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Figs. 37-47. Leafhoppers that probably act as vectors of *Xylella fastidiosa* if it gets accidentally introduced into India. 37. *Anagonalia melichari* (Distant); 38. *Anatkina helena* (Distant); 39. *Atkinsoniell aopponens* (Walker); 40. *Bothrogonia albidicans* (Walker); 41. *B. sclerotica* Young; 42. *Cofana lineata* (Distant); 43. *C. unimaculata* (Signoret); 44. *C. spectra* (Distant); 45. *Kolla ceylonica* (Melichar); 46. *K. paulula* (Walker); 47. *Signoretia* sp.
For details see page No. 1179 of this issue

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FIRST RECORD OF CITRUS LEAF ROLLER *ARCHIPS MACHLOPIS* (MEYRICK) ON SWEET ORANGE IN BANGLADESH

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ABSTRACT

The occurrence of *Archips machlopis* (Meyrick) (Tortricidae: Lepidoptera) for the first time on sweet orange (*Citrus sinensis*) at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh during 2021-22 is reported herein. Larva bears a brown prothoracic shield and a thin white line which delineates the reddish-brown head capsule. Moths are dark grey, 8-10 cm long, while pupa are dark brown to black with elongate cremaster. DNA barcoding with mtCOI gene confirmed it as *A. machlopis.* Larvae are observed attacking young leaves, and being voracious cause severe foliage damage. It folds leaf around them and feeds inside the folded leaf. Approximately 21.5% young leaves were infested. More investigations are suggested to assess the pest status, hosts, biology and other aspects.

Key words: *Archips machlopis*, new record, Bangladesh, citrus, leaf roller, sweet orange, pest, DNA barcoding, mtCO1, morphological, molecular characterization

Archips machlopis (Meyrick) is a polyphagous lepidopteran pest belonging to the family Tortricidae (Razowski, 2009). It is reported as a serious pest to several important crops and generally roll or tie the leaves of host plant (Soumia et al., 2019). Meyrick (1912) had first recorded this insect pest from Assam. Recently, it has been extensively disseminated in southern Asia containing Thailand, Vietnam, Malaysia, Indonesia, Sumatra, China, Pakistan, India, Nepal and Myanmar. It was detected to feed on different hosts such as *Acacia nilotica, Araucaria cunninghamii, Ipomoea batatas, Cassia fistula, Medicago sativa, Litsea monopetala, Stylosanthes guianensis, Cedrela toona, Lansium domesticum, Toona ciliate, Morus indica, Coffea liberica, Citrus sinensis, Citrus medica, Santalum album, Litchi chinensis, Nephelium lappaceum, Theobroma cacao, Camellia sinensis, Lantana camara, Toona ciliate, Daucus* sp., *Psophocarpus tetragonolobus*, *Vigna unguiculata*, *Persea americana*, *Allium sativum, Gossypium herbaceum*, *Donax* sp., *Jasminum sambac*, *Averrhoa carambola, Cedrela* sp., *Aglaia* sp., *Araucaria cunninghamii, Durio zibethinus* and *Gloriosa superba* (Bhumannavar et al., 1991; Fletcher 1932; Robinson et al., 2010; Soumia et al., 2019; Tuck, 1990; Kuznetzov 2000; Pathania et al., 2020).

Citrus including sweet orange, mandarin, lemon, lime and pummelo are widely cultivated in Bangladesh. Citrus cultivation in Bangladesh is plagued with various problems including high incidence of insect pests (Bhuiyan et al., 2016). Several researchers reported that different insect pests such as citrus leaf miner (*Phyllocnistis citrella*), Asian citrus psyllid (*Diaphorina citri*), lemon butterfly (*Papilio demoleus*), lemon butterfly (*Papilio polytes*), spined citrus bug (*Biprorulus bibax*), citrus red mite (*Panonychus citri*), thrips (*Scirtothrips citri*), citrus red scale (*Aonidiella aurantii*), citrus leaf roller (*Platynota stultana*), citrus leaf folder (*Psorosticha zizyphi*) red fire ant (*Solenopsis invicta*), citrus mealy bug (*Pseudococcus filamentosus*), citrus stem borer (*Chelidonium cinctum*), citrus aphid (*Toxoptera aurantii*), bark and stem borer (*Indarbela quadrinotata*), citrus red scale (*Aonidiella aurantii*), citrus yellow scale (*Aonidiella citrina*), citrus blackfly (*Aleurocanthus woglumi*), citrus whitefly (*Dialeurodes citri*), termite (*Odontotermes obesus*) and oriental fruit fly (*Bactrocera dorsalis*) had been infested different citrus crops in Bangladesh (Bhuiyan et al., 2016; Uddin et al., 2014). Recently, citrus leaf roller (*Archips rosanus*) (Lep.: Torticidae) has become a serious insect pest of citrus crops in Iran (Toorani and Abbasipour,

2019). However, there is no previous report of its presence from Bangladesh. This study, therefore, made a survey to determine the presence of *A. rosanus* from Bangladesh and first occurrence of citrus leaf roller *A. machlopis* infesting sweet orange (*Citrus sinensis*) leaf is documented at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh confirmed by morphological and molecular characterization.

MATERIALS AND METHODS

Regular survey and monitoring was done on sweet orange and the infestation was noticed on leaf at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh during rabi, 2021-22. For morphological and molecular diagnostics, a large number of larvae were collected from infested leaf and reared on petri dishes at the laboratory feeding the leaves of host plant. The total DNA was extracted from adult using GeneJET Genomic DNA Purification Kit (Catalog number: K0721, https://www.thermofisher.com/) following the manufacturer's protocol and 50 µg/ µl stock DNA solution was prepared and stored. Study was targeted to amplify approximately 411 bp long fragment at the 3′ end of the barcoding region by using the forward primer BF2 (5'-GCHCCHGAYATRGCHTTYCC-3') and the reverse primer BR2 (5'-TCDGGRTGNCCRAARAAYCA-3') (Elbrecht and Leese, 2017). The PCR reaction conditions were as follows: 94°C for 180 s, 35 cycles of 94°C for 30 s, 50°C for 30 s, and 65°C for 150 s, and then a final incubation at 65°C for 5 min. The amplified PCR DNA was visualized on 1.5% agarose gel. The purified DNA was sent for sequence determination (BTSeq™ Sequencing Service, https://www.celemics.com/, Korea). After sequencing of CO1 gene amplicons, homology of the obtained sequence was evaluated with Basic Local Alignment Search Tool (BLAST) tool of National Center for Biotechnology Information (NCBI) for comparison and identification of the sample. BioEdit software was used to check the quality of sequenced data. Sequence was submitted to GenBank. MEGA (Molecular Evolutionary Genetic Analysis) tools (version 11) software was used for contracting phylogenetic tree (Tamura et al. 2021). Maximum likelihood (ML) and neighbor-joining (NJ) methods were used for reconstruction of phylogeny using bootstrap with 1000 times of repetition. Some relevant sequences were downloaded from NCBI GenBank for phylogenetic tree analysis.

RESULTS AND DISCUSSION

Citrus leaf roller *Archips machlopis* extensively infests sweet orange young leaves causing approximately 21.5% damage. The larva wraps the young leaves and continues to feed insight into the wrapped leaves (Fig. 1A, B). This insect's attack causes the infested leaves to drop off hampering the fruit production severely. Larva were collected from infested sweet orange leaves and reared artificially to adults in the laboratory. The developed adults were identified *A. machlopis* according to Soumia et al. (2019) description. The larva is greenish grey to whitish grey and 10-12 cm long (Fig. 1C). Head reddish brown which is demarcated from the prothorax by a narrow white line. Pupa are dark brown to black and 12-14 cm long (Fig. 1D, E). It has cremaster elongate and becomes adult inside the wrapped leaf. The adult is 8-10 cm long (Fig. 1F). Head and thorax are dark purplish-fuscous. Wings are held like a flattened roof similar to an arrowhead. The identity of *A. machlopis* was also confirmed by DNA sequencing. DNA was extracted from adult and oligonucleotide primer pair BF2 and BR2 were used for partial (~411bp) mitochondrial cytochrome c oxidase subunit I (mtCOI) gene amplification (Fig. 2)

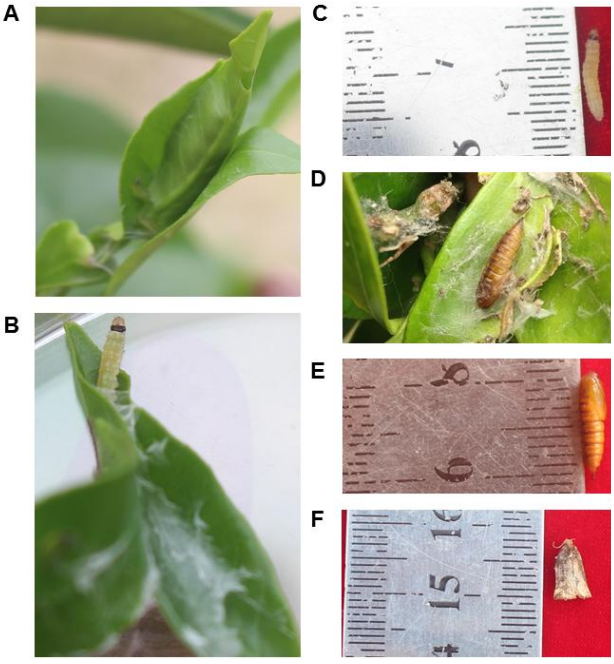


Fig. 1. Infested leaf of sweet orange and life stages of *A. machlopis*. A. Folded leaf of sweet orange. B. *A. machlopis* infested sweet orange leaf. C. *A. machlopis* larvae. D. *A. machlopis* pupa at infested sweet orange leaf. E. *A. machlopis* pupa. F. *A. machlopis* adult

(Elbrecht and Leese, 2017). The obtained partial COI gene sequence was submitted to the NCBI-GenBank with the accession number was OP574698 and also to BOLDSYSTEMS, DNA barcode with Process ID "ARMBD001-23" was successfully generated (Fig. 3). Phylogenetic analyses were conducted using the MEGA 11 program. The phylogenetic tree was generated using the most identical sequences of *A. machlopis* available in the GenBank database (Fig. 4). Phylogenetic analysis comparing with publicly available CO1 sequences revealed that our isolate was identified as *A. machlopis*.

Thus, it is confirmed the presence of *A. machlopis* as the new insect pest of sweet orange from Rangpur, Bangladesh. Since, sweet orange is an important fruit in Bangladesh, a comprehensive study covering biology, host range, identification of parasitoids, insecticide efficacy, etc. should be undertaken to develop sustainable management options.

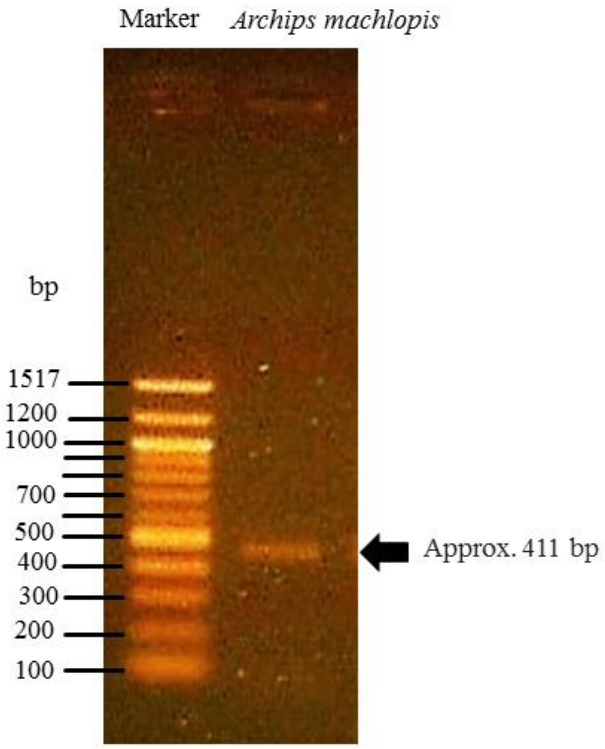


Fig. 2. Agarose gel electrophoresis (1.5%) of amplified COI (~411 bp) PCR products of *Archips machlopis* sample

