectively. 15 species of predacious spiders belonging to 8 families under the order of Araneae were also recorded (Fig 2). The food preferences of these were mostly identical. The effect of insecticides on these are given in Table 2. At maximum tillering stage, both at 3 and 14 DAS, predator diversity was maximum in untreated control. Among the insecticides emamectin benzoate (1.75 to 1.76) treatment recorded maximum Shannon index. Least diversity was recorded in lambda cyhalothrin 5%EC and chlorpyrifos 20%EC treated plots. Similar trend was observed in case of evenness index and emamectin benzoate 5%SG showed the



Ophionia indica, *Ophionia ishii ishii*, *Paederus fuscipes* (first row); *Micraspis discolor*, *Cheilomenes sexmaculata*, *Brumoides suturalis* (second row); *Camponotus compressus*, *Solenopsis geminata*, *Andrallus spindens* (Third row)

Fig. 1. Important insect predators



Tetragnatha maxillosa, *Tetragnatha bengalensis*, *Phidippus indicus* (first row); *Lycosa pseudoannulata*, *Pardosa sumatrana*, *Neoscona bengalensis* (second row); *Marpisa* sp (immature), *Oxyopes* sp. (Immatur), *Argiope aemula* (third row)

Fig. 2. Important spiders

Table 2. Effect of different insecticides on the diversity indices of predators on rice (Rainy season, 2020 and 2021)

Sl. No.	Name of insecticides	Dose a.i. g/ ha	Diversity indices of predators							
			Maximum tillering stage				Booting stage			
			3D		14D		3D		14D	
			H	J	H	J	H	J	H	J
1.	Chlorpyriphos 20%EC	315	1.46	0.70	1.47	0.70	1.42	0.66	1.68	0.76
2.	Lambdacyhalothrin 5%EC	50	1.02	0.57	1.19	0.49	0.90	0.55	1.00	0.62
3.	Imidacloprid 17.8%SL	25	1.70	0.81	1.73	0.83	1.72	0.82	1.74	0.83
4.	Cartap hydrochloride 50%SP	300	1.71	0.82	1.72	0.82	1.71	0.82	1.71	0.82
5.	Fipronil 5%SC	75	1.45	0.70	1.46	0.70	1.40	0.64	1.66	0.75
6.	Spinosad 45%SC	75	1.72	0.83	1.74	0.95	1.72	0.82	1.74	0.83
7.	Emamectin benzoate 5%SG	10	1.75	0.84	1.76	0.84	1.73	0.83	1.74	0.83
8.	Sulfoxaflor 24%SC	93.75	1.74	0.83	1.75	0.84	1.72	0.82	1.75	0.83
9.	Indoxacarb 14.5%SC	30	1.70	0.82	1.72	0.95	1.70	0.94	1.71	0.95
10.	Chlorantraniliprole 18.5%SC	30	1.71	0.82	1.72	0.82	1.72	0.82	1.72	0.82
11.	Buprofezin 25%SC	250	1.73	0.81	1.73	0.83	1.72	0.83	1.73	0.83
12.	Pymetrozine 50%WG	125	1.74	0.83	1.76	0.84	1.74	0.83	1.74	0.83
13.	Untreated control	0	1.88	0.90	1.95	0.93	1.91	0.91	1.92	0.92

H =Shannon index, J=Evenness index

maximum value. During boot leaf stage, maximum diversity was recorded in sulfoxaflor 24%SC followed by pymetrozine 50%WG (1.74), emamectin benzoate 5%SG, spinosad 45%SC, imidacloprid 17.8%SL. Minimum diversity was recorded in lambdacyhalothrin 5%EC. Evenness index revealed similar trend and while it was reverse with lambdacyhalothrin 5% both at maximum tillering and boot leaf stages, respectively. Thus, insecticides reduced predator population both at maximum tillering and boot leaf stages except emamectin benzoate 5%SG followed by pymetrozine 50%WG and spinosad 45% treated plots. Baehaki et al. (2017) also reported that emamectin benzoate and pymetrozine were highly safe to coleopteran predator viz *Paederus fuscipes* and *Coccinella* sp. Applications of lambdacyhalothrin 5%EC and chlorpyrifos 20%EC were relatively unsafe to predators (Fritz 2013). In the present study, the spider population was more in sulfoxaflor 25%SC followed by pymetrozine 50%WG and emamectin benzoate 5%EC. Baehaki et al. (2017) reported on toxicity and persistence of emamectin benzoate 5%SC vis a vis wolf spider *L. pseudoannulata* recording spider mortality rate less than 25%. Deekshita et al. (2017) also reported that sulfoxaflor was the safest for spider followed by pymetrozine 50%WG Kumar et al. (2019) in rice found that lambdacyhalothrin and chlorpyriphos are not safe. Kumar and Veluswami (1997) reported that chlorpyriphos was highly toxic to spiders. Diversity of predator in rice was more in sulfoxaflor 24%SC treated plot followed by

pymetrozine 50%WG and emamectin benzoate 5%SG. Sarao and Mahal (2012) also reported that relatively less toxic chemicals showed maximum population and diversity of natural enemies.

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CONFLICT OF INTEREST

No conflict of interest.

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BIOSAFETY OF BOTANICALS TO *TRICHOGRAMMATOIDEA BACTRAE* NAGARAJA

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ABSTRACT

Bioassay was conducted with nine botanical extracts at various concentrations viz., azadirachtin 300 ppm, 1500 ppm, 3000 ppm, 10000 ppm, neem seed oil 1%, 2%, 3%, neem seed extract 5% and dashparniark 4% on *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) in completely randomized design. Assessment of these on % parasitization, reduction in parasitism and adult emergence of *T. bactrae* on *Corcyra* eggs was done. The results revealed that dashparniark (ten plant leaves extract) 4% was found safer with maximum parasitization (53.3%) and minimum reduction in parasitization (27.9%). Azadirachtin 10000 ppm was found harmful resulting in reduced parasitization (12.6%) and adult emergence (32.6%); and maximum reduction in parasitism (82.9%) on *Corcyra* eggs was observed. Maximum % adult emergence was recorded when parasitized eggs were treated with neem seed oil 1% (64.3%).

Keywords: *Trichogrammatoidea bactrae*, parasitization, *Corcyra cephalonica*, azadirachtin, neem seed oil, adult emergence, dashparni ark, neem seed extract, biosafety, bioassay.

Trichogrammatoidea bactrae Nagaraja (Hymenoptera: Trichogrammatidae) is the most important and effective egg parasitoid of pink bollworm, *Pectinophora gossypiella* (Saunders). In 1993, *T. bactrae* was introduced from Australia into California and Arizona for control of the pink bollworm in cotton (Naranjo, 1993). This parasitoid is widely distributed in the Oriental region, adapted to terrestrial humid habitats and parasitise various insect pests of cotton, sugarcane, fruits and vegetables (Nagaraja, 1978). This parasitoid was found to attack eggs of many lepidopteran insect pests such as *Helicoverpa armigera* Hubner and *Atherigona soccata* Rondani (Rao et al., 1987), *Earias vitella* F., *Achanthodelta janata* L. (Rao et al., 1980), and *Pectinophora gossypiella* (Saunders) (Hutchinson et al., 1990). In cotton, against the pink bollworm *T. bactrae* is recommended @ 60000/acre (Nagrare et al., 2018). Twelve sequential releases of *T. bactrae* showed a significant decrease in pink bollworm incidence (Kumara et al., 2022) and thus the release was as effective as insecticide (Naik et al., 2019). Due to the increased awareness regarding the deleterious effect of pesticide cotton cultivation is slowly shifting to organic agriculture. According to latest reports, the organic cotton production has increased (PIB, 2022). Hence, for the management of pests and diseases the botanicals and bioagents play prominent role, mostly dominated by neem-based botanicals (Mageshwaran et

al., 2019). The active ingredient of neem formulations is azadirachtin and it is used as antifeedant. Apart from these several plant products have been utilized for the management of insect pests. The botanicals used in such ecosystem can affect parasitization or activity of natural enemies. The exposure of *T. bactrae* to different botanicals used in cotton ecosystem is possible which might affect the parasitizing behaviour and emergence of the parasitoid. Hence, the present study the focusing on the biosafety aspects of botanicals on *T. bactrae*.

MATERIALS AND METHODS

The experiments were conducted at the Biological Control Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Dr PDKV, Akola, Maharashtra during 2020-2021. For the rearing of factitious *Corcyra cephalonica* standard procedure was followed and reared in plastic containers, and oviposition chambers. The eggs were collected from the base of oviposition chamber daily and used for rearing of *Trichogramma*. For the rearing of the parasitoid *T. bactrae*, the nucleus culture was procured from the National Bureau of Agricultural Insect Resources (NBAIR) Bangalore, India. The gum arabic was smeared on the trichocards uniformly. Then, one cc of *Corcyra* eggs was sprinkled evenly on the trichocards with mesh and cards were allowed to dry under the blower. Cards were kept under the UV light for 1 to

1.5 hr to kill the embryo. After UV treatment, the cards were exposed to *T. batrae* adults for oviposition up to 24 hr. Then cards were removed and placed separately. On 4th or 5th day after oviposition the eggs turn black indicating parasitization. Then 3 to 4 days after parasitization the adults emerged from the eggs. The host insect *C. cephalonica* and the parasitoid *T. batrae* reared (25±2°C) 65-75% RH. The commercial formulations of botanical insecticides were obtained from market and their concentrations were fixed based on recommendation of the Central Insecticide Board and Registration Committee (CIBRC, 2021).

The card dip bioassay methodology was followed as given in Consoli et al. (2000), Bastos et al. (2006) and Chen et al. (2013). Irradiated fresh eggs of *C. cephalonica* were glued to the egg cards separately (@50 eggs per card strip). Each card strip dipped in test botanicals for 5 sec., and for control water was used. These treated card strips were dried under shades and was kept in glass vials (15.0×2.5 cm) @ 1 card strip/vial. Each treatment was then labelled properly with details such as name of treatment, concentration of botanicals, date and time of application. The treated strips were exposed to adults of *T. batrae* (@ 5:1 host: parasitoid ratio) for 24 hr for parasitization. The eggs cards were examined for parasitization after four to five days and the parasitized eggs were counted under the stereozoom microscope. For adult emergence, the already parasitized eggs (@50 eggs/ card strip) of *Corcyra* by *T. batrae* were treated with test botanicals by same procedure as mentioned above. Such treated

strips were then shade dried and placed individually in plastic vials (2.5×6 cm). Each treatment was labelled properly and observations on number of adults emerged made. The data ($\bar{x} \pm SE$) were subjected to ANOVA in completely randomized design and mean values were compared by Tukey's post-hoc test, using SPSS program, version 23 (SPSS 2015) and treatment means were separated at $p=0.05$.

RESULTS AND DISCUSSION

The results given in Table 1 reveal significant differences among the treatments in parasitization, reduction in parasitization and adult emergence. Significantly maximum parasitization and adult emergence of 74 and 86.33% was in control; among the treatments, T₉ (dashparniark-ten plant leaves extract) was the safest (53.33% parasitization). It was followed by treatment T₈ (NSE 5%). Minimum % parasitization was recorded in T₄ (azadirachtin 10000 ppm) with 12.67% parasitization. The treatment T₉ (dashparniark) was thus found significantly safer followed by treatment T₈ (NSE 5%). Among the botanicals tested, treatments T₅ (neem seed oil 1%), T₈ (NSE 5%), T₉ (dashparniark) and T₆ (neem seed oil 2%) were found significantly superior with 64.33, 62.33, 60.33 and 55% adult emergence, respectively and were at par.

Rao and Raguraman (2005), the aqueous neem seed extract 5% was safer to natural enemies. Observed that Singh (2015) has also reported that neem seed extract 5% and neem gold 5% were safer to *T. chilonis*. However, Narendra et al. (2013) found that azadirachtin

Table 1. Effect of botanicals on *T. batrae* reared on *C. cephalonica* eggs

Treatment details	Dose ha ⁻¹ (gm. or ml/ l)	% parasitization	% reduction in parasitization	% adult emergence	Score
T ₁ Azadirachtin 300 ppm	10 ml/ l	19± 2.30 ^{de}	74.40± 2.77 ^{ab}	54.33± 0.88 ^{bc}	Slightly harmful
T ₂ Azadirachtin 1500 ppm	10 ml/ l	15± 1.2 ^{de}	79.32± 1.27 ^a	45± 2.30 ^{cd}	Moderately harmful
T ₃ Azadirachtin 3000 ppm	4 ml/ l	17.33± 0.33 ^{de}	76.56± 0.39 ^{ab}	31.67± 1.85 ^d	Slightly harmful
T ₄ Azadirachtin 10000 ppm	3 ml/ l	12.67± 1.45 ^e	82.93± 1.70 ^a	32.67± 2.72 ^d	Moderately harmful
T ₅ Neem seed oil 1%	10 ml/ l	30± 2.08 ^c	59.42± 2.83 ^c	64.33± 2.02 ^b	Slightly harmful
T ₆ Neem seed oil 2%	20 ml/ l	25.33± 4.33 ^{cd}	65.69± 5.88 ^{bc}	55± 1.00 ^{bc}	Slightly harmful
T ₇ Neem seed oil 3%	30 ml/ l	13.33± 1.20 ^e	81.90± 1.94 ^a	34± 2.51 ^d	Moderately harmful
T ₈ Neem seed extract 5%	5 ml/ l	46± 2.00 ^b	37.89± 1.49 ^d	62.33± 1.45 ^b	Slightly harmful
T ₉ Dashparni ark 4%	4 ml/ l	53.33± 1.33 ^b	27.88± 1.88 ^d	60.33± 8.00 ^{bc}	Harmless
T ₁₀ Control		74± 1.52 ^a	---	86.33± 2.7 ^a	
F value		F= 102.10*	F=55.39*	F= 29.14*	
		df= 9,20	df= 8,18	df= 9,20	

Treatment columns bearing different letters significantly different from other treatments according to Tukey's Post-hoc test* ($p=0.05$); Means values represents 3 replicates; Scores given according to % reduction in parasitization as suggested by IOBC classification which is harmless <30%, slightly harmful 30-79% moderately harmful 79-99% harmful >99%. (Sterk et al., 1999, Pawar et al., 2020)

1 ml/ l has significantly reduced parasitization by *T. chilonis*. Azadirachtin formulations were found to affect the parasitization adversely. Rao and Raguraman (2005) reported that neem oil 3.0% was relatively toxic when compared to other botanicals against *T. chilonis* Ishii and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), which corroborates present findings. Neem seed oil 3% was found harmful for parasitization by *T. bactrae*. Raguraman and Singh (1999) also reported that the neem seed oil at 0.3% deterred oviposition (parasitization) by *T. chilonis*. Babasaheb et al. (2009) stated that neem seed oil @ 1% reduced the parasitization but it did not affect the development and emergence. Parreira et al. (2018) revealed that all oils reduced the parasitism rate. Rampelotti-Ferreira et al. (2017) found that the neem oil treatment showed 67% reduction in parasitism by *T. pretiosum*. Earlier Selvamuthukumaran and Jayakumar (2017) reported 76.29% adult emergence of *T. chilonis* when treated with NSE 5%, which is in line with present findings. It was also revealed that five leaf extracts at 10% had 69.32% adult emergence of *T. chilonis*. In the present study, dashparni showed 60.33% adult emergence of *T. bactrae*. Singh (2015) revealed that neem seed extract 5% and neem gold 5% were safer for adult emergence of *T. chilonis*. In the present study, neem seed oil 1% (T_5) recorded maximum % adult emergence (64.33%) followed by T_6 , neem seed oil 2% (55%) and T_7 , neem seed oil 3% (34%). These findings derive support from Selvamuthukumaran and Jayakumar (2017), on the neem seed oil. Narendra et al. (2013) revealed 78.95% adult emergence with *T. chilonis*, when parasitized eggs were treated with azadirachtin 1 ml/ l. Saber et al. (2004), reported that the effect of neemazal (azadirachtin 10000 ppm) on the egg parasitoid was tested by dipping parasitized *Sitotroga cerealella* (Olivier) and *Cydia pomonella* (L.) eggs at the field recommended concentration. These revealed that adult parasitoids are adversely affected. Sharma and Agarwal (2019) observed the effect of azadirachtin 10000 ppm on adult emergence of *T. chilonis* and *T. japonicum*. These studies revealed that two-fold doses significantly disrupted the adult emergence of *T. chilonis* and *T. japonicum*. Alcántara-de la Cruz et al. (2021) stated that essential of *A. indica* affected at-least one biological attribute of *Trichogramma galloi*. Asrar et al. (2022) concluded that neem seed oil is relatively safer to *T. chilonis*. Gladenucci et al. (2020) reported that botanical extracts initially reduced total number of parasitized eggs (*T. pretiosum*) and female longevity. Parreira et al. (2019) revealed that essentials oils are harmful to parasitism.

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AUTHOR CONTRIBUTION STATEMENT

JSVM carried out the experiments, collected and analysed the data, and wrote the manuscript. NSS supervised the experiments, provided technical support, and critically revised the manuscript. DBU and SKB substantively revised it. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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INSECT GUT MICROBIOTA AND PESTICIDE DEGRADATION IN RESPONSE TO INNATE METABOLITES- A REVIEW

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ABSTRACT

Insects host a diverse microbiota in their gut, encompassing bacteria, fungi, viruses, and archaea, influencing their physiology, nutrition, and overall health. The composition of these microbial communities varies with factors like insect species, diet, and the environment. Insect gut microbiota serve pivotal roles such as aiding digestion, synthesizing essential nutrients, safeguarding against pathogens, and detoxifying toxins, including insecticides. A particularly promising facet of their function lies in influencing the metabolism of insecticides. These gut microbiotas can either augment or diminish insecticide toxicity through mechanisms like enzymatic breakdown, sequestration, target site alteration, or modulation of the insect's immune response. Understanding these interactions is paramount for devising sustainable pest management strategies. This review explores into insect gut microbiota, their impact on insecticide susceptibility, and the potential use of microbial metabolites in ecofriendly pest control. We explore pesticide degradation mechanisms, the consequences of microbiota disruption on susceptibility, and the role of microbiota-produced metabolites in shaping pesticide efficacy. Ultimately, we highlight the potential of microbiota manipulation as a strategy to enhance insecticide effectiveness and combat pesticide resistance in pest management.

Key words: Insect gut, microbiota, *Drosophila*, *Bactrocera*, pesticides, degradation, primary and secondary symbionts, enzymes, metabolites, physiology, insecticides, microorganisms, xenobiotics

Insects are hosts to various microorganisms which inhabit their gut and play a significant role in their physiology and health (Cheng et al., 2017). The gut microbiota aids in nutrient acquisition, digestion, and synthesis of essential compounds (Jing et al., 2020). Insecticides are chemicals designed to target insect pests and disrupt their physiological processes leading to their death (Khan and Ahmad 2019). However, there is evidence that the gut microbiota can metabolize and degrade some insecticides, leading to reduced efficacy and increased resistance (Mahas et al., 2023; Siddiqui et al., 2022). Additionally, exposure to insecticides can also disrupt the gut microbiota, leading to altered metabolites and potential health impacts (Jaffar et al., 2022). This review will explore the interaction between insect gut microbiota, insecticides, and innate metabolites and their influence on insect physiology and management. Pesticides are the most widely used tools for controlling insect pest populations in agriculture and public health. However, their extensive

use has led to widespread environmental contamination, with potential negative effects on human health and biodiversity (Khan et al., 2023). Furthermore, the emergence of pesticide-resistant pest populations poses a major challenge for pest management (Li et al., 2021). Insects have a complex gut microbiota, which can play a significant role in the metabolism of xenobiotic compounds, cytochrome; monooxygenases (P450; P450s) including pesticides, phytochemicals and environmental hazardous substances (Chaitra and Kalia 2020; Lu et al., 2021). The gut microbiota can break down and detoxify pesticides, as well as produce innate metabolites that can further influence pesticide metabolism and efficacy (Muñoz-Benavent et al., 2021; Schmidt and Engel 2021). Hence, understanding the interactions between insect gut microbiota and pesticides can provide insights into novel strategies for pest management that are both effective and environmentally sustainable (Giambò et al., 2021). Recent advances in molecular and sequencing

technologies have greatly expanded our understanding of the diversity and function of insect gut microbiota. Moreover, the discovery of novel metabolites produced by these microbial communities has opened up new avenues for the development of biologically-based pest management tools (Huang et al., 2019). Insect gut microbiota plays a crucial role in the metabolism of xenobiotic compounds, including pesticides. The disruption of gut microbiota due to pesticide exposure can lead to altered metabolism and bioavailability of these compounds, potentially affecting insecticide efficacy and resistance. Additionally, the gut microbiota produces innate metabolites that can further influence pesticide degradation and susceptibility.

Insects can host a wide variety of symbiotic relationships with microorganisms, including both primary and secondary symbionts. The location of these symbionts within the insect's body can vary depending on the species of insect and the specific symbiotic relationship (Martin et al., 2023). Here's a general overview of primary and secondary symbionts are typically found in insects.

Primary Symbionts (PS)

PS are typically essential for the insect's survival and often have a long coevolutionary history with their host. They are usually located within specialized cells or tissues of the insect, such as bacteriocytes (specialized host cells for harboring bacteria) or mycetocytes. Moreover, commonly found in the digestive system, specifically the midgut or hindgut, where they aid in the digestion of complex nutrients or provide essential nutrients that are lacking in the insect's diet. Aphids, for example, have PS called *Buchnera aphidicola* located

in specialized cells called bacteriocytes within their bodies (Martin et al., 2023).

Secondary Symbionts (SS)

SS are not as tightly integrated into the insect's physiology as primary symbionts and are often facultative, meaning their presence is not essential for the insect's survival. They can be found in various locations within the insect's body, and their distribution can vary. SS can inhabit the insect's gut, reproductive organs, or other tissues. Some SS are found within specialized cells similar to bacteriocytes, while others may be more loosely associated with the insect's cells or circulatory system. The location of SS can also vary between different insect species and even within populations of the same species. Symbiotic relationships can vary widely among different insect species. The presence and location of symbionts depend on factors such as the insect's diet, life history, and environmental conditions. Furthermore, ongoing research continues to discover new and captivating understandings into the complexity of insect-microbe interactions (Li et al., 2023).

Role of primary and secondary endosymbionts in pesticide degradation

The use of pesticides in agriculture has played a pivotal role in increasing crop yields and protecting crops from pests and diseases. However, the widespread application of pesticides has raised concerns about their environmental impact, including their persistence in soil and water ecosystems. In recent years, researchers have been exploring novel and sustainable approaches to mitigate the negative effects of pesticides. One promising avenue of research involves the utilization of primary and secondary endosymbionts in pesticide

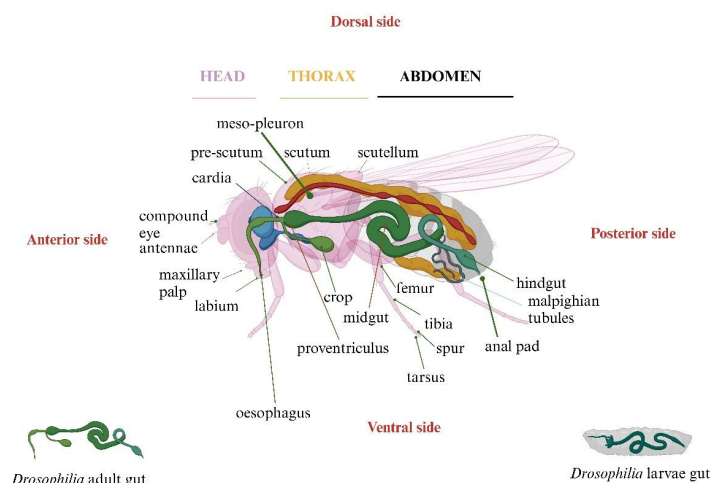


Fig. 1. Insect gut a schematic diagram of model *Drosophila*

degradation. These microscopic organisms, often found within the bodies of insects, have shown remarkable capabilities in breaking down and detoxifying various pesticides some are listed as. *Burkholderia gladioli* (in darkling beetle), *Snodgrassella alvi* (in honey bee), *Sulcia muelleri* and *Pantoea agglomerans* (in leafhopper), *Hamiltonella defensa* and *Buchnera aphidicola* (in aphids), *Wolbachia* spp. and *Asaia* spp. (in mosquitoes), *Wigglesworthia glossinidia*, and *Sodalis glossinidius* (in Tsetse fly), This comprehensive review explores the roles of primary and secondary endosymbionts in pesticide degradation and their potential applications in sustainable pest management strategies (Liu and Guo 2019; Martin et al., 2023).

Primary endosymbionts (PE): PE are micro-organisms that have co-evolved with their host insects over millions of years. They reside primarily in specialized cells or organs of their insect hosts and often have a mutualistic relationship with them. These endosymbionts are crucial for the survival and reproduction of the host insects (McCutcheon et al., 2019). Role in Pesticide Degradation: PE have been found to possess various enzymatic pathways that can metabolize and detoxify pesticides. These enzymes can break down specific chemical compounds found in pesticides, rendering them less toxic to the host insect. In some cases, PE can even confer resistance to certain pesticides. Examples: a). *Buchnera aphidicola*: Found in aphids, *Buchnera* has been shown to play a role in detoxifying organophosphate and carbamate insecticides. b). *Wolbachia*: Although primarily known for its reproductive manipulation effects, *Wolbachia* can also influence pesticide susceptibility in certain insects (Martin et al., 2023; Štarhová Serbina et al., 2022).

Secondary endosymbionts (SE): SE are typically not as tightly integrated into their host's biology as SE. They may be present in only certain populations of a host species or under specific environmental conditions. Role in pesticide degradation: SE often exhibit more diverse metabolic capabilities compared to PE. Some SE possess enzymes that can break down a wider range of pesticide compounds, making them potential candidates for bioremediation purposes. Examples: a). *Hamiltonella defensa*: Found in aphids, *H. defensa* has been associated with increased resistance to parasitoid wasps and certain pesticides. b). *Serratia symbiotica*: This secondary endosymbiont has been linked to increased tolerance to various environmental stresses, including pesticides, in aphids (Csorba et al., 2022; Martin et al., 2023).

Applications in pest management or as biological control: Utilizing primary and secondary endosymbionts that can metabolize pesticides could enhance the natural pest control mechanisms. This approach could reduce the need for chemical pesticides and minimize their environmental impact. Bioremediation: Linking the pesticide-degrading capabilities of endosymbionts for bioremediation purposes offers a sustainable approach for cleaning up pesticide-contaminated environments. Genetic Engineering: Researchers are exploring ways to transfer pesticide-degrading genes from endosymbionts into crop plants or beneficial insects to confer pesticide resistance (Gomes et al., 2023).

Removal with antibiotics and degradation in insects

PS are often intimately associated with their insect hosts and play critical roles in their biology. Eliminating PS in insects like aphids can have significant consequences for the insect's fitness and survival e.g., Aphids and *Buchnera aphidicola* and camellia spiny whitefly, *Aleurocanthus camelliae*. Several studies have conducted experiments to eliminate PS using antibiotics. For instance, when aphids are treated with antibiotics targeting *Buchnera aphidicola*, the primary symbiont, it disrupts the essential nutritional mutualism. Aphids deprived of *Buchnera* may exhibit reduced reproduction, slowed development, and increased susceptibility to environmental stressors. In some cases, for degradation of PS can be selectively degraded or eliminated by the host's immune system. For example, aphids possess a specialized mechanism called the bacteriocyte-infiltrating conoid, which allows them to selectively eliminate *Buchnera* when necessary. This occurs during the production of specialized dispersal forms (alates) that do not require the symbiont-dependent traits needed for regular reproduction (Tan et al., 2023).

SS often have more variable relationships with their hosts and can be facultative. Eliminating SS may have less severe consequences for the insect's survival compared to primary symbionts e.g., whiteflies and rickettsia. SS can be eliminated with antibiotics as well. For instance, whiteflies can harbor SSs like Rickettsia. When treated with antibiotics targeting Rickettsia, researchers have observed altered reproductive ratios and various fitness effects in whiteflies. However, these effects are generally less critical for the insect's survival compared to primary symbionts. The degradation of secondary symbionts may occur through evolutionary processes. Some insect populations may naturally lose SS over time, leading to changes in host-symbiont

interactions. This can be due to the selective advantage of carrying or losing the SS in different environments or under changing ecological conditions. Eliminating or degrading symbionts can vary depending on the specific insect-symbiont system, the antibiotic used, and the environmental context. In several cases, the relationship between insects and their symbionts for example in whitefly an intricate and dynamic, and complete elimination of symbionts can have far-reaching consequences for both the insect and the surrounding ecosystem. Further researchers needed to investigate these relationships to better understand their ecological and evolutionary implications (Tan et al., 2023).

Transient symbionts (TS) play a distinct role in insects compared to primary or secondary symbionts. While primary and secondary symbionts are typically long-term and often vertically transmitted, transient symbionts have a shorter association with their host, and their transmission is usually horizontal. Here's an overview of the role of TS in insects, along with examples:

Role of TS

In the environmental adaptation TS can help insects adapt to changing environmental conditions. Insects often encounter various microorganisms in their environment, and some of these TS can provide short-term benefits that enhance the insect's fitness (Frago et al., 2020). These benefits may include improved tolerance to specific stressors, such as heat, toxins, or pathogens. For the nutritional supplementation in some transient symbionts can assist insects in obtaining essential nutrients or metabolizing non-standard food sources. It can be particularly beneficial when the insect's primary diet lacks certain nutrients. TS can contribute to the insect's ability to exploit diverse food resources. Protection in the pathogen defense of insects may acquire TS that produce antimicrobial compounds or compete with harmful pathogens for resources. These transient symbionts can help protect the insect from infections by pathogens and parasites. In the reproduction and Fitness TS can influence various aspects of insect reproduction and fitness. They may impact mating behavior, fecundity, or the quality of offspring produced by the insect host. In many cases, TS can alter the sex ratio of the insect's offspring, affecting population dynamics. Some examples of TS in Insects are *Wolbachia* is a well-known example of a transient symbiont in insects. It is a bacterium that infects a wide range of insect species and is known for its ability to

manipulate host reproduction (Miller 2013). *Wolbachia* can induce cytoplasmic incompatibility, where infected males are unable to successfully reproduce with uninfected females or females infected with a different strain of *Wolbachia*. This reproductive manipulation can lead to the rapid spread of *Wolbachia* within insect populations. *Spiroplasma* is another TS found in various insect species. It can influence host reproductive patterns, alter insect development, and confer resistance to certain pathogens. For example, *Spiroplasma* in *Drosophila* fruit flies can affect the fly's ability to resist parasitoid wasp infections. *Arsenophonus* is a TS that has been identified in several insect groups. It is known for its potential role in providing resistance to parasitoid wasp attacks in aphids and other insects. *Arsenophonus* may contribute to the defense mechanisms of its insect host against parasitic wasps (Blow and Douglas 2019; Nováková et al., 2015).

Mechanisms of pesticide degradation by gut microbiota

Insecticides are widely used in agricultural production to control pests and increase crop yields (He et al., 2022; Jaffar et al., 2023; Li et al., 2022c). However, their extensive use has resulted in environmental contamination, which poses significant risks to human health and the environment. The gut microbiota of insects plays a crucial role in the degradation of pesticides, as they possess enzymes capable of breaking down these chemicals. Understanding the mechanisms involved in pesticide degradation through insect gut microbiota is therefore important for developing sustainable pest management strategies (Jang and Kikuchi 2020). Several studies have demonstrated the involvement of gut microbiota in pesticide degradation (Gomes et al., 2020; Li et al., 2022b), the gut microbiota of fall armyworm *Spodoptera frugiperda*, cotton bollworm *Helicoverpa armigera* were shown to degrade the insecticide (chlorpyrifos) have been identified in several bacterial strains that were capable of degrading synthetic pesticides and promotes sex pheromones, including *Bacillus* (Gui et al., 2023; Singh et al., 2022), *Enterobacter*, *Lactobacillus plantarum*, *Enterobacter cloacae*, *Photobacterium luminescens*, *Wolbachia*, and *Pseudomonas* species. Further investigation revealed that the gut microbiota produced several enzymes involved in pesticide degradation, such as esterase and cytochrome P450. Similarly, in many comprehensive studies (Bai et al., 2019; Cheng et al., 2017; Siddiqui et al., 2022; Zhu et al., 2023), the gut microbiota of silkworms *Bombyx mori*, cotton leaf worm *Spodoptera littoralis*, cotton aphid *Aphis gossypii* Glover, Colorado

potato beetle *Leptinotarsa decemlineata* (Say), diamondback moth *Plutella xylostella*, parasitic wasp *Eretmocerus mundus* (Mercet), bedbug *Cimex lectularius* (Linnaeus), fruit fly *Bactrocera dorsalis*, honey bee *Apis mellifera*, was shown to degrade the insecticide chlorantraniliprole. The study identified several bacterial strains, such as *Enterobacter cloacae* (*E. cloacae*), *Enterococcus* and *Lactobacillus*, that were capable of degrading chlorantraniliprole. Further analysis revealed that the gut microbiota produced several enzymes involved in pesticide degradation, such as carboxylesterase and glutathione S-transferase. Several other studies have explored the role of gut microbiota in the degradation of herbicides. In a study by (Wang et al., 2018), the gut microbiota of honey bees was shown to degrade the herbicide glyphosate. These studies identified several bacterial strains, including *Lactobacillus* and *Bifidobacterium*, that were capable of degrading glyphosate. Further analysis revealed that the gut microbiota produced several enzymes involved in pesticide degradation, such as amino-methyl-phosphonic acid (AMPA) lyase and glycine oxidase. In addition to the gut microbiota, innate metabolites produced by insects have also been shown to play a role in pesticide degradation. In a study (Cohn et al., 2022), the larvae of the black soldier fly were shown to produce metabolites that were capable of degrading the insecticide imidacloprid. Moreover, identified several metabolites, including 6-chloronicotinic acid and nicotinic acid, that were involved in the degradation of imidacloprid. Further analysis revealed that the metabolites were produced by the gut microbiota of the black soldier fly. The mechanisms involved in pesticide degradation in insect

gut microbiota are complex and involve the production of several enzymes and metabolites. However, several features, such as diet, environmental factors, and host genetics, can influence the composition and activity of gut microbiota, which can affect pesticide degradation. For example, a study (Engel and Moran 2013), showed that the composition of gut microbiota in honey bees was affected via exposure to the herbicide glyphosate. The exposure resulted in a significant reduction in the abundance of several bacterial strains capable of degrading glyphosate (Motta et al., 2022). In the gut microbiota of insects plays a crucial role in the degradation of synthetic pesticides, and several studies have identified the bacterial strains and enzymes involved in pesticide degradation. In addition, innate metabolites produced through insects have also been shown to play a role in pesticide degradation (Jang and Kikuchi 2020; Yun et al., 2014). However, the composition and activity of gut microbiota can be influenced by several factors, which can affect pesticide degradation. Understanding the mechanisms involved in pesticide degradation, insect gut microbiota is important for developing sustainable pest management strategies that minimize environmental contamination and reduce the risk to human health (French et al., 2021; Siddiqui et al., 2022).

Impact of microbiota disruption on insecticide susceptibility

Insects are the most abundant and diverse group of faunas on the earth, and they play an indispensable role in ecosystems (Holtot et al., 2019). However, they can also be significant pests in agriculture, causing billions of dollars in crop damage annually

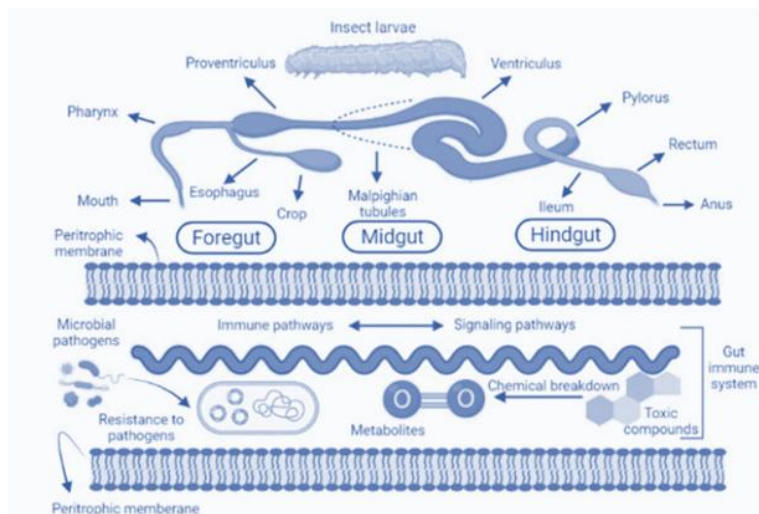


Fig. 2. Insect gut microbiota resistance to pesticides. A graphical representation.

(Scudder, 2017). Insecticides are commonly used to control insect pests, but the overuse of these chemicals can lead to resistance development, environmental contamination, and negative effects on non-target organisms (Naqqash et al., 2016). Insect gut microbiota have been identified as an essential factor in the detoxification of pesticides, and their disruption can lead to increased insecticide susceptibility (Gao et al., 2022; HS and KKalia, 2022). Recent investigate has shown that gut microbiota enzymes can detoxify various classes of insecticides, including organophosphates, pyrethroids, and neonicotinoids. The gut microbiota of insects can degrade these pesticides through various mechanisms, including hydrolysis, oxidation, and reduction (Jaffar et al., 2022; Sogorb and Vilanova, 2002). For example, the gut microbiota of the diamondback moth, *Plutella xylostella*, can degrade the pyrethroid insecticide deltamethrin through the production of detoxification enzymes such as esterases and cytochrome P450s (Li et al., 2022a; Zhang et al., 2013). The gut microbiota of insects has been shown to play an important role in various biological processes, including digestion, immunity, and reproduction. New studies have also indicated that the gut microbiota can have a significant impact on insecticide susceptibility and resistance. In this review, we will discuss the impact of microbiota on insecticide susceptibility in *Bactrocera dorsalis*, one of the most economically important pests of fruits and vegetables. Several research have explored that the gut microbiota can influence insecticide susceptibility in *B. dorsalis*. For example, it has been shown that antibiotic, and synthetic pesticides treatment can alter the gut microbiota composition, leading to increased resistance to insecticides such as chlorpyrifos and fipronil. This effect is thought to be due to the disruption of the gut microbiota's role in metabolizing and detoxifying insecticides, leading to higher levels of toxic metabolites in the insect's body (Huang et al., 2013; Koc et al., 2022; Li et al., 2007). Moreover, research has shown that the gut microbiota can also affect the expression of insecticide resistance genes in *B. dorsalis*. Studies have found that certain gut bacteria can induce the expression of insecticide resistance genes, leading to decreased exposure to insecticides. On the other hand, some gut bacteria have been shown to inhibit the expression of these genes, leading to increased susceptibility to insecticides (Xia et al., 2018; Yang and Cong 2021).

Manipulating the gut microbiota of *B. dorsalis* has the potential to improve pest management strategies by increasing susceptibility to insecticides. Approaches

such as using selective insecticides, biocontrol programs and antibiotics to selectively decrease the growth of certain gut bacteria or using bacteriophages to target specific bacteria could be explored as potential pest management tools. The gut microbiota of *B. dorsalis* plays a significant role in insecticide tolerance, and further research on the mechanisms involved could lead to novel pest management strategies (Bai et al., 2019; Zhang et al., 2022a). Disrupting the gut microbiota through antibiotics or other means has been shown to increase insecticide susceptibility in many insect species. For instance, a study found that the administration of antibiotics to the gut bacteria of mosquito *Culex quinquefasciatus* and *Anopheles arabiensis* increased its susceptibility to the streptomycin, erythromycin and insecticide permethrin (Barnard et al., 2019; Seal and Chatterjee 2019; Yang et al., 2021). Another study demonstrated that the disruption of gut microbiota through antibiotic treatment increased the susceptibility of the cotton bollworm, *Helicoverpa armigera*, to the Bt-resistant, endosulfan, carbamates and organophosphate insecticide chlorpyrifos (Myint Khaing et al., 2018; Torres-Vila et al., 2002). The impact of microbiota disruption on insecticide susceptibility has also been studied in the context of insecticide resistance. Research has shown that the gut microbiota of resistant insects can play a role in the detoxification of pesticides, and that disrupting this microbiota can increase insecticide susceptibility in resistant populations (Sato et al., 2021). For example, a study found that disrupting the gut microbiota treatment increased the susceptibility of a resistant strain of the tobacco budworm, *Heliothis virescens*, to the chlorantraniliprole, organophosphate, canavanine, an arginine antimetabolite, *Bt* Vip3Aa, and l-2-amino-4- butyric acid (Blanco et al., 2009; Pickett et al., 2017; Zilnik and Burrack 2021).

It is imperative note that the relationship between gut microbiota and insecticide susceptibility is complex and can vary depending on the insect species, the type of insecticide, and other factors. For example, some studies have shown that disrupting gut microbiota can increase insecticide susceptibility, while others have shown no effect or even a decrease in susceptibility. Additionally, the composition of gut microbiota can differ between insect populations, which can affect pesticide detoxification and susceptibility. Further research is needed to better understand the mechanisms underlying the relationship between gut microbiota and insecticide susceptibility, as well as to develop effective and sustainable pest management strategies that take into account the role of gut microbiota.

Innate metabolites produced by gut microbiota and their influence on pesticide metabolism

In recent years, there has been growing interest in the role of gut microbiota in the metabolism of insecticides. It is now widely recognized that the microbiota inhabiting the gut of insects can play a key role in the detoxification of insecticides (Jaffar et al., 2022), thus affecting their efficacy and ultimately impacting pest management strategies. The gut microbiota can directly degrade insecticides or produce metabolites that aid in their detoxification. The latter mechanism is particularly intriguing, as it suggests that gut microbiota may serve as a reservoir of metabolites with potential applications in pesticide metabolism (Siddiqui et al., 2022). We explore the role of innate metabolites produced by gut microbiota and their influence on pesticide metabolism. The gut microbiota in insect digestive system is a complex ecosystem consisting of a diverse array of microorganisms, including archaea, bacteria, protozoa, fungi, and viruses (Muñoz-Benavent et al., 2021). These microorganisms can interact with each other and with their host in a variety of ways, influencing various physiological processes, including digestion, immune function, and metabolism (Wang et al., 2020). Recent studies have shown that gut microbiota can also plays significant role in the metabolism of insecticides. For example, the gut microbiota of some insects can degrade pyrethroid insecticides, such as permethrin, by producing esterase that hydrolyze the ester bonds in these insecticides (Cruse et al., 2023; Gómez-Govea et al., 2022). Other gut microbes can produce carboxylesterases that can also degrade pyrethroids and indoxacarb (Ramya et al., 2016). In addition to direct degradation of insecticides, gut microbiota can also produce metabolites that indirectly aid in the detoxification of insecticides (Sogorb and Vilanova 2002). For example, gut microbiota can produce enzymes such as cytochrome P450s, which can modify the insecticidal potential, making them more easily excreted by the host (Lu et al., 2021). Additionally, gut microbiota can produce metabolites that act as antagonists of insecticide target sites, reducing their efficacy. For example, some gut microbes can produce γ -aminobutyric acid (GABA), which can bind to and inhibit the GABA receptor, the target of some insecticides such as fipronil (Nauen et al., 2022; Rashmi et al., 2018; Zhou et al., 2018).

The gut microbiota can also modulate the expression of insecticide resistance genes in their host. For e.g., some gut microbes can induce the expression of

detoxification genes in the host, leading to increased resistance to insecticides. On the other hand, some gut microbes can also suppress the expression of resistance genes, leading to increased susceptibility to insecticides (Amezian et al., 2021; Marcombe et al., 2009).

Advanced research has shown that the gut microbiota of insects can produce a variety of innate metabolites that have potential applications in pesticide metabolism (Engel and Moran 2013). As an instance, gut microbes have ability to produce short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which have been shown to enhance the detoxification of insecticides in the host. SCFAs have also been shown to induce the expression of genes involved in detoxification pathways in honey bees and other insects (Thakur et al., 2023; Wang et al., 2018), further enhancing their efficacy as detoxification agents. Another class of innate metabolites produced by gut microbiota is indole derivatives, which have been shown to enhance the activity of detoxification enzymes such as cytochrome P450s. Indole derivatives have also been shown to modulate the expression of genes involved in insecticide resistance, leading to increased susceptibility to insecticides (Schmidt and Engel 2021; Zhang et al., 2022b). Furthermore, gut microbiota can produce other metabolites, such as phenolics, alkaloids, and terpenoids, which have been shown to play a role in insecticide metabolism (Zhao et al., 2022). Phenolics, for example, have been shown to enhance the activity of detoxification enzymes such as glutathione S-transferases, which are involved in the detoxification of organophosphate insecticides (Vaish et al., 2020). Alkaloids and terpenoids have also been shown to enhance the activity of detoxification enzymes and modulate the expression of genes involved in insecticide resistance (Muñoz et al., 2020).

Potential applications of gut microbiota manipulation in pest management

Insect gut microbiota plays a necessary role in nutrient acquisition, digestion, and metabolism. The gut microbiota comprises bacteria, fungi, viruses, and archaea, which coexist in a symbiotic relationship with their insect host. Microbes in the gut produce essential vitamins and amino acids, digest complex polysaccharides, and degrade xenobiotics. Recent studies have also revealed the gut microbiota's role in regulating immune responses and behavior, making it an essential factor in insect physiology. The gut microbiota of insects has been shown to play important roles in many aspects of their biology, including digestion,

immunity, and behavior. In recent years, researchers have begun to investigate the potential of manipulating the gut microbiota to improve pest management strategies (Jang and Kikuchi 2020; Yun et al., 2014). Manipulation of the gut microbiota can be achieved through a variety of methods, including the use of probiotics, prebiotics, and antibiotics. This review will discuss the advantages and current research on potential applications of gut microbiota in pest management. Management of the gut microbiota has several potential advantages in pest control. Firstly, it could provide a more targeted and sustainable approach to pest control compared to traditional chemical insecticides, which can have negative impacts on non-target organisms and the environment. Secondly, it could help to overcome issues of insecticide resistance by targeting multiple aspects of insect biology. Finally, manipulation of the gut microbiota could also have broader applications in agricultural production, such as improving nutrient utilization and stress tolerance in crops (Schmidt and Engel 2021). Developing methods for employed the gut microbiota research has focused on insects to improve pest management. One approach is the use of probiotics, which involves the application of beneficial bacteria to the insect gut to enhance their health and reduce susceptibility to pests. For example, *Lactobacillus*, *Saccharomyces*, *Streptococcus*, *Bacillus* and *Lactobacillus plantarum* has been shown to improve the survival of honeybees infected with the parasite *Nosema ceranae*, which causes significant losses in bee populations (Bloemendaal et al., 2021; Engel and Moran 2013; Guarner et al., 2012). Prebiotics, which are substances that promote the growth of beneficial gut bacteria, have also been investigated for their potential in pest management. In a study on the tomato fruit

borer *Helicoverpa armigera*, probiotic supplementation increased the abundance of beneficial gut bacteria and reduced larval growth and survival (Paramasiva et al., 2014; Tian et al., 2023).

An antibiotic treatment has also been explored for their potential in pest management. Various studies on the diamondback moth *Plutella xylostella* found that the antibiotic levofloxacin, rifampicin, streptomycin sulfate, ciprofloxacin, and metronidazole reduced the abundance of gut bacteria and increased susceptibility to the insecticide chlorpyrifos and *Bacillus thuringiensis*. Manipulation of the gut microbiota has the potential to provide a more sustainable and targeted approach to pest management, with potential applications in both agricultural production and public health (Lin et al., 2015; Xia et al., 2018). Nevertheless, further research is needed to develop effective and practical methods for manipulating the gut microbiota of insects, and to understand the broader impacts of these interventions on insect and environmental health.

Insecticides and their interaction with gut microbiota

Insecticides target specific physiological processes in insects, leading to their abnormality or mortality. However, some insecticides can be metabolized and degraded by the gut microbiota, leading to reduced efficacy and increased resistance (Siddiqui et al., 2022). An example, pyrethroid insecticides are metabolized by gut bacteria, leading to reduced toxicity in certain insects. Similarly, organophosphate insecticides are degraded by gut bacteria, leading to increased resistance in many species of insects. This phenomenon highlights the importance of considering the role of the gut microbiota in insecticide resistance management.

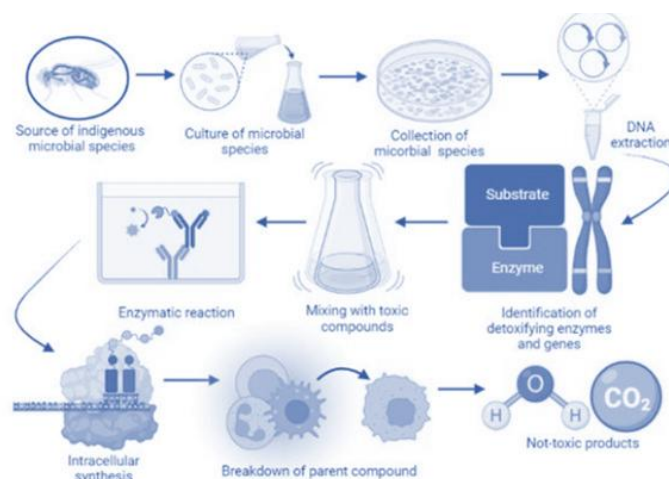


Fig. 3. Isolation techniques from insect gut microbial community and their roles in degradation (Jaffar et al., 2022)

Gut microbiota disruption and their influence on innate metabolites

Exposure to insecticides can also disrupt the gut microbiota, leading to altered metabolites and potential health impacts (Jaffar et al., 2022). Disruption of the gut microbiota can lead to increased susceptibility to pathogens, reduced nutrient acquisition, and transformed behavior. Furthermore, changed metabolites produced by the gut microbiota can also impact insect physiology and behavior (Gómez-Govea et al., 2022). For example, some bacteria in the gut produce short-chain fatty acids, which can regulate host metabolism and immune responses. Therefore, disruption of gut microbiota can lead to a significant impact on insect physiology and strength in insects (Engel and Moran 2013; Muñoz-Benavent et al., 2021).

The insect gut microbiota has a significant impact on the host's susceptibility to insecticides. Gut microbiota composition can alter the expression of host genes involved in detoxification pathways and insecticide targets. This paper aimed to review the literature on the impact of microbiota disruption on insecticide susceptibility in and its potential applications in pest management. The use of insecticides has been the primary control method, but insecticide resistance has emerged as a significant challenge (Jaffar and Lu 2022). Recent studies have shown that gut microbiota composition plays a crucial role in the susceptibility of insects to insecticides. For example, the gut microbiota of *B. dorsalis* is dominated by Proteobacteria and Firmicutes, which have been shown to play a role in the metabolism of insecticides (Wang et al., 2011). Alternative study found that the depletion of gut microbiota through antibiotic treatment increased the susceptibility of *B. dorsalis* to the insecticide chlorpyrifos (Zhang et al., 2022a). The authors suggested that the depletion of gut microbiota reduces the expression of genes involved in the detoxification of insecticides, leading to increased susceptibility. Gut microbiota of invasive ants associated with gut bacteria nesting preference (Huang et al., 2020) and *B. dorsalis* can also confer resistance to insecticides and oviposition aversion (Li et al., 2020). The investigators identified bacterium, trimethylpyrazine (TMP), tetramethylpyrazine (TTMP), and *Klebsiella oxytoca*, that metabolizes the pheromone in midgut insecticide chlorpyrifos, thereby reducing its toxicity to *B. dorsalis* and degradation also effected (Guentzel 1996; Koc et al., 2022; Ren et al., 2021). Manipulating gut microbiota to enhance insecticide susceptibility or to reduce insecticide resistance offers several advantages. First, it

provides an alternative to chemical insecticides, which are increasingly ineffective due to the development of resistance. Second, it offers a targeted approach that does not affect non-target organisms, reducing the environmental impact of pest control measures. Finally, it could potentially be used in combination with other control methods, such as biological control, to enhance their efficacy. However, there are also potential disadvantages to deploying gut microbiota. One concern is the potential for unintended consequences, such as the spread of antibiotic resistance genes or the unintended killing of beneficial microorganisms (Lillehoj et al., 2018; Lin et al., 2021; Muñoz-Benavent et al., 2021). Additionally, it is unclear how stable and effective gut microbiota manipulation methods are in the field, as laboratory studies may not accurately reflect real-world conditions.

CONCLUSIONS

In conclusion, gut microbiota composition plays a central role in the susceptibility of insects to insecticides, including *B. dorsalis* (Liu et al., 2022). Eliminating of gut microbiota to enhance insecticide susceptibility or reduce insecticide resistance offers several advantages, including a targeted approach that does not affect non-target organisms and the potential for use in combination with other control methods (Francis and Aneesh 2022; Meng et al., 2019). However, potential disadvantages include unintended consequences and uncertainty regarding the efficacy of gut microbiota manipulation methods in the field. Future research should focus on developing safe and effective gut microbiota manipulation methods that can be applied in the field to enhance pest management efforts. The gut microbiota plays a critical role in insect physiology and health. Exposure to insecticides can disrupt the gut microbiota and alter metabolites, leading to potential health impacts. Additionally, some insecticides can be metabolized and degraded by the gut microbiota, leading to reduced efficacy and increased resistance. Therefore, it is essential to consider the role of the gut microbiota in insecticide resistance management and insect physiology. Future research should focus on understanding the interactions between the gut microbiota, insecticides, and innate metabolites to improve our understanding of insect physiology and management.

AUTHOR CONTRIBUTION STATEMENT

SJ performed, conceived and designed review research. SJ revised and conceived the overall study.

All authors have read and approved the manuscript being submitted.

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CONFLICT OF INTEREST

No conflict of interest.

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THE POTENTIAL OF VISUAL AND OLFACTORY SIGNALS IN GALL DEFENCE

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ABSTRACT

Avoiding attacks is clearly better than suffering, or even overcoming attacks. Here we discuss the signalling ways by which galls (i.e., gall-inducers) may defend themselves from damage by avoiding attacks. Many colourful galls that are simultaneously chemically and/or physically defended, and/or omit repelling odours fulfilling the general criteria to be tentatively considered as aposematic. It has been shown experimentally that chemically defended galls also emit volatiles that repel relevant herbivores. Thus, both visual and olfactory gall traits may serve as adaptive signals that have been usually overlooked. It is also highly probable that the conspicuous colours (red, yellow) of many galls may also serve physiological functions, such as defence from reactive-oxygen production, from UV, and from excess visible light, or serve other, unknown functions. The certain role of camouflage (especially by being green), in defence from enemies, thus potentially increasing a gall-inducers' fitness, was not given the attention it deserves. Detailed comparative and especially experimental studies on the adaptive role of gall shape, colouration and odours can further shed light on these phenomena.

Key words: Aposematic, camouflage, defence, extended phenotype, galls, herbivory

Galls are abnormal plant growths induced by various insects. Insects induce galls on plant organs such as, leaves, shoots, flowers, and fruits. Galls are not just the typical developmental wound responses of damaged plants, but are rather specialised organs induced by specialised parasites that serve as nurseries for the developing stages of gall-inducing insects, providing a nutrient supply and protection from both abiotic factors (e.g., sun irradiation, wind, desiccation, rain and snow), and from natural enemies, such as, pathogens, predators, parasitoids, and non-gall-inducing herbivores (Price et al., 1987; Stone and Schonrogge, 2003; Miller and Raman, 2019; Harris and Pitzschke, 2020; Kurzfeld-Zexer and Inbar, 2021). The gall-inducing habit has evolved independently numerous times among diverse insect lineages, indicating that it has a high adaptive value. Since it seems that the gall-inducing insects control gall induction up to the smallest details, galls are commonly considered their extended phenotype, *sensu* Dawkins (1982). As such, the gall inducing insects control the physiological, anatomical, chemical and visual properties of the gall-bearing plant organ (e.g., Favery et al., 2020). Defensive gall traits against natural enemies are primarily plant-mediated, including chemical and structural traits, and

secretion of nectaries that attract mutualistic ants (e.g., Cornell, 1983; Ferreira et al., 2019; Nicholls et al., 2017; Schonrogge et al., 1999). Some gall-inducing insects such as- the Aphidoidea and Phlaeothripidae may actively and aggressively protect galls from natural enemies (Abbot, 2022; Crespi et al., 1997; Inbar, 1998). Galls are typically armed with high levels of defensive secondary metabolites originating in their host-plant metabolism (Rand et al., 2014, 2017; Hall et al., 2017; Martinson et al., 2022). However, some of these defences operate only after enemy attack. Herein, the visual and olfactory defences signals that allow gall-inducing insects in reducing the rate of predation are discussed, an issue that received little attention.