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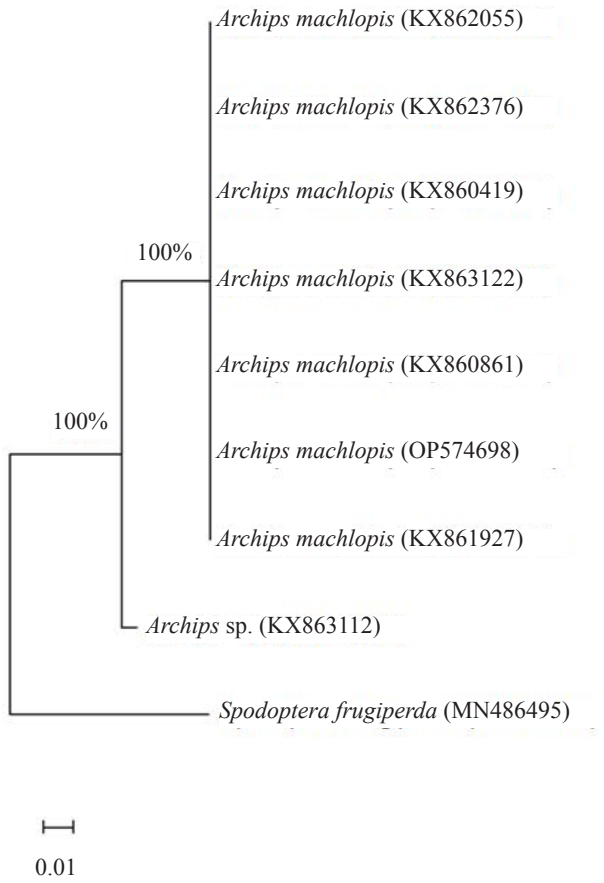


Fig. 4. The phylogenetic relationship of the Archips machlopis according to the Neighbor-Joining method using gene sequences of mitochondrial cytochrome oxidase I (COI) regions of the rDNA. Numbers in parentheses indicated the accession numbers in GenBank. Our deposited the NCBI-GenBank accession number is OP574698. The bar represents sequence divergence

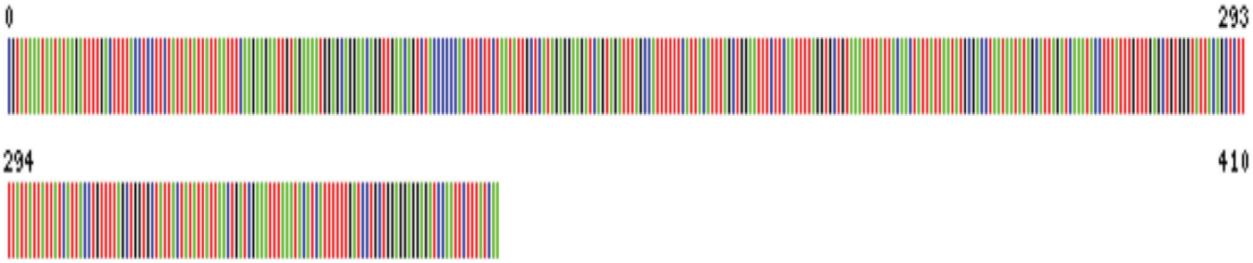


Fig. 3. DNA Barcode of *Archips machlopis* with Process ID: ARMBD001-23

AUTHOR CONTRIBUTION STATEMENT

MHR planned and designed this study. MTHH performed molecular diagnosis. MHR analyzed data. MAS, NKD and AKS revised the draft. MHR and MTHH drafted and revised original manuscript.

FINANCIAL SUPPORT

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CONFLICT OF INTEREST

The authors declare that the research work was carried out without any financial or commercial dealings that could be raised as a probable conflict of interest.

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ACCEPTANCE OF INSECTS AS FOOD: AN EXPLORATORY STUDY OF YOUNG CONSUMERS IN MACAU

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ABSTRACT

Substitute foods are increasingly popular to reduce our environmental footprint and promote food security. As the world population is expected to grow and food resources become scarce, insects as food have recently gained attention as a viable alternative. In the present study, a model grounded on the Theory of Planned Behavior (TPB) is proposed and analyzed through structural equation modeling software (SmartPLS) to assess consumers' intentions toward insects as food. Except for subjective norm, both attitude and perceived behavioral control were key determinants of intention and, in turn, of actual use behaviour. Despite insects being consumed in nearly 1/4 of the sample (for instance in Chinese medicine), the study found that respondents were on average relatively unwilling to use them as a dietary habit. Also, it appeared that men were more likely to consume insects as food than women. The insights of our study have important implications for practitioners and policymakers seeking to promote sustainable nutritional practices among consumers. This study is particularly relevant for Macau, as the city positions itself as a "UNESCO Creative City of Gastronomy" with the aim to develop internationally a unique and sustainable food image.

Key words: Insects as food, entomophagy, edible insects, consumer behaviour, environmental footprint, food security, theory of planned behaviour, attitude, behavioural control, dietry habit, nutritional practice

Some ideas are difficult to accept even when they bring advantages (Rogers, 2003). Ideas can take several years to become apparent, and, in practice, many of them fail to be widely accepted for several causes (Tidd, 2010). The reason for this is that consumers are often resistant to change and need time to get used to new concepts. For instance, when plant-based meat alternatives first came on the market, many consumers were initially skeptical, but over time they accepted the idea (Alcorta and Porta, 2021). As a means of reducing our environmental footprint and promoting human welfare, food substitutes have become increasingly popular. In particular, due to the anticipated increase in world population and scarce food resources, insects have recently drawn attention as an alternate source of food. According to the Food and Agricultural Organization (FAO), a viable way to address worldwide food security is through insect farming. This is because insects are everywhere and reproduce quickly, they have high growth and feed conversion rates with a minimal environmental footprint (FAO, 2013). Human diets have always included insects. Before people had the tools for hunting or farming, insects constituted an important component of the human diet (Kouřimskáa and Adámková, 2016).

Around the world, >2000 insect spices are considered edible (Mariod, 2020), and some are even part of the local cuisine. In Thailand, for example, worms and crickets are common street food (InsectGourmet, 2023), while in Mexico a specie of grasshopper served with beef and beans is considered a national dish (Mariod, 2020).

Similarly, in many areas of China, roasted larvae from worms, water beetles, or bee cocoons are consumed as gourmet foods (InsectGourmet, 2023). Eating insects in China has been an integral part of the culture since ancient times. In addition to providing nutrition, insects are the sources of active ingredients for traditional Chinese medicine (Xie et al., 2021) with a significant role in improving people's health and quality of life (Hong and Lampo, 2022). Currently, over 300 species of insects are documented as edible in China, but the number is growing (Guiné, Correia, Coelho and Costa, 2021). Although insects are becoming increasingly popular as food, there is a lack of research being conducted in Macau. An ex-Portuguese enclave where gambling is the pillar of the economy (Lampo, 2023), Macau was chosen as a "UNESCO Creative City of Gastronomy" in 2017. This international recognition,

in the government's plans, will help Macau become a diverse, truly unique, and sustainable city (The Macau Post Daily, 2017). However, while many studies have focused on consumers in Europe and the US (Liu et al., 2020) little is known about the intention toward edible insects in Macau. To fill the gap in the literature, we collected data from local consumers to assess the factors that influence their insect-related consumption decisions. Using the Theory of Planned Behavior (TPB) (Fishbein and Ajzen, 2009), a basic model to assess consumers' intentions was developed and analyzed by structural equation modeling (SmartPLS). To the best of our knowledge, this is the first quantitative study that examines consumers' intentions toward insects in Macau. This is especially relevant considering the city's plan to develop a sustainable image in the food sector. Additionally, this study's findings have implications for policymakers and practitioners who want to encourage local consumers to adopt sustainable nutritional alternatives.

The term "Entomophagy" is used to describe the eating of insects as food (Collins, 2023), a common practice in many countries around the world but predominantly in parts of Asia, Africa, and Latin America (FAO, 2013). Insects account for 80% of the world's species and are the most diverse group of organisms (Chantawannakul, 2020). From a nutritional standpoint, insects have significant protein content that varies from 20 to 76% of dry matter depending on the type and development stage of the insect (Kouřimskáa and Adámková, 2016). Insects are also rich in amino acids, fatty acids, and various minerals such as copper, iron, magnesium, manganese, phosphorous, selenium, and zinc as well as the vitamins riboflavin, biotin, and, in some cases, folic acid (Chantawannakul, 2020). Despite the benefits, consumer aversion is the largest barrier to adopting insects as viable sources of nutrients (FAO, 2013). When associated with food, insects are viewed as pests and a sign of filth due to food neophobia, which is the fear of eating unfamiliar foods Field (Bao and Song, 2022), however, many would be pleasantly surprised by their taste (Sjøgren, 2017). It is argued that the shape and appearance of insects play a significant role in consumers' responses (Liu et al., 2020). Research shows that curiosity drives people to try edible insects, while disgust and negative opinions from family and friends prevent them from doing so (Sogari, 2015). An additional barrier to consumption is the possibility that some insects produce or contain toxic compounds, or may contain residues of pesticides (Kouřimskáa and Adámková, 2016). While the West has been reluctant to adopt edible insects as food because insects are typically associated with uncleanliness (Bao and Song, 2022), studies suggest that Chinese consumers are more accepting of consuming insects due to their familiarity with the idea (Liu et al., 2020). However, China has only a limited amount of scientific research available. Past studies on entomophagy have demonstrated the environmental benefits of substituting meat protein with edible insects (Bao and Song, 2022). Livestock production accounts for around 15% of global greenhouse gas emissions and pollutes both the land and surrounding water (Sjøgren, 2017). Due to lower greenhouse gas emissions, water pollution, and land use, breeding insects appears to be more environmentally friendly than raising livestock (Lange and Nakamura, 2023). Therefore, it appears as insect-based foods could contribute to the achievement of sustainable development goals and help solve the looming issue of global food insecurity. Next, we examine the research model for assessing our sample's behavioral intentions toward edible insects.

MATERIALS AND METHODS

To assess the intention of Macau residents of consuming edible insects, exploratory study was conducted based on the Theory of Planned Behaviour (TPB) (Fishbein and Ajzen, Predicting and changing behaviour: The reasoned action approach, 2009). Developed upon the theory of reasoned action (TRA) (Fishbein and Ajzen, 1975) which is regarded as one of the most fundamental theories of human behaviour (Momani and Jamous, 2017). The TPB has been used in a variety of domains and represents an effort to predict and explain virtually any human behaviour (Lampo, 2022). At its core, the theory poses that the performance of a behaviour (for example using edible insects as food) is the consequence of the behavioural intention to perform that behaviour. The intention is in turn influenced by three predictors: i.e., attitude, subjective norm, and perceived behavioural control concerning the behaviour in question. As described in Lampo (2022), a person's attitude refers to positive or negative feelings about performing a behaviour. The subjective norm describes the perceived expectation of relevant others (e.g., family members, friends, etc.) as to whether an individual should perform a specific behaviour. Lastly, perceived behavioural control represents the perceived ease or difficulty of performing a specific behaviour. The TPB suggests that when the perceived behavioural control closely reflects an actual control, it may directly predict the behaviour (Fishbein and Ajzen, 2009),

thus behavioural control and behavioural intention can directly predict the actual behaviour. As a general rule, the stronger the attitude, the perceived norm, and the perceived behavioural control towards a specific behaviour, the higher an individual's intention to perform the behaviour in question. However, the relative importance of these three determinants is expected to vary depending on the behaviour and population under consideration (Fishbein and Ajzen, 2009). Figure 1 illustrates the conceptual model, while the associated hypotheses are subsequently reported in Table 1.

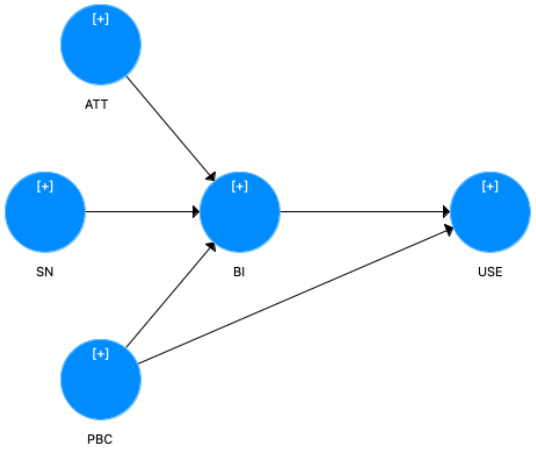


Fig. 1. Analytical framework (Smart PLS output)

To test our hypotheses about respondents' behaviour regarding the consumption of insects as food, a cross-sectional approach was adopted. To collect data, we used a self-administered survey based on the TPB framework. Each item was assessed using a 5-point Likert scale with anchors ranging from 1 (Disagree) to 5 (Agree). For better understanding, items were developed in English and then translated into traditional Chinese using the back-translation technique (Son, 2018). To gather insights quickly, convenience sampling and snowball (Flick, 2009) methods were used to recruit participants. The survey design included a pilot test with 10 respondents to identify problematic items and further improve the flow of the survey. Participants were provided with a link to a survey hosted in Google form, and their responses were downloaded at the end of the fieldwork. Regarding the minimum sample, we used the application G*Power (Erdfelder, Faul and Buchner, 1996). The software (settings: .15 effect size, .05 probability error, .80 power, and three predictors in the model) returned 77 cases as the minimum sample size.