Defensive signalling

Plant-borne visual and chemical signals are an important component of pollination and seed dispersal by animals, two better studied systems that illuminate the many possibilities and mechanisms of defensive signalling. In this article, the same explanation is used in discussing galls. Aposematic (warning) signalling is a biological phenomenon in which poisonous, dangerous, unpalatable or unprofitable organisms including plants, visually, chemically or vocally advertise these qualities

to potentially attacking animals (Ruxton et al., 2004; Lev-Yadun, 2016), as defence from potential enemies. The evolution of aposematic signalling is based on the ability of target enemies to associate signals with the risk, or non-profitable handling, and later to avoid such organisms as prey. In certain instances, there is even an innate tendency to avoid objects with certain colours or colour patterns (Ruxton et al., 2004), a character indicating the long duration of the interactions and the strength of selection. Usually, colours of aposematic plants and animals are yellow, orange, red, purple, black, white, brown, and their combinations (Ruxton et al., 2004; Lev-Yadun, 2001, 2016). Olfactory plant aposematism, whereby poisonous plants deter mammalian or insect herbivores by production of signalling volatiles (e.g., Eisner and Grant, 1981; Massei et al., 2007; Holopainen, 2008; Lev-Yadun et al., 2009; Karban, 2015) is well known. It is possible, that similarly to pollination (Faegri and van der Pijl, 1979) and seed dispersal (van der Pijl, 1982; Schaefer and Ruxton, 2011), where many plants simultaneously use visual and olfactory signals for animal attraction, that such double signalling is also true in diverse instances of plant aposematism. Certain toxic and colourful autumn leaves that could be considered visually aposematic (Lev-Yadun and Gould, 2007; Lev-Yadun, 2022), probably simultaneously use olfactory aposematism to deter various herbivorous insects (Holopainen, 2008).

The aposematic gall hypothesis

The general key factors behind the aposematic gall hypothesis, formulated by Inbar et al. (2010a) are:

1. Galls are valuable and irreplaceable asset to their inducers.
2. Because the galls can easily be detected by their natural enemies (invertebrates and vertebrates), and because the gall-inducers cannot actively flee, there is a strong selection for efficient gall defensive traits.
3. Galls are often defended by physical structures and accumulate high levels of chemical defensive compounds compared to intact plant parts.
4. The gall-inducers control gall traits including their colour, shape, chemistry, and volatiles emission.
5. Many galls are visually and/or chemically conspicuous.
6. The colours and scents of plant organs often

interact (advertisement) with animals: they attract pollinators, seed dispersers, and deter herbivores.

Visual conspicuousness is a striking and common gall trait. Many galls are visually conspicuous because of their size and shape, which differ from the background of the plant organs that form or carry them. In addition, quite often galls are characterized by bright or contrasting colours such as red and yellow colours (Fig. 1) (e.g., Russo, 2007), as a result of accumulation of plant-derived pigments in their tissues (Czeczuga, 1977), and can be clearly distinguished visually from the surrounding host plant organs. Galls may change colour during their development and aging, especially from green to red, or express such a change in association with exposure to strong sunlight (e.g., Wool, 2004; Isaías et al., 2013). The aposematic gall hypothesis suggests that chemically or otherwise protected galls that are also conspicuous (visually or by odours) are potentially aposematic: warning potential enemies and thus preventing gall destruction before any physical contact and initial damage can be made. The galls, which are made of host plant tissues, are manipulated by their inducing parasites to form both direct defences and signalling. Hence, both the gall-inducing insects and the attacking enemies benefit from this strategy.

The predictions of the aposematic gall hypothesis as proposed by Inbar et al. (2010a) were formulated from several life history traits that are thought to promote aposematism in general *sensu* Mallet and Joron (1999) and Ruxton et al. (2004). Concerning the defence level, chemically and possibly also physically well-defended galls are expected to be usually colourful. Galls that are less well defended, especially from avian predators, will tend to be more visually cryptic. Alternatively, it could be argued that gall's conspicuousness is a defence strategy of the plant to attract potential enemies of the gall-inducing insects. Inbar et al. (2010a) proposed that if the aspect of indirect plant defence by gall colouration, which may attract the gall's enemies is true, less-defended galls should be more colourful and conspicuous to enhance learning of their predators. This explanation awaits deeper examination. They also suggested that the ability to overcome initial and partial damage (gall repair), and thus accelerate enemies' avoidance learning without self scarifying, should promote the evolution of gall aposematism. Many poisonous plants emit characteristic volatiles that may deter herbivores (Eisner and Grant, 1981; Rothschild, 1986; Massei et al., 2007). It is thus expected that well-



Fig. 1. The signalling galls (see Rostás et al 2013) of the aphid *Slavum wertheimae* that induced large (as a tennis ball) galls on *Pistacia atlantica*. (Photo: Moshe Inbar).

defended galls will emit a distinct blend of volatiles that will serve as a warning olfactory signal.

Conspicuous galls: alternative explanations

The aposematic gall hypothesis (Inbar et al., 2010a) encouraged researchers to pay attention to the potential adaptive role of gall signals or to propose alternative explanations.

Gall colouration is related to senescing: White (2010) argued that a gall's colour is related to the senescing status of the galled tissue. He suggested that dying gall tissues release nutrients to the benefit of the gall inducer. Furthermore, he argued that the gall inducers induce the plant organ to initiate the gall around the gall inducer's feeding action, after the rest of the plant body has stopped growing (White, 2010). However, gall colour has limited association with the senescing state of the galled tissue, and redness can also be seen in young and fast-growing galls (Inbar et al., 2010b). The tissues of young red galls are commonly meristematic and not dying, and when galls grow, the rest of the host plant, and even the immediate gall-bearing organ (e.g., leaf, leaflet) grows as well. One should keep in mind that redness and yellowing are not synonymous with senescence (Inbar et al., 2010b). Many young and growing flowers are red and yellow (Lee, 2007) and many young leaves are red

(Richards, 1996; Dominy et al., 2002; Lev-Yadun et al., 2012). Nevertheless, gall colour probably could have evolved along physiological routes such as stress-related colouration or because of pleiotropic effects of defensive genes. Still, White's (2010) hypothesis may theoretically be relevant for at least some gall inducers, but certainly not for all.

There are numerous colourful galls, representing thousands of different gall-inducing species. They represent many repeated but independent events of galling habit evolution. They are found in different environments and on a great variety of plant taxa, host life stages and organs. It is unreasonable to assume that all these galls have only a single and ubiquitous function for their conspicuous colour. Indeed, Inbar et al. (2010a) discussed several alternative explanations for this phenomenon. Moreover, gall conspicuousness and signalling are dependent not only on colour, but also on size and shape (Stone and Schonrogge, 2003) and even on odour (see Rostás et al., 2013). A single physiological explanation, such as the level of light exposure, also cannot explain all the variation in gall colouration, as colourful galls are also common in less illuminated microhabitats (Inbar et al., 2010b).

Gall colour is a by-product of gall induction: Connor et al. (2012) argued that the redness of many

galls is simply a by-product of the galling process (“a fabrication noise”). They suggested the following line of reasoning: (1) Many gall-inducing bacteria (and some fungi) are known to produce cytokinins, similarly, gall-inducing insects have been found to harbour high levels of cytokinins (Yamaguchi et al., 2012), which may differ from the host plant’s cytokinins. (2) Insect-induced galls are mobilization sinks for photosynthates and cytokinins are known to be involved in strengthening mobilization sinks in active plant sinks. (3) Cytokinins and high-sugar concentrations are involved in the production of flavonoids, including anthocyanins, and therefore, the colouration is due to accumulation of anthocyanins as a side-effect of the combination of high sugar and cytokinins. (4) Sugars suppress carotenoid synthesis, suggesting that it is unlikely that the colouration is due to carotenoid accumulation. (5) Connor et al. (2012) referred to White (2010), accepting that reddening is associated with senescence.

In spite of these suggestions, the biology of plants in general and that of galls in particular are not so simple (Gerchman et al., 2013). However, galls may come in many colours: red, yellow, pink, purple, green, brown, and their combinations, and since gall colouration can change, more than one factor determines the evolution and expression of gall colour. Some galls change their colour during their development or when exposed to sunlight (see photos in Russo, 2007; Redfern, 2011), while others redden without being exposed to direct sun light, even when induced on the shaded abaxial side of the leaf. Others, however, will always remain green, both inside and outside. This variation of colours suggests that anthocyanins could be only one of the multiple pigment types involved in gall colouration. For example, many insect-induced galls are yellow (e.g., Russo, 2007). This challenges Connor et al.’s (2012) suggestion that carotenoids are unlikely to be involved in gall pigmentation because sugars suppress the synthesis of carotenoids. Moreover, the fact that there are so many yellow–orange, sweet ripe fruits that change colour from green to yellow or orange, along with a dramatic increase in their sugar content (e.g., mango, apricot, peach, and various date varieties), demonstrate that sugars do not suppress carotenoid synthesis in principle.

Another problem with Connor et al.’s (2012) suggestions is that in addition, anthocyanin and cytokinin levels do not often correlate (Gerchman et al., 2013). Anthocyanins are pigmented flavonoids common in many plant tissues. To date more than 700

anthocyanins have been reported (e.g., Wallace and Giusti, 2015). Anthocyanin content has been found to increase under various conditions, some of which, but definitely not all, and not even the majority, are sink tissues. Elevated levels of anthocyanins were demonstrated in both young expanding and in senescing foliage (Richards, 1996; Dominy et al., 2002; Lev-Yadun et al., 2012), in flowers (Grotewold, 2006), in many ripe fruits (Allan et al., 2008), and as a response to low temperatures (Hughes, 2011). While some of these (namely young expanding leaves) could be considered as sink tissues, others, such as senescing leaves, are source tissues and not sinks. Altogether, the connection between anthocyanin content and high cytokinin levels is far from being deterministic. Young and growing fruits, a definite sink tissue, include high levels of cytokinins, but are green. A similar trend was reported for nitrogen starvation conditions (Close and Beadle, 2003), where anthocyanins accumulate but cytokinin levels do not, and in some instances even decline (e.g., Yong et al., 2000). Young roots, strong cytokinin producers, are usually pale. Finally, some cytokinin-secreting organisms are known to induce ‘green islands’ on senescing colourful leaves (Walters et al., 2008), which are, as their name implies, green. Altogether, there is no universal trend of cytokinins inducing reddening.

Connor et al. (2012) also pointed out in their opposition to the aposematic gall hypothesis that in a given gall system, insectivorous birds and mammals attack galls in the cold winter months, when galls have lost their redness. This might be true in some systems, but in the Mediterranean habitats, for example, galls induced by the Aphidoidea are attacked by birds in the summer and fall (August–December) (Burstein and Wool, 1992; Inbar personal observations). One could easily adopt a counter explanation to Connor et al.’s (2012) proposal; accordingly, this can be regarded as a suitable example for red (aposematic) colouration’s defensive role, i.e., when the galls are red and signal their defence, they are not attacked. Gerchman et al. (2013) concluded that the hypothesis proposed by Connor et al. (2012), although stimulating and possibly even true in certain instances, was an over simplification, in general. Even if the cytokinin byproduct hypothesis *sensu* Connor et al. (2012) is the mechanism of gall reddening in certain taxa, it is not a universal rule, and does not provide a negative proof for other explanations of gall colouration.

Overall, the suggestion that aposematic signalling does not operate in galls (White, 2010; Connor et al.,

2012) referred only to the visual aspect (see Rostás et al., 2013). However, olfactory aposematism seems to operate both independently of, and/or simultaneously with the visual one (e.g., Damasceno et al., 2010; Klimm et al., 2020; Villagra et al., 2021). We stress that as shown in many fungi (e.g., *Amanita* spp) (Sherratt et al., 2005), olfactory aposematism may be especially important as a defence against either colour blind or nocturnal enemies, or within or under dense canopies that are not well illuminated, environments highly appropriate for many gall species. Olfactory aposematism may be especially important for galls as a defence against predatory and parasitic insects and mammalian herbivores. Clearly, gall-inducers can control the olfactory signals of the galls (if needed) to prevent their detection by natural enemies (Tooker et al., 2008). Another alternative explanation for galls being red is that anthocyanins are known to defend leaves and fruits from fungal attacks (Coley and Aide, 1989; Schaefer et al., 2008; Schaefer, 2011; Tellez et al., 2016), and since the internal cavities of many galls are humid, defence from fungal attacks is probably equally relevant for the gall inducing insects as well.

Testing the aposematic gall hypothesis

Inbar et al. (2010a) proposed several models to test the aposematic gall hypothesis. However, comparative surveys and analyses (within and between species) of gall colouration, their chemical defence levels, and gall position (e.g., shaded v directly exposed to the sun) in varying ecosystems are needed. Nevertheless, controlled experiments (field and laboratory) in which accelerated associative learning of relevant enemies, i.e., insect and mammalian herbivores (see Berman and Inbar, 2023), predators (vertebrates and invertebrates) and parasitoids needs to be tested. Additional analyses of the unique volatile emission by the galls and their impact on potential enemies are also needed. Alternatively, proven aversion, be it inherited or of experienced individuals from colourful or odorous galls, can also indicate gall aposematism. Clearly, descriptive, theoretical and experimental data are still needed to evaluate the generality of gall aposematism. Moreover, the possibility of Müllerian and Batesian mimicry should also be considered in defensively signalling galls.

Several studies have considered, but not necessarily supported the aposematic plant gall hypothesis (e.g., Dias et al., 2013; Álvarez, 2012; Patankar et al., 2012; Bomfim et al., 2019; Luz et al., 2015; Miyazaki et al., 2020; Fernandes et al., 2022). So far, only one

study (Rostás et al., 2013) experimentally tested the visual aposematic gall hypothesis. The aphid *Slavum wertheimae* (Hemiptera: Fordini) (Fig. 1) induces large and conspicuous, often red, galls on the lateral shoots of *Pistacia atlantica* (Anacardiaceae). The galls included nearly four times higher levels of tannins and nearly two times greater concentration of terpenes compared with non-galled *P. atlantica* leaves (Rostás et al., 2013). These galls also emit distinct and higher levels of volatile plant terpenes than the neighbouring leaves. They experimentally showed that goats sensed these emitted volatiles, in particular the combination of α -pinene, sabinene, and limonene as olfactory signals serving as feeding deterrents. As a result, goats consumed all the leaves of *P. atlantica* but most of the galls on the consumed shoots remained intact (Rostás et al., 2013).

Whatever be the reason for the evolution of gall colouration, we still have to explain its variability and maintenance. It is widely accepted that gall inducers regulate gall traits ('extended phenotype'). Thus, if conspicuousness would have had a strong negative effect on gall survival (e.g., by attracting predators and parasitoids) it is expected to impose a strong selection against it (Inbar et al., 2010b). Inbar et al. (2010b) thus explained that the conspicuousness of galls is sound evidence of its potential beneficial role, or at least for the lack of strong selection against it. An interesting example of the sophisticated ability of the inducing insect to control gall signalling trait has been reported by Tooker et al. (2008). They demonstrated that gall-inducing insects can 'silence' the emission of conspicuous odour (volatiles) from the gall-bearing organs, which thus reduces its detection by potential odour-oriented enemies.

CONCLUSIONS

In conclusion, there is potentially strong selection for gall defence from a variety of natural enemies: pathogens, parasitoids, herbivores, and predators, both arthropods and vertebrates (Price et al., 1987; Wool and Burstein, 1991; Schultz, 1992; Zamora and Gómez, 1993; Inbar et al., 2003; van Hezewijk and Roland, 2003; Gerchman and Inbar, 2011; Kurzfeld-Zexer and Inbar, 2021). Many gall traits are influenced by such selection pressures (Price et al., 1987; Stone and Schonrogge, 2003). Gall signalling (colouration, shape, and scent) may be part of their defensive arsenal. Insects can manipulate the chemical production and accumulation of defensive substances in the galls

(Martinson et al., 2022; Davidovich-Rikanati, 2022), alter volatile emission from the galls to reduce the level of parasitism or deter enemies (Tooker et al., 2008; Rostás et al., 2013; Barônio and Oliveira, 2019), and manipulate gall phenotypes including pigmentation (Korgaonkar et al., 2021; see also Maderspacher, 2021). Considering the selective evolutionary forces on the gall systems, and having the ecological, behavioural, biochemical and molecular tools available, the adaptive significance (or not) of gall signalling could be readily addressed, and many new and exiting discoveries are expected.

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MANAGEMENT OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J E SMITH) IN SOUTH ASIA-CURRENT STATUS AND FUTURE STRATEGIES

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ABSTRACT

Fall armyworm (FAW) *Spodoptera frugiperda* (J E Smtih) is a native of the neotropics and it has invaded Nigeria region of Africa in 2016 and reached India in May 2018. Since then it has spread to most of the South and Southeast Asian countries, Australia and recently reached New Zealand and some South Pacific islands. Even though FAW has many hosts in its native region, the population that reached Africa and spread to Asia and Oceania prefers maize the most. In its biology, the capability of adults to fly more than 100 km in a night, females laying eggs in clusters of 100 or more to a maximum of 2,000 eggs in five days of their lifetime, later instar larvae becoming cannibalistic, pupating in soil, not diapausing and inability to survive at temperatures below 10°C, offers a unique opportunity to devise an effective management strategy. The IPM package for maize incorporating management of FAW involve: seed treatment with a systemic insecticide to protect young plants up to three weeks after planting; release of egg parasitoids *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and *Telenomus remus* (Hymenoptera: Platygasteridae) immediately after finding FAW moths caught in the pheromone traps in the field; and release of larval parasitoids *Bracon* (*Habrobracon*) *hebetor*, and *Bracon* (*Habrobracon*) *brevicornis* (Hymenoptera: Braconidae) and treating with bio- and botanical pesticides, when whorl damage is observed.

Key words: *Spodoptera frugiperda*, fall armyworm, maize, IPM, South Asia, insecticides, seed treatment, biology, egg laying, pupation, diapause, survival, temperature, IPM package, biological control, biopesticides

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a native of tropical and subtropical North and South American continents. Outside its native range, it was first reported in Nigeria region of West Africa in January 2016 (Goergen et al., 2016). By the end of 2017 it has covered most of the Sub-Saharan Africa (Njuguna et al., 2021). In May 2018, it was found in the Shivamogga region of the Karnataka state in southern India (Sharanabasappa et al., 2018a) and before the end of that year, it has spread to Bangladesh (Alam et al., 2018), Myanmar (FAO, 2019), Thailand (FAO, 2018), and China (Sun et al., 2021). In 2019, it was reported from Nepal (Bajracharya et al., 2019), Sri Lanka (Perera et al., 2019), Pakistan (EPPO, 2020), Indonesia (Trisyono et al., 2019), the Philippines (Navasero et al., 2019) and Vietnam (Hang et al., 2020). In 2020, it was reported from Australia (GRDC, 2020), Papua New Guinea (Pacific Community, 2020) and New Caledonia in 2020 (FAO, 2021a), in 2021 from Solomon Islands (FAO, 2021b) in the Pacific and New Zealand in 2022 (RNG News, 2022).

Biology

Fall armyworm is a migratory pest. The adults are nocturnal and capable of flying about 100 km per

night. Two genetic strains namely corn/maize and rice are known to occur in the Americas and over 350 species of plants have been recorded as hosts. The corn/maize strain prefers maize, cotton, and sorghum, and the rice strain rice and pasture grasses (Dumas et al., 2015). The population that reached Africa and then to Asia seems to be a hybrid and prefers maize over other crops. Female moth lays 100 to 200 eggs in batches covered with scales on the leaves, with an average of about 2,000 eggs/ moth in its three days lifetime (Barnard, 2022). Eggs hatch in two to three days and the emerged larvae feed on the shells of the eggs before start feeding on the leaves. Young larvae feed by scraping the leaves and leaving the epidermis intact exhibiting window like appearances. Wind helps them in dispersal from the plant on which they emerged from eggs to the neighboring plants when they produce silken threads and hang from the leaves. Later instars devour chunks of leaves and are strongly cannibalistic resulting one larva causing major damage to the plant by remaining in the whorl. The larva hides deep in the whorl during day time and feeds on the leaves at night. Pupation takes place in the soil and it lasts for 8 to 9 days in the tropics (Sharanabasappa et al., 2018b). There is no diapause stage in its life cycle.

Effect of abiotic factors

Long distance dispersal of FAW is aided by wind currents and their directions. In North America, every summer FAW moths migrate from Florida, Texas and Mexico regions in the south to as far north as Canada covering over 2700 km (Mitchell, 1979). In India, FAW was first observed in the Southwestern part in May 2018 and it spread eastwards to Bangladesh by November 2018, and by December, it reached China, a distance of over 5,500 km, was aided possibly by the summer southwest monsoon winds. However, its migration towards north and northeasterly directions was comparatively slow as it reached Nepal, about 1,750 km and Pakistan, about 2,500 km from southern India, in May 2019 (Bajracharya et al., 2019) and March 2019 (EPPO 2020), respectively. Northeasterly monsoon winds coincide with winter season and the survival rate of the moth in Nepal and Pakistan, which are north of India, is low because of cold weather and lack of preferred host plants. It took only seven months to move from southern India to China in the easterly direction, however, it took ten to twelve months to reach Pakistan and Nepal, even though they are only about a third of the distance compared to China from southern India.

Rainfall significantly affects the population of FAW in the field as it dislodges egg masses and young larvae from the plants. Dry and warm weather favors population increase. According to Suby et al (2020) FAW may not establish in places where the temperature drops below 10°C and raises above 40°C.

Economic damage

Even though over 350 host plant species have been recorded for this pest, the population established in Asia prefers maize. Maize is the third most important crop in Asia after rice and wheat (Prasanna et al., 2021). Food and Agriculture Organization (FAO) cautioned that FAW can cause a loss of 80 million tonnes of maize worth \$18 billion annually, impacting 600 million people in Africa, Asia-Pacific, and the Near East countries (FAO 2020). Day et al. (2017) predicted FAW to cause 21 – 53% annual loss in maize production in Africa but Harrison (1984) estimated 14.3 – 22.7% yield reduction in the Americas. In India, Suby et al (2020) estimated the damage to be 5 – 10% leading to a reduction in maize output by 37,000 – 75,000 tonnes. The annual loss of FAW attack is at least \$300 million for farmers in the U.S., and billions of dollars around the globe (Barnard, 2022).

Management

Fall armyworm is a polyphagous migratory pest but the strain that invaded Africa and Asia prefers maize as its primary host. The management option is to identify and develop compatible and effective technologies, and incorporate them in the Integrated Pest Management program for maize ensuring that these technologies to complement rather than disrupt technologies included for management of other maize pests and diseases in the regions. Different management options available for consideration are: scouting and use of pheromone traps for monitoring, mechanical control, cultural control, resistant varieties, GMOs, biological control including natural enemies and bio- and botanical pesticides, mating disruption, and use of chemical pesticides.

Scouting and use of pheromone traps for monitoring: McGrath et al. (2021) have reviewed scouting, action thresholds, and monitoring for FAW. While scouting is common in developed countries, its adoption is minimal among the small-scale farmers in developing countries. Several private companies, both in developed and developing countries produce pheromone lures and traps for FAW and their use has become popular in Asia. FAW being a highly mobile insect and it can migrate from one region to another depending on the intensity and direction of the wind (Day et al., 2017), and its preference to maize, setting up pheromone traps in the fields immediately after sowing and monitoring will lead to immediate recognition after its occurrence. Scouting for egg masses soon after observing the first moth in the trap will further confirm its establishment in the field however it is not mandatory.

Mechanical control: Squashing egg masses and handpicking larvae in Kenya and Ethiopia in the early years after FAW invasion in 2017 under panic conditions was carried out, however, it is labor intensive and not economical. Additionally, this activity results in killing parasitoids in the already parasitized eggs leading to negating early establishment of the parasitoids in the field. Placing of sand and ash in the whorls to kill larvae was also practiced in some African countries during the same period (Hruska 2019) which was a desperate attempt to save the crop in the absence of availability of other management tactics.

Cultural control: Van Huis (1981) in Nicaragua and Hailu et al. (2018) in Uganda found intercropping maize with beans reduced incidence of FAW. Midega et al. (2018) reported push-pull system using *Desmodium*

uncinatum (Leguminaceae) and Napier grass (*Pennisetum purpureum*) or *Brachiaria* sp. (Poaceae) in maize fields to be effective in reducing FAW incidence and plant damage caused by it.

Host plant resistance: Prasanna et al. (2021) have reported progress made in genetic and transgenic breeding for resistance to FAW in Africa and Asia. A few lines have been identified for resistance but the resistance need to be incorporated in locally accepted varieties for widespread adoption. Transgenic maize varieties are available but currently accepted to be cultivated only in the Philippines and Vietnam in Asia.

Chemical Control: McGrath et al. (2021) have reviewed chemical pesticides and their safe use for FAW control. There is concern for some of the chemicals being recommended for Asia and Africa such as Flubendiamide, whose registration has been cancelled in the U.S.A. in 2016 (Fitchette 2016). USAID has prepared a Pesticide Evaluation Report and Safe Use Action Plan (PERSUAP) listing pesticides authorized for use in management of FAW which is the most reliable among recommendations currently exist. https://ipmil.cired.vt.edu/wp-content/uploads/2022/09/Pages-from-USAID_2019_PERSUAP_GLOBAL_FALL_ARMYWORM_MANAGEMENT_4.pdf

Biological control: In the Americas, native range of FAW, over 150 natural enemies have been recorded (Molina-Ochoa et al., 2003). Of these, the promising ones identified for augmentative control of FAW were the native egg parasitoids, *Trichogramma pretiosum*, (Hymenoptera: Trichogrammatidae) and the introduced egg parasitoid from Asia, *Telenomus remus* (Hymenoptera: Playgastridae). *Telenomus remus* was described from Malaysia in 1937 and it is a native to peninsular Malaysia and Papua New Guinea. In 1963 it was introduced to India from Papua New Guinea for biological control of castor semilooper, *Achaea janata* (Lepidoptera: Noctuidae) (Sankaran 1974). Later, it was sent to Israel from India in 1969 (Rao et al., 1971). It was introduced to Australia in 1982 from Southeast Asia and Dominican Republic and to New Zealand from Pakistan (Waterhouse and Norris, 1987). Cave (2000) reviewed its introduction to Barbados in 1971-1972 and other Caribbean Islands, Central and South America and Florida for control of FAW resulting in a classic example of supporting theory of New Association proposed by Hokkanen and Pimentel (1984). Currently it is being used in augmentative control of FAW in Central and South American countries (Cave, 2000). Recently it has

been found to occur fortuitously in most of the countries in Africa (Kenis et al., 2019) and Asia (Elibariki et al., 2020; Liao et al., 2019; Muniappan, Pers. Obser.).

In Asia, several natural enemies of FAW have been identified with China leading in this list with more than three dozen organisms, followed by India about two dozen and others actively surveying for natural enemies. The common natural enemies considered for augmentative biological control in Asia are *Tr. pretiosum* and *T. remus*. In Bengaluru, India, *Bracon brevicornis* (Hymenoptera: Braconidae) was reported to attack fourth and fifth instar larvae of FAW and 54% average reduction in infestation after its release in the field (Ghosh et al., 2022) and in Hyderabad, India under laboratory conditions, *Bracon hebetor* (Hymenoptera: Braconidae) attacked larvae of FAW but it preferred *Corcyra Cephalonia* (Lepidoptera: Pyralidae) first followed by *Helicoverpa armigera* (Lepidoptera: Noctuidae) and then FAW (Sree Latha et al., 2019). However, studies in Bangladesh showed *Habrobracon hebetor* (syn. *Bracon hebetor*) to sting, host feed and kill the larvae of FAW but no parasitoid progenies were produced (Alam et al., 2021). Based on these studies it could be speculated that there are three different species of *Bracon* occurring in the subcontinent which need to be determined through molecular studies. The egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) currently mass reared and field released for FAW control in Kenya, Nepal and other countries is a poor choice as it is not an effective parasitoid of FAW. Instead *Tr. pretiosum* should be considered. Predators such as *Eocanthecona furcellata* (Hemiptera: Pentatomidae) and earwig *Euborellia annulata* (Dermaptera: Carcinophoridae) are also being studied and evaluated.

IPM for maize incorporating management of FAW

1. Select varieties that are resistant to pests and diseases and locally acceptable, if available.
2. Treat seeds with *Trichoderma* sp. and a systemic insecticide. *Trichoderma* sp. provides protection against soil borne fungal diseases and also induces resistance in plants. Systemic insecticide normally provides protection of the young crop up to two to three weeks against caterpillar pests (caterpillars/ larvae emerged from the eggs laid on the treated plants will die when they feed on the leaves but the eggs of the moths are unaffected).
3. Set up a FAW pheromone trap in the field one week after sowing seeds.

4. Survey the field for FAW egg masses once a moth is found in the pheromone trap, but not mandatory.
5. Release egg parasitoids *T. remus* and *Tr. pretiosum* in the field when FAW moths are noted in the trap (and egg masses are found in the field). Absence of the need for chemical sprays to control larval stages of FAW in the first three weeks of the crop due to seed treatment with a systemic insecticide, presence of live FAW eggs in the field laid by the invading populations of FAW, and early inoculation with egg parasitoids leads to establishment of a healthy population of natural enemies.
6. Avoid treating with chemical pesticides when window damage is noted on leaves as the maize plants overcome and compensate this damage and also leads to protect existing natural enemies in the field.
7. Release late larval instar attacking parasitoids *B. hebetor* or *B. brevicornis*, when whorl damage is observed.
8. Treat only the whorls not the whole plant with bio- or botanical pesticides to kill the late instar larvae. Use a safe chemical insecticide for whorl treatment as a last resort.
9. Spraying chemical pesticides when the plants are above two feet in height is not recommended under Asian small holder farm conditions as most farmers lack access to personal protective clothing and are not prepared for safe application of these pesticides.
10. Follow USAID approved PERSUAP (Pesticide Evaluation Report and Safe Use Action Plan) as some of the published literature lack thorough scrutiny of approved pesticides in their recommendations.
11. Use bio- or botanical pesticides for treating plants at the tasseling stage, if needed.

Future priorities

To implement the IPM package for maize incorporating FAW management activities, countries should register one of the safe systemic insecticide that could provide protection for early stages of the crop. Set up public and private laboratories for rearing and release of egg parasitoids *Tr. pretiosum* and *T. remus* and larval parasitoids *B. hebetor* or *B. brevicornis*. Observe conservation biological control to preserve natural enemies in the field. Follow the IPM package template for maize crop.

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BIOECOLOGY AND MANAGEMENT OF MAJOR INSECT PESTS OF RICE IN WEST BENGAL

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ABSTRACT

West Bengal is considered a large producer state of rice in India with an output of nearly 55.48% of total crop production of Bengal. In West Bengal, rice has grown in three different cropping seasons such as Aus (autumn rice), Aman (winter rice) and Boro (summer rice), contributing about 20.69% to the total net State Domestic Product (SDP). Rice production in West Bengal hampered by many biotic stresses mostly by stem borers [*Scirphophaga incertulas* (Walker, 1863), *Sesamia inferens* (Walker, 1856), *Chilo polychrysus* (Meyrick, 1932), *Chilo suppressalis* (Meyrick, 1863)] leaf folder [*Cnaphalocrocis medinalis* (Guenee, 1854)], plant hoppers [*Nilaparvata lugens* (Stal, 1854), *Sogatella furcifera* (Horvath, 1899)], leaf hoppers [*Nephotettix virescens* (Distant, 1908), *Nephotettix nigropictus* (Stal, 1870)], rice hispa [*Dicladispa armigera* (Olivier, 1808)], rice bugs [*Leptocorisa acuta* (Thunberg, 1783), *Leptocorisa oratorius* (Fabricius, 1794), *Brevennia rehi* (Lindinger, 1943)], gall midge [*Orseolia oryzae* (Wood-Mason, 1889)], etc. Demographic parameter has shown that rice pests are greatly affecting rice host either caused directly by creating dead heart (DH), hopperburn, onion leaf, silver shoot during different stages of harvesting or by indirectly transmitting grassy stunt virus (RGSV), rice ragged stunt virus (RRSV), rice tungro viruses (RTV), rice tungro bacilliform virus (RTBV) etc. resulting 9.5% production loss. For minimize the infestation farmers use different rice varieties which provide resistance effect in pest population. For controlling rice pest population farmers mostly use chemical insecticide [e.g. fipronil, acephate, fipronil, monocrotophos] which exhibit good results against rice pests. Beside those other techniques including using biological and biochemical pesticides [neem oil, karanj oil, mahua oil, conidial suspension of *M. anisopliae*], pheromones [(Z)-11-hexadecinal, (Z)-9-hexadecenal], allomones [5: 1 mixture of 2-(E)-octenyl acetate and octanol 3-octenal], cultural methods [synchronized planting, using of trap crop etc.] are effective for controlling rice pests. The population ecology based sustainable management of such pest species will support E³ strategy [Ecosystem service-based ecological engineering for ecological pest management (ESS-EE-EPM)] of pest management for successful cultivation of rice in the near future.

Key words: *Oryza sativa*, state domestic product (SDP), West Bengal biotic stresses, sustainable management, E³ strategy, chemical, pests, status, cultural, biopesticides, biological resistant variety, pheromones, population based control

Rice (*Oryza sativa* L., Gramineae or Poaceae, 2n=24) continues to remain as the staple food of West Bengal by contributing approximately 55.48% of total crop production (Adhikari et al., 2012; Bag et al., 2011; Dey et al., 2005). Several limiting factors such as flood, pest infestation, damage by animal and human activity causing yield loss thereby narrow down the profit of Bengal farmer (Dutta and Roy, 2022; Bag et al., 2011). Among them insect pests have appeared as the most important limiting factors during pre- and post-harvest stage of rice cultivation (Arora et al., 2019;

Adhikari et al., 2019). For controlling rice insect pest in Bengal, farmers have been most frequently using synthetic pesticides in broad-spectrum which shown good efficacy against herbivorous insect pests (Adhikari et al., 2019; Tigga et al., 2018). Besides that, using of biochemical pesticides, biological agents, pheromone trap, repellent allomone, cultural strategies and several other modern strategies like selection of high yielding resistant varieties, sterile male technique by irradiation or genetically engineered pests in management of respective pest species in the field also shown good result

for controlling rice insect pests (Sankar and Rani, 2018; Gunawardena and Bandumathie, 1993; Padhan and Raghuraman, 2018). All of these management processes have imparted negative impact also (Chatterjee et al., 2008). Sometime pest polulation create resistance against applied pesticide and also these pesticide tagets benifitful faunal biodiversity (pollinators, natural enemy of pest etc.) which create distabilizing agro ecosystem (Keary, 2023; Roy, 2020, 2021, 2022). Beside it has observed that all of rice pest management techniques did not consider economic threshold levels (ETLs) limit for pest that create ecological imbalance (Carvalho, 2017; Mobarak et al., 2020). These result into secondary pest outbreak, pest resurgence, and emergence of new pest biotypes, distabilized prey (rice pest)-predator (natural enemy of rice pest) stability, which ultimately leads regulatory complications with several constrains and limitations on the agro ecosystem (Dutta and Roy, 2016; Kakde and Patel, 2018; Kakde et al., 2014; Jeevanandham et al., 2020; Chávez et al., 2018; Roy, 2020, 2021). Therefore, this study emphasis that the life cycle of major rice pests, their active and passive infestation and their management that will very helpful to point out a possible scope for sustainable ecological management towards climate smart pest management (CSPM) through supporting E³ strategy [Ecosystem service-based Ecological engineering for Ecological pest management (ESS-EE-EPM)] of pest management for successful cultivation of rice crop in the near future.

Status of cultivation

Agriculture is the leading occupation of West Bengal and rice is the state's principal food crop (Chatterjee et al., 2008; Adhikari et al., 2012). Rice cultivation in West Bengal has occurred in diversified ecosystems such as irrigated, upland, rainfed low land, semi deep water and deep water as well as coastal saline regions in three different cropping seasons, [Aus (autumn rice), Aman (winter rice) and Boro (summer rice)] (Dhaliwal et al., 2010; Azgar and Hembram, 2018; Aryal et al., 2018). Rice was cultivated 61% of the total geographical area in West Bengal, contributing about 20.69% of State Domestic Product (Adak et al., 2020). Rice in West Bengal generally cultivated in three different seasons Boro, Aus and Aman crop season (Dutta and Roy, 2022; Bag et al., 2011). Six different agro-climatic zones (Northern Hill Zone, Terai-Teesta Alluvial Zone, Gangetic Alluvial Zone, Red and Laterite Zone, Vindhya Alluvial Zone, Coastal Salaine Zone) of West Bengal is a producer of high yielding (Annada, Jamini, Bhupen, IR 36, IR 64, and IR 22 etc.), traditional or local

(Bhadoi, Kabiraj Sal, and Shatia etc.), and aromatic rice varieties [Basmati, Badsabhog, Gobindabhog, Tulaipanji, etc.] (Bag et al., 2011; Mandal et al., 2022; Chatterjee et al., 2008; Dutta and Roy, 2022). This rice production was mostly hampered by insect pest infestation, accounted 7.37% of total rice production (Ghosh et al., 2015; Aryal et al., 2018) (Table 1, 2).

Status of rice insect pests

At the present agricultural scenario, loss in production of rice crop is due to a variety of major insect pest infestation (Chakraborty and Deb, 2012; Ghule et al., 2008). Generally, the crop loss at the farm level during pre and post harvest level occurred by major insect pests like stem borers, gall midge, plant hoppers, leaf folders, rice hispa, and rice bug etc. (Sarkar et al., 2016; Chakraborty and Deb, 2012) (Table 1, 2).

A. Stem borers

The rice production in West Bengal mostly hampered by infestation causing Lepidopteran stem borer species which include yellow stem borer (YSB), *Scirphophaga incertulas* (Walker, 1863) (Lepidoptera: Pyralidae), pink stem borer (PSB), *Sesamia inferene* (Walker, 1856) (Lepidoptera: Pyralidae), stripped stem borer (SSB) *Chilo suppressalis* (Meyrick, 1863) (Lepidoptera: Pyralidae), dark headed stem borer (DHSB) *Chilo polychrysus* (Meyrick, 1932) (Lepidoptera: Pyralidae), (Shivasharanappa et al., 2010; Dutta and Roy, 2022; Chakraborty, 2012). *Scirphophaga incertulas* (Walker) encompasses egg, 5-6 instars larvae, pre- pupa, pupa and adult during their life cycle (Hamsein et al., 2020; Dutta and Roy, 2018b). *Sesamia inferene* (Walker) encompasses egg, 6-8 instars larvae, pre pupa, pupa and adult during their life cycle (Baladhiya et al., 2018; Jeevanandham et al., 2020). *Chilo suppressalis* (Meyrick) has passed egg, 4 instars larvae, pupa to become full grown adult (Pathak and. Khan, 1994; Dutta and Roy, 2022). *Chilo polychrysus* (Meyrick) also shown same development pattern except 3 instars larva has shown instead of 4 instars of larva (Bhatt et al., 2018; Luo et al., 2016). Damage caused by stem borer has encountered due to internodal penetration by newly hatched larva during vegetative phase specially in booting or flowering stage which remain inside or behind leaf sheath in groups and feed on the epidermal layer of leaf sheath formation of oblong holes in parallel rows in the unfolded leaves as a result central leaf turns brownish, a condition known as 'Dead Heart' (DH) (Bhatt et al., 2018; Pathak and Khan, 1994). When infestation was severe resulting folding of older rice

Table 1. Biological parameters and host range of different rice pests

Rice pest	Common name and Scientific name	Stages in life cycle	Parameters of life cycle	Period	Reference
Stem borer	Yellow stem borer <i>Scirphophaga incertulas</i>	Egg, larvae (5-6 instars), pre-pupa, pupa and adult	Oviposition period	26.0 hours	Hamsein et al., 2020; Dutta and Roy, 2018b; 2022.
			Larval period	20-40 days	
			Pre-pupal period	1-2 days	
			Pupal period	6-12 days	
			Number of days in life cycle	52-64 days	
	Pink stem borer <i>Sesamia inferenze</i>	Egg, larvae (6-8 instars), pre-pupa, pupa and adult	Number of generation each year	5-8	Baladhiya et al., 2018; Jeevanandham et al., 2020; Dutta and Roy, 2022.
			Oviposition period	2 days	
			Larval period	21-39 days	
			Pre-Pupal period	1-2 days	
			Pupal period	8-14 days	
Leaf folder <i>Cnaphalocrocis medinalis</i>	Stripped stem borer <i>Chilo suppressalis</i>	Egg, larvae (4 instars), pupa and adult	Number of days in life cycle	45-57 days	Pathak and Khan, 1994; Dutta and Roy, 2022; Bhatt et al., 2018.
			Number of generation each year	4-5	
			Oviposition period	5 days	
			Larval period	23-36 days	
			Pupal period	7-8 days	
	Dark headed stem borer <i>Chilo polychrysus</i>	Egg, larvae (3 instars), pupa and adult	Number of days in life cycle	35-57 days	Dutta and Roy, 2022; Bhatt et al., 2018; Luo et al., 2016.
			Number of generation each year	6	
			Oviposition period	3 days	
			Larval period	23-39 days	
			Pupal period	7 days	
Planthoppers <i>Sogatella furcifera</i>	Leaf folder <i>Cnaphalocrocis medinalis</i>	Egg, larvae (5-6 instars), pupa, adult	Number of days in life cycle	35-60 days	Bhatt et al., 2018; Gangwar, 2015.
			Number of generation each year	2	
			Oviposition period	3 days	
			Larval period	14-23 days	
			Pupal period	6-10 days	
	Brown planthopper <i>Nilaparvata lugens</i>	Egg, Five nymphal stage and Adult	Number of days in life cycle	15-28 days	Catindig et al., 2009; Bottrell and Schoenly, 2012.
			Number of generation each year	11	
			Oviposition period	10 days	
			Nymphal period	18-32 days	
			Number of days in life cycle	25 to 44 days	
White backed planthopper <i>Sogatella furcifera</i>	Egg, five nymphal instar, adult	Number of days in life cycle	12-13	Kaur et al., 2015; Kumar, 2019.	
		Number of generation each year	2 days		
		Nymphal period	15-28 days		
		Number of days in life cycle	20-31 days		
		Number of generation each year	5-6		

(contd.)

(contd.)

(Table 1 contd.)

Leafhopper	Green leafhopper <i>Nephotettix virescens</i> and <i>Nephotettix nigropictus</i>	Egg, five instars nymph and adult	Oviposition period	9-14 days	Bunawan et al., 2014; Bhatt et al., 2018; Dey, 2016.
			Nymphal period	12-18 days	
			Number of days in life cycle	21-35 days	
			Number of generation each year	11	
Rice hispa	Rice hispa <i>Dicladispa armigera</i>	Egg, four instars larva, pupa. adult	Oviposition period	12 days	Iqbal et al., 2015, Bhattacharjee and Ray, 2017.
			Larval period	11-14 days	
			Pupal period	7-8 days	
			Number of days in life cycle	18-36 days	
			Number of generation each year	6	
			Oviposition period	12 days	
Rice bug	Gundhi bug <i>Leptocoris oratorius</i>	Egg, five nymphal stage and adult	Nymphal period	24-36 days	Khare and Prakash, 2020; Arora et al., 2019.
			Number of days in life cycle	24-36 days	
			Number of generation each year	2-5	
			Oviposition period	5 days	
			Nymphal period	22-24 days	
			Number of days in life cycle	30 to 60 days	
			Number of generation each year	4-5	
			Oviposition period	26 hours	
Gall midge	Rice mealy bug <i>Brevinnia rehi</i>	Egg, Four nymphal instars and Adult	Nymphal period	14-30 days	Bhatt et al., 2018; Pathak and Khan, 1994.
			Number of days in life cycle	17-37 days	
			Number of generation each year	12-13	
			Oviposition period	2 days	
			Larval period	9-26 days	
			Pupal period	2-8 days	
			Number of days in life cycle	15-34 days	
			Number of generation each year	4-5	
	Rice gall midge <i>Orseolia oryzae</i>	Egg (3 instar), larva, pupa and adult	Oviposition period	2 days	Ogah and Nwile, 2017; Bhatt et al., 2018; Ouattara et al., 2020.
			Larval period	9-26 days	

Table 2. Infestation parameters of rice pests

Rice pest	Name of pest	Most infectious stage	Economic threshold level	Nature of damage	Symptom of damage	Reference
Stem borer	Yellow stem borer <i>Scirphophaga incertulas</i>	Larva	5–10% damaged tillers	Due to larval boring and feeding activity inside leaf sheath resulting in yellowing of leaves.	Dead Heart (DH)	Bhatt et al., 2018; Pathak and Khan, 1994; Baladhiya et al., 2018; Asghar et al., 2009; Cork et al., 1998.
	Pink stem borer <i>Sesamia infernce</i>	Larva	1.9-3.0% WHs			
	Stripped stem borer <i>Chilo suppressalis</i>	Larva	2-8% hill or damage tillers	On severe condition folding of older rice leaves became drying off and turns whitish in colour.	White Head (WH)	
	Dark headed stem borer <i>Chilo polychrysus</i>	Larva	5% DHs			
Leaf folder	Leaf folder <i>Cnaphalocrocis medinalis</i>	Larva	3% folded leaves or 0.2-5 adult per hill	Scrapping the green mesophyll tissue affecting on photo-system II activity in photophosphorylation process resulting in scorching and drying of the leaves affecting in plant growth and reduce yield.	Leaf folding	Padmavathi et al., 2012; Pathak and Khan, 1994; Bhatt et al., 2018; Asghar et al., 2009.
	Brown planthopper <i>Nilaparvata lugens</i>	Nymphs and adults	3-8 nymphs/hill	Sucking phloem sap from stem and leaf sheath resulting yellowing of leaves and by ovipositional activity causing damage to plant tissue. On severe infestation results complete drying of the plants. Transmit Rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV).	Hopperburn, Grassy Stunt (GS), Ragged Stunt (RS), Wilt Stunt (WS).and Rice Yellowing Syndrome (RYS)	Catindig et al., 2009; Bhatt et al., 2018; Kaur et al., 2015; Bottrell and Schoenly, 2012; Matharu and Tanwar, 2020.
Plant-hoppers	White backed planthopper <i>Sogatella furcifera</i>	Nymphs and adults	4% hopperburn or 10-20 hoppers/hill	Sucking phloem sap from stem and leaf sheath resulting yellowing of leaves and by ovipositional activity causing damage to plant tissue and encourages the growth of sooty mould eg. <i>Cladosporium</i> sp and <i>Dematium</i> sp. On severe infestation results complete drying of the plants. Transmit Southern rice black-streaked dwarf virus (SRBSDV).	Hopperburn, Southern rice black-streaked dwarf disease (SRBSD)	Catindig et al., 2009; Kaur et al., 2015; Bhatt et al., 2018.
	Green leafhopper <i>Nephotettix virescens</i> and <i>Nephotettix nigropictus</i>	Nymphs and adults	2-20 insects per hill	Suck up sap from vascular vessels and by ovipositional activity damage cortex and epidermal layer of leaf resulting yellowing plant, drying up of panicle. Transmit Rice Tungro Viruses (RTV), Rice Tungro Bacilliform Virus (RTBV), Rice Tungro Spherical Virus (RTSV), Rice Dwarf Virus (RDV), Rice Waika Virus (RWV) along with bacterial and fungal infection.	Milder Tungro Symptoms (MTS), Mild Stunting Symptoms (MSS), Dwarf Syndrome (DS), Yellow-Orange Leaf Symptom (YOLS)	Pathak and Khan, 1994; Dey, 2016; Sabale et al., 2010.

(contd.)

(Table 2 contd.)

Rice hispa	Rice hispa <i>Dictyodonta armigera</i>	Grubs and adults	2 damaged leaves/plant or 1-2 adults/hill	By feeding upon the chlorophyll content of leaves and also through scraping the upper and lower surfaces of leaves resulting appearance of blisters and blotches or burned appearance on surface of leaves with parallel white lines on the leaf surface.	Leaf folding	Iqbal et al., 2015; Rahman et al., 2020.
Rice hug	Gundhi bug <i>Leptocoris oratorius</i>	Nymph and adult	2 bugs/ m ² or 2 adults/ hill	Through piercing and sucking mouth part suck up the milky juice of the developing rice grains resulting partially filled grains. Severe infestation resulting chaffy and empty grains remain small in size. Passively transmit <i>Sarocladium oryzae</i> and <i>S. attenuatum</i> (fungi).	Panicles discoloration, Sooty Mould, Sheath Rot	Dutta and Roy, 2016; Khare and Prakash, 2020; Bhatt et al., 2018; Arora et al., 2019; Dutta et al., 2020, 2021; Bhatt et al., 2018.
	Earhead bug <i>Leptocoris acuta</i>	Nymph and adult	0.5-1 individuals (adult and nymph)/ hill	Sucking the sap from stems and leaf sheaths resulting in leaves become yellowish, dried off and curled, also affects the panicle-bearing capacity of plant. Act as a vector for chlorotic streak virus and <i>Sarocladium oryzae</i>	Stunting and wilting plant, Sheath Rot Disease	Bhatt et al., 2018; Pathak and Khan, 1994; Rath et al., 2020b.
	Rice mealy bugs <i>Brevinnia rehi</i>	Nymphs and adults	3-5% affected tillers	Feeding secretes a chemical known as cecidogen from its saliva which affecting nutrient supply and resulting central leaf elongation and tubular gall-like structure is formed. Larval feeding on the meristematic tissue of terminal and auxiliary shoot apices resulting in gall formation.	Onion leaf Silver shoot	Ogah and Nwile, 2017; Bhatt et al., 2018; Kumari et al., 2020.
Gall midge	Rice gall midge <i>Orseolia oryzae</i>	Larval	5-10% onion shoots or silver shoot			

leaves longitudinally with the abaxial surface inside the folds, while younger instars partially rolled the leaf edges and folds were made along the middle portion of upper leaves with drying off central leaf which turn into whitish in colour and condition is known as 'White Ears or White Head' (WH) (Baladhiya et al., 2018). Agrosystem analysis estimated that economic threshold level was 5 to 10% damaged tillers for *Scirphophaga incertulas* (Walker) and 1.9-3.0% white heads for *Sesamia inferens* (Walker) whereas 2-8% hill or damage tillers for *Chilo suppressalis* (Meyrick) while 5% dead hearts recorded for *Chilo polychrysus* (Meyrick) (Asghar et al., 2009; Cork et al., 1998). Stem borers activity mostly has been recorded mostly on rice, beside that they also aggregate on wild grasses like *Panicum* sp., *Echinochloa* sp., *Cyperus* sp., *Dactylis* sp., *Scirpus* sp., *Saccharum* sp. etc. grown in and around crop fields (Bhatt et al., 2018; Dutta and Roy, 2018b; Jeevanandham et al., 2020).

B. Leaf folder

Rice leaf folder, *Cnaphalocrocis medinalis* (Guenee, 1854) (Lepidoptera: Pyralidae) were predominant defoliator pests during nursery to harvest (Bhatt et al., 2018). It encompasses egg, 5-6 larval instars, pupa and adult (Gangwar, 2015). Damage generally done due to voracious feeding by fourth and fifth instar inside leaf by scrapping the green mesophyll tissue of upper epidermis but the lower epidermis becomes dry and falls off and longitudinal folding of leaves by stitching the leaf margins with the help of salivary secretions (Padmavathi et al., 2012). Within the folded leaves reduction in chlorophyll content occur, influencing on photo-system II activity in photophosphorylation process in photosynthesis resulting membranous patches resulting in scorching and drying of the leaves which ultimately affecting in plant growth and reduce yield (Pathak and Khan, 1994). Beside that faecal matter of insect inside the folded leaf can cause development of fungal and bacterial infections (Bhatt et al., 2018). Researches have shown that economic threshold level which are 3% folded leaves or 0.2-5 adult per hill (Asghar et al., 2009). This deleterious leaf folder mainly infested rice with that they aggregated on plant like maize, *Panicum decompositum*, *Leersia hexandra*, *Echinochloa crusgalli*, *Leptochloa chinensis*, *Isachne dispar*, *Eleusine coracana*, and *Pennisetum typhoides* (Hurali et al., 2020; Asghar et al., 2009).

C. Planthopper

Herbivorous sap-feeding homopterans brown

plant hopper (BPH) *Nilaparvata lugens* (Stal, 1854) (Hemiptera: Delphacidae) and white backed plant hopper (WBPH) *Sogatella furcifera* (Horvath, 1899) (Hemiptera: Delphacidae) have posed a serious threat in summer Amon rice of Bengal (Matharu and Tanwar, 2020). Generally, two morphological types of plant hopper, macropterous and brachypterous have noticed in agrosystem (Catindig et al., 2009). Both morphological types of *N. lugens* (Stal) and *S. furcifera* (Horvath) encompass egg, six nymphal stages and adult (Bottrell and Schoenly, 2012). Damages caused by nymphs and adults of *N. lugens* (Stal.) and *S. furcifera* (Horvath) actively in growing plants during tillering stage and flowering stage by sucking phloem sap from stem and leaf sheath resulting yellowing of leaves and by ovipositional activity during booting and heading stages causing damage to plant tissue (Catindig et al., 2009; Bhatt et al., 2018). Severe infestation results complete drying of the plants, known as hopperburn (Kaur et al., 2015; Catindig et al., 2009). Feeding punctures and lacerations caused by ovipositor predispose of white-backed planthopper on plants to pathogenic organisms and honey dew excretion encourages the growth of sooty mould such as *Cladosporium* sp. and *Dematium* sp. (Kaur et al., 2015). Passively *N. lugens* (Stal.) can damage by transmitting Rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) result in rice yellowing syndrome (RYS) in rice (Bottrell and Schoenly, 2012). Similarly, *S. furcifera* (Horvath) act as an important vector of fiji virus, Southern rice black-streaked dwarf virus (SRBSDV) causing of Southern rice black-streaked dwarf disease (Bhatt et al., 2018). ETLs have encountered for brown planthopper 3-8 nymphs/hill (Matharu and Tanwar, 2020) whereas 4% hopperburn or 10-20 hoppers/ hill have recorded for white-backed planthopper (Bhatt et al., 2018). Generally, the rice is considering the best suitable host system for planthopper development but they had also aggregate on a weed *Leersia* sp., *Digitaria* sp., *Echinochloa* sp., *Isachne globosa*, *Poa annua*, *Poa repens* (Bhatt et al., 2018; Matharu and Tanwar, 2020).

D. Leafhopper

The rice green leafhoppers (GLHs) *Nephotettix virescens* (Distant, 1908) and *Nephotettix nigropictus* (Stal, 1870) (Hemiptera: Cicadellidae) have considered another devastating herbivorous pest of Kharif crop season (Bhatt et al., 2018). During life cycle it encompasses Egg, five instars nymph and adult stages (Dey, 2016). Damage mainly cause by either directly with their needle shaped piercing and sucking mouth

part sucking the sap and plugging the vascular bundles (xylem and phloem) with their feeding stylet sheaths results in wilting and pieces of tissue pushed into these vessels during exploratory feeding and oviposition or indirectly by transmitting virus diseases during feeding caused by Rice Tungro Viruses (RTV), Rice Tungro Bacilliform Virus (RTBV), Rice Tungro Spherical Virus (RTSV), Rice Waika Virus (RWV), Rice dwarf virus (RDV) responsible for Milder Tungro symptoms, Mild stunting Symptoms Wilting, Dwarf syndrome, Transitory yellowing syndrome, Yellow dwarf syndrome and Yellow-orange leaf symptom and also transmit fungal and bacterial infection responsible for Sooty Moulds in infected plant (Pathak and Khan, 1994; Dey, 2016). Economic threshold level of leafhopper has found 10 ± 9 insects per hill (Sabale et al., 2010). Generally, the rice is considering the best suitable host system for planthopper development but they had also aggregate on *Leersia* sp., *Digitaria* sp., *Echinochloa* sp., *Isachne globosa*, *Poa annua*, and *Panicum repens* etc. (Sarao and Bentur, 2018; Dey, 2016).