The online survey-based data collection resulted in 137 responses. We had to discard 9 responses because of straight-lining (Shmueli, et al., 2019). In total, 128 valid and usable responses were obtained from fieldwork and deemed sufficient for an exploratory study. The contribution was voluntary and respondents were not remunerated for their participation. Besides guaranteeing anonymity, respondents were informed about the purpose of the research and that the findings would be published in aggregated form.

RESULTS AND DISCUSSION

The respondents were evenly divided between females (50.00%) and males (50.00%). The large majority of participants were below 34 of age (78.12%) while the remaining were under 44 (21.88). A majority of respondents reported having a bachelor's degree (31.25%) or a master's degree (31.25%). In 22% of the sample, it was found that insects were already consumed in some form (e.g., in Chinese medicine). SmartPLS version 3 (Ringle, Wende and Becker, 2022) was used to analyze the data. Before running the PLS algorithm, several factors were examined, including missing data, outliers, non-normality, and multicollinearity; and there was no cause for concern. It was initially found that the model explained 63.8% ($R^2 = 0.638$) of BI's variance and 31.9% ($R^2 = 0.319$) of USE's variance. A weak path was also observed in the relationship SN-BI ($\beta = 0.025$). However, to interpret these findings properly a 2-step process was necessary (Hair et al., 2021). Hence, the first step consisted in testing our data for reliability, internal consistency, convergent validity, and discriminant validity to determine whether the indicators of each construct measured the same underlying concept and

Table 1. Summary of hypothesis

H1	There is a positive and significant relationship between attitude (ATT) and behavioral intention (BI).
H2	There is a positive and significant relationship between subjective norm (SN) and behavioral intention (BI).
H3	There is a positive and significant relationship between perceived behavioral control (PBC) and behavioral intention (BI).
H5	There is a positive and significant relationship between perceived behavioral control (PBC) and use behavior (USE).
H4	There is a positive and significant relationship between behavioral intention (BI) and use behavior (USE).

Authors' table.

were different from other constructs in our model. In the evaluation of the measurement model, a common rule is that the standardized outer loading should be 0.708 or higher (Hair et al., 2021). In our case, the analysis reported a lower loading for the items PBC1 (0.460) with an adverse impact on the variance explained. By removing this item, BI's variance increased by 0.8%, thus bringing the explanatory power of the model to 64.6%. (R^2 = 0.646). There were no changes in USE as a result of the removal of the item. Then, because all of the outer model's measurements fell within the recommended thresholds (Shmueli et al., 2019). More precisely, all the loadings exceeded the critical value of 0.708, all the construct reliabilities (i.e., Cronbach's alpha, rho_A, and the composite reliability) had values above 0.70, and the average variance extracted (AVE) was higher than the threshold value of 0.50. Table 2 presents the reliability and validity of the construct in our model.

According to the literature, the heterotrait-monotrait (HTMT) criterion is the preferred method in PLS-SEM for evaluating the discriminant validity (Hair, Hult, Ringle and Sarstedt, 2021), testing that a construct does not relate to other variables in the model. All the values were below the conservative threshold of 0.85. As a result, the constructs in our model were significantly different and measured what they were supposed to measure. Table 3 reports the HTMT results. As all of the measurements fell within the suggested values (Garson, 2016; Hair, Hult, Ringle and Sarstedt, 2021), the outer model was successfully evaluated.

The second step consisted in examining the structural (or inner) model. To ensure that collinearity issues didn't affect the regression results, the constructs' VIF values were examined. In our case, VIF values reached a maximum of 2.906 and, therefore, below the recommended value of 3.3 (Garson, 2016). As mentioned, the revised model explained 64.6%. (R^2 = 0.646), of the dependent variable BI; this is regarded as a substantial explanatory power (Hair et al., 2021). On the other hand, the model explained 31.9% (R^2 = 0.316) of USE's variance; a result that is regarded as more than satisfactory as USE is solely dependent on the variable BI. A closer examination of the structural paths indicated that ATT (β= 0.608) had the strongest effect on BI, followed by PBC (β= 0.284). It was also

Table 3. Constructs discriminant validity

	ATT	BI	PBC	SN	USE
ATT	-				
BI	0.820				
PBC	0.836	0.816			
SN	0.764	0.594	0.697		
USE	0.459	0.589	0.504	0.363	-

Evaluating criterion: HTMT <0.85

Table 2. Constructs reliability and validity

Construct	Items	Loadings	Cronbach's Alpha	rho_A	C.R.	AVE
Behaviour intention (BI)	BI1	0.911	0.906	0.911	0.941	0.842
	BI2	0.943				
	BI3	0.898				
Attitude (ATT)	ATT1	0.830	0.896	0.916	0.917	0.766
	ATT2	0.806				
	ATT3	0.734				
	ATT4	0.910				
	ATT5	0.878				
	ATT6	0.812				
Subjective norm (SN)	SN1	0.898	0.886	0.891	0.929	0.735
	SN2	0.901				
	SN3	0.875				
Perceived behavioural Control (PBC)	PBC2	0.989	0.711	0.791	0.809	0.714
	PBC3	0.888				
Use behaviour (USE)	USE1	1.000	1.000	1.000	1.000	1.000

Evaluation criteria: Loadings >0.708; Cronbach's Alpha: >0.70; Rho_A: >0.70; Composite Reliability: >0.70; AVE: >0.50.

observed that BI had a strong impact on USE (β= 0.510). The bootstrap routine validated that these results were significant at the 5% level. The relationship SN→BI (β= -0.025) was negatively correlated and, together with the relationship PBC→USE (β= 0.079) was found not significant. The structural results are reported in Fig. 2.

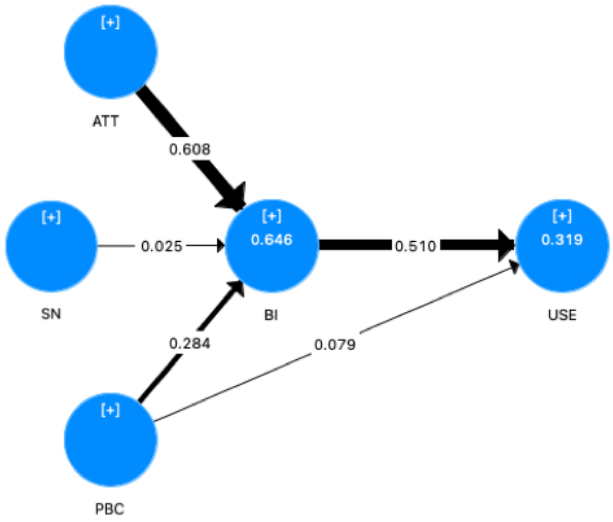


Fig. 2. PLS-SEM structural results

Additionally, the f^2 effect sizes were calculated to determine whether an omitted construct had a fundamental influence on intention and, in turn, on use behaviour. An effect size of approximately 0.02, 0.15, and 0.35 is considered small, medium, and large, respectively (Hair et al., 2021). The analysis reported a large effect in the case of removing ATT (0.435) and a small to medium effect in removing PBC (0.125) from the model. On the other hand, SN (0.001)) could be dropped from the model as it does not significantly impact the dependent variable BI. The structural analysis concluded with the assessment of the approximate model fit. Despite reservations about using this metric (Hair et al., 2021), this test in SmartPLS is measured by the standardized root mean square residual (SRMR). The SRMR value of 0.078 was lower than the suggested threshold of 0.80 (Garson, 2016), indicating that the model fits the data well.

The structural analysis indicated the importance of attitude (ATT) as a predictor of behaviour, however, the mean (M=2.64, SD=0.89) suggested an unclear attitude concerning the intention to use insects as food. Subjective norm (SN) did not contribute to the intention of consuming edible insects. The construct values (M=2.70, SD=1.13) also indicated that respondents tend to neither agree nor disagree about the impact of society on their behavioural intentions. About the construct of perceived behavioural control (PBC) (M=2.75, SD=0.92) the analysis showed that respondents perceived having some degree of control of the situation. As for behavioural intention (BI), the strong structural path suggested that respondents with higher intentions were more likely to use edible insects. As a matter of fact, it was found that insects were already consumed in some form in 22% of the sample, for example as an ingredient of Chinese medicine (Hong and Lampo, 2022). However, the values of the construct (M=2.15, SD=1.05) indicated that respondents, on average, were somehow resistant to the idea of consuming edible insects as part of their diet soon. Additionally, in the sample, men (M=2.29, SD=1.02) tend to score higher than female (M-1.99, SD=1.08) concerning intention, however, an independent t-Test showed that this difference was not significant (p>.05). For the survey items in BI, the detailed results are shown in Table 4.

Table 4. Items of behavioural intention

Items		Mean	SD
BI1	I intend to eat insects one day	2.27	1.24
BI2	In the future, I predict I will eat insects	2.33	1.28
BI3	I plan to eat insects soon	1.86	1.05

Items measured on a 5-point Likert scale, and adapted from literature (Fishbein and Ajzen, 2009).

Assessment done by the study found that three of the five proposed hypotheses were supported; their path relationships were significant at the 0.05 level and had signs in the expected directions with beta coefficients ranging from 0.282 to 0.609. On the other hand, subjective norm (SN) and perceived behavioural control (PBC) displayed a non-significant correlation with Bi, and the associated hypothesis H2 and H4 were rejected. The following Table 5 summarizes these results.

This work explored the intention and use behaviour of a sample of young consumers in Macau regarding edible insects. Present model explained 64.6% of behavioural intention and 31.9% of use behaviour, and three of the five proposed hypotheses were accepted. Taking into account that the construct of subjective norm did not contribute to the model, present results are considered to be satisfactory and provide valuable insights. There is a possibility that the construct of subjective norm was not significant and negatively correlated to intention because most people haven't tried eating insects yet. Therefore, the majority of consumers were not able to persuade family members and friends to use edible insects. It is assumed that the impact of

Table 5. Assessment of hypotheses

Hypothesis	Path	Coefficient	t-Value	*p*-Value	Supported
H1	ATT→BI	0.608	6.900	0.000	YES
H2	SN→BI	-0.025	0.309	0.757	**NO**
H3	PBC→BI	0.284	3.405	0.001	YES
H4	PBC→USE	0.079	0.646	0.519	**NO**
H5	BI→USE	0.510	7.516	0.000	YES

Hypotheses evaluation criteria: t-value>1.96; p-value<0.05

the social norm may become increasingly relevant over time as individuals become familiar with the idea of consuming insects as food; this is because social factors typically play important roles in the decision to adopt new ideas (Lampo, Silva and Duarte, 2022). Different from the mainstream theory (Fishbein and Ajzen, 1975), perceived behavioural control did not perform as a direct predictor of the actual behaviour. It is possible that consumers' perceptions may not reflect an actual control since they did not know where to find insect-based food products in Macau or that insects were not compatible with their dining habits, thus precluding an actual behaviour. On the other hand, attitude and perceived behavioural control were key determinants of intention in the prsent model and, indirectly, of actual use.

The detailed analysis of the construct of behavioural intention showed that respondents in Macau, on average, were relatively unwilling to consume insects as food at the time of the survey, even though the practice of using insects (for example in the case of traditional Chinese medicine) is well established in the region. In particular, present sample hints that men tend to be more inclined to consume insects than women, but a larger sample should be used for this conclusion. To conclude, the findings are relevant as an exploratory study, taking into account Macau's plan to promote internationally a unique and sustainable image in the food sector.

Throughout the literature, it is apparent that entomophagy has the potential for benefiting society on different levels, including social (reducing hunger), environmental (reducing the footprint of meat consumption), and health (nutritious food). Yet, consumers on average are hesitant about insects as food. However, history has shown that food habits can change. For instance, the rapid acceptance of eating raw fish in the form of sushi is a good example (ScienceDirect, 2023). Similarly, the culture of entomophagy should be disseminated where it is not widespread yet, such as in Macau. The food industry should play a significant role in raising the status of insects as food, through the creation of original recipes, gourmet dining, or events. It is also suggested that to increase the acceptance of insects as food, research and practice should focus on optimizing the experience for early adopters rather than trying to convince reluctant consumers, so as to enhance the effects of social norms and promote diffusion. Ultimately, consumers decide whether insects will become a common food source in society.

The present findings provide insights into consumers' intentions toward eating insects in Macau, but some limitations exist. For instance, the design opted for convenience sampling techniques assuming that the participants were similar to the overall target population, and based the analysis on a relatively limited sample. Most importantly, the research examined the intentions of consumers at a single point in time and used a specific theoretical framework. Therefore, future research should draw from a larger sample to take advantage of more statistical tests, make use of different theoretical frameworks, and include new constructs that may help to shed light on the individual peculiarities when it comes to considering insects as food.