E. Rice hispa

The rice hispa or spiny beetle, *Dicladispa armigera* (Olivier, 1808) (Coleoptera: Chrysomelidae), has occupied a status of major pest of boro rice cultivation (Bhattacharjee and Ray, 2017). This pest encompasses egg, four instars larva or grubs, pupa and adult during their development (Iqbal et al., 2015). During vegetative stage of plant, the grubs feed upon the chlorophyll content of leaves and leaves became dry and give rise to blisters or blotches (Bhattacharjee and Ray, 2017). Adults also damaged through scrapping the upper and lower surfaces of leaves result in white streaks parallel to the midrib of leaves (Iqbal et al., 2015). During infestation photosynthetic activity of the leaves gets affected and leaves became dry and give rise blisters and blotches or burned appearance on surface of leaves with parallel white lines on the leaf surface (Rahaman et al., 2020). It has reported that ETL of this delitorious pest recorded 2 damaged leaves/plant or 1-2 adults/hill (Iqbal et al., 2015). This notorious pest also aggregated on *Andropogon gayanus*, *Sorghum halepense*, *Imperata cylindrica* and *Themeda anathera* (Rahaman et al., 2020).

F. Rice bug

The rice bugs have considered prevalent major pests for rainfed wetland or upland rice belong to *Leptocoris* sp., *Brevennia rehi* (Lindinger, 1943) (Rath et al., 2020a, 2020b). *Leptocoris acuta* (Thunberg, 1783) (Hemiptera: Alydidae) and *Leptocoris*

oratorius (Fabricius, 1794) (Hemiptera: Alydidae) are known as Gundhi Bug (GB) due to releasing of characteristic graceless, bothersome, incursive odour on inciting caused by chemicals released from the bug's abdominal glands (Dutta et al., 2020). Rice Mealy Bugs (RMB), *Brevennia rehi* (Lindinger, 1943) (Hemiptera: Pseudococcidae) are plant-sucking, sporadic insect pest of rainfed rice (Bhatt et al., 2018). Life cycle of *Leptocoris oratorius* (Fabricius) include egg, five nymphal stage and adult (Khare and Prakash, 2020; Arora et al., 2019) but *Leptocoris acuta* (Thunberg) also shown developmental stages except they have six nymphal stages instead of five stages (Dutta and Roy, 2016, 2018a). *Brevennia rehi* (Lindinger, 1943) has encompassed egg, four nymphal instars and adult during their development (Bhatt et al., 2018; Pathak and Khan, 1994). Damage occurs generally during milk stage to dough stage by nymphs and adults of *L. acuta* (Thunberg) and *L. oratorius* (Fabricius) through piercing and sucking mouth part suck up the milky juice of the developing rice grains resulting partially filled grains (Dutta and Roy, 2016, Khare and Prakash, 2020; Dutta et al., 2020). Severe infestation resulting chaffy and empty grains remain small in size (Bhatt et al., 2018). The *L. acuta* (Thunberg) and *L. oratorius* (Fabricius) passively transmit *Sarocladium oryzae* and *S. attenuatum*, the cause of sheath rot disease which results in damaging of the panicle of the rice plant and infected plant may not produce rice grains (Arora et al., 2019). Economic threshold level has encountered for *L. acuta* (Thunberg) 2 bugs/ m² or 2 adults/ hill (Dutta et al., 2020) whereas that level for *L. oratorius* was 0.5-1 individuals (adult and nymph)/ hill (Bhatt et al., 2018). Similarly, damages occur by *B. rehi* (Lindinger) by both nymphs and adults feed by sucking the sap from stems and leaf sheaths resulting leaves become yellowish, dried off, and curled (Bhatt et al., 2018). The infestation also affects the panicle-bearing capacity of plant and produces chaffy grains and also stunting and wilting infected plant leading growth and development of the affected plant with that infested fields show isolated patches of stunted plants (Rath et al., 2020b). Presence of this bug well marked by protruding white waxy fluff between the leaf sheath and the stem and mealybug-infested grains have not developed properly, having bitter taste with spoil, flavour (Pathak and Khan, 1994). Passively this insect pest is also known to transmit chlorotic streak virus and *Sarocladium oryzae* responsible for sheath rot disease (Bhatt et al., 2018). Economic threshold level of *B. rehi* (Lindinger) has estimated 3-5% affected tillers (Rath et al., 2020b). *L. acuta* (Thunberg), *L. oratorius* (Fabricius) and *B. rehi*

(Lindinger) are generally aggregated on rice host (*Oryza sativa*) beside that they also aggregated on non rice hosts also like, *Echinochloa glabrescens*, *Paspalidium flavidum*, *Paspalum scrobiculatum*, *Echinochloa crusgalli*, *Echinochloa colona*, *Paniculum* spp. *Cyperus* sp. (Dutta and Roy, 2018a; Rath et al., 2020b).

G. Gall midge

Asian rice gall midge, *Orseolia oryzae* (Wood-Mason, 1889) (Cecidomyiidae: Diptera) were considered as one of the major destructive pests of lowland to upland rice (Nacro et al., 1996). Development of *O. oryzae* (Wood-Mason) involves egg, three larval instars, pupa and adult completed in 15-34 days (Ogah and Nwilene, 2017). Gravid females are laid tubular or rod-shaped eggs in vicinity of plant below or above ligules, on leaf blade, central whorl, standing water near the plant (Mathur and Rajamani, 1984). Infestation by *O. oryzae* (Wood-Mason) has seen from nursery to the tillering stage (Bhatt et al., 2018). Larvae enter the shoot apex and larval feeding secretes a chemical known as cecidogen from its saliva which affecting nutrient supply and resulting central leaf elongation and tubular gall-like structure is formed, resembles as “onion leaf” beside that due to larval feeding on the meristematic tissue of terminal and auxiliary shoot apices resulting in gall formation the characteristic symptom is known as “silver shoot” due to the shiny appearance of the leaf (Ogah and Nwilene, 2017). Through mathematical model that ETLs are 5-10% onion shoots or silver shoot (Bhatt et al., 2018; Kumari et al., 2020). Rice *Oryza sativa* has considered as most favourable host but this pest also aggregated in *Cynodon dactylon*, *Echinochloa colona*, *Paspalum scrobiculatum*, *Brachiaria mutica*, *Heteropogon contortus*, *Paspalidium geminatum* and *Leersia hexandra* (Bhatt et al., 2018).

H. Other pests

There are other insect pests of rice in West Bengal having regional significance such as army worm *Mythimna separate* (Walker), grasshoppers *Hieroglyphus banian* (Fabricius), white stem borer *Scirpophaga innotata* (Walker) and rice thrips *Stenchaetothrips biformis* (Bagnall), etc. (Chatterjee et al., 2008).

Management strategies (Table 3)

In this modern era with increasing human population there is a need to increase rice production per unit of land through sustainable management strategies (Dutta et al., 2021; Chávez et al., 2018). To minimize

yield losses and to increased productivity farmers use different management strategies with minimum cost to control pest population in agro ecosystem as described below (Roy, 2019a, 2019b; Kunal et al., 2020).

A. Chemical control

Most popular method used by farmers for controlling insect rice pest was through using synthetic chemical pesticide (Roy, 2019a, 2019b). Application of aqueous or oily spray of fenvalrate, acephate, quinalphos, cartap hydrochloride, flubendiamide, carbofuran, carbosulfan, chlorantraniliprole, fipronil, thiamethoxam, chlorpyrifos, spinosad, and N-((2,6-dimethyl-4-(heptafluoropropyl-2-yl)phenyl) carbamoyl)-2,6-difluorobenzamide has shown best efficacy against adult and larva of rice leaf folder, gall midge, planthopper, leafhopper, gandhi bug, mealybug and stem borers (Shyamrao and Raghuraman, 2019; Dutta and Roy, 2022; Zhao et al., 2019; Baladhiya et al., 2018). Application of soil granular formulation of diazinon, propaphos, endrin chlorfenvinphos, disulfoton, padan, deltamethrin, propenphos, propenphos, chlopyrifos, monocrotophos, imidacloprid, buprofezin, cycloxaprid, pymetrozine, sulfoxaflo, indoxacarb, flonicamid, malathion, dimethoate, phosphamidon, dinotefurain, chlorpyrifos, cypermethrin, lindane, triazophos, spinosad, rynaxypyr, and endosulfan during seedling plantation have shown good result for managing adult and larval incidence caused by major insect pests (Dutta and Roy, 2022; Zhao et al., 2019; Achiri et al., 2020; Matharu and Tanwar, 2020; Hurali et al., 2020; Luo et al., 2023; Adhikari et al., 2019; Bhattacharjee and Ray, 2017; Gupta et al., 2019; Kumari et al., 2020; Adhikari et al., 2019). Monocrotophos, imidacloprid, and triazophos were most economical on basis of cost benefit ratio (CBR) (Tigga et al., 2018; Adhikari et al., 2019).

B. Cultural control

For controlling deleterious rice pests, popular mechanical and cultural techniques used by farmers was based on crop sanitation because in most of cases it has observed that source of infestation occurred by egg, larva and adult of phytophagous rice insect pests that persist during seedlings (Ayelo et al., 2021). Another popular method was based on plant quarantine technique which includes raising crop nurseries away from light source avoids the early infestation have done by rice insect pests (Seleiman et al., 2021). Most commonly used crop sanitation technique are seed soaking with fertilizer along with insecticide, clipping

Table 3. Management strategies for rice pests

Rice pest	Common name and scientific name	Management agents	Management strategies	References
		Carbofuran, carbosulfan, fipronil, acephate, fipronil, chlorpyrifos, quinalphos, flubendiamide.	Chemical	Shyamrao and Raghuraman, 2019; Dutta and Roy, 2022
	Yellow stem borer <i>Scirphophaga incertulas</i>	Leaf extract of neem (<i>Azadirachta indica</i>), tobacco (<i>Nicotina tobacum</i>), Karanja (<i>Pongamia glabra</i>), bishkatali (<i>Polygonum hydropiper</i>), lantana (<i>Lantana camara</i>) and neem oil, mahogany (<i>Swietenia macrophylla</i>) oil, etc.	Botanicals	Shyamrao and Raghuraman, 2019.
		Extract of <i>Trichogramma japonicum</i> .	Biological	Dutta and Roy, 2022.
	Pink stem borer <i>Sesamia inferens</i>	Fenvalrate, thiamethoxam, spinosad, chlorantraniliprole, carbofuran.	Chemical	Baladhiya et al., 2018; Zhao et al., 2019.
		Isolates of earwig <i>Euborellia stali</i> (Dohrn), Bt protoxin CryIca. Isolates of <i>Beauveria bassiana</i> , NBAIL- Bb-1, 4, 8, 11, 15, 39 and 59 isolates.	Biological	Baladhiya et al., 2018.
	Dark headed stem borer <i>Chilo suppressalis</i>	Fipronil, carbofuran, diazinon, disulfoton Padan, TXH09 [N- ((2,6-dimethyl-4-(heptafluoropropyl-2-yl)phenyl)carbamoyl)-2,6- difluorobenzamide]	Chemical	Zhao et al., 2019; Khan et al., 2020.
		Extract of rice variety TKM-6, Pentadecanol TKM-6	Botanicals	Prakash et al., 2008.
		Extract powder of <i>Trichogramma japonicum</i> , <i>Trichogramma chilonis</i> , <i>T. dendrolimi</i> , and <i>T. ostriniae</i>	Biological	Hajjar et al., 2023.
	Stripped stem borer <i>Chilo polychrysus</i>	Spinosad, deltamethrin, lambda-cyhalothrin, emamectin-benzoate, indoxacarb, chlorpyrifos-ethyl, chlorantraniliprole, novaluron	Chemical	Achiri et al., 2020.
		Extract of <i>Neorautanenia mitis</i> , <i>Derris elliptica</i> , <i>Metarhizium anisopliae</i> , <i>Beauveria bassiana</i>	Biological	January et al., 2020.
	Leaf folder <i>Cnaphalocrocis medinalis</i>	Fipronil, propenphos, chlorpyrifos, monocrotophos, carbofuran, carbosulfan, imidacloprid	Chemical	Hurali et al., 2020.
		Azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, deacetylhimbin	Botanicals	Nathan et al., 2005.
		Pseudomonas stain (Pfl + TDK1 + PY15), Cnme GV, (isolated from <i>Yangzhou</i>)	Biological	Jian et al., 2019.
		Endrin, carbofuran, disulfoton, chlorfenvinphos, phorate, methyl-demeton, dicrotophos, monocrotophos, imidacloprid, flonicamid, etc.	Chemical	Matharu and Janwar, 2020.
	Brown planthopper <i>Nilaparvata lugens</i>	Extract of <i>Adhatoda vasica</i> , <i>Vitex negundo</i> , <i>Azadirachta indica</i> , <i>Ricinus communis</i> and <i>Pongamia glabra</i> leaves, neem seed kernal extract, etc.	Botanicals	Longkumer and Misra, 2020.
		Conidial suspension of <i>Metarhizium anisopliae</i> , <i>M. flavoviride</i> , <i>Beauveria bassiana</i> , and <i>Hirsutiellatiriformis</i>	Biological	Sharma and Sharma, 2021
		Imidacloprid, buprofezin, thiamethoxam, chlorpyrifos, isoprocarb, cycloxaprid, buprofezin	Chemical	Luo et al., 2023.
	White backed planthopper <i>Sogatella furcifera</i>	Leaf crude extract of neem, notch (<i>Vitex negundo</i>), palmarosa (<i>Cymbopogon mauritii</i>) oil, jatropa (<i>Jatropha carcus</i>) oil, neem oil, etc.	Botanicals	Raj et al., 2020.
		Jinggangmycin (JGM) [product of <i>Streptomyces</i> var. <i>jinggangensis</i>]	Biological	Ge et al., 2017.

(contd.)

(Table 3 contd.)

Leafhopper	Green leafhopper <i>Nephotettix virescens</i> and <i>Nephotettix nigropictus</i>	Pymetrozine, imidacloprid, dinotefuran, sulfoxaflor, flonicamid, buprofezin, propaphos, disulfoton	Chemical	Adhikari et al., 2019.
		Karanj oil, mahua oil, pinnai oil, neem oil, custard-apple oil, seed oil and leaf and fruit extract of <i>Annona squamosa</i>	Botanicals	Kumar et al., 2021.
		Conidia and blastospores of <i>Beauveria bassiana</i>	Biological	Sharma and Sharma, 2021.
		Quinalphos, malathion, dimethoate, phosphamidon, endosulfan, chlorpyrifos, monocrotophos, cypermethrin, fenvalarate	Chemical	Bhattacharjee and Ray, 2017.
Rice hispa	Rice hispa <i>Diurapha armigera</i>	Neem oil, Azacel, Calpaste, Larvocat, dk-bioneem, Multineem	Botanicals	Kumar et al., 2021.
		Powder formulation of <i>Beauveria bassiana</i> (Bals.) Vuill, spore extraction of <i>B. bassiana</i>	Biological	Purnima et al., 2013.
		Imidacloprid, triazophos, monocrotophos, thiamethoxam, acephate, carbaryl, malathion, lindane, etc.	Chemical	Gupta et al., 2019.
		Extract of <i>Chromolaena odorata</i> , <i>Azadirachta indica</i> , <i>Ricinus communis</i> , neem seed kernel powder (NSKP), etc.	Botanicals	Raveau et al., 2020.
Rice bug	Earhead bug <i>Leptocoris acuta</i> , gundhi bug <i>Leptocoris oratorius</i> , rice mealy bug <i>Brevinnia rehi</i>	Isolates of entomopathogens (<i>Beauveria bassiana</i> and <i>Metarhizium anisopliae</i>), cow urine	Biological	Kumar and Sarada, 2020.
		Acephate, rynaxypyr, dinotefuran, fipronil, thiamethoxam, buprofezin, spinetoram, methoxyfenozide, acephate, dinotefuran, triazophos,	Chemical	Kumari et al., 2020.
Gall midge	Rice gall midge <i>Orseolia oryzae</i>	Neem Seed Kernel Extract (NSKE)	Botanicals	Ogah and Nwile, 2017.
		Parasitized galls of <i>Platyaster diplosisae</i>	Biological	Minab et al., 2023.

of leaves of rice plants during highest tillering stage, removal of stubbles through plough and then burn at ground, drain out water in infested paddy field, seedling root dipping, removal of alternate grassy and other weeds hosts from seedbed, crop field and adjacent area shown very effective against leaf folder, stem borer, gall midge, rice hispa and rice bug (Bhatt et al., 2018; Kunal et al., 2020). Cultivation of certain susceptible aromatic rice cultivars like Pusa Basmati-1, Gobindabhog near main crop can be used as trap crop to reduce damage to the main crop (Katti et al., 2023). Crop rotation with leguminous pulse seed, potato, oil seed in low land and cultivation of maize in upland area are also shown good efficacy against different insect pests (Breidenbach et al., 2017; Chen et al., 2018). Uses of oil or electric light traps are very helpful against stem borer and planthopper (Bhatt et al., 2018). Even, use of fish like, *Puntius conchoni* (F. Hamilton, 1822), *Clarias batrachus* (Linnaeus, 1758), *Puntius ticto* (F. Hamilton, 1822), frogs like, *Euphyllotis cyanophyllotis*, *Duttaphrynus melanostictus* (Schneider, 1799) etc. and *Anas poecilorhyncha* (Forster, 1781) are preyed on adult of rice pest thereby reduce the population (Khatriwada et al., 2016; Yuan et al., 2022).

C. Biopesticides

Plant formulations, so called 'green pesticide' were also effective and have minimal residual activity (Raveau et al., 2020). Natural oil, cake and crude extract of different part of neem plant [*Azadirachta indica* (Juss, 1830)] neem oil, neem oil soap solution, neem seed oil, cake extract, leaf extract, leaf crude extract, seed kernel extract, limonoid, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, deacetyl nimbin, azadirachtin, neemgold, nimbidine, neemax, azacel, and larvocel have shown good efficacy against deleterious pest incidence (January et al., 2020; Shyamrao and Raghuraman, 2019; Dougoud et al., 2019). Application of extract of different part from other plants like seeds of *Pongamia pinnata*, *Madhuca longifolia*, *Calophyllum inophyllum*, *Neorautanenia mitis*, *Derris elliptica*, *Nicotinatabacum*, *Pongamia glabra*, *Chromolaena odorata*, *Ricinus communis*, *Adhatoda vasica*, *Andrographis paniculata*, *Prosopis juliflora*, *Polygonum hydropiper*, *Lantana camara*, *Vitex negundo*, mixture of *Polygonum hydropiper*, *L. camara* and ginger-garlic-chili effectively reduced incidence caused by herbivorous insect pests (Raj et al., 2020; Longkumer and Misra, 2020). Oil formulation from Mahogany, Palmarosa, Jatropa, custard-apple seed, Karanj (*Pongamia pinnata*), Eucalyptus (*Eucalyptus*

obliqua), Cedar Wood, Mahua (*Madhuca longifolia*), Pinnai (*Calophyllum inophyllum*), Citronella, Vetiver, Catnip, were effective against *Dicladispa armigera* (Olivier), *Sogatella furcifera* (Horvath), *Nephotettix virescens* (Distant), *Nephotettix nigropictus* (Stal), *Scirphophaga incertulas* (Walker) population (Raj et al., 2020; Kumar et al., 2021; Raveau et al., 2020). Animal derived cow urine also acts as antifeedant and disinfectant against rice pests and their transmitting pathogens (Kumar and Sarada, 2020).

D. Biological control

Most popular biological controlling agents include mycobial powder and pesticide extracted from *Bacillus thuringiensis*, *Trichogramma japonicum*, *T. chilonis*, *T. dendrolimi*, *T. ostrinae*, *Lecanicillium lecanii*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Skermanella* sp., *Pseudomonas* stain, CnmeGV galls of *Platygaster diplosis* and Jingga mycin (JGM) had shown good antibiosis effects through egg parasitic, larvicidal activity on rice insect pests (Dutta and Roy, 2022; Hajjar et al., 2023; January et al., 2020; Kumar and Sarada, 2020; Minab et al., 2023; Jian et al., 2019; Ge et al., 2017). Using of conidial suspension of *Metarhizium anisopliae*, *M. flavoviride*, *Beauveria bassiana*, and *Hirsutella citriformis*, preparations of marcescent mycelium of *Paecilomyces lilacinus*, wettable powder formulation, spore extract, conidia and blastospores extract of *Beauveria bassiana* had shown egg parasitoid activity on *C. polychrysus* (Meyrick), *D. armigera* (Olivier), plant and leafhopper (Purnima et al., 2013; Sharma and Sharma, 2021). Bio-pesticides Btprotoxin CryIca, NBAIL-Bb-1, 4, 6, 8, 11, 15, 39 and 59 isolates, NBAIL-Ma-1, 6, 10, 13, 15, 23, 25, 26, 41 and 42 isolates effective against *D. armigera* (Olivier) population (Baladhiya et al., 2018).

E. Resistant variety based control

Rice variety D518, IR-42, ASD7, and IR46 were effective against incidence by *N. lugens* (Stal.) and *S. furcifera* (Horvath) (Gao et al., 2020). Infestation potential of *C. medinalis* (Guenee) has recorded low in cultivar Ptb-12, TKM-6, Prasanna, Radha-4, Ramdhan, IRRI-6, KSK-282, DR-83, Bas-385 (Adhikari et al., 2022; Chen et al., 2022; Chatterjee et al., 2021). Similarly, GAR-13, IR-22, Gontra Bidhan 3, MTU-2020, IR-50, TKM 6, KMD1, KMD2, MTU15, and IR50 have shown good efficacy against *S. incertulas* (Walker), *C. suppressalis* (Meyrick) and *C. polychrysus* (Meyrick) (Jha et al., 2023; Chavan and Patel, 2018a, 2018b; Xu et al., 2022). Pankhari 203, WRC25,

WRC34, WRC37, WRC40, WRC6, WRC7, WRC48, and WRC55 has recorded effective against *N. virescens* (Distant) and *N. nigropictus* (Stal) (Thein et al., 2019). Rice variety like IET-6286, Surekha, Usha, Sahbhagi Dhan, Sudha, Abhisek, ARC-5833, ARC-5984, TKM-6, and PTB-10 were effective against *O. oryzae* (Wood-Mason) and *S. inference* (Walker) incidence (Sanchit and Sanchit, 2019). Rice cultivar like Garem and Bengaubisi found effective against *D. armigera* (Olivier) infestation (Dutta and Hazarika, 1994). GR-9, Jaya, Dandi, Pawana, Gurjari, Narmada, Pawana, and GR-104 have shown good efficacy against *B. rehi* (Lindinger), *L. acuta* (Thunberg) and *L. oratorius* (Fabricius) (Khalifa and El-Rewainy, 2012; Morya and Kumar, 2019; Rath et al., 2020b).

F. Phoromone and allomone based control

Now a days farmers often use attractant sex pheromones (Z)-11-hexadecinal, (Z)-9-hexadecenal, (Z)-9-hexadecenal and (Z)-11-hexadecenal with PVC matrix, Z11-16 Acetate, (Z)-hexadec-9-enal, (Z)-11-octadecenal, (Z)-11-hexadecenyl acetate, (Z)-13-octadecenyl acetate, pymetrozine, 2-(E)-octenyl acetate, octanol, n-tridecane and 2S,6S-diaxetoxiheptane which help in attract male and female mass trapping and mating disruption of insects for management of rice pest (Gunawardena and Ranatunga, 1989; Dutta and Roy, 2022; Liu et al., 2020; Dang et al., 2016; Song et al., 2022). Some repellent allomone 5: 1 mixture of 2-(E)-octenyl acetate and octanol 3-octenal, 1-octanol, (Z)-3-octenyl acetate, extracts of Bamboo (*Bambusa levis* L.), Ginger (*Catimbium haenkei* L.), Malubago (*Hibiscus tiliaceus* L.) repel herbivore stem borer, leaf folder and gandhi bug population (Gunawardena and Bandumathie, 1993).

G. Population based control

Several demographical models described the biotic and abiotic mortality factors affecting its abundance, development, survivorship, reproduction, in agroecosystem (Mobarak et al., 2020). Developing of age-specific (horizontal) and stage-specific (vertical) life tables for assessing population density, fluctuation, most vulnerable stage or stage having higher mortality rate of different rice pests under different agroclimatic field conditions (Dutta and Roy, 2016; Dutta et al., 2020, 2021). Population growth and reproductive parameters [probability of survival from birth to age x (l_x), survival rate (s_x), average population alive in each stage (L_x), total individuals at age x and beyond k (T_x), life expectancy (e_x), generation survival

(GS), age specific fertility or fecundity (m_x), gross reproductive rate (GRR), net reproductive rate (NRR or R_0), intrinsic rate of population increase (r_m), finite rate of population increase (λ), weekly multiplication rate (λ^7), increase rate per generation (λ^{T_c}), total fertility rate (F_x), population growth rate (PGR), and population momentum factor of increase (PMF)], mortality profiles [proportion dying each age (d_x), mortality rate (q_x), and mortality coefficient (MC)], span of generation time (T_c) and doubling time (DT) depend on temperature, nutritional quality [quantity of carbohydrates, protein, lipid, phenol, phytate, saponin etc.] host system [rice host (*O. sativa*), non rice host (eg. *E. colona*, *C. rotundus*, etc.)], presence of egg and adult predator parasitoid (Babamir-Satehi et al., 2022; Schoonhoven et al., 2005; Dutta and Roy, 2018a, 2018b; Harborne, 1973, 1984; Manikandan et al., 2015; Havelka and Zemek, 1999). By analyzing life table data of rice pests in different host and agro-climatic condition type -II survivorship has shown by *S. furcifera* (Horvath) but type-III survivorship obtained in *S. incertulas* (Walker), *N. lugens* (Stal), *L. acuta* (Thunberg) and even, type IV exhibited by *C. suppressalis* (Meyrick) and *C. polychrysus* (Meyrick) (Dutta and Roy, 2016, 2018b; Shivasharanappa et al., 2010). By studying population dynamics, sixth nymphal stage of *L. acuta* (Thunberg), first instar larva of *S. incertulas* (Walker), *C. suppressalis* (Meyrick), *D. armigera* (Olivier), fourth nymphal stage of *N. lugens* (Stal) and first nymphal stage and adult of green leafhopper have identified most vulnerable stages (Win et al., 2009, 2011). Ecological limiting factors like relative humidity in winter, low moisture, predator density and dispersal loss during larval stage, egg predator [eg. *Conocephalus chinensis* (Redtenbacher)], high temperature, low relative humidity, parasitisation by the entomopathogenic nematode, adult migration, parasitoids of egg, nymphal and adult, moulting failure of larva into pupa and host nutritional quality appeared as limiting factors *N. virescens* (Distant), *N. nigropictus* (Stal), *S. furcifera* (Horvath), *D. armigera* (Olivier), *C. suppressalis* (Meyrick), *L. acuta* (Thunberg) and *O. oryzae* (Wood-Mason) (Kumar et al., 2012; Dutta and Roy, 2016; Win et al., 2011; Suzuki et al., 1996; Roy, 2022). Parasitoids and disease on 3rd and 4th instar larva and pupal parasitism also appeared as limiting factor for *C. medinalis* (Guenee) (Huang et al., 2020; Padmavathi et al., 2008). By identifying most vulnerable stages and population dynamics along with limiting factors of rice insect pest are very essential for the timely adoption of different management practices (Mobarak et al., 2020; Roy, 2019a, 2019b, 2020, 2021).

CONCLUSIONS

Rice, one of the most consumable staple crop of most of Bengal household cultivated 56.63 lakh hectare areas with a production of 274.37 lakh tones, contributing about 20.69% to the total net State Domestic Product (SDP) (Dutta and Roy, 2022; Jha et al., 2023; Adak et al., 2020; Chakraborty and Deb, 2012). Rice in West Bengal has cultivated in the six agro-climatic zone during three different cropping seasons, Aus (autumn rice), Aman (winter rice) and Boro (summer rice) (Adhikari et al., 2012; Chakraborty and Deb, 2012). But this rice production in West Bengal was hampered by different biotic stresses causing 33% production loss (Aryal et al., 2018; Jha et al., 2023). Most important biotic stress caused to hamper production of rice in West Bengal was through direct or indirect infestation by phytophagous rice pest (Adhikari et al., 2012; Dutta and Roy, 2022). The remarkable deleterious rice insect pest fauna has observed mostly are *S. incertulas* (Walker), *S. inferens* (Walker), *C. polychrysus* (Meyrick), *C. suppressalis* (Meyrick), *C. medinalis* (Guenee), *N. lugens* (Stal.), *S. furcifera* (Horvath), *N. virescens* (Distant), *N. nigropictus* (Stal), *D. armigera* (Olivier), *L. acuta* (Thunberg), *L. oratorius* (Fabricius), *O. oryzae* (Wood-Mason) etc. (Azgar and Hembram, 2018; Dhaliwal et al., 2010; Jha et al., 2023).

It has observed that most of polyphagous major rice pests in rice mainly beside that they were also aggregated on some alternative non rice weeds found in and around rice field (Bhatt et al., 2018; Dutta and Roy, 2018a, 2018b). Population growth, reproductive and mortality parameters of different rice pests are greatly varied depending on host system and agroclimatic conditions (Roy, 2017, 2022; Reji and Chander, 2008). Modern agriculture concerned with ecofriendly and sustainable climate smart agriculture (CSA) as a part of ecological pest management (EPM) based integrated pest management (IPM) for of different economic crops (Pedigo et al., 1986; Pedigo and Higley, 1992; Aryal et al., 2018; Chávez et al., 2018; Roy, 2019b). Therefore, basic information regarding pest ecology is necessary before deciding any strategy to combat with deleterious rice pest (Rahaman et al., 2014; Roy, 2019b). Commonly most of farmers use different synthetic pesticides for controlling pests (Aktar et al., 2009; Bais et al., 2017). But some plant extract botanicals are also seen against rice pests (Aryal et al., 2018; Shitiri et al., 2014; Dutta and Roy, 2022). Beside these most popular controlling methods usually mechanical and cultural control strategies include mechanical synchronised

planting, cultivation of certain rice varieties like Pusa Basmati-1 as trap crop, soaking sprouted seed in synthetic pesticide, seedling root dipping in pesticide and fertilizer, remove, burn or plough the stubbles, and using of fish, frog ducklings etc. also seem to be useful for controlling these pest population (Ayelo et al., 2021; Khatiwada et al., 2016; Bhatt et al., 2018). Some attractant sex pheromones like, (Z)-11 hexadecinal, (Z)-9-hexadecenal, (Z)-11-hexadecenal, (Z)-9-octadecenal are show good efficacy in mass trapping and mating disruption of deleterious insect pests (Liu et al., 2020; Dang et al., 2016; Gunawardena and Ranatunga, 1989). Beside that repellent allomones like, 5: 1 mixture of 2-(E)-octenyl acetate and octanol 3-octenal, 1-octanol and extracts of resistant rice varieties distracts rice pests' population from cultivated field (Gunawardena and Bandumathie, 1993; Pathak and Khan, 1994). Farmers some time use biological agents as pesticides derived from strains of *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae*, *M. flavoviride* etc. which were effective against major paddy pests (Dutta and Roy, 2022; Purnima et al., 2013; Sharma and Sharma, 2021). Certain resistant rice cultivars like, Pankaj, Triveni, IR-19362-183, IR-20, H4, IR-36, IR-50, IR-9828-23-1, IR-13429-57-1, IR-44, IR-22, and GR-9 etc. have impart resistance effect against rice pest population growth, development and reproductive parameters (Sanchit and Sanchit, 2019; Huang et al., 2019; Rath et al., 2020b).

Recent days, farmers generally use synthetic chemical insecticides indiscriminately against those pests for the hope of high production of rice, but it has seen that these pesticide not only target phytophagous rice pests but also targeted beneficial natural enemies of pest, pollinator resulting destabilizing of biodiversity, decrease the sustainability by bio-magnification and bio-accumulation of toxic substance which result in ecologically and environmentally imbalance and creates health hazards (Katti et al., 2023; Biswas et al., 2006; Aktar et al., 2009; Kumari et al., 2020). It has seen that excessive use of insect lethal insecticides leads to increase the amount of primary nutrient like amino acids and sucrose available in the phloem of rice plants results in insecticide-induced resurgence, and cross resistance leads to of periodic outbreaks pest populations, known as "Moran effect" (Bottrell and Schoenly, 2012; Dutta and Roy, 2016, 2022). To overcome this constrains caused by rice pest researchers usually use insecticide by considering several parameters like cost: benefit ratio, economic threshold level (Selvaraj et al., 2012), population dynamics by exploring of allelopathy-based

strategies and enhancing use of green pesticides and cultural methods for appropriate management strategies for rice pests (Dutta and Roy, 2022). Thus, population ecology based sustainable management of such pest species will support E³ strategy [Ecosystem service-based Ecological engineering for Ecological pest management (ESS-EE-EPM)] of pest management for successful cultivation of rice in the near future.

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VARIATIONS IN THE BIOLOGICAL AND ECOLOGICAL ATTRIBUTES OF INSECTS DUE TO CLIMATE CHANGE: A REVIEW

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ABSTRACT

Climate change is causing a shift in long-term weather patterns and altering parameters such as temperature, atmospheric CO₂, and precipitation patterns. It is likely to be an indirect effect of greenhouse gases and their enhancement in the atmosphere largely induced by human activities. As poikilothermic animals, insects are highly dependent on environmental conditions, particularly temperature, which affects various aspects of their life. Climate change have a direct impact on insect pests by affecting their population dynamics, diurnal activity, growth rate and diapause. It may lead to a range expansion, increased overwintering survival, more generations per year, elevated risk of invasive insect species and insect-borne plant diseases, and changes in their interactions with host plants and natural predators. Indirectly, changes in climate can modify host plants and competitors, further complicating the effect on insect biology and phenology. Impact of elevated CO₂ levels on their host plants can affect growth rates, fecundity, and population densities of insects. Altered precipitation patterns, such as droughts and floods, affect insect survival and diapause. Further, there is significant implications of climate change on different aspects of insect ecology, including insect distribution, behavior, and communication. The climate changes are predicted to alter the geographic ranges and migration behavior of insect species, creating new ecological niches and removing low-temperature barriers. These changes can threaten food security and increase the latitudinal and altitudinal range of crop pests. It also affects natural enemies, which can decrease the plant defense system against insect pests. Additionally, climate change can affect pheromonal communication, including production to behavioral response, in insects that rely on long-range chemical signals for communication. The review paper highlights the potential impacts of climate change on insect biological, phenological and ecological aspects.

Key words: Global warming, climate change, GHG, Temperature, precipitation, CO₂, diapause, population dynamics, voltinism, phenology, distribution, chemical ecology, pheromone communication, insect pests

Climate change refers to a gradual shift in the long-term patterns of weather in a particular area. It involves a range of parameters such as an increase in temperatures, atmospheric CO₂ levels, and changing precipitation patterns. The rise in temperatures, increased CO₂ and other greenhouse gases, and altered precipitation patterns are causing climate extremes, which are a serious threat to global food production (Shrestha, 2019). Mean temperature has been increasing as a result of ongoing climate change and is predicted to rise by 1.5°C within the next two decades than preindustrial levels (the average over the years 1850-1900) (IPCC, 2022). It is suggested that the average temperature may increase by 1.7–5.3°C as a result of increasing greenhouse gases (CH₄, CO₂, and N₂O) concentration within the coming 60-100 years (IPCC, 2022; IPCC, 2014). The agriculture sector is responsible

for approximately 21% of global GHG emissions (Lamb et al., 2021), with significant variability worldwide. By 2030, greenhouse gas emissions are expected to rise nearly 40%, mainly through direct emission as CH₄ and N₂O or indirectly in the form of energy consumption by the agricultural system (CO₂) (Vermeulen et al., 2012). The complexity of climate change is evident in the fact that night-time temperatures are warming faster than day-time temperatures across much of the planet, as study on warming from 1983 to 2017 found a difference in mean annual temperature of more than 0.25°C between daytime and night-time warming (Cox et al., 2020; Speights et al., 2017).

Insects are cold-blooded animals whose body temperature varies with that of the surrounding environment. Therefore, insects are highly dependent

on the thermal conditions of their environment which influences various aspects of their life (Kocmankova et al., 2010). Climate conditions are one of the primary factors that determine insect distribution ranges, along with food plants. Climate change is affecting the distribution and severity of crop pests (Macfadyen et al., 2018; Lamichhane et al., 2015), with regions at higher latitudes facing severe increases that could disproportionately impact insects (Bale and Hayward, 2010). So, climate change is likely to have a significant impact on insect pests, posing a serious threat to sustainable food production (Andrew and Terblanche, 2013). Predicting the impacts of climate change on insect pests is challenging due to the complex interactions of increasing atmospheric CO₂ concentration, changing climatic regimes, and altered intensity/frequency of extreme weather events (Bebber et al., 2013; Gregory et al., 2009). Climate change can also indirectly affect insects through the differing responses of host plants and natural enemies of insect pests. Further, different thermal preferences for insect pests and their natural enemies can negatively affect the synchronization between them and cause a risk of pest outbreaks (Furlong and Zalucki, 2017; Hance et al., 2007).

Agricultural research is focused on understanding the impacts of climate change and associated phenomena on agricultural production and agricultural insect pests. Temperature rise directly affects the survival, reproduction, spread, and population dynamics of the pest, as well as the relationships between pests, the environment, and natural enemies (Prakash et al., 2014). This review provides an overview of the possible effects of climate change on insects in various ways. Here the impact of some of the predicted climate changes, especially the increase in temperatures and atmospheric carbon dioxide concentrations along with changing precipitation patterns on the biology, phenology, ecology, and physiology of insects, especially agricultural pest species is reviewed.

Response of insects to rising temperatures, CO₂ and changes in precipitation

Insects are known to be highly adaptable organisms that respond differently to various impacts of climate change (Skendžić et al., 2021). The physiology of insects is highly sensitive to temperature fluctuations (Dukes et al., 2009). With temperature being the most crucial environmental factor affecting the dynamics of insect populations by altering fertility, it is anticipated that a warming climate could lead to a range expansion,

increased overwintering survival, more generations per year, an elevated risk of invasive insect species and insect-borne plant diseases, as well as changes in their interactions with host plants and natural predators (Skendžić et al., 2021; Bale et al., 2002). It can accelerate insect consumption, development, and mobility, and overall population size (Bale et al., 2002). Species that can adapt to changing temperatures will thrive, while those that cannot will decline. The rising CO₂ level has various impacts on insects, mainly by affecting their host plants. Elevated atmospheric CO₂ levels can impact the distribution, abundance, and overall performance of herbivory. Such changes may affect the consumption rates, growth rates, fecundity, and population densities of insect pests (Fuhrer, 2003). The feeding response of herbivory is also likely to be uneven on C₃ and C₄ plants (Lincoln et al., 1984). Elevated CO₂ levels can alter the nutritional quality and palatability of leaves, affecting the leaf-feeding insects' consumption and survival (Lincoln, 1993). Due to climate change, precipitation patterns have altered, with decreased frequency and increased intensity. This change has favored the incidence of droughts and floods, affecting insect survival and diapause (Shrestha, 2019). During severe rainfall, small-bodied insects like jassids, aphids, mites, and whiteflies can be washed away (Pathak et al., 2012).

Impact of climate change on biology and phenology of insects

Climate change and weather alterations have significant impacts on the biology and phenology of insect pests. As generally adaptable organisms, insect pests respond differently to different factors of climate change (Skendžić et al., 2021). These changes affect insect population dynamics, diurnal activity, growth rate, reproduction, winter performance, and diapause in complex ways. They also indirectly impact insects by modifying their host plants and competitors (Bale et al., 2002). Furthermore, research suggests that tropical insects are more vulnerable to warming situations because tropical areas have experienced less climatic variability historically, and insects in these regions are already closer to detrimental thermal maxima than temperate insects (Garcia-Robledo et al., 2016; Deutsch et al., 2008). Insect pests are impacted directly and indirectly by climate change. Direct effects include changes to the pests' biology, phenology, and distribution, while indirect effects involve modifications to the host plant on which they feed, as well as interactions with natural enemies, other insect species,

competitors, and the environment (Prakash et al., 2014). Although it is challenging to predict the precise effect of climate change on insect biology and phenology, different species respond differently to climatic variation even within the same landscape (Nice et al., 2019). Nonetheless, if the changes in environmental factors are observed in isolation, the following impacts on insect biology and phenology can be identified.

Population dynamics and voltinism

An increase in average temperature can accelerate the growth and reproduction of insect species, resulting in shorter life cycles and higher numbers of larvae on a single host plant, as well as an increased frequency of outbreaks (Jönsson et al., 2007). This change in population dynamics due to climate change is likely to escalate the population size and pest activities in many insect groups (Puri and Ramamurthy, 2009). Temperature changes can also affect the survival and development rates of insects, as well as their voltinism (the number of generations produced in a year), which can impact population density, size, and genetic composition, as well as the level of damage inflicted on host plants (Bale et al., 2002). The population model of the grape berry moth, *Paralobesia viteana*, predicts that a temperature increase of more than 2°C can have a significant impact on its voltinism, causing a shift in the timing of its ovipositional period that is currently controlled by photoperiod-induced diapause (Tobin et al., 2008). Under current climate conditions in Brazil, the voltinism of *Spodoptera eridania* as reported to range from 2.9 to 9.2 generations, with fewer cohorts in low temperature regions and more in warmer ones, and a considerable increase in the number of generations of *S. eridania* was projected under climate change scenarios (Sampaio et al., 2021). In one of the study from India, with mean temperature is predicted to increase by 0.51°C, 1.03°C, 1.57°C and 2.1°C in climate years 2030, 2050, 2070 and 2090, escalated annual *Helicoverpa armigera* generation to 12.9, 13.3, 13.8 and 14.2, respectively. There will be 3-12% increase in *H. armigera* generations in future climate years in India (Bapatla et al., 2022).

Diurnal activity response and insect phenology

One of the most well-known effects of recent global warming is alterations in phenology, or the timing of seasonal lifecycle events (Radchuk et al., 2019; Thackeray et al., 2016; Menéndez, 2007). Many species are now appearing earlier in the spring and remaining active for longer periods, which can have significant

impacts on population dynamics and abundance (Macgregor et al., 2019; Roy et al., 2015). As host plant phenology is also affected by climate change, insects must rapidly adapt their phenology to synchronize with these critical resources for survival and fitness (Van Asch et al., 2013; Van Asch et al., 2007). However, if microclimate temperatures become too warm for insects to feed and forage, they may alter their behavior and activity patterns to avoid these conditions, which can be challenging depending on the species' level of plasticity in their responses to temperatures and photoperiod, as well as any ecological interactions that may limit their ability to adapt (Andrew and Terblanche, 2013).

Growth rate, reproduction and abundance

The response of insects to rising temperatures can be positive, such as increased reproductive potential, as long as species growth maxima are not surpassed. As ambient temperatures generally increase toward optimal temperatures for growth and development of many insect species, possibly reducing thermal constraints on population dynamics, the abundance of insects expected to increase under global warming scenarios (Skendžić et al., 2021). Higher CO₂ levels cause changes in the C: N ratio of plants, leading to an increase in the consumption of plants by insect pests, as nitrogen is essential for insect growth (Bezemer et al., 1998). This increased consumption can result in greater plant damage, as insect need to consume more plant tissue to obtain the same energy. When nitrogen levels drop, as expected due to CO₂ fertilisation, leaf feeders increase their consumption rates to compensate nutrient deficiency (DeLucia et al., 2008; Hamilton et al., 2005). Under increased temperature conditions, the egg, larva, and pupal stages' developmental periods shorten, as demonstrated by Karolewski et al. (2007) that the growth period of the gypsy moth *Lymantria dispar* (L.) was significantly reduced in their laboratory experiment as a result of an increase in temperature. Warmer weather shortens the life cycle of insects, resulting large number of generations and faster life histories. This causes more generations in a cropping season and can aggravate the pest complex (Puri and Ramamurthy, 2009). This quicker development means shorter exposure to adverse conditions such as low temperatures, parasitoid attacks, predator attacks, and entomopathogens. This shorter exposure can lead to the reproductive success of many insect species. However, crossing a specific optimum temperature range can reduce growth rates, fecundity, and increase mortality rates for many species (Rouault et al., 2006).

Increased overwinter survivability and winter performance

Insects face a significant challenge in overwintering in temperate regions, as highlighted by Williams et al. (2015). With climate change, winters are anticipated to become more variable in addition to warmer. The severity of cold stress is determined by extremely low temperatures, which disrupt ion balance in crucial tissues, leading to membrane depolarization and eventually programmed cell death and chilling injury (Bayley et al., 2018; Overgaard and MacMillan, 2017). Crossing the minimum threshold temperature has been shown to have additional energetic and fitness costs for insects (Marshall and Sinclair, 2018). So, warming of winter may lead to higher winter survival in insect due to lower survival cost. Furthermore, increased mean temperatures during the winter can cause energy depletion for insects unable to feed during this time, leading to decreased fitness (Sinclair, 2015). So, winter performance may have mixed performance in different species based on their adaptation strategy and cold hardiness mechanism.

Diapause

Insects have developed several survival strategies to overcome unfavorable winter conditions, including diapause, a dormant period during which an insect's metabolic and physiological processes are slowed down. In temperate insects, the initiation and termination of winter diapause are critical life-history changes that regulate the time spent on development and population growth (Kostal, 2006). Several studies have shown local adaptation in the timing of diapause in both insect pests and natural enemies (Lindstad et al., 2019; Posledovich et al., 2015; Paolucci et al., 2013), indicating that they may evolve in response to changing climate conditions (Bradshaw et al., 2001). Studies on genome-wide association suggest that local adaptation in diapause induction and termination timing is linked to variation in circadian clock genes of insects (Kozak et al., 2019; Pruischer et al., 2018). Typically, short day lengths at the end of the growing season and the beginning of harsh winter conditions are used as a cue for diapause induction (Lindstad et al., 2019; Kostal, 2006; Hodek, 1996). However, the adaptive value of photoperiodic induction relies on historical relationships between photoperiod and specific value of climatic variables such as temperature and rainfall. Rapid climate change can distort these correlations, leading to unsuitable diapause timing (Kerr et al., 2020; Van Dyck et al., 2015). Additionally, the timing of

diapause termination and subsequent spring phenology is generally affected by temperature during winter (Lehmann et al., 2017; Kostal, 2006; Hodek, 1996). Therefore, climate change can impact diapause patterns and spring phenology in complex ways, altering the phenological synchronization to essential resources and enemies, thus affecting both ecological and evolutionary processes in insect populations (Kharouba et al., 2018; Forrest, 2016).

Impact on insect ecology

Climate change is expected to have a significant implication for different aspects of insect ecology. It can create favorable conditions for pests to thrive and expand their range. Insects have distinct life stages that can vary in their responses to environmental factors (Kingsolver et al., 2020). Some pests, which are currently restricted to small areas or low densities, may be able to exploit the changing conditions and distribute more widely, leading to damaging population densities (Bale et al., 2002; Porter et al., 1991). Climate change can also affect the synchronization within and between insect species. Even though parasitoids respond to climate change in similar ways to herbivores, their close relationship with their hosts and their particular life cycle make host-parasitoid synchrony and population cycles challenging under changing climate (Tougeron et al., 2020). Insect communication, which relies on volatile compounds, may also be impacted by changes in temperature and humidity. These changes can affect the production, release, and detection of these compounds, thereby altering the behavior and survival of insects. Understanding these potential impacts is also important for developing effective strategies to control insect pest.

Distribution: Geographic range expansion and migration behaviour

The extent and range of occurrence of an insect species is very much influenced by the climate of a region (Ramamurthy, 2009). So, unfavourable climate is one of the factors that decides the boundary for the distribution of a particular insect species. Insects have species-specific climatic requirements that are crucial for their development and survival. Climate change may have a significant effect on the geographic distribution of insect species, and low temperatures are often more critical in determining their geographic range than high temperatures (Skendžić et al., 2021). Upper latitudinal margins limit the distribution of a species due to relatively cool climate (Ramamurthy,

2009). Insect species are moving towards polar areas whose expansions were previously restricted by acute low temperatures, and the range boundary is determined by climate extremes rather than mean temperatures (Lynch et al., 2014; Battisti et al., 2005). Northern and southern range shifts are predicted for many insect species towards higher altitudes and elevations (Menéndez, 2007; Parmesan and Yohe, 2003). Many of the species expanding their geographic range are thermal generalists, and their expansions are driven by declined winter mortality (Lancaster et al., 2016). Predicted changes in temperature, CO₂, and precipitation patterns resulting from climate change will influence the distribution, geographic ranges, and migration behavior of insect species (Fand et al., 2012). These changes may create new ecological niches and remove low-temperature barriers, providing opportunities for insect pests to spread across physical and political boundaries, threatening food security (FAO, 2008). Climate change and the resulting changes in land use are expected to cause an overall global pattern of increasing latitudinal and altitudinal range of crop pests (Barzman et al., 2015; Gregory et al., 2009). Over longer timescales, insect species in their current habitat may respond to changing climates in various ways. They may adapt to new climatic conditions in their existing habitats through genetic, physiological, or behavioral changes. Alternatively, they may adapt to new climatic conditions in their present range while simultaneously extending their range to remain within their current climatic envelope. Populations may also not adapt to the changing local climatic situations and shift their range entirely to stay within their optimal climatic envelope or go locally extinct, leading to the population's eventual extinction (Andrew and Terblanche, 2013). The ability of a species to respond to the increasing temperature and expand its range will depend on number of life history characteristics, making the possible response quite variable among species (Subrahmanyam et al., 2009).

Tritrophic (interspecific) interaction and chemical Ecology

Climate change is changing ecosystems by altering composition of communities, and trophic interactions. Climate change can increase exposure to heat stress and result in phenological and morphological mismatches with adjacent trophic levels (Abarca and Spahn, 2021). Increasing temperatures and CO₂ have a profound impact on the insect physiology which in turn, can have significant consequences on interactions between crops and herbivores (Caulfield and Bunce, 1994). As

climate change alters plant phenology, it also affects the growth and abundance of herbivores feeding on these plants, which consequently affects natural enemies by altering their availability of prey and hosts. Due to changes in plant phenology, there may be a reduction in predation and parasitism by natural enemies, since an increase in plant foliage and variations in herbivore life cycle can have a significant impact on their availability as prey (Coviella and Trumble, 1999). Changes in flowering and growth pattern of host plant due to climate change can create a serious imbalance in insect host plant relationships and their tri-trophic interactions (Puri and Ramamurthy, 2009). While changing synchrony with their host plant, insect same time adapting to the climate variables (Ramamurthy, 2009). Further, numerous studies conducted in a controlled environment have shown that changes in temperature, CO₂, precipitation, relative humidity, and other environmental factors can alter the production of secondary metabolites and defensive traits of host plants, which can have a considerable effect on insect-plant interactions (DeLucia et al., 2012; Zavala et al., 2008; Zvereva and Kozlov, 2006). These changes can decrease the plant defense system against insect pests, thus making them vulnerable to damage (Dhaliwal et al., 2010). Moreover, elevated temperature and CO₂ levels can affect the herbivore-induced plant volatile (HIPV) and the olfactory perception of volatiles, which can affect the host-finding ability of natural enemies and the efficiency of biological control (Bruce and Pickett, 2011; Thomson et al., 2010; Gouinguéné and Turlings, 2002). Several studies have also shown poor synchronization between parasitoids and their hosts (Chen et al., 2007; Grabenweger et al., 2007). Phenological shifts within and in between trophic levels are changing biotic interactions. These asynchronies contribute to restructuration of insect communities (Damien and Tougeron, 2019). So, climate change has far-reaching consequences on the interactions between plants, herbivores, and natural enemies.

Efficacy of behavioural method: Pheromone communication

It is likely that higher temperatures and changing atmospheric CO₂ and O₃ levels will affect the entire process of pheromonal communication, from production to behavioral response. Insects that rely on long-range chemical signals for communication are expected to be most affected, as these signals may be exposed to oxidative gases during dispersal (Boullis et al., 2016). Unsaturated terpenes, which are constituents of sexual,

alarm, or aggregation pheromones in several insects, such as aphids (Boullis and Verheggen, 2016; Francis et al., 2005), bark beetles (Taft et al., 2015), and fruit flies (Sarles et al., 2015), may be altered by ozone. Moreover, raised temperatures are expected to impact pheromone biosynthesis both qualitatively and quantitatively, as insect's enzymatic activities are influenced by its changing body temperature with the environment (Neven, 2000). For example, the ratio of compounds in the sex pheromone may change with temperature in the potato tuber moth *Phthorimaea operculella* (Ono, 1993). Additionally, warmer rearing conditions of the larvae of *Philanthus triangulum* increased the production of pheromonal secretions in adult males and the percentage of compounds with high molecular weight in pheromones (Roeser-Mueller et al., 2010). Temperature changes may also alter the shape of scent plumes and affect the efficiency of insects reaching their target in pheromones dispersed over long distances (McFrederick et al., 2009). Furthermore, elevated CO₂ levels can alter the ability of aphids to produce and/or respond to the alarm pheromone, which impacts their defensive behaviors (Sun et al., 2010). Several reports demonstrate that climate change by increases in temperature and atmospheric gas concentrations can interfere with insect pheromone communication in insects (Renou and Anton, 2020). As climate change may also modify the behavioral reaction of insects to pheromones and allelochemicals, all semiochemical-based insect management strategies may be impacted. Evidence suggest that climate change affects pheromone molecule stability, thus reducing its biological efficacy under field, so climate change could be a general problem for chemical communication in insect species (El-Sayed et al., 2021).

Effect on insect indirectly through host plants

The effect of climate change on insects will by indirect too due to the change in vegetation affected by climate change. Vegetation not only responds to climate change but also create a distinct microclimate pattern (Ramamurthy, 2009). Insect's life cycle and development are intrinsically linked to their host plant phenology, which is primarily governed by temperature conditions in the surrounding environment (Bale et al., 2002; Szujekki, 1998). So, alterations in temperature, CO₂ and humidity levels can impact insects by changing the host plant's physiology and metabolism (Netherer and Schopf, 2010; Moore and Allard, 2008; Stevens et al., 2004). Furthermore, an increase in average temperature can affect plants and phytophagous insects

differently, potentially disrupting the synchronization of critical processes at different trophic levels within an ecosystem (Visser and Holleman, 2001). Increased CO₂ levels are expected to affect plant physiology by enhancing photosynthetic activity, leading to improved plant growth. While C4 plants are less sensitive to increased CO₂ and less likely to be impacted by changes in insect feeding behavior, C3 plants are more likely to be positively affected by elevated CO₂ levels and negatively influenced by insect feeding (Lincoln et al., 1984). Plants' carbon: nitrogen ratio changes with increased CO₂ levels since they tend to store more sugars and starches in their leaves (Cotrufo et al., 1998) which may further affect insect feeding behavior and metabolism. Changes in the frequency, duration, and intensity of rainfall are predicted under climate change (Chen et al., 2019). This rainfall change will affect the soil moisture, which is important for many soil dwelling insects (Ramamurthy, 2009).

CONCLUSIONS

Greenhouse gases emission are considered major cause of climate change and agriculture sector responsible for approximately 21% of global greenhouse gas emissions. Insects, being highly dependent on the thermal conditions of their environment, are highly vulnerable to climate change. Predicting the impacts of climate change on insect pests is challenging due to the complex interactions of increasing atmospheric CO₂ concentration, changing climatic regimes, and altered intensity/ frequency of extreme weather events. Increasing of temperatures, atmospheric CO₂ levels, and altered precipitation patterns impacting insect directly and indirectly. While insects are adaptable organisms that respond differently to various impacts of climate change, the rising temperature can lead to a range expansion, increased overwintering survival, and changes in their interactions with host plants and natural predators. The rising CO₂ level can affect the consumption rates, growth rates, fecundity, and population densities of insect pests. Altered precipitation patterns can favor the incidence of droughts and floods, which can affect insect survival and diapause. Some species are better adapted to changing climates and may proliferate and become invasive while a large number of species are unable to adjust their complex life event to changing dimensions of climate. Even though impacts on insect pests, induced by climate change are variable and subject to various interacting factors, it is expected to cause an increase in the latitudinal and altitudinal distribution range of insect species. The

distribution and range of insect species are shifting, with many pests spreading beyond their traditional boundaries. Further, it may also impact phenological synchronization and patterns of species interactions, and species complex of a region. Additionally, the impact of climate change on insect ecology is significant and multifaceted, affecting insect communication, behavior, and interactions with plants and natural enemies. Changes in plant phenology and defensive traits, coupled with altered insect communication systems, pose further challenges to plant-herbivore-natural enemy interactions, potentially affecting the efficiency of biological control. Also, climate change together with continuously increasing trade can be favourable for invasive phytophagous insect species. Understanding these complex interactions is crucial for developing effective strategies to correctly predict the future trend of insect pest and successfully managing them.