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AUTHOR CONTRIBUTION STATEMENT

All authors have contributed equally to the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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GENETIC DIVERSITY OF MAJOR POLYPHAGOUS SPIDER MITE SPECIES ACROSS HOST PLANTS AND SPATIAL DISTRIBUTION

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ABSTRACT

Different agroecosystems of southern Karnataka sampled for plant associated mites recorded six genera of tetranychid mites viz., *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus* and *Tetranychus*. Neutrality test revealed a lowest haplotype diversity (0.993) of *T. neocaledonicus* population and Tajima's D and Fu's Fs test values were negatively significant for *T. fijiensis, E. orientalis* and *S. baltazari* populations, indicating the greater expansion of populations. Genetic analysis with ITS2 (rDNA) sequences revealed highest similarity between mite genera *Petrobia* and *Aponychus* showing lowest genetic distance of 0.51. *Schizotetranychus* has sister relationship with *Eutetranychus* which in turn clustered with *Petrobia* and *Aponychus. Tetranychus* and *Oligonychus* genera with lower genetic distance of 0.60 are clustered together. The study confirmed the clustering of morphologically related genera and lower genetic distances observed between related genera, expressed sister relationship. Molecular phylogenetic analysis of species under genus *Oligonychus* showed clustering in line with the morphology-based species taxonomic keys, while polyphagous species under the genus *Tetranychus* did not express form this grouping. Genetic diversity study of ten polyphagous mite species across their host plants and geographical occurrence revealed grouping according to locations in the phylogenetic tree. However, populations of *Oligonychus tylus* with a narrow host range showed close relationship for host plants than geographical locations which was evident in phylogenetic tree branching.

Key words: Tetranychid mites, *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus, Tetranychus* genetic diversity, ITS2, neutrality test, phylogenetic analysis, host plants, cluster analysis, host range, polyphagous

Acari is a species rich subclass under the class Arachnida of subphylum Chelicerata under the phylum Arthropoda with more than 50,000 described species worldwide (Zhang, 2003). The Acarine diversity is such that, the currently described species represent only a small fraction of the present species and half to one million species in the world are yet to be discovered (Walter and Proctor, 1999). Apart from this vast diversity, spider mites are known to exhibit morphological variations and phenotypic plasticity, perceived as environmental variations (Meyers and Bull, 2002). Hence, molecular methods are increasingly being accepted for accurate taxonomic identification of mites as well as delineation of species. DNA sequences of mitochondrial COI (Cytochrome Oxidase I) gene and ITS (Internal Transcribed Spacer) regions of rDNA genes are widely used for phylogenetic analyses at different taxonomic levels, to ascertain intergeneric relationships in Tetranychidae (Navajas et al., 1996), interspecific studies (Navajas et al., 1997, 1998; Navajas and Boursot, 2003; Hinomoto et al., 2007b) and interpopulation analyses within a species (Navajas, 1998; Navajas et al., 1999; Hinomoto and Takafuji, 2001&2004; Hinomoto et al., 2001, 2007a). Using ITS2 DNA sequences Navajas et al. (1992) investigated phylogenetic relationships between six tetranychid mite species viz., *Eotetranychus carpini E. pruni, Tetranychus pacificus, T. mcdanieli, T. turkestani* and *T. urticae*. Detailed reassessments of the data could be used in studying the biogeography of the spider mite genera and for understanding their evolutionary relationships. Indian tetranychid mite fauna is lacking in such studies, except for the molecular phylogenetic analysis of *Tetranychus urticae* and *Tetranychus macfarlanei* by Zeity (2015). Considering the diversity of spider mites both within and between species, present study focused on the genetic diversity of few agriculturally important tetranychid mite pests was conducted using ITS2 sequences owing to its importance in the studies concerning intraspecific relationships. The noncoding

rDNA spacer ITS2 comprise fastest-evolving tool used frequently for validation of species status (Abdel-Mawgood, 2012) and an effective diagnostic tool for quarantine and decision making (David et al.,2007). Intraspecific variation among the populations of 10 economically important polyphagous spider mite species viz., *Eutetranychus orientalis* (Klein), *Oligonychus biharensis* (Hirst), *Oligonychus tylus* Baker and Pritchard, *Schizotetranychus baltazari* Rimando, *Tetranychus fijiensis* Hirst, *Tetranychus ludeni* Zacher, *Tetranychus macfarlanei* Baker and Pritchard, *Tetranychus neocaledonicus* Andre and *Tetranychus truncatus* Ehara was studied. Outcome of the study be extrapolated to understand their genetic relationship, expansion of pest status and the pest scenario in relevance to changing agricultural practices and the climate change as well.

MATERIALS AND METHODS

Mite samples collected from different locations and host plants were suitably preserved in absolute alcohol in airtight plastic vials (Table 1). After initial extraction of genomic DNA using CTAB method, ITS2 sequence region was amplified using 5.8S (5'-GGGTCGATGAAGAACGCAGC-3') and 28S (5'-ATATGCTTAAATTCAGCGGG-3') primers. PCR was performed in 25- µl reaction volume with 10X Scigenomics® Taq buffer (15 mM $MgCl_2$, 100 mM Tris pH 9), 2.5 mM of each nucleotide dNTP, 10 pmol. each of 5.8S and 28S primers, Genei® Taq polymerase (5 units/µl) and 1 µL DNA template in Bio-Rad® DNA Engine thermocycler, with 1 cycle of initial denaturation for 60 s at 94 °C followed by 35 cycles of denaturation (60 s at 94 °C), annealing (90 s at 52 °C) and extension (72 °C for 10 min), with a final extension period of 72°C for 10 min units. Amplified DNA segments were sequenced (Applied Biosystems® ABI 3130 XL), subjected to BLAST analysis and were then deposited in NCBI GenBank database (Accession No. given in supplementary data as Supplementary Table S1). The genetic diversity of populations was calculated using DnaSP 5.0 and neutrality tests, including Tajima's D (Tajima 1989) and Fu and Li's F (Fu 1997) were implemented for each population in DnaSP 5.0 software. Phylogenetic analysis with rDNA (ITS2) sequences of different populations across hosts and locations was carried out. The sequences were aligned using MEGA-X 64 software and genetic distances were calculated. Molecular phylogenetic reconstructions were executed using UPGMA (for inter and intrageneric studies) and Maximum Likelihood method (for intraspecific studies) with 1000 bootstrapping replications. Further, the aligned sequences were used in the assessment of genetic diversity by computing divergence as well as pairwise distance values.

RESULTS AND DISCUSSION

Neutrality test and genetic diversity analysis of spider mite populations revealed that the genetic diversity of 10 spider mite species collected from geographic regions in Karnataka. The haplotype diversity (Hd) of *T. neocaledonicus* population was the lowest (0.993) compared to other populations (1.000) and similar phenomenon was also detected for nucleotide diversity (Pi) of 0.71102, followed by 0.71236 for *T. truncatus* population. Under the hypothesis of selective neutrality and population equilibrium, Tajima's D and Fu's Fs test values tend to be negative under an excess of recent mutations, which is regarded as evidence of population expansion (Tajima, 1989; Fu, 1997). Tajima's D and Fu's Fs tests values of populations showed negative, indicating recent mutations and population expansion. The populations of *T. fijiensis*, *E. orientalis* and *S. baltazari* were significant for both tests, indicating that the expansion of these species was greater than that of the other species (Table 1). The *O. biharensis* and *O. tylus* populations also showed significant signs of expansion based on Tajima's D test; the other populations showed no significant signs of expansion. Genetic diversity data of tetranychid populations relevant to Indian situations of host plants and geographical locations is obsolete of source. The lowest haplotype diversity (Hd) of *T. neocaledonicus* population and negatively significant values for Tajima's D and Fu's Fs test for *T. fijiensis, E. orientalis* and *S. baltazari* populations in the present study indicates the greater population expansion than that of the other groups as claimed by Cai *et al.* (2019). Similar trend was observed in the field surveys, where *T. neocaledonicus* species found taking over the host plants of *T. urticae,* which was considered as serious mite pest for decades. Also, *E. orientalis* and *S. baltazari* species found expanding their host range in the field study confirming the results of genetic diversity analysis.

ITS2-rDNA sequences of six genera of Tetranychidae viz., *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus* and *Tetranychus* were subjected to phylogenetic analysis using *Raoiella* representing mite Family Tenuipalpidae as out group. Highest similarity was evident between *Petrobia* and *Aponychus, Tetranychus* and the out group *Raoiella*. Genus *Schizotetranychus* showed sister relationship with *Eutetranychus* which clustered with genera *Petrobia*

Table 1. Details of GPS location and GenBank accession number of mite populations analysed

Sl. No.	Species	Location	GPS coordinates		Host plants	GenBank Accession
			Latitude (N)	Longitude (E)		
1.	*Aponychus corpuzae* Rimando	Bengaluru	13°04'40"	75°25'03"	*Bambusa* sp.	KT361606
2.	*Eutetranychus orientalis* (Klein)	Tarikere	13°45'13"	75°52'06"	*Nerium oleander*	MW981326
		Chitradurga	14°13'00"	76°23'49"	*Wrightia tinctoria*	OM248464
			14°13'00"	76°23'49"	*Azadirachta indica*	OM214534
3.	*Oligonychus bellarensis* sp. nov.	Ballari	15°10'23"	76°19'31"	*Saccharum officinarum*	MG677944
4.	*Oligonychus biharensis* (Hirst)	Harihara	14°30'27"	75°49'24"	*Militia pinnata*	MW709398
		Koppa	13°31'50"	75°21'28"	*Rosa chinensis*	MZ618719
		Thirthahalli	13°41'56"	75°13'59"	*Rosa chinensis*	MZ615524
		Hassan	12°58'17"	76°15'42"	*Rosa chinensis*	MW909770
5.	*Oligonychus plegas* Baker & Pritchard	Bengaluru	13°04'38"	77°34'39"	*Megathyrsus maximus*	MN986931
6.	*Oligonychus thelytokus*	Chikkamagaluru	13°31'50"	75°21'28"	*Syzygium jambos*	MZ604933
7.	*Oligonychus tylus* Baker & Pritchard	Honnalli	14°14'00"	75°38'33"	*Zea mays*	MZ618679
			14°14'00"	75°38'33"	*Pennisetum glaucum*	MW709399
		Hassan	12°58'17"	76°15'42"	*Cocos nucifera*	MW909769
8.	*Petrobia hartii*(Ewing)	Mudigere	13°07'15"	75°37'01"	*Oxalis* sp.	MW714337
9.	*Schizotetranychus baltazari* Rimando	Channagiri	14°07'42"	75°53'08"	*Azadirachta indica*	MW14336
		Hiriyur	13°53'06"	76°29'26"	*Murraya koenigii*	OM248459
		Chitradurga	14°13'00"	76°23'49"	*Azadirachta indica*	OM214535
10.	*Schizotetranychus* sp.	Chitradurga	14°11'35"	76°23'38"	*Bambusa* sp.	OM219636
11.	*Tetranychus bambusae* Wang and Ma	Hassan	12°58'17"	76°15'42"	*Bambusa* sp.	MW909782
12.	*Tetranychus fijiensis* Hirst	Mudigere	13°05'00"	75°39'40"	*Cocos nucifera*	MT582419
		Shivamogga	13°58'33"	75°34'40"	*Areca catechu*	MW459972
		Tarikere	13°45'13"	75°52'06"	*Areca catechu*	MW980051
		Chikkamagaluru	13°16'43"	75°48'06"	*Citrus* sp.	MN963777
13.	*Tetranychus lombardinii* Baker and Pritchard	Shivamogga	13°58'35"	75°33'06"	*Jasminum* sp.	MW980043
14.	*Tetranychus ludeni* Zacher	Hassan	12°58'17"	76°15'42"	*Parthenium hysterophorus*	MW911625
		Munirabad	15°17'47"	76°19'00"	*Ocimum* sp.	MN963774
		Shikharipura	14°14'05"	75°22'15"	*Bidens pilosa*	MZ618720
		Soraba	14°17'51"	75°15'32"	*Parthenium hysterophorus*	MZ540269
		Kadur	13°32'54"	76°00'36"	*Impatience balsamina*	MW704096

(contd. Table 1)