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A REVIEW ON VECTOR BORNE DISEASES AND VARIOUS STRATEGIES TO CONTROL MOSQUITO VECTORS

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ABSTRACT

Mosquito vector transmit serious infectious diseases that include dengue, chikungunya, malaria, filariasis, leishmaniasis, Japanese encephalitis, west Nile fever, yellow fever and rift valley fever. Insecticides-based control measures have historically and currently been an important control approach against major mosquito-borne diseases. Chemical pesticides, on the other hand, are non-selective and can harm other beneficial organisms. Controlling mosquitoes with entomopathogenic bacteria is a convincing, ecologically acceptable alternative to chemical pesticides. *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* are insecticidal microorganisms which are spore forming and these bacteria are the most extensively utilised alternative mosquito control agents, considering the rapid development of resistance, especially to *B. sphaericus* by the larvae of *Culex* spp. Under this circumstances, it is necessary to find an alternative biological control agents from natural resources. The present review focus mainly on the various strategies to control mosquito vectors.

Key words: *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles*, vector ecology, vector borne diseases, vector control, pesticides, biological control, *Bacillus thuringiensis*, *Bacillus sphaericus*

Vectors are living organisms that can spread infection or illness among human or between animals and humans. Many of these vectors fall under arthropods which feed on disease-causing microbes while feeding on blood from infected host (animal or human) and then spread it to another victim during their next blood meal. Dengue fever, malaria, leishmaniasis, schistosomiasis, yellow fever, Chagas disease, onchocerciasis and lymphatic filariasis are vector-borne diseases caused by infection of virus, bacteria and parasites in human populations, infecting over one billion people each year and killing over one million people (WHO, 2014). Disease causing vectors include mosquitoes, ticks, flies, sandflies, fleas, beetles, and freshwater snail. Mosquitoes are the most common disease vector (WHO, 2014). It has long been known that mosquitoes are the most significant arthropods that affect human wellness (Foster and Walker, 2019). Mosquitoes are significant vectors of a variety of arboviruses, protozoa, and helminths, and their negative consequences are far reaching. Mosquitoes provide a significant public health risk by transmitting multiple tropical diseases. Mosquitoes can be found in a variety of water-related habitats. Malaria, filariasis, yellow fever, and dengue fever are mosquito-borne diseases that cause significant morbidity and

mortality, as well as a significant economic burden in disease-endemic nations (Jose, 2021). *Ae. albopictus* and *Ae. aegypti* are capable vectors of five important arboviruses (viruses of YF, Zika, RVF, DEN and CHIK) and are broadly distributed, making these species a significant contributor to the global burden of infectious diseases (Moyes et al., 2017). Malaria, yellow fever, dengue, haemorrhagic fever, and filariasis are spread by *Anopheles*, *Aedes*, and *Culex* species (WHO, 2016). As a result, managing these species of mosquitoes is critical for controlling the mosquito-borne diseases. Controlling the proliferation of vector and non-vector mosquito populations is an urgent need in order to prevent vector-borne diseases and their nuisance using appropriate management strategies. There are no vaccinations for various vector-borne diseases, and medication resistance is on the rise (WHO, 2014). Vector control is acknowledged as an effective technique to prevent all these diseases, but it is not being done to its full potential (WHO, 2014). Vector control is a critical and promising method to prevent spreading of vector-borne diseases. Every year, hundreds of millions of dollars are spent globally to protect people from being bitten by mosquitoes, which are present on all continents except Antarctica (Diagne et al., 2020).

Vector ecology

The study of disease-carrying organisms, their behaviours, and the settings in which they thrive is known as vector ecology. Climate elements such as temperature, rainfall, humidity, wind, and daylight duration have a significant impact on mosquito ecology, development, behaviour, and survival, as well as the transmission dynamics of the diseases they spread (Reiter, 2001). Except in deserts and cold places, mosquitoes can adapt to a wide range of environments (Becker et al., 2003). Climate change has raised concerns that the spread of mosquito-borne diseases would become more severe due to higher vector survival and bite rates, faster pathogen replication inside vectors, shorter reproduction rates, and longer transmission seasons (Mordecai et al., 2019). Compared to urban environments, natural and rural areas have greater levels of mosquito diversity and abundance (Ferraguti et al., 2020). There are about 3000 or more mosquito species worldwide (Hillary and Ceasar 2021). Except a few species, mosquitoes are mostly harmless to humans. Among mosquito species *Aedes*, *Anopheles*, and *Culex* are the three major mosquito vectors capable of spreading illnesses. Ecology of *Aedes*, *Anopheles* and *Culex* are given below.

Aedes

Some *Aedes* species breed in ground pools and marshes, such as snowmelt pools in arctic regions, particularly tropical species, numerous *Aedes* are encountered in natural habitats like leaf axils, tree holes, stumps of bamboo, rock pools, or artificial habitats such as open tin cans, tyres and water storing pots that are prone to drying out; thus, the ability of eggs to endure moisture loss is clearly helpful (Service, 2012). *Ae. aegypti* is the main vector of dengue as well as Zika, Yellow fever and Chikungunya (WHO, 2022). In Southeast Asia, the secondary dengue vector *Aedes albopictus* breeds both in wild and man-made container habitats (Service, 2012). Both *Aedes aegypti* and *Aedes albopictus* feed during the day; their prime feeding phases are early morning and before sunset (Service, 2012). They rest indoors, mostly in bedrooms and living rooms. This increases human-vector contact while minimising contact with insecticides applied outdoors, making it more challenging to manage this vector. *Aedes aegypti* is capable of breeding in dirty water or small container having little collections of water. Once a female has set her eggs, the eggs can survive in arid conditions over several months before hatching and hatch when exposed to water (WHO, 2022).

Anopheles

Anopheles mosquitoes are medically significant due to their links to malaria, filariasis, and other arbovirus diseases (WHO, 2022). Only a few of *Anopheles* species are vectors of human diseases, with 30–40 *Anopheles* species transmitting malaria in nature (Nicoletti, 2020). Adult anophelines favoured small habitats such as man-made ponds, natural ponds, drainage ditches, swamps, tyre tracks, and puddles for breeding, while increased vegetation cover lowered *Anopheles* larval counts (Hinne et al., 2021). *Anopheles* is mainly encountered in pure, pollution free streams, and is rarely seen in settings with rotting vegetation or faeces (Service, 2012). Rainfall alters the amount of breeding habitats, as more habitats are generated during the rainy season, which enhance the richness of *Anopheles* mosquitoes in all geographical areas.

Culex

In humans, the *Culex* mosquito transmits Japanese encephalitis, Lymphatic filariasis, and West Nile disease. *Culex* mosquitoes breed in a variety of stagnant water conditions. Catch basins rainwater barrels, septic tanks and storm drains are preferred oviposition habitats because they are rich in organic material (Valent Biosciences, 2022). *Culex* mosquitoes are associated with urbanisation, particularly in areas with poor drainage and sanitation. *Culex* species bite people and other hosts at night (Service, 2012).

Vector borne diseases (VBDs)

Human diseases caused by bacteria, parasites and viruses which are carried from individual to individual by vectors are known as vector-borne diseases (WHO, 2020). Each year, almost 700,000 people die from diseases like Dengue, Leishmaniasis, Malaria, Schistosomiasis, African Trypanosomiasis, Japanese Encephalitis, Chagas Disease, Onchocerciasis, and Yellow Fever (WHO, 2020). Many VBDs have been found to have expanded geographic distribution and a rise in the frequency of epidemic transmission (Muller et al., 2019). The two major mosquito-borne diseases, malaria and dengue, are reemerging in locations where they had previously receded for decades and are gradually expanding their geographic range (Watts et al., 2019). Due to an expanded geographic range and prolonged transmission season brought on by climate change, malaria and dengue transmission could increase (Messina et al., 2019; Iwamura et al., 2020). Almost 40% of the world's population is at risk from mosquito-borne diseases, including human malaria, dengue

fever, chikungunya fever, Zika virus (ZIKV) disease, and lymphatic filariasis which are a major worldwide health issue (Franklinos et al., 2019; Jones et al., 2021). More than 1 million people globally die each year from mosquito-borne diseases such as malaria, dengue, Zika, chikungunya, yellow fever, and others, with the majority of these deaths occurring in undeveloped areas (Yang et al., 2021).

Malaria

Malaria is a prominent vector-borne disease in tropical and subtropical areas killing significantly more people than all parasitic diseases combined (Talapko et al., 2019). Plasmodium parasites causing Malaria are spread to humans by female *Anopheles* mosquitoes that have been already infected by them (WHO, 2022). Variations in mosquito vectors and malaria parasites, as well as ecological conditions affect malaria transmission. Only 30–40 *Anopheles* species spread malaria in nature, out of approximately 530 species (Nicoletti, 2020). With more than 200 million cases in 2018, malaria remains a significant public health burden worldwide. Around 400,000 people die each year from malaria despite there being viable treatments and control measures, mostly in sub-Saharan Africa (WHO, 2019). Based on the most recent worldwide report of malaria data, there was a rise of 241 million cases of malaria in 2020 from 227 million cases in 2019. (WHO, 2022). Vector control is an important aspect of malaria control and eradication methods since it is a highly effective approach to minimise malaria transmission. In the previous year, two different initiatives—the Lancet Commission on Malaria Eradication and the World Health Organization’s Strategy Advisory Group for Malaria Eradication—analyzed potential futures and came to the conclusion that malaria eradication is possible while identifying critical priorities (Feachem et al., 2019; WHO, 2020).

Chikungunya

Chikungunya is a tropical viral illness which is caused by the Chikungunya virus, belonging to Togaviridae family (WHO, 2022). It is spread by *Aedes*, primarily *Aedes aegypti* (Cunha et al., 2017). The main vectors of chikungunya virus in the regions of Indian Ocean and Asia are *Aedes aegypti* and *Aedes albopictus*, and disease outbreaks occur during the rainy season, when vector population rises (Pialoux et al., 2007). It is believed that the virus of chikungunya originated in Africa and was first found during an outbreak in Tanganyika in 1952–1953. (Presti et al., 2014). The

first urban epidemics were noticed in 1967 in Thailand (Wimalasiri-Yapa et al., 2019). Chikungunya epidemics have been observed in India from 1963 to 1973 and 2005 to 2019. Chikungunya is indigenous in twenty-four Indian states and six union territories, showing that it is a significant public health issue in our country (Translational Research Consortia, 2021). Maharashtra, Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka, Madhya Pradesh and Kerala are the states affected by chikungunya. Vaccine and drugs for chikungunya are not available to prevent or treat the infection (National Centre for Vector Borne Diseases control, 2022). Therefore, the most available efficient measures for preventing chikungunya are mosquito management and personal safeguarding from mosquito bites (Pialoux et al., 2007; National Centre for Vector Borne Diseases control, 2022).

Lymphatic filariasis

Elephantiasis is a neglected vector-borne tropical illness caused by infection with the filarial worms such as *Brugia timori* (*Brugia* spp.), *Brugia malayi* and *Wuchereria bancrofti* transmitted by mosquito species. It damages the lymphatic system and can cause hydrocele and lymphoedema (elephantiasis) in affected people (WHO, 2022). Globally, it is estimated that lymphatic filariasis affects about 15 million people with lymphoedema and 25 million men with hydrocele (WHO, 2022). Infection with parasitic nematodes (roundworms) of the family Filariodidea causes lymphatic filariasis (WHO, 2022). Lymphatic filariasis is a primary factor of long-term illness that affects people in tropical and subtropical regions of Asia, the Western Pacific, Africa, and parts of the Americas (Bockarie et al., 2008). It is spread by several mosquito species, including the *Culex*, *Anopheles*, and *Aedes* mosquitoes. *Culex quinquefasciatus* is the most common filariasis vector. Around 1 billion people were at risk of contracting lymphatic filariasis (LF) from 72 nations before the World Health Organization began the Global Program to Eradicate Lymphatic Filariasis. Approximately 36 million people were affected by the related morbidity (Fang and Jang, 2019).

Dengue

The most typical arthropod-borne viral disease with severe public health effects is dengue fever. Dengue is a disease that can affect over 2.5 billion people and is present in over 100 nations (Fonseca et al., 2002). *Ae. aegypti* and *Ae. albopictus* mosquitoes both spread the virus that causes it (WHO, 2022). Humans are the

primary host. The disease is prevalent in the warm temperate and tropics regions (Chan et al., 2006). Humans contact the virus by bites from infected female mosquitos. Dengue haemorrhagic fever and dengue fever are two well-known clinical manifestations. There are 4 different dengue viruses (serotypes) namely, Dengue virus-1, Dengue virus-2, Dengue virus-3, and Dengue virus-4 that are antigenically related (WHO, 2022). The Americas, Southeast Asia, and the Western Pacific regions are severely affected, with Asia accounting for 70% of the worldwide disease load (WHO, 2022). Globally, in the year 2019, the highest number of dengue cases was reported. All regions were affected, for the first time, Afghanistan was affected by dengue (WHO, 2023). Dengue fever has been endemic throughout Southeast Asia since the 1950s, when DHF/DSS was first identified (Fonseca et al., 2002). Dengue fever and its severe version, DHF or DSS, became one of the world's leading infectious diseases as the mosquito, *Aedes aegypti*, rapidly colonised urban areas in tropical countries (Halstead, 2002). Sanofi-dengue Pasteur's vaccine (Dengvaxia) has received approval in a number of nations, although there are safety concerns for mass delivery (Aguiar et al., 2016). Although dengue vaccine has been developed, WHO recommends nationwide dengue control programmes that use environmental management methods and pesticides (WHO, 2016).

Yellow fever

Because it is transferred among vertebrate hosts by arthropods, yellow fever virus is called as 'arbovirus'. YF is an acute severe sickness in humans that causes vomiting, hepatitis with jaundice, shock, epigastric pain, fever, nausea, renal failure, bleeding, and death in 20–60% of cases (Monath, 2015). YF is caused by the genus *Flavivirus*, which encompasses roughly 70 positive-strands, single-strand RNA viruses, most of which are spread by mosquitoes and ticks (Monath, 2015). Female *Aedes aegypti* is the principal yellow fever vector. The virus is spread from one host to another, primarily between monkeys, people and monkeys and humans (WHO, 2014). From the 18th century to the early twentieth century, YF was a major hazard to public health, with epidemics recurring after introductions to coastal towns and cities far from endemic areas in the Caribbean, Europe and North America. (Monath, 2015). Yellow fever is prevented by an effective vaccine, which is safe and affordable (WHO, 2023). One of the first viral vaccines, the yellow fever vaccine, has proven to be safe and convincing (WHO, 1998). The virus employed in this vaccination study was virus 17 D. In 1951, the Nobel Prize of

Physiology or Medicine had been given to Max Theiler for the discovery of a convincing vaccine against yellow fever (Norrby, 2007). The best yellow fever treatment is prevention through a vaccine (WHO, 2022). A total of 203 confirmed cases, 252 probable cases, 40 deaths (a case fatality ratio of 9%), and 13 countries in the WHO African Area reported cases to WHO between January 1 and December 7, 2022 (WHO, 2023).

Japanese encephalitis

The Japanese encephalitis virus is a zoonotic flavivirus infecting a huge spectrum of vertebrate animals, especially large waterfowl birds and swine, in an enzootic cycle (Endy et al., 2002). The Japanese encephalitis virus is a flaviviridae virus that is spread between animals and humans via *Culex* mosquitoes. Although Japanese encephalitis virus has been isolated from over 30 species, the virus is primarily transmitted by mosquitoes breeding in paddy fields of the *Cx. vishnui* subgroup, especially *Cx. tritaeniorhynchus* (Van den Hurk et al., 2009). Temperature and abundant bodies of water, such as those found in paddy fields across most of Asia, provide an excellent habitat for vectors and viral multiplication within the mosquito hosts (Endy et al., 2002). Historical perspective epidemics of Japanese encephalitis was documented in Japan since 1871 (Erlanger et al., 2009), but the first big epidemic occurred in 1924 (Endy et al., 2002), including approximately 6000 patients (Endy et al., 2002; Van den Hurk et al., 2009). Children and young people are the most vulnerable to this disease. JE epidemics have been documented in various places of India, and it is considered a major paediatric issue (Tiwari et al., 2012).

Although JE remains a main public health issue in Asia, the number of cases reported is reducing, with only 10,426 cases reported in 2011. This is thought to be caused by JE immunisation (particularly in China and India), agricultural pattern changes, urbanisation, and advanced living environment (Wang and Liang, 2015). Estimation of the real global burden of disease and development of effective JE-control approaches must be strengthened in Japanese encephalitis endemic region of India in order to avoid JE-related morbidity, mortality, and sequelae. JEV transmission can be prevented and controlled by one of the three tactics, each of which targets a different stage of the transmission cycle: (a) human vaccination, (b) control of increasing hosts via swine immunisation or changing livestock breeding style, or (c) vector management (Van den Hurk et al., 2009). Mosquito repellents, insecticide-impregnated mosquito bed nets, and clothing that cover the full

body and prevents from bug bites are all used in vector management.

Mosquito vector control measures

Vector control is crucial in the fight against pandemic vector-borne diseases. In many cases, vector control is the only method to avoid disease epidemics (WHO, 2014). Vector control measures aim to limit disease transmission by reducing the quantity of disease vectors or interactions between animals and disease vectors, as well as people (WHO, 2016). Indoor Residual spraying, insecticide-treated nets, or self-protection measures, depending on the parasite-vector species, may help to protect individuals from infection (WHO, 2022). Classically, mosquito control efforts have centred on killing mosquitoes with a range of insecticides. Insecticide-based vector control is crucial for the prevention and treatment of infectious diseases like malaria, dengue fever, and filariasis (Gillbert and Gills, 2010). Controlling mosquitoes can be achieved using different measures.

Chemical control

Chemical techniques have been promising in limiting disease spread to endemic regions (Demirak and Canpolat, 2022). Chemical insecticides were once the main method of controlling mosquitoes, however in recent years, mosquitoes have developed widespread chemical resistance (Peng et al., 2022). Arsenicals, which are stomach poisons, are among the first generation of insecticides. The second generation consists of contact insecticides including chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids (Becker et al., 2003). Organophosphates (OPs), Organochlorines (OCs), Carbamates (Cs), and Pyrethroids (PYs) are the most common insecticides. They work by damaging the neurological system of insects. Insecticides may harm nontargeted organisms, due to similarities in nervous system components that make it nearly impossible to find insecticides that exclusively impact insect pests (Prasad et al., 2013). There are three types of insecticides: natural organic, inorganic and synthetic organic. The majority of pesticides used to control mosquito vectors are synthetic compounds (Becker et al., 2003). To stop the spread of mosquito-borne diseases, man-made insecticides are used widely due to the appearance or re-emergence of mosquitoes in endemic, non-endemic, and new areas of the world (Demirak and Canpolat, 2022). The widespread usage of insecticides in recent decades has had a negative impact on the environment

and public health (Tembhare, 2016). Some insecticides degrade water quality or have other negative effects on organisms (Prasad et al., 2013). Majority of synthetic pesticides are neurotoxic to the pests (Olson et al., 2015). The emergence of physiological resistant in vectors, environmental degradation that causes biomagnification of food chain contamination, and deleterious effects on beneficial animals have all made the use of chemical insecticides difficult (Poopathi et al., 2010). A sustained and high selection pressure as a result of repeated applications of a mono class of insecticide (or another with the same mechanism of action) is the primary source of resistance development in insects in the field (Gillbert and Gill, 2010). Alternatives to conventional mosquito control methods that are safer, biodegradable, and target-specific have been researched due to the emergence of resistant mosquitoes and worries about toxicity to both target and beneficial organisms. (Demirak and Canpolat, 2022). Insecticide resistance has been observed in various mosquito species of *Anopheles*. The emergence of mosquito resistance to extensively used pesticides in dengue and malaria control has a significant negative impact on the management of these disease mosquito vectors (Poopathi et al., 2010). Current mosquito control research is aimed at better understanding of mosquito resistance to synthetic insecticides and devising new tactics to combat resistance (Demirak and Canpolat, 2022).

Biological control

Biopesticides are non-toxic, environment friendly pesticides made from living organisms including microorganisms, animals and plants that can control severe plant destructing insect pests and diseases causing vectors. They are becoming increasingly important around the world. Biological control has emerged as the most viable alternative pest control strategy for controlling vectors while avoiding negative consequences for the environment and nontarget organisms. Fungus spores were first utilised as a biopesticide in the late 1800s to manage insect infestations. One of the earliest documented uses of a biopesticide is when Agostine Bassi, an Italian entomologist showed in 1835 that the white-muscadine fungus spores (*Beauveria bassiana*) could infect silkworms and kill them by causing white muscardine disease. Since then, the use of biopesticides has persisted uninterrupted throughout the history of modern agriculture, but it has only ever represented a minor industry in comparison to traditional crop

protection. The mode of action, which includes mating disruption, anti-feeding, asphyxia, and desiccation, is what distinguishes biopesticides from synthetic pesticides (Olson et al., 2015). It is hoped that in the future, in addition to parasites and predators, more focus will be placed on biological control through introducing microbial agents (Tembhare, 2016). Natural molecules that are more effective and less hazardous than man-made compounds continue to gain popularity among scientists (Demirak and Canpolat, 2022). Compared to traditional synthetic pesticides, crude bacterial extracts are far less likely to lead to the development of mosquito resistance because they almost always contain numerous compounds with insecticidal activity (Engdahl et al., 2022). To lessen the selection pressure for pesticide resistance, environmentally friendly alternatives have been investigated. These numerous biocontrol strategies aim to target distinct stages of the mosquito lifecycle in an environment friendly and long-lasting way.

As effective treatments for diseases like dengue and Zika are not yet broadly available, conventional insecticide-based vector control methods continue to be a majorstay (Namias et al., 2021). Contrary to pesticides, bio-control agents are host-specific, environmentally safe, easier to implement in the field, more affordable to manufacture, non-pathogenic to mammals, including humans (Prasad et al., 2013). Microbes are one of the current natural sources being investigated for the development of novel mosquitocidal treatments (Caragata et al., 2020; Dahmana et al., 2020). Organisms including fungi, viruses, bacteria, nematodes, protozoa, invertebrate predators and fish have been investigated for controlling mosquito vectors. However, copepods, some spore-forming bacteria and fish have been successfully tested in the wild (Poopathi et al., 2010). Although while research into natural sources for novel compounds with mosquitocidal action has resurged, relatively few natural products have succeeded in becoming approved vector control agents (Marrone, 2019). Typically, one or more issues with toxicity, strict regulatory requirements, efficacy, prices and availability are the cause of this failure. In addition to that, a new product must be outstanding to the ones that are already on the market from a marketing perspective (Demarque & Espindola, 2021).

Biopesticides and other insecticides have been developed, mostly for the control of mosquito larvae. Apart from being safe for the environment and non-target creatures such as humans, these microorganisms

have certain significant advantages over traditional insecticides in mosquito control operations (Poopathi et al., 2010). *Bacillus thuringiensis* (Brühl et al., 2020) and *Bacillus sphaericus* are already utilised as effective pesticide alternatives. They have been quite efficient against *Anopheles*, *Culex*, and *Aedes* species for the past two decades, though *Bacillus sphaericus* is particularly powerful against *Culex* (Prasad et al., 2013). Despite the fact that few mosquito species (such as *Culex quinquefasciatus* in the lab and *Culex pipiens* in the field) have developed resistance to *Bt* and *Bs*, other species, such as *Aedes vexans*, never had developed resistance despite being exposed to the pesticide for more than 25 years. Hence, in the field of microbial pesticides, *Bt* and *Bs* are still maintaining their reliability (Hegazy et al., 2022).

Bacillus thuringiensis

Bacillus thuringiensis is a naturally occurring, spore forming bacterium. During the sporulation phase, this bacterial species produces insecticidal proteins concentrating in a parasporal body (PSB) termed the protein crystal, which mostly consists of more than one protein named Cry and Cyt toxins (Gilbert and Gill, 2010). When consumed by vectors, *Bacillus thuringiensis* (*Bt*) damages the insect gut (Olson et al., 2015). The entomopathogenic features of *Bt* separate it from other members of the *B. cereus* group, and *Bt* entomopathogenic activity is mostly attributable to Cry toxins (Gilbert and Gill, 2010).

The parasporal body (PSB) of *B. thuringiensis israelensis* is responsible for the insecticidal impact. The PSB comprises of four main toxin proteins with various molecular weights: Cry4A (125kDa), Cry4B (135kDa), Cry10A (58kDa), and Cry11A (68kDa) (Delecluse et al., 1996). This bacterial bio-pesticide seems to remain longer in the environment, especially in dirty water, and hence could be a suitable choice for long-term mosquito control (Prasad et al., 2013).

Bacillus sphaericus

This bacterium produces either terminal or sub-terminal endospores and is an aerobic, gram-positive, rod-shaped spore-forming bacterium. It is found in both soil and aquatic habitats. It produces binary toxic proteins during sporulation with molecular weights of 51.4kDa and 41.9kDa that are harmful to mosquito larvae (Becker et al., 2003). *Anopheles* and *Culex* species are highly susceptible to the binary toxin of *Bs* strains, however most *Aedes* species are less susceptible

(Poopathi et al., 2004). When these poisonous proteins are consumed, they destroy the intestinal epithelial cells of the larvae, resulting in death. *Bs* is fully safe for humans, animals, wildlife, and the environment, and they are excellent for community use (Bendary, 2005). Contrary to *Bti*, for which there have been no cases of known field resistance, *Bacillus sphaericus* crystal toxin resistance has been noted in *Culex* larvae (Gilbert and Gill, 2010). As a result, the recent emergence of resistance has hampered mosquito control efforts.

Because these bacteria are safe for animals, the environment, humans, numerous formulations in the form of Granules (G), Flowable concentrate (FC), Water Dispersable Concentrate (WDC), Emulsifiable Concentrate (EC), Wettable Powder (WP) and Dust (D) have been developed to control a variety of mosquito species. Tests have been conducted extensively for these formulations in the United States, Zaire, Brazil, France, India, and Bangladesh (Poopathi et al., 2004).

Physical control

Physical control, in its broadest definition, aims to reduce mosquito breeding sources by improving sanitation, water management, changing the water-air interface, and limiting human-mosquito interaction (Becker et al., 2003). Water supply management can limit the growth of mosquito vector populations and hence can reduce the transmission of mosquito-borne diseases. Scarcity of water can increase public health issues including vector-borne infections. Water scarcity may allow non-indigenous mosquito vector incursions, increase the number and quality of mosquito habitats, and prolong the “mosquito season” by providing a perfect habitat year-round during normally dry season (Akanda et al., 2020).

Personal protection

Personal protection from mosquito vectors is considered to be a significant strategy of avoiding infection from any mosquito-borne diseases, in addition to all of the above-mentioned mosquito vector control methods. Insecticide-impregnated bed nets, for example, were successfully used in Thailand, China, Latin America, and certain African nations to combat malaria and other vector-borne infections (WHO, 1996). Additionally, repellents on the skin or clothing, mosquito coils, vapourising mats, and liquid vapourisers all play important roles in personal protection against blood-sucking mosquito vectors and the transmission of vector-borne diseases.

CONCLUSIONS

The importance of mosquitoes as disease carriers in medical field has put them at the centre of entomological research (Becker et al., 2003). Even though chemical insecticides were effective for decades to control mosquitoes, the occurrence of resistant mosquitoes (Peng et al., 2022), as well as an understanding of the long-term negative effects of chemicals on beneficial living things and concerns about chemical accumulation in the environment, has hastened the need to develop alternatives (Deng et al., 2019). As a result, finding more sustainable, environmentally friendly, and low-cost alternative biological control strategies became a necessity (Deng et al., 2023).

Controlling mosquitoes with entomopathogenic bacteria has become a convincing, ecologically acceptable alternative to synthetic pesticides (Park and Federici, 2009). *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* are insecticidal spore-forming microorganisms which are the most extensively utilised alternative mosquito control agents (Park et al., 2010). Considering the development of resistance, especially by *B. sphaericus* against *Culex* larvae (Gilbert and Gill, 2010), isolating new indigenous and more potent mosquitocidal bacterial strains is necessary. Every natural ecosystem contains bacteria and other microorganisms, and soil is no exception. An attempt can be made to find a novel mosquito larvicidal bacteria to control mosquito vectors from soil samples of different places.

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AUTHOR CONTRIBUTION STATEMENT

KG has prepared the manuscript, collected literatures and has contributed in compilation of the manuscript, BB, SM, YA,KA, SM, JL and PH have contributed in literature search, reference work and assisted in compiling the manuscript. AM and KV have contributed in interpretation of background work by the various

authors in the subject. SP has given the background ideas, conception of the studies, reviewing the literatures revision and modification of the manuscript. KG has written the manuscript. All authors have read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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PESTICIDES USAGE AND ITS TOXIC EFFECTS-A REVIEW

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ABSTRACT

The urbanization of agriculture sector leads to an increased chemical affliction on the environment. Pesticides are the chemicals used in agriculture to protect the plants from weeds, pests or diseases and human being from various diseases. In this review, the literature of different types of pesticides, pesticide usage and its toxic effects on mammalian systems are summarized. Most commonly used pesticides are fungicides, insecticides and herbicides to control the weeds and pest from agricultural sites. In the usage of pesticides, India stands 12th in the world even though the use of pesticides in India is low as compared to other nations in the world. However due to their negative impacts on human and the environment there is a requirement for an ecological and sustainable approach, as the public needs the execution of the latest concept of agriculture that is safe for humans and the environment. For the sustainable agriculture development and to protect the environment from adverse effects of chemical pesticides, formulation and utilisation of bio-pesticides should be encouraged.

Key words: Human health, pesticides environment, agriculture, toxicity, india, biopesticides, humans, weeds, diseases, insecticides

Pesticides are the substances used to control pests, weeds and diseases from plants. Pesticides are important for agriculture as many farmers depend on them to keep pests out of their crops. These are known to be associated with human health and environmental issues. These cause various human diseases like skin, olfactory and reproductive due to their accumulation in human body (Aggarwal and Sharma, 2010). Pesticides can pollute different compartments of environment like soils, water or air depending on the mode of application, movement of compound and persistence of particular contaminant in the environment (Chourasiya et al., 2015). Application of pesticides in the field is mainly done by spraying which enters into the environment by different means (WHO, 1990). Direct entry of pesticides into the atmosphere can occur via spray drift and indirectly through vaporization of insecticides from vegetation or wind erosion for so many days after application into the field (Savary et al., 2019). Deposits of pesticides can be formed on lands or shallow waters and then make their way into ground waters by transportation of pesticides through long distances (Samada and Tambunan, 2020). Because of this, there is occurrence of adulteration of the different ecological milieu and the off-targeted environments and organisms may also be exposed, including humans (FOCUS, 2008). Typical examples of pesticides are fungicides, herbicides, insecticides and weedicides (WHO, 1990).

Pesticides may be metabolized, stored, excreted or bio accumulated in human body (Pirasaheb, 2015). Pesticide residues can be found in a wide variety of foods, meals, fruit juices, beverages, wine, refreshments and animal feeds (McGill et al., 1968; Chourasiya et al., 2015).

According to FAO four million tons of pesticides are used annually on a global basis, of which herbicides are (56%), insecticides (19%), fungicides (25%) and others nematicides and rodenticides (FAO, 2018). Detrimental effects are produced by indiscriminate and injudicious use of pesticides, as its residues can enter into food chains when the concentration surpasses the maximum limit (McLachlan, 2001; Ghosh and Philip, 2006). With the development of industrialization and urbanization various potential toxic chemicals are liberated into the environment and their exposures to animals have drastically changed its surroundings and lifestyle (Banerjee et al., 1999). Pesticides are one of the most effective measures of pest eradication in the entire world, but their utilization causes several adverse effects on non-target species involving humans by causing toxicity (Ghosh and Philip, 2006). Utilization of pesticides in agriculture has gradually increased to fulfil the rising demands of the population. But inadvertently these harmful chemicals have transcended the tolerance level, creating an imbalance in the system

(Bhardwaj et al., 2014). Acute and chronic exposure to pesticides was also noxious to the human reproductive system. Pesticides induce oxidative stress and amend the defence mechanisms of detoxification (Scandalios, 2005). These harmful composites hinder function of cell, enzyme activity and produce toxicity through the formation of reactive oxygen species (Milatovic et al., 2006; Agarwal and Sharma, 2010). This review article will discuss about the classification of pesticides, their usage in India and worldwide and the toxic effects of pesticides on human health.

I. Classification of pesticides

A. IRAC or MoA classification (Sparks and Naunen, 2015)

1. Acetylcholine esterases: Includes subgroup like carbamates and organophosphates
2. Chloride channel antagonists and GABA gated channels: Include subgroups like organochlorines and phenyl pyrazoles
3. Sodium Channel Modulators: include subgroups like pyrethroids, pyrethrins, DDT and methoxychlor
4. Nicotinamide acetylcholine receptor antagonists: include subgroups like neonicotinoids, nicotine and sulfoxaflor
5. Chloride channel activators: include subgroups avermectins and milbemycins
6. Juvenile hormone mimics: Include subgroups like fenoxycarb and pyriproxyfen
7. Mitochondrial Complex I electron transport inhibitors: Include subgroups like acaricides, insecticides and rotenone
8. Octopamine receptor agonists: Includes amitraz
9. Miscellaneous nonspecific inhibitors: include subgroups like alkyl halides, chloropicrin, sufuryl fluoride
10. Growth regulators includes clofentezine, hexythiazox and diflovidazin

B. Classification on the basis of pest organism it kills (Akashe et al., 2018)

1. Target organism (e.g., rodenticides, fungicides, herbicides, insecticides, pesticides),
2. Chemical pesticides (e.g., biopesticides, inorganic, organic, synthetic)
3. Physical Pesticides (e.g. gaseous)
4. Biopesticides

C. Classification based on nature- natural, synthetic and chemical (Tudi et al., 2021)

1. **Natural-** These pesticides are present in nature. These are environmentally friendly and safe. e.g. Pesticide extracted from *Chrysanthemum* plants (pyrethrin) and insecticide extracted from the neem tree (*Azadirachtin*). Rotenoids are the group of flavonoids used to control insects. These are synthesized from plant extract rotenone.
2. **Synthetic-** Synthetic pesticides are not natural these are human made pesticides and an inorganic fertilizer, e.g. Potassium chloride (KCl), Ammonium nitrate (NH_4NO_3), sodium phosphate (Na_3PO_4), calcium sulfate (CaSO_4).
3. **Inorganic-** It contains carbon and elemental sulphur. This included compounds containing arsenate and fluorine.
4. **Organic-** This contains carbon and has different types as nereils toxin isolated from marine annelids, neonicotinoids from plants, organophosphorus, organochlorines, and asynthetic organic insecticides including carbamates, pyrethroids, neonicotinoids, pyrazoles, oxadiazine, spinosyns, avermectins and diamides.
5. **Chemical-** Chemical pesticides include fungicides, herbicides, rodenticides and insecticides. These are harmful to human health as well as plants.

D. On the basis of chemical nature (Nayak et al., 2020)

1. **Organochlorine-** Organochlorine pesticides include endosulfan, dieldrin, dicofol, heptachlor and methoxychlor. Organochlorine pesticides are associated with a huge number of health effects, such as endocrine disruptors (Lemaire et al., 2004; Mnif et al., 2011), effects on fetal development (Tieman, 2008), haematological (Karami-Mohajeri et al., 2011) and hepatic damage (Calle, 2002), cancer effects (Chourasiya et al., 2015), metabolism of lipids (Karami et al., 2011), changes in liver and hematology (Freire et al., 2015). These pesticides act on nervous system by deactivating the nerve membranes and inhibit the GABA chlorine complex thus causing the accumulation of acetylcholine at the nerve endings thereby disrupting the nerve impulse transmission and leads to uncontrolled neural excitation (Bannen et al., 1998).
2. **Organophosphorus-** Organophosphorus pesticides can be used as an alternative to organochlorine pesticides (Jaga and Dharmani, 2003). Examples of organophosphorus pesticides are malathion,

parathion and dimethioate etc. The most common organophosphorus pesticide is glyphosate (Thongprakaisang et al., 2013). Glyphosate is the herbicide widely used in agriculture and it disrupts the endocrine system, affects the hematology and is carcinogenic (Swanson et al., 2013). These pesticides inhibits AChE through a chemical reaction in which serine hydroxyl moiety is blocked by phosphorus group and is unable to take part in the hydrolysis of Ach. These pesticides can have effects on function of acetylcholine esterase enzymes, endocrine disruption potential (Gasnier et al., 2009) and distraction of cellular metabolism of proteins, cytotoxic and genotoxic effects (Li et al., 2015), also have effects on cellular functions and causing oxidative stress and other problems related to endocrine system (Karami-Mohajeri et al., 2011).

3. **Carbamate-** Carbamate pesticides also act by inhibiting the AChE in a chemical reaction where inhibition is done by carbamylation of serone hydroxyl moiety. Examples of carbamate pesticides are carbofuran, aldicarb and ziram. These types of pesticides are associated with endocrine disrupting activity, disorders of reproductive system (Jamal, 2015) and effects on cellular metabolism. Carbamate pesticides have the ability to cause cytotoxic, oxidative stress and genotoxic effects. Carbamates can also induce programmed cell death and DNA damage in humans (Li et al., 2011). There are some studies which revealed that carbamate pesticides have also caused effects on brain (Wesseling et al., 2002).
4. **Pyrethroids and neonicotinoids-** Pyrethroids are the pesticides which cause respiratory allergies and these compounds deteriorate rapidly in the presence of light. Pyrethroid pesticides mainly act on voltage gated sodium channels thereby interfering process of cell to cell communication. Neonicotinoids mainly include imidacloprid, acetamiprid, clothianidin and other insecticides or pesticides which mainly have effect on acetyl choline receptors of brain (Sogorb and Vinalova, 2002). These pesticides generally bind with acetylcholine receptor site and thereby inhibiting the acetyl choline esterase enzyme (AChE) and causes overestimation of nerve cell.

E. On the basis of mode of action (MoA) by target sites (Sparks and Naunen, 2015)

1. Nerve and muscle targets: Includes

Acetylcholinesterase (AChE) inhibitors, chloride channel antagonists, sodium channel antagonists, chloride channel activators and Octopamine receptor agonists

2. Respiration targets: Includes inhibitors of mitochondrial ATP synthase.
3. Midgut targets: Includes microbial pesticides
4. Growth and development targets: Includes juvenile hormone mimics and ecdysone receptor agonists

II. Pesticides usage

Number of registered pesticides in India is 293. And India is using around 104 pesticides being prohibited in one or more nations in the world. In the usage of pesticides India stands 12th in the world (GOI (RC), 2021). According to 2017 data use of pesticides in India is low as compare to other nations in the world (Roser, 2019). The usage of pesticides in India differs from that of world (Figs. 1, 2). Herbicides, fungicides and insecticides are used in India and insecticides are used in majority in total. And in the world pesticide use pattern, herbicides are at highest point, followed by fungicide & bactericide, rodenticide and insecticide. In the world, India is the fourth largest producer of insecticides and pesticides. In 2017 usage of pesticides is low as compared to other countries like Saint Lucia, Hong

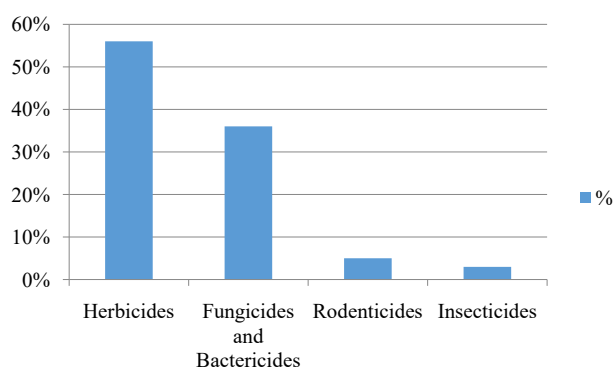


Fig. 1. Pesticide usage worldwide (FAO, 2018)

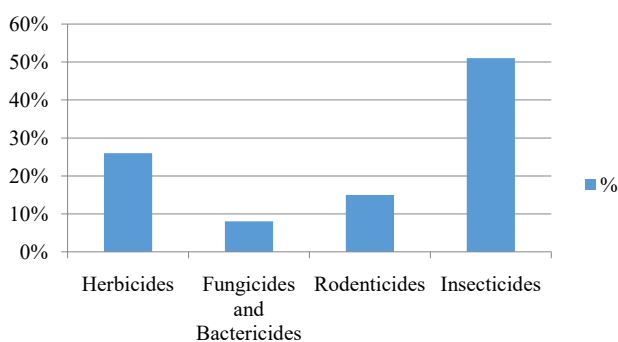
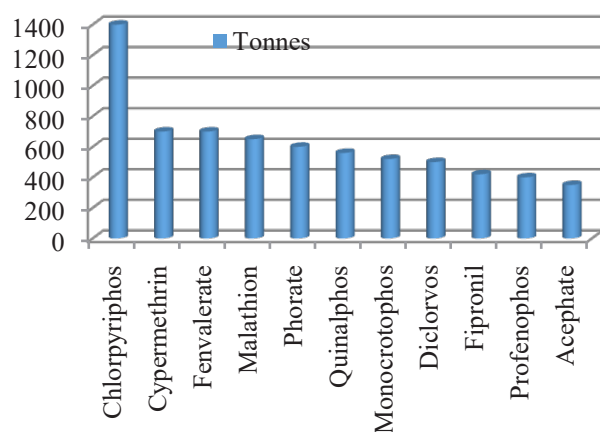


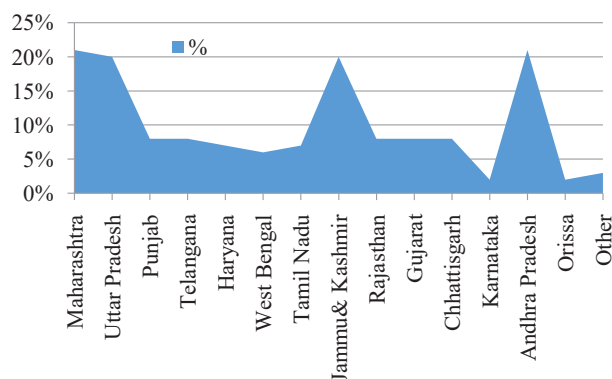
Fig. 2. Pesticide Usage India (FAO, 2018)

Kong, China and America (Roser, 2019). Among all the pesticides, chlorpyrifos is the most commonly used in India. Its usage has increased over the years. The trend followed for usage of insecticides is organophosphates, neonicotinoids and pyrethroids (GoI, 2020). Figure 3 shows the most commonly used pesticides in India during 2019-20. Among these chlorpyrifos is the most widely used followed by cypermethrin, malathion and acephate. Figure 4 shows the statewide pesticide consumption in India. Maharashtra is the highest in pesticide consumption in India followed by Uttar Pradesh, Punjab and Haryana. In developing countries, number of deaths of people due to pesticide consumption in food every year is approximately around 20,000 (Bhardwaj, 2013). Although the usage of chemical pesticides has declined in India from 72,130 tonnes to 56,090 tonnes in the past few years. These fluctuations over the use of pesticides are due to their availability in the market or its relation with weather parameters. Pesticide consumption intensity was found to be highest in Punjab, Haryana and Jammu and



Source: (Ministry of Agriculture & Farmers Welfare)

Fig. 3. Most commonly used pesticides- India (2019-20)



Source: (GoI, 2020)

Fig. 4. State wise pesticide usage- India (2019-20)

Kashmir. Because these are the agricultural prominent states and have negative growth pattern in the usage of pesticides.

III. Toxic effects of pesticides on human health

There are many evidences that showed potential risks of pesticides to environment and living organisms specially human beings (Devi, 2010). The economy of India mainly depends on agriculture sector. Farm production can be improved by safe use of pesticides and these can also be used to protect stored products. Fruits and vegetables contain the highest lethal concentration of pesticides. Epidemiological studies had revealed that variations in enzymatic concentration, oxidative stress is proposed methods through which health disorders can be linked to pesticide exposure (Muniz et al., 2008). Toxic pesticides primarily affect the kidney and liver, as they are the most sensitive organs of the body and are predominantly responsible for the metabolism and elimination of pesticides (Kalender et al., 2007). In the past few years the augmented prevalence of diseases are attributed due to aging and chemical pesticides exposure (Debost et al., 2016). Toxicity of pesticides is related to the level of exposure of pesticides and children and elder people are prone to exposure indirectly. (Meenakshi et al., 2012). Oral exposure of pesticides produces more lethality as compared to other routes of exposure (Matthews et al., 2015). Legrand et al. (2016) reported some cancer incidences due to exposure of population to certain cancer-causing agents.

Acute and chronic toxicity- Acute toxicity generally appears after the short time exposure to pesticides. Chronic toxicity develops after prolonged exposure to pesticides. Pesticide exposure from agricultural fields, during application or unintentional poisoning leads to acute toxicity or illness in human beings (Lee et al., 2011). Symptoms of acute toxicity include headache, dizziness, nausea, panic attacks and in several cases coma (Pan-Germany, 2012). Severity of toxic symptoms in acute toxicity can be related with the mode of application, action and quantity of pesticide used at the time of application (Richter, 2002). In case of chronic toxicity, symptoms will appear after long time or at later stage after pesticide exposure. Farmers are more exposed to the pesticides as compare to general population which is exposed through contaminated food and water (Pan-Germany, 2012). Lu et al. (2018) reported a study to observe the chronic and acute toxicity of pesticides triadimenol and triadimefon on tadpole larva. Results revealed that development of tadpole was inhibited due to triadimenol and triadimefon pesticides.

This was because of disruption of hormonal pathways due to pesticides.

Hepatotoxic and neurotoxic effects- Acute or chronic exposure to pesticides may affect the functioning of liver and kidney of mammals. Several studies showed that the continuous exposure to pesticides may lead to increased marker enzymes of liver such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine phosphatase (ALP) and alkaline phosphatase (AKP) (Azmi et al., 2006; Harnandez et al., 2006). Increased activity of ALT and AST indicates liver dysfunctions (Yousaf et al., 2006). And inhibition of acetylcholine esterase by pesticides may cause deposition of neurotransmitter at nerve endings that causes exhaustion of synapses. A number of studies showed that long exposure of pesticides in agricultural workers have caused several neurotoxic effects (Tapi et al., 2005).

Pesticides as endocrine disruptors- Pesticide exposure is associated with various reproductive effects in women like abortions, decreased fertility, low birth weight, premature birth, teratogenicity and disruption in hormonal function (Schettler, 2003; Henderson, 1995). Pesticides that causes hormonal disturbance are called endocrine disrupting chemicals. Some pesticides have the ability to inhibit hormone synthesis (Vinggaard et al., 2000). Dithiocarbamates like thiram and sodium N-methyl dithiocarbamate (SMD) are the pesticides which causes the reduced conversion of dopamine to norepinephrine and ultimately destroys the dopamine receptor activity (Goldman et al., 1994). Some pesticides have shown estrogenicity in vivo, e.g. methoxychlor (Goldman et al., 1999), and toxaphene, DDT, endosulfan, dieldrin, fenarimol, kepone and triadimefon (Hodges, 2000). Some pesticides like dieldrin, methiocarb, fenarimol and endosulfan showed less estrogenicity, but showed their effect when used in combination or mixing with some other pesticide. When used additively, they may induce estrogenicity at lower concentrations than those required individually. Receptors of estrogens also show less sensitivity through prolonged exposure to high concentrations endocrine disruptors (Tudi et al., 2021). Pesticides can act as endocrine disruptors because these can affect the reproductive hormone pathway (Fig. 5). The endocrine disrupting pesticides can reduce or alter the gonadotropin releasing hormone and then alter the male and female reproductive hormones (FSH, LH). There are so many pesticides which are endocrine disruptors. Among them 31% are

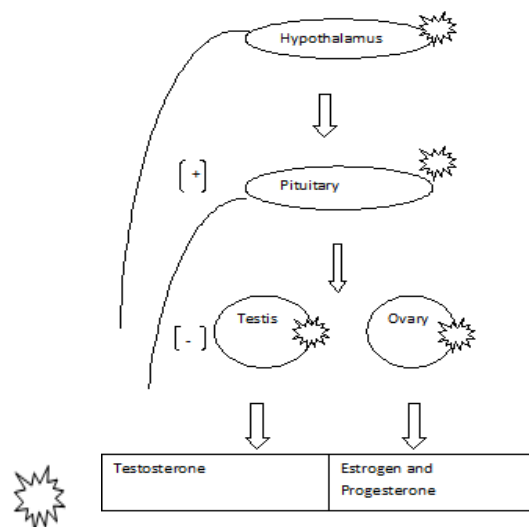


Fig. 5. Endocrine disruption pathway after exposure to pesticides

fungicides, 21% herbicides and 46% are insecticides. Endocrine disruptor pesticides mainly act by interfering the mechanism of natural hormones as they have the potential to bind to androgen receptors. Then they activate the hormone receptors and imitate the natural hormone action. Endocrine disruptor pesticides can also interfere the production, transportation and metabolism of hormones (Tabb and Blumberg, 2006). Endocrine disruptor chemicals or pesticides cause damage at the time of gamete formation and early foetal development in humans, these foetuses got great dose of pesticides from the mother at the time of pregnancy (Birnbbaum and Fenton, 2003, Goldman et al., 2004, Sharpe et al., 2006). Table 1 shows the different pesticides and their hormone disturbance effects. The pesticides given in the table bind to the androgen receptors and thereby interfering the mechanism of natural hormone.

Exposure to pesticides leads to endocrine disruption which has the following effects- reduced GnRH release, altered GnRH responsiveness, and FSH and LH levels. Effects on male reproductive system include reduced testicular weight, reduced testosterone level, reduced sperm motility, altered steroidogenesis and spermatogenesis, and Sertoli cell degeneration. Effects on female reproductive system include reduced ovarian weight and follicles, altered steroidogenic enzymes, and arrested estrous cycle

Developmental and reproductive toxicity- Reproduction is an important process for producing new individuals from parents, which is adversely affected by the use of pesticides (Ngoula et al., 2012). Chronic and subchronic exposure of pesticides

Table 1. Different pesticides and their hormone disturbance effects

Pesticide	Hormone Disturbance effects
Aldicarb	Inhibition of progesterone and estrogen
Aldrin	Testosterone receptor binding
Carbofuran	Increase in estradiol and progesterone and decrease in testosterone
Dieldrin	Androgen receptor binding
Deltamethrin	Increased estrogens
Chlordane	Androgen receptor binding
Fenoxycarb	Decrease in luteal progesterone, increase in androgens
Lindane	Testosterone metabolism disruption
Parathion	Inhibition of gonadotrophic hormone synthesis
Methoxychlor	Estrogen antagonism in females
Tetramethrin	Estrogenic effects

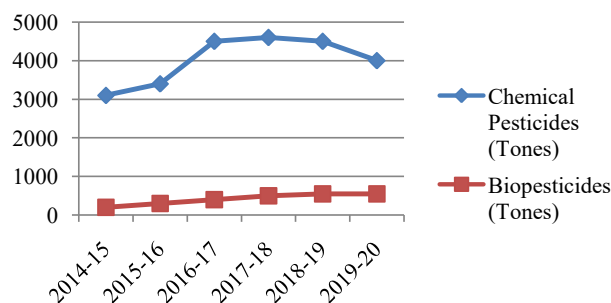
can cause damage to ovarian and testicular tissue. Insecticide intoxication may result in anomalies in a reproductive system such as disturbed estrous cycle/ ovarian cycle irregularities, infertility, reduction in weight of ovary, testis, epididymis, seminal vesicles and prostate gland and change in other fertility parameters (Tiemann et al., 2000); (Verma et al., 2007). Pesticides are reported to shorten gestation period, have effect on the growth of uterus, fetal neurodevelopment and other male and female reproductive cells. Pesticide-induced male infertility can be adjudged from disturbed spermatogenesis, variations in reproductive hormonal pathways, deteriorated sperm quality and movement (Ghuman et al., 2013). Toxic effect of pesticides on male reproductive system can be observed directly from histopathological changes in reproductive organs and hormonal imbalance in endocrine system (Ngoula et al., 2012). Organophosphates can alter the spermatogenesis, abnormalities in sperm function and lesser levels of testosterone or inhibition of testosterone synthesis. Carbamates can also cause reduced testicular weight and deterioration of epididymis and seminiferous tubules (Archana et al., 2007, Shalaby et al., 2010). Qian et al. (2018) conducted a study on the embryos of zebra fish to observe the acute toxicity and sublethal toxicity of boscalid. Results showed that the sublethal effects of boscalid have affected the development in zebrafish. This developmental toxicity could have assessed through altered lipid metabolism, melanin synthesis, and deposition of pesticide within the embryos of zebrafish.

Genotoxicity and cytotoxicity- Genotoxicity is the damage caused to genetic material or DNA by any biological or chemical agent thereby causing alterations or damage in the genesis or polymerization of proteins or enzymes involved in the process of

chromosome or chromatin formation. This ultimately leads to genetic mutations (Vinggaard et al., 2000). Cytotoxicity is the damage or injury caused at cellular level thereby harming the overall physiology of the cell. Genotoxicity can be caused due to organophosphates, organochlorines, carbamates and neonicotinoids and can lead to various diseases. Amal et al. (2005) reported a study to show the acute toxicity of organophosphorus pesticides through the bio markers of oxidative stress and apoptosis. In this study assays for different antioxidants enzymes were performed. Results revealed significant alterations in the malonyldialdehyde (MDA) and glutathione (GSH) level and significant reduction in the levels of catalase, glutathione (reduced) and increase in MDA level was found in organophosphorus treated patients (Melchiorri et al., 1996). Negative correlation was observed between cholinesterases and MDA and positive between cholinesterases and reduced glutathione (GSH). Thus showed that organophosphate caused production of free radicals as shown by increased MDA level and decreased glutathione and catalase level. As MDA level is the indicator of oxidative damage and lipid peroxidation to cells and tissues (Verma et al., 2007; Maellaro et al., 1990). Pandey et al. (2018) conducted a study to induce genotoxicity by use of profenofos (PFF) (organophosphate pesticide) in namely *Channa punctatus* which is a fresh water fish. Tunnel assay and comet assays were used to study the DNA damage. The DNA damage was measured in comet tails in erythrocytes in terms of the percentage of DNA damage.

IV. Biopesticides as an alternate to chemical pesticides

Biopesticides are pesticides that have their origin in natural sources such as plants, animals and microorganisms. Biochemical pesticides and microbial



Source: GoI (2020)

Fig. 6. Chemical and biopesticide consumption in India

pesticides are included in this category (Samada and Tambunan, 2020). Bacteria, fungi and viruses can be used as biological pesticides or agents for the control of pests in place of chemical pesticides as these have no effect on other organisms. Figure 6 shows the use of chemical and bio-pesticide in India during the last six years (GoI, 2020). Although the usage of bio-pesticides is lesser as compare to chemical pesticides but bio-pesticides are eco-friendly than the chemical pesticides. Bio pesticides are healthier, safer, cheap, biodegradable, target specific and harmless.

Agriculture is facing lot of problems caused by pests and it has been controlled by the use of pesticides. Initially pesticides were used to increase the agriculture production by controlling the infectious diseases, pests and weeds. But due to indiscriminate use of pesticides several health and environmental issues have been raised. Therefore there is a need for some alternative which is eco-friendly with environment. Pesticides are not only one substance, but consist of different substances having diverse toxic effect and may act through different mechanisms. Bio-pesticides is an alternate to chemical pesticides as bio-pesticides are eco-friendly and sustainable alternatives. It is anticipated that the viable and public zone work collectively to help farmers at the grassroots level by evolving an integrated guidelines and policies for the use of chemical pesticides and bio-pesticides. More and more agricultural research is required to incorporate bio pesticides into production system which can provide better quality and low cost product availability to the customers.

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CONFLICT OF INTEREST

No conflict of interest.

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