15.	*Tetranychus macfarlanei* Baker and Pritchard	Shivamogga	13°58'33"	75°34'40"	*Abelmoschus esculentus*	MW714334
			13°58'33"	75°34'40"	*Vicia faba*	MN963776
			13°58'33"	75°34'40"	*Impatience balsamina*	MT582418
			13°58'33"	75°34'40"	*Vigna unguiculata*	MT580908
			13°58'33"	75°34'40"	*Abelmoschus esculentus*	MW714335
			13°58'33"	75°34'40"	*Glycine max*	MT576576
			13°58'33"	75°34'40"	*Boerhavia diffusa*	MW332262
			13°58'33"	75°34'40"	*Tagetes erectus*	MW784003
		Bavikere	13°45'13"	75°52'06"	*Desmodium ganageticum*	MW459984
		Honnalli	14°14'00"	75°38'33"	*Pennisutum glaucum*	MZ618639
		Chamarajanagar	11°55'32"	76°56'44"	*Abelmoschus esculentus*	MT023425
		Hassan	12°58'17"	76°15'42"	*Phaseolus vulgaris*	MW911626
			12°58'17"	76°15'42"	*Lucas* sp.	MW911628
		Bidadi	12°47'55"	77°23'28"	*Abelmoschus esculentus*	MN794988
		Bramhavara	13°25'27"	74°45'24"	*Piper betel*	OM680002
16.	*Tetranychus neocaledonicus* Andre	Bengaluru	13°04'23"	77°34'56"	*Acalypha wilkasiana* var. inferno	MT023426
		Bengaluru	13°04'10"	77°36'45"	*Tagetes erectus*	MT020380
		Mudigere	13°05'00"	75°39'40"	*Piper betel*	MT576583
		Chikkamagaluru	13°16'43"	75°48'06"	*Hibiscus rosa-chinensis*	MW704021
		Tarikere	13°45'13"	75°52'06"	*Tinospora cardifolia*	MZ620637
		Mandya	12°34'06"	70°50'09"	*Macrotyloma uniflorum*	MW439310
			12°34'06"	70°50'09"	*Polyscias scutellaria*	MZ6020636
		Honnalli	14°14'00"	75°38'33"	*Tectona grandis*	MZ618670
		Davanagere	14°21'20"	75°44'30"	*Vigna mungo*	MW704022
		Thirthahalli	13°41'56"	75°13'59"	*Clitoria turnatea*	MZ618715
			13°41'56"	75°13'59"	*Syndrella nodiflora*	MZ618680
			13°41'56"	75°13'59"	*Codiem variegatum*	MZ618721
		Soraba	13°41'56"	75°13'59"	*Tinospora cardifolia*	MZ620637
		Hassan	12°58'17"	76°15'42"	*Acalypha wilkasiana*	MW915439
			12°58'17"	76°15'42"	*Rosa chinensis*	MW916311
			12°58'17"	76°15'42"	Dicot weed	MW911835
			12°58'17"	76°15'42"	*Carica papaya*	MW916308

(contd. Table 1)

(contd. Table 1)

17.	*Tetranychus okinawanus* Ehara	Hebri	13°07'57"	74°46'19"	*Hibiscus rosa-sinensis*	OM248465
18.	*Tetranychus truncatus* Ehara		13°16'43"	75°48'06"	*Parthenium hysterophorus*	MW332245
		Chikkamagaluru	13°16'43"	75°48'06"	*Amaranthus* sp.	MW748069
			13°16'43"	75°48'06"	*Crassocephalum crepediodus*	MW789347
		Harihara	14°30'27"	75°49'24"	*Tinospora cardifolia*	MZ542294
		Bhadravati	13°59'18"	75°41'43"	*Vicia faba*	MW704097
		Mudigere	13°06'53"	75°37'55"	*Solanum melongena*	MW332246
		Surahonne	14°08'22"	75°33'07"	*Rosa chinensis*	MW488928
		Soraba	14°17'51"	75°15'32"	*Tinospora cardifolia*	MZ618617
		Shikharipura	14°14'05"	75°22'15"	*Solanum melongena*	MZ618678
			14°14'05"	75°22'15"	*Tinospora cardifolia*	MZ618717
		Kaapu	13°00'30"	74°58'27"	*Amaranthus dubius*	MW704097
19.	*Tetranychus udaipurensis* Gupta and Gupta	Tarikere	13°45'13"	75°52'06"	*Ricinus communis*	MW979818
20.	*Tetranychus urticae* Koch	Shikharipura	14°14'05"	75°22'15"	*Solanum lycopersicum*	MZ618718

Neutrality test and genetic diversity metrics for populations of spider mite species

Population	Hd± SD	Pi	Tajima's D	Fu's Fs test
Eutetranychus orientalis	1.000± 0.177	0.73672	-2.11817*	-0.95124*
Oligonychus biharensis	1.000± 0.177	0.73100	-2.06570 *	-0.92096
Oligonychus tylus	1.000± 0.177	0.73621	-2.05782*	-0.92096
Schizotetranychus baltazari	1.000± 0.177	0.73200	-2.08446*	-0.95609*
Tetranychus fijiensis	1.000± 0.177	0.73262	-2.12850*	-0.98502*
Tetranychus ludeni	1.000± 0.126	0.71720	-1.65189	-0.77852
Tetranychus macfarlanei	1.000± 0.027	0.73015	-0.87879	0.52857
Tetranychus truncatus	1.000± 0.039	0.71236	-1.07616	0.04190
Tetranychus neocaledonicus	0.993± 0.996	0.71102	-0.66557	0.76135

Hd: haplotype diversity; SD: standard deviation; Pi: nucleotide diversity; *: significant difference ($p < 0.001$)

and *Aponychus*. While, *Oligonychus* clustered with *Tetranychus* and the outgroup *Raoiella* (Fig. 1). Genetic distance was lowest for *Aponychus* with *Petrobia* (0.51) and *Eutetranychus* (0.61), which clustered together. These genera were morphologically similar and excepting *Petrobia,* other two genera together form a tribe Eurytetranychini. *Tetranychus* and *Oligonychus* had lower divergence value of 0.60 and clustered together. Genetic distance of the out group *Raoiella* was lowest with *Tetranychus* (0.53) with which it had clustered as a sister group. While it was highest with *Petrobia* (1.12). The study revealed clustering of morphologically related genera together and lower genetic distances observed between related genera showing their sister relationship. This phylogenetic analysis of ITS2 region (rDNA sequences) of six genera confirmed the clustering of more morphologically related genera together. Lower genetic distances were observed between related genera evidencing their sister relationship.

Phylogenetic analysis of species under *Oligonychus* genus with *T. macfarlanei* as outgroup showed clustering of *O. tylus* and *Oligonychus bellarensis* sp. nov. together i.e., prompting to morphology-based species taxonomic keys. *O. biharensis* and *O. thelytokus* which were morphologically near similar having similar host range appeared in the common clade (Fig. 2). No similarity was observed in species clustering under the genus *Tetranychus* for their morphological taxonomic keys. But *T. bambusae* which is a grass feeding species clustered outside all other polyphagous species associated with broad leaved plants (Fig. 3). Genetic distances of *E. orientalis* populations harbouring neem, *W. tinctoria* and *N. oleander* at different locations of Chitradurga and Tarikere were estimated by using Mega-X software. The genetic distance within *E. orientalis* ranged from 0.053 to 0.087. The lowest distance between *N. oleander* and *W. tinctoria* host populations (0.053) and the maximum distance among populations from hosts of *Azadirachta* and *W. tinctoria* (0.87). Genetic distances between *E. orientalis* and out-group *Eutetranychus* sp. ranged from 0.056 to 0.085, of which least was in *N. oleander* population and maximum in *W. tinctoria* population. Phylogenetic tree of *E. orientalis* revealed deeper relationship among populations for host plants. Populations of *N. oleander* and *W. tinctoria* clustered together. Similarly, populations from neem plants clustered (with bootstrap value of 96) with the out group *Eutetranychus* sp. which also associated with neem plants in other locations (Fig. 4).

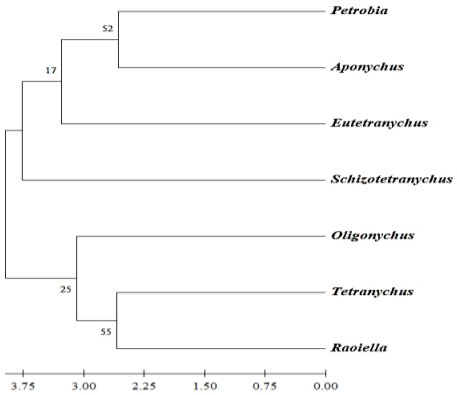


Fig. 1. Phylogenetic tree of tetranychid mite genera collected in the study for the ITS2 sequences using UPGMA method

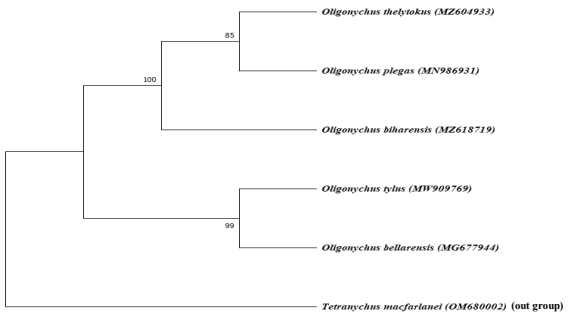


Fig. 2. Phylogenetic grouping of the genus *Oligonychus* of ITS2 sequences using UPGMA

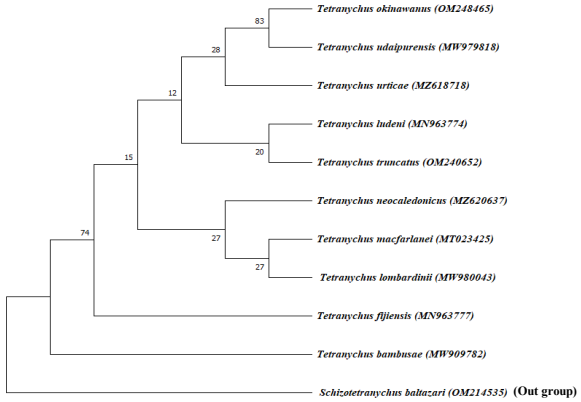


Fig. 3. Phylogenetic grouping of the genus *Tetranychus* of ITS2 sequences using UPGMA

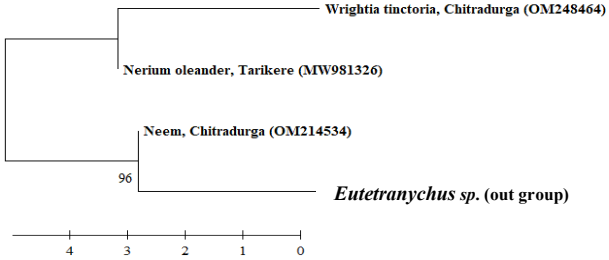


Fig. 4. Phylogenetic tree inferred from ITS2 sequences of *Eutetranychus orientalis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

For *Oligonychus biharensis* (Hirst, fgenetic distance within the species ranged from 0.051 to 0.068, with a lowest of 0.051 for population on *Pongamia* from Davangere and on rose plant from Thirthahalli followed by Hassan (0.057) population. Distance within *O. biharensis* species and out group *O. thelytokus* was the lowest for the populations of rose from Hassan (0.053) followed by rose population from Koppa (0.056). Genetic distance was the highest for populations of *Pongamia* (0.088). The variation in the distance of populations from same host plant across different locations signifies the genetic diversity of *O. biharensis* attributed to the difference in locations. Phylogenetic analysis exhibited similar pattern, as the populations on rose plant in different locations clustered together (bootstrap value = 78) showing sister relationship. Also, Koppa and Thirthahalli rose populations fall in the same cluster (Fig. 5) owing to closer geographical distribution compared to distant location evident with Hassan population. For *Oligonychus tylus* Baker and Pritchard: Among the populations of *O. tylus,* genetic distance ranged from 0.054 to 0.080, the lowest distance between bajra and maize populations from Honnalli and the highest of 0.080 distance between populations occurring on coconut. This suggested that genetic distance between *O. tylus* populations occurring on grasses was lower (0.054-0.064) compared to that occurring on tall coconut tree (0.080), indicating its diversity for host plants. Genetic distance of *O. tylus* and the out-group *O. biharensis* was low for maize population (0.056) and high for bajra population. Phylogenetic analysis of ITS2 sequences confirmed the genetic diversity of *O. tylus* for host plants. Bajra and maize populations showed sister relationship with bootstrap value of 60 which clustered with *I. cylindrica* population (bootstrap value = 46). Whereas, coconut population clustered as a group well separated from population occurring on plants of grass species (Fig. 6).

With *Schizotetranychus baltazari* Rimando, genetic distance between populations ranged from 0.067 to 0.14, with the minimum distance observed between neem and curry leaf populations from locations of Hiriyur and Chitradurga. It was maximum for neem population from Channagiri location. This confirmed the genetic diversity of *S. baltazari* populations for geographical locations. The out group *Schizotetranychus* sp. exhibited minimum genetic distance (0.075) for neem population (from Channagiri) and maximum of 0.181 for curry leaf population (from Hiriyur). *Schizotetranychus baltazari* populations occurring on curry leaf and neem from different locations in Chitradurga district clustered with bootstrap value of 96 and separated from Channagiri population (Fig. 7).

Regarding *Tetranychus fijiensis* Hirst, arecanut population from Shivamogga and citrus population from Chikkamagaluru had lower genetic distance of

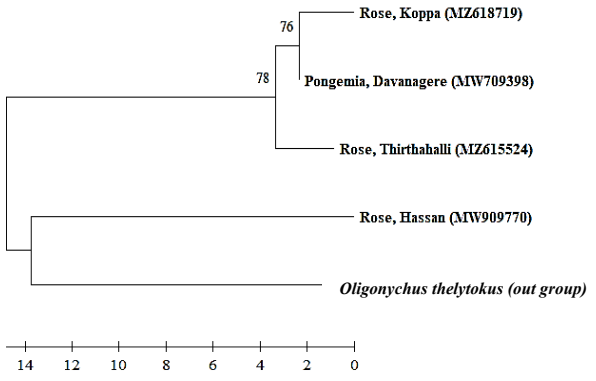


Fig. 5. Phylogenetic tree inferred from ITS2 sequences of *Oligonychus biharensis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

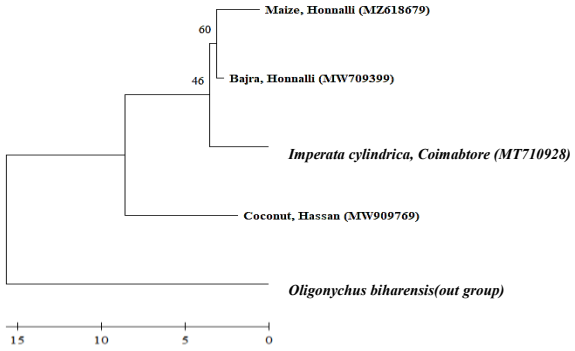


Fig. 6. Phylogenetic tree inferred from ITS2 sequences of *Oligonychus tylus* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

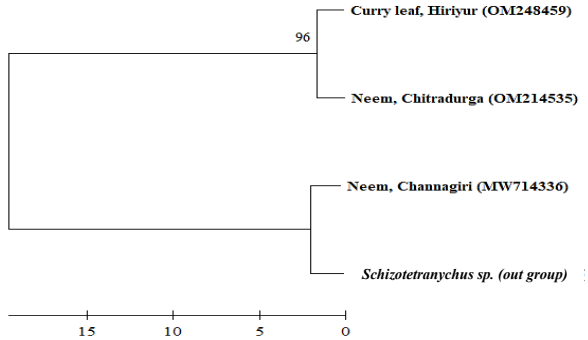


Fig. 7. Phylogenetic tree inferred from ITS2 sequences of *Schizotetranychus baltazari* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

0.050, while higher distance observed among coconut populations from Mudigere and arecanut population from Tarikere. This genetic difference could probably due to the environmental factor, the former two populations were from Southern Transitional zone, while the latter populations were from two extreme environments of hilly (Mudigere) and dry zones (Tarikere), accounting the genetic diversity of *T. fijiensis* to geographical locations. The out-group *T. ludeni* showed maximum genetic distance for citrus populations from Chikkamagaluru (0.094) and highest to arecanut population from Shivamogga (0.045). Phylogenetic analysis exhibited a distinct separation of *T. fijiensis* from the out-group *T. ludeni.* Arecanut population from Tarikere and citrus population from Chikkamagaluru clustered (with bootstrap value of 88) and formed a sister clade with arecanut population from Shivamogga (Fig. 8). Coconut population from Mudigere exhibited sister relationship with all other populations. With *Tetranychus ludeni* Zacher, populations showed a wider range of genetic distance (0.052 to 0.090). Lower distance of 0.052 was between *Ocimum* population from Munirabad and *Parthenium* population from Hassan.While the highest (0.090) distance was between balsam population from Kadur and black-jack population from Shikharipura. The outgroup *T. fijiensis* showed highest genetic distance of 0.102 with black-jack population and the while lowest distance of 0.056 was with *Parthenium* weed plant population from Hassan. Similarly, the populations with lower genetic distances clustered together in the dendrogram with the outgroup *T. fijiensis*. *Ocimum* population from Munirabad; parthenium population from Hassan and parthenium population from Soraba; balsam population from Kadur together with bootstrap values of 54 and 31, respectively (Fig. 9). Black jack

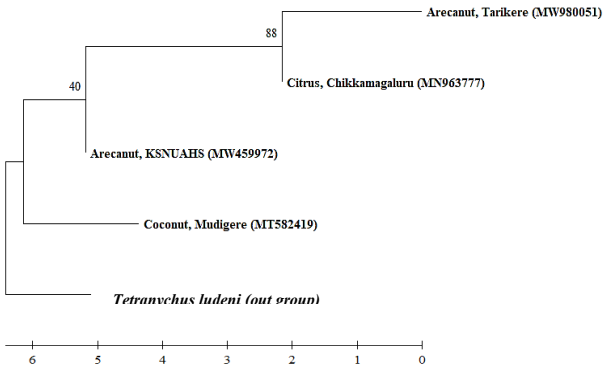


Fig. 8. Phylogenetic tree inferred from ITS2 sequences of *Tetranychus fijiensis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

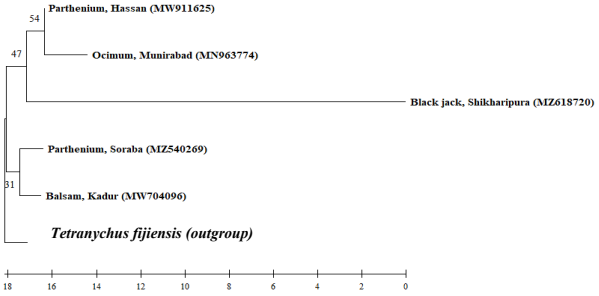


Fig. 9. Phylogenetic tree inferred from ITS2 sequences of *Tetranychus ludeni* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

population formed a sister group with populations from former two host plants of *Ocimum* and parthenium.

As far as Tetranychus macfarlanei Baker and Pritchard is concerned, genetic distance across 15 populations ranged from 0.038 to 0.096. Among these populations, French bean population exposed highest distance with *D. gangeticum* population and the lowest with okra population. Genetic distance of *T. macfarlanei* and the out-group *T. fijiensis* ranged from 0.042 to 0.077. It was the lowest for okra (Shivamogga) population and highest for *Leucas* population. Phylogenetic analysis showed sister relationship among okra and balsam populations from Shivamogga, cowpea and soybean populations from Shivamogga (Fig. 10) and between bajra and *Lucas* population (Hassan). These populations showed lower genetic distances of 0.055, 0.044 and 0.043, respectively and clustered accordingly. Clustering of populations from different host plants from the same location indicated the similarity for locations and or divergence for host plants. With *Tetranychus neocaledonicus* Andre, genetic distance between the populations*s* ranged from 0-0.036. *T. cardifolia* population exhibited no variation for hibiscus population, while it was the lowest with *Acalypha* population (0.015). Rose (Hassan) and undetermined weed (Hassan) populations showed no variation with zero distance. Highest distance was observed between papaya and *Acalypha* populations of Hassan. This confirmed the genetic similarity of *T. neocaledonicus* populations harbouring different host plants in the same location and was diverse for populations occurring on different host plants. The out-group *T. udaipurensis* exhibited the lowest genetic distance of 0.015 for horse gram population, followed by marigold population (0.017), while distance was maximum for *Acalypha* (Hassan) population. Phylogenetic analysis of 18 populations of *T. neocaledonicus* showed distinct

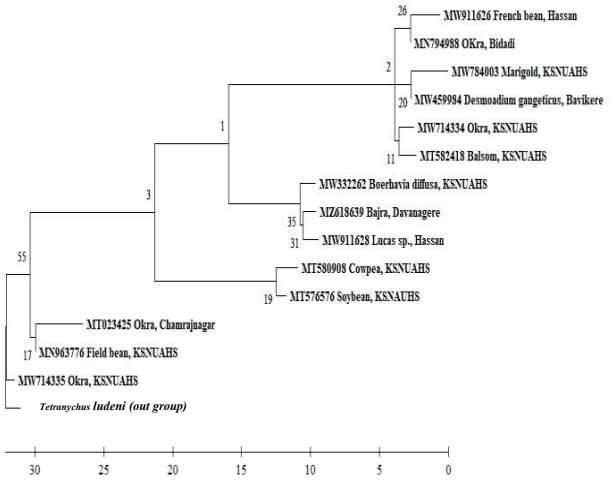


Fig. 10. Phylogenetic tree inferred from ITS2 sequences of Tetranychus macfarlanei by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

clustering of *T. cardifolia* (Soraba) and hibiscus (Amble) hosts; *Clitoria* and *Acalypha* (Hassan); rose (Hassan) and weed (Hassan); *Synedrella* (Thirthahalli) and horse gram (Mandya); croton (Thirthahalli) and black gram (Honnalli) showing sister relationship. The genetic distances corresponding to these populations were low 0, 0.032, 0, 0.022, 0.018 and hence clustered in a sister clade. Of which closer populations with zero distances i.e., *T. cardifolia* (Soraba); hibiscus and rose (Hassan); weed (Hassan) clustered with a high bootstrap value of 99 and 96, respectively (Fig. 11). The populations from Hassan, Mandya and Bengaluru clustered as a separate clade and those from Davanagere, Shivamogga and Chikkamagaluru formed separate clade. This confirmed the genetic similarity in *T. neocaledonicus* populations to geographical locations and its diversity for host plants.

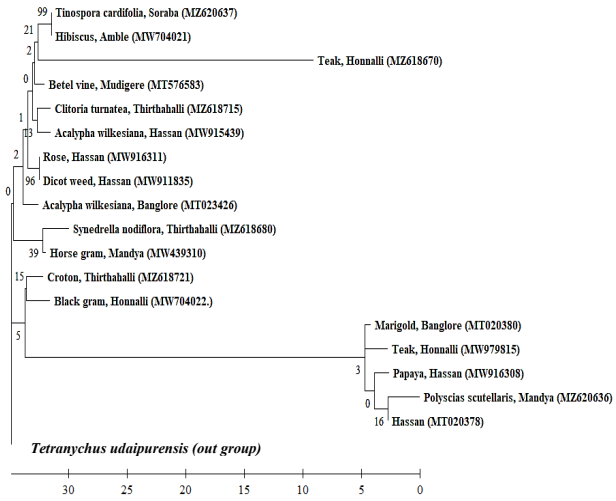


Fig. 11. Phylogenetic tree inferred from ITS2 sequences of Tetranychus neocaledonicus by Maximum Likelihood method. (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from 1000 samples)

Tetranychus truncatus Ehara revealed that the genetic distance of the populations ranged from 0 to 0.117 with zero distance between rose and *T. cardifolia* populations, followed by *Amaranthus* populations from Kaapu and Chikkamagaluru (0.052). While the highest distance of 0.117 was observed between populations of field bean & *T. cardifolia*. Genetic distance between the populations of *T. truncatus* and the out group (*T. neocaledonicus*) was the lowest (0.057) for field bean population (Bhadravati) and the highest (0.117) for *Amaranthus* population (Kaapu), thus showed their nearness and relationships, respectively. The phylogenetic analysis showed distinct clustering of populations from Southern Transitional zones, hilly & coastal zones as separate clades. Populations of Shikaripura, Sorahonne, Davangere and Chikkamagaluru formed a clade with bootstrap value of 19 and Mudigere and Kaapu in a clade with Bhadravati and Chikkamagaluru populations (bootstrap value = 27), branching represented Southern Transitional zone with populations from Soraba, Shikaripura and Chikkamagaluru (bootstrap value = 67). Rose (Surahonne) and *T. cardifolia* (Shikharipura) populations with zero genetic distance clustered with bootstrap value of 92 (Fig. 12). This confirmed the diversity of *T. truncatus* for host plants and similarity for location.

Variation in ITS2 sequences for populations of most of the tetranychid (spider mite) species was more related to locations indicating their genetic separation by geographical borders that are barriers of gene flow and prompting the population structure. As a result, different clades in the phylogenetic tree were closer

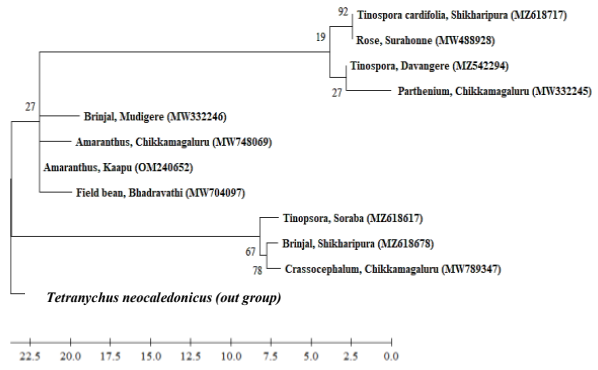


Fig. 12. Phylogenetic tree inferred from ITS2 sequences of Tetranychus truncatus by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

to near-locations. For populations of *Tetranychus turkestani* geographical locations of France appeared to be a factor determining its genetic structure with no distinct variations for populations harbouring different host plants (Bailly *et al* 2004). However, in our study *Oligonychus tylus* populations showed close relationship for host plants than for geographical locations with clades in the phylogenetic tree distinctly separated for host plants such as bajra, maize and *I. cylindrica*. In Japan Nishimura *et al.* (2007) distinguished different populations of *Tetranychus kanzawai* (using rDNA fragments of ITS1 region) and attributed significant variations to associated host plants. Mirza *et al* (2020) also concluded that genetic variation in ten different haplotypes of *E. orientalis* was more for host plants in Saudi Arabia. Host plant selection by herbivores could be a major factor separating different gene pools of tetranychid mites inhabiting specific host plants. The spatial distribution of mite populations and strength of gene flow are essential in ascertaining their future pest invasiveness. Spider mites display their astounding dispersal mechanism and management of weeds which serve as efficient host plants of spider mites during the off-cropping season may be of prime significance. Ecologically linked molecular studies would explicate the genetic structure of populations, that could be used in predicting pest outbreaks and thus may be utilised in scheming effective management strategies.

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AUTHOR CONTRIBUTION STATEMENT

Contributed in molecular confirmation, diversity studies (SM, NS, CC, RHP); Field surveys and host studies (SM, RK, RHP).

CONFLICT OF INTEREST

No conflict of interest.

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BIOCHEMICAL MECHANISM OF INSECTICIDE RESISTANCE IN *SPODOPTERA LITURA* (F) POPULATIONS FROM UTTARAKHAND

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ABSTRACT

***Spodoptera litura* populations were collected from different lowland areas and doon valley situated in Uttarakhand and insecticide resistance effect was studied for indoxacarb 14.5% SC, chlorantraniliprole 18.5%SC, fipronil 5% SC, chlorpyriphos 50%+cypermethrin 5% EC and profenofos 40%+cypermethrin 4% EC, also the specific activity of carboxylesterase, acetyl choline esterase, mono-oxygenase and glutathione-s-transferase was studied for biochemical analysis of detoxification enzymes. The scale of infestation was measured and related to temperature and humidity. High infestation has been observed in areas with optimum temperature and humidity. Highest resistance in the study was found against profenofos 40%+chlorpyriphos 4% and fipronil 5%SC with LC_{50} 243.63 µg/ml and 214.22 µg/ ml respectively. On biochemical analysis of detoxification enzymes, it was observed that the highest activity of all the enzymes was observed in Mota Haldu population. Hence, overproduction of detoxification enzymes was found to be responsible for insecticide resistance in *S. litura* populations studied.**

Key words: *Spodoptera litura*, carboxylesterase, acetyl choline esterase, mono-oxygenase, glutathione-S-transferase, Uttarakhand, insecticide resistance, Himalayan lowlands, LC_{50}, detoxification enzymes.

Spodoptera litura (F) is an economically important polyphagous pest on field crops and horticultural crops and is widespread in tropical and subtropical regions (Ferry et al., 2004). Losses from *S. litura* are estimated to vary from 26% to 100% under field conditions (Su et al., 2012). According to datasheet of CABI (2022), *S. litura* is a polyphagous pest and covers about 120 host range species, soybean having one of the important hosts. In Uttarakhand, *S. litura* is an important defoliator moth, causing significant crop losses (Singh and Sachan, 1992; Joshi et al., 2022). Chemical method of management has been the main control method till now, but recently most of the insecticides are ineffective on *S. litura* due to the development of insecticide resistance (Vengateswari et al., 2020). It has been documented in IRAC Newsletter 2017 that India ranks 7th among the top 20 countries and *S. litura* ranks 7th among the top 20 arthropods, in terms of number of cases of resistance. The most basic and classical detection of resistance is the dose-mortality experiments carried out in the laboratory in a controlled environment (Brown, 1976). There are primarily three types of assays, namely bioassays, biochemicals and molecular assays, which focus on phenotypic, biochemical, and genetic modifications (R4P Network, 2016). In biochemical analysis it was revealed that the main enzyme families involved in pesticide catabolism and/or sequestration are cytochrome P450-dependent mixed-function oxidases (MFOs), glutathione-S-transferases (GST), carboxylesterases (CarE) and UDT-glycosyltransferase (UGT) (Kranthi, 2005). So far, there has been no systematic study of *S. litura* in lowlands of Himalayas to study baseline resistance data from different geographical areas. The current study was conducted in 2019-2020 and 2020-2021 to study the insecticide resistance in *S. litura* in different areas situated in lowlands and valley of N-W Himalayas, India. The results of this study will facilitate insecticide resistance management practices.

MATERIALS AND METHODS

Field populations of *S. litura* egg masses and larvae were collected from four different lowland regions of Uttarakhand, namely Mota Haldu (District Nainital, 29.1473N, 79.5352E, 424 masl), Bichai, Tanakpur (District Champawat, 29.0875N, 80.0133E, 255 masl), Mandawar (District Haridwar, 30.039N, 77.7327E, 314 masl) and Bimora, Dakpathar (District Dehradun, 30.4691N, 77.7931E, 452 masl) in 2019-2020 and 2020-2021. Larvae were reared on artificial diet (Ballal, 2003) and adults were fed on 10% honey solution

under laboratory conditions (27± 1°C and 65± 5% relative humidity) with a photoperiod of 16:8 hours light: dark. Adults were kept in jars with butter paper attached for oviposition by females and reared to F1 generation. A susceptible population of *S. litura* was obtained from NBAIR, Bangaluru, Karnataka, India for comparative studies. Based on the survey information on insecticides used by farmers in the selected regions, five insecticides were used in the study, namely indoxacarb 14.5% SC, chlorantraniliprole 18.5%Sc, fipronil 5% SC, chlorpyriphos 50%+cypermethrin 5% EC and profenofos 40%+cypermethrin 4% EC.

The bioassay method of IRAC Method No. 7 (IRAC, 2010) was performed on third instar larvae of *S. litura*. The insecticide solution was prepared from a 1% stock solution by serial dilution. Fresh castor leaves of uniform size i.e., 5x5cm were taken and dipped in the insecticide solution for 10 seconds. Excess liquid was drained off and the leaves were then air dried for half an hour. Ten third instar larvae from the F1 progeny (after being starved for 6 hr) were transferred to each petri dish and mortality was recorded at 24, 48 and 72 hours post exposure. Tests were conducted at a controlled temperature of 27± 1 °C, 65± 5 % relative humidity and a 16:8 L:D photoperiod in Pulse Entomology Lab, Deptt of Entomology, College of Agriculture, GBPUA&T, Pantnagar. To investigate the potential of the detoxification enzymes, biochemical analysis was performed to quantity the total protein and estimate the activity of Monooxygenase (P450) (Kranthi, 2005), Glutathione-S-transferase (GST) (Van,1962 and Booth et al., 1961), Carboxylesterase (CarE) (Devonshire, 1977 and Van, 1962) and Acetyl choline esterase (AChE) (Kranthi, 2005). Polo Suite Leora Software LLC was used to estimate the LC_{50} values of the chemical bioassays, and the specific activities of the biochemical studies were analysed using ANOVA.

RESULTS AND DISCUSSION

The present study was conducted in four locations situated in lowlands and valley in the Uttarakhand. The survey was conducted in 2019-2020 and 2020-2021 and the locations selected are important agricultural areas in the region and exposure to various insecticides was observed. To determine the degree of infestation, a visual estimation methodology was used. The scale of infestation was determined based on the analysis of Vennila et al. (2010). Four scales were given for infestation: no insect/scares appearance (scale 0); scattered appearance of few (scale 1); severe incidence on only one branch (scale 2); severe incidence on more than one branch (scale 3); and complete severe incidence (scale 4). It was observed that at the time of collection there was complete severe incidence of *S. litura* in Tanakpur; severe incidence on more than one branch in Mota Haldu; severe incidence on only one branch in Dehradun and Haridwar. The infestation scale could be related with temperature, humidity, and availability of abundant host since the favourable conditions generated due to these factors are important for the growth and development of *S. litura* (Fand et al., 2015) (Table 1). Data for the previous month and the month of collection showed that areas such as Tanakpur had constant favourable climatic conditions in addition to host abundance. On the other hand, in areas like Dehradun and Haridwar the lack of availability of host could be the major factor for scarce appearance of *S. litura*.

Samples of *S. litura* collected at different locations and reared in F1 progeny bioassays were subjected to using third instar larvae (Table 2). Insecticide resistance results according to insecticide used and locations visited were classified according to Shen et al. (1991) insecticide resistance level classification. The study found that the degree of resistance varied from susceptible to high. For indoxacarb 14.5%SC, almost all sites were found to be sensitive, except for Mota Haldu, which exhibited a moderate level of resistance. For other insecticides, there was low to high level of resistance in the study areas. Fipronil 5%SC showed resistance at almost all locations ranging from a low level of resistance to an extremely high level of resistance, except

Table 1. Meteorological data and scale of infestation of areas from where *S. litura* was collected

S.No.	Name of place	Average temperature °C		RH (%)		Scale of infestation of *S. litura*
		(Previous month)	(Collection month)	(Previous month)	(Collection month)	
1	Mota Haldu (Nainital)	27.375	25.89	75.70	84.81	3
2	Bichai, Tanakpur, (Champawat)	22.21	21.57	78.47	84.92	4
3	Mandawar, (Haridwar)	25.56	21.45	80.41	64.08	2
4	Bimora, Dakpathar, Dehradun	25.56	21.45	80.41	64.08	2

Table 2. Dosage mortality response of *S. litura* against insecticides

Insecticides	Population	LC50 (µg/ ml) (95%FL)	Heterogeneity Chi square ($\chi 2$)	Regression Equation Y=a+bx	Resistance Ratio (RR)
Indoxacarb 14.5%SC	Mota Haldu (Nainital)	3.81(1.84-6.79)	0.378	Y=0.82+1.42x	10.89 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	0.87(0.44-1.84)	1.154	Y=0.11+1.25x	2.49 (Susceptible)
	Mandawar, Haridwar	0.79(0.47-1.32)	1.157	Y=0.25+1.75x	2.26 (Susceptible)
	Bimora, Dakpathar, Dehradun	0.71(0.38-1.29)	1.842	Y=0.29+1.49x	2.03 (Susceptible)
	Lab population	0.35(0.14-0.64)	0.435	Y=2.97+1.87x	
Chlorantraniliprole 18.5%SC	Mota Haldu (Nainital District)	18.74(8.38-49.23)	0.702	Y=1.28+1.02x	18.93 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	10.76(4.50-21.70)	0.211	Y=1.16+1.11x	10.87 (Moderate level of resistance)
	Mandawar, Haridwar	12.51(5.48-25.98)	0.238	Y=1.22+1.11x	12.64 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	1.74(0.65-3.30)	0.949	Y=0.31+1.22x	1.75 (Susceptible)
	Lab Population	0.99(0.62-1.64)	0.342	Y=0.05+1.84x	-
Fipronil 5%SC	Mota Haldu (Nainital District)	88.51(46.34-180.19)	0.384	Y= 2.43+1.25x	19.08 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	214.22(108.44-757.29)	0.621	Y=2.51+1.08x	46.17 (High level of resistance)
	Mandawar, Haridwar	81.05(42.51-223.57)	1.668	Y=2.28+1.2x	17.47 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	26.24(13.33-46.91)	0.157	Y=1.96+1.38x	5.66 (Low level of resistance)
	Lab Population	4.64(2.45-8.78)	0.391	Y=0.89+1.37x	
Chlorpyriphos 50%+ cypermethrin 5%EC	Mota Haldu (Nainital District)	64.92(28.85-122.82)	0.127	Y=2.21+1.22x	6.83 (Low level of resistance)
	Bichai, Tanakpur, Champawat	280.88(150.05-594.88)	0.586	Y=3.12+1.28x	29.57 (Moderate level of resistance)
	Mandawar, Haridwar	156.61(79.29-286.19)	0.517	Y=3.05+1.39x	16.49 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	48.11(26.92-107.43)	0.654	Y=2.23+1.34x	5.06 (Decreased Susceptibility)
	Lab Population	9.50(5.48-18.27)	0.075	Y=1.41+1.45x	-
Profenophos 40%+ cypermethrin 4% EC	Mota Haldu (Nainital District)	243.63(142.06-492.79)	0.616	Y=3.64+1.52x	56.53 (High level of resistance)
	Bichai, Tanakpur, Champawat	35.69(19.16-94.62)	0.773	Y=1.9+1.24x	8.28 (Low level of resistance)
	Mandawar, Haridwar	228.29(123.47-529.69)	0.106	Y=2.98+1.46x	52.97 (High level of resistance)
	Bimora, Dakpathar, Dehradun	103.78(59.12-176.77)	0.442	Y=3.24+1.62x	24.08 (Moderate level of resistance)
	Lab Population	4.31(2.32-8.29)	0.440	Y=0.83+1.31x	

for Dehradun which showed decreased susceptibility. For both the profenophos 40%+cypermethrin 4%EC and chlorpyriphos 50%+cypermethrin 5%EC in which OP and pyrethroids are in a ratio of 1:10 a good level of resistance was observed. Also, chlorantraniliprole 18.5%SC showed a moderate to high level of resistance for almost all locations except for Dehradun which showed susceptibility and *S. litura* population tested with Indoxacarb 14.5%SC was found to be susceptible to almost all populations. It was revealed in the study that fipronil 5%SC showed resistance at almost all locations except Dehradun and the possible cause of such a different outcome in the Dehradun may be the possible absence of a potential host and the selection pressure which is otherwise different from other locations.

The current studies are similar to the studies of Ahmad and Mehmood (2015) where a moderate level of resistance was developed in population of Pakistan in a gap of 8 years and increased from 5.5-5.6 folds resistance in 1998 to 28-35 folds in 2006. Ahmad et al. (2008) also reported 224 folds resistance for fipronil 5%SC and this high level of resistance was explained as result of multiple resistance mechanism. The results of profenophos 40%+cypermethrin 4%EC and chlorpyriphos 50%+cypermethrin 5%EC in the study are in in accordance to the study of Ahmad (2009), El-Guindy et al. (1983), Goebel and Jacquemard (1990) and Joshi et al. (2023) suggested that profenophos and chlorpyriphos show antagonism with cypermethrin in 1:10 ratio. For chlorantraniliprole 18.5% SC, Muthusamy et al. (2014) reported an 80.07 resistance ratio to the susceptible population of NBAIR in insecticide resistance class 4, Che et al. (2023) and Wang et al. (2019) also reported 77.0-fold and 22.3-fold resistance, justifying the present result. Similar findings were reported by Saleem et al. (2016) for Indoxacarb 14.5% SC who found very low to high levels of resistance ranging from 7-87 folds. Babu and Singh (2023) also reported a low to moderate level of resistance ranging from 4-11.88 fold for *S. litura* population of Southern Rajasthan, for indoxacarb from 2017-2019.

Levels of detoxification enzymes were studied in different places and compared to susceptible populations of NBAIR, Bengaluru (Table 3). Mota Haldu, Nainital showed the highest specific activity for all the detoxification enzyme studied i.e., acetylcholinesterase (13.47± 1.24 nmol/ min/ ml enzyme), carboxylesterase (1.48± 0.02 µmoles 1-naphthol/min/mg protein),

Table 3. Specific activity of AChE, CarE, GST and P450 for *S. litura*

S.No.	Places	AChE (nmoles/ min/ ml of enzyme)		CarE (µmoles of 1-naphthol formed/ min/ mg of protein)		GST (µmoles/ min/ mg of protein)		P450 (nmoles/ min/ ml of enzyme)	
		Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population
1	Mota Haldu Nainital	13.47± 1.24	168.38	5± 0.01	62.5	0.41± 0.08	16.4	1.48± 0.02	98.67
2	Bichai, Tanakpur, Champawat	5.115± 0.11	63.94	1.795± 0.05	22.44	0.215± 0.02	8.6	1.3± 0.09	86.67
3	Mandawar, Haridwar	2.355± 0.08	29.44	0.405± 0.11	5.06	0.095± 0.01	3.8	0.06± 0.01	4
4	Bimora, Dakpathar, Dehradun	3.28± 0.04	41	0.18± 0.01	2.25	0.07± 0.01	2.8	0.065± 0.02	4.33
5	Lab Population	1.0.01	1	1.0.01	1	1.0.01	1	0.015± 0.01	1
	CD	2.073		0.156		0.192		0.137	
	p value	<0.05		<0.05		<0.05		<0.05	

glutathione-S-transferase (5± 0.01 µmoles/ min/ mg protein) and monooxygenase P450 enzymes (0.41± 0.08 nmol/min/ml enzyme). It was observed in the studies that the resistance in all the insecticides could not be directly correlated with activity of any detoxification enzymes, however, resistance in chlorantraniliprole could be related with enhanced expression of GST, which has also been reported that detoxification by gluthione-S-transferase might be responsible for chlorantraniliprole resistance in *P. xylostella* (Eziah et al., 2008; Tang et al., 2011, Di et al., 2014). According to Hilliou et al. 2021, Nehare et al. 2010 and Muthusamy et al. 2013 MFO, esterase and GST are significant detoxification enzymes in the metabolism of old and novel insecticides in *S. litura.*

Populations of *S. litura* studied in different lowlands and doon valley regions of Uttarakhand demonstrated widespread spatial variation in insecticide resistance, making it a serious polyphagous pest. A basic knowledge of insecticide resistance is required since there are multiple mechanisms involved in insecticide resistance viz., excretion, sequestration, degradation of toxin, contact and ingestion avoidance and target site mutation (Despres et al., 2007). Different levels of detoxification enzyme production demonstrate the mechanism of insect homeostasis by changing enzyme levels in *S. litura* (Sreelakshmi et al., 2019). However, the resistance phenotypes may be the result of an overproduction of detoxification enzymes or due to an additive effect of multiple loci (ffrench-Constant et al., 2004; Shi et al., 2019). Thus, the detection of insecticide resistance by proper pest scouting and monitoring in the remotest areas is required for timely management of insecticide resistance. Resistance management-based strategies and timely, selective, and controlled use of insecticides in field could ensure successful management of *S. litura*.

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AUTHOR CONTRIBUTION STATEMENT

Dr Rashmi Joshi is the main author and did complete research with Sudha Mathpal in field study, under guidance and mentorship of Dr. Neeta Gaur.

COFLICT OF INTEREST

No conflict of interest.

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COMPETITIVE REGULATION OF DOMINANT EXOTIC WHITEFLIES ON COCONUT PALMS AS INFLUENCED BY BIOTIC AND ABIOTIC FACTORS

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ABSTRACT

In the recent past, exotic whiteflies viz., *Aleurodicus rugioperculatus, Paraleyrodes bondari, Paraleyrodes minei* and *Aleurotrachelus atratus* are infesting coconut plantations in India. Based on the damage potential, the predominant species are the rugose spiralling whitefly, *A. rugioperculatus* and the Bondars nesting whitefly, *P. bondari.* A study was undertaken to determine the seasonal incidence and population dynamics of these in the new invasion region at Karungalakudi block of Madurai district from 33rd standard meteorological week (SMW) of 2021 to 32nd SMW of 2022. The results indicated that *A. rugioperculatus* incidence rose considerably from the first week of April (10.9 adults/ leaflet) to third week of May (8.3 adults/ leaflet; while *P. bondari* reached maximum during the third week of March (71.99 adults/ leaflet) and extended up to first week of June (67.57 adults/ leaflet) coinciding with summer months with high temperature and limited precipitation. Subsequently, their incidence declined with the onset of north east monsoon coupled with parasitism by *Encarsia guadeloupae* and predation by chrysopids from third-week of August to fourth-week of December in 2021. The extent of parasitism by *E. guadeloupae* on *A. rugioperculatus* nymphs was positively correlated and ranged from 40% (third week of August, 33rd SMW, 2021) to 84% (second week of June 2022, 24th SMW). Chrysopids revealed a highly significant positive correlation with the incidence.

Key words: *Aleurodicus rugioperculatus*, *Paraleyrodes bondari*, invasive whiteflies; weather factors, parasitism, predation, *Encarsia guadeloupae*, green lacewings

Currently, many species of exotic whiteflies have invaded and caused heavy damage to coconut plantations in India. During 2016-2019, four non-native whiteflies namely rugose spiralling whitefly (*Aleurodicus rugioperculatus* Martin) (Shanas et al*.,* 2016; Selvaraj et al., 2016; Srinivasan et al*.,* 2016); Bondars nesting whitefly (*Paraleyrodes bondari* Peracchi) (Josephrajkumar et al., 2019); Neotropical nesting whitefly (*P. minei* Iaccarino) (Chandrika Mohan et al., 2019) and Neotropical palm whitefly (*Aleurotrachelus atratus* Hempel) (Selvaraj et al*.,* 2019; Josephrajkumar et al., 2020) were predominantly observed on coconut palms in southern states of Tamil Nadu. Climax modeling was performed for *A. rugioperculatus* to predict its invasiveness and the damage inflicting zones which included coastal regions of Maharashtra, Karnataka, Goa, parts of Kerala extending up to Gujarat border. In south India, Karnataka (Bengaluru, Mysuru, Tumakuru, Hassan, Belagavi, Shikaripura and Birur) and Tamil Nadu (Coimbatore) had favourable weather conditions to expand the invasive potential of the pest (Chakravarthy et al., 2017).

MaxEnt studies further revealed that Eastern coastal parts of Tamil Nadu, North-Eastern parts of Andhra Pradesh, Eastern coastal belts of Odisha, north-western coastal belts of Kerala, south-western coastal parts of Karnataka and western coastal belts of Maharashtra and Gujarat were predicted for highest habitat suitability regions for *A. rugioperculatus* (Selvaraj et al., 2022). Maruthadurai et al. (2023) also highlighted MaxEnt model that forecasted a possible *A. rugioperculatus* distribution with the yearly mean temperature (Bio 1, 28.9%), mean diurnal range (Bio 2, 19.5%) and annual precipitation (Bio 12, 19.1). India's coastal and southern states were found as the most ideal places for *A. rugioperculatus* establishment. Though, *A. rugioperculatus* sustained well in hot

humid weather, the tropics, subtropics and temperate zones were found as the best places to proliferate and invade. In Kerala, deficit in south-west monsoon during 2016 increased the *A. rugioperculatus* population whereas subsequent heavy rains subdued the whitefly population. Furthermore, increase in the temperature up to 2°C was found as a pre-disposing factor and 7% decline in the relative humidity favoured the *A. rugioperculatus* population (Chandrika Mohan et al., 2016). As the invasion of *A. rugioperculatus* and *P. bondari* is relatively new to the Indian lands there is a need to understand the pestiferous nature of these exotic whiteflies in relation to the biotic (parasitoids and predators) as well as abiotic factors (temperature, rainfall and relative humidity). In this research paper the population dynamics of these whiteflies as influenced by biotic and abiotic factors were observed and presented.

MATERIALS AND METHODS

The population variation and seasonal modulation of *A. rugioperculatus* and *P. bondari* were studied in Karungalakudi block of Madurai district, Tamil Nadu on four-year-old highly susceptible Dwarf x Tall coconut trees. Surveillance was done at weekly interval for a year starting from August 2021 to August 2022, which coincided with 33rd standard meteorological week (SMW) of 2021 to 32nd SMW of 2022. Ten coconut trees were randomly selected. In each selected tree, five mature bottom fronds were chosen in which five leaflets were marked to collect data on the population dynamics of *A. rugioperculatus* and *P. bondari*. Number of nymphs and adult whiteflies were recorded on the selected leaflet and inter-related with weather factors. The coconut trees were maintained in pesticide-free environment as well as the trees were supplied with recommended dose of macronutrients, micronutrients and irrigation as per recommended package of practices. The correlation studies of exotic whiteflies population with biotic and abiotic factors was taken up. Biotic factors included natural parasitism of *A. rugioperculatus* by *Encarsia guadeloupae* (X1), predation by chrysopids (X2), coccinellids (X3) and spiders (X4) while the abiotic factors included were maximum temperature (X5), minimum temperature (X6), minimum relative humidity (X7), maximum relative humidity(X8) and rainfall (X8). In case of *P. bondari*, excluding the parasitoid (X1) all other factors were observed and correlated. The weather parameters were collected from the Google earth engine software (Satellite derived data). Multiple regression analysis was also performed for *A. rugioperculatus* and *P. bondari* relationship with predators, parasitoid and weather parameters. Using the SPSS 16.0 statistical software, simple correlation and multiple regression analyses were done between both dependent and independent variables to know the significant relationship.

RESULTS AND DISCUSSION

The population of exotic whiteflies on coconut was maximum during summer season i.e., March, 2022 to April, 2022. *A. rugioperculatus* population was high from fourth week of April, 2022 (10.9 adults/ leaflet) concurred with 12th SMW of 2022. Later, *A. rugioperculatus* population was moderate throughout the study period and it ranged from 5.4 to 10.9 adults/ leaflet. *P. bondari* was found maximum from third week of March, 2022 (71.99 adults/leaflet) to second week of June, 2022 (67.49 adults/ leaflet) and coincided with 11th to 23rd SMW of 2022. Among these two exotic whiteflies, the population of *P. bondari* was seven-fold higher when compared to *A. rugioperculatus* (Fig. 1). Similar results were observed in Kerala where *P. bondari* increased to four-fold with less numbers of *A. rugioperculatus* during July, 2020 (CPCRI, 2020). Further no two species cannot exist in the same limiting ecosystem and our study clearly states the increasing phase of *P. bondari* and receding phase of *A. rugioperculatus* due to higher parasitism rates of *E. guadeloupae* as reported by Chandrika Mohan et al. (2019). Chavan et al. (2022) reported peak population of *A. rugioperculatus* during 9th April to 15th April, whereas, Elango and Jeyarajan Nelson (2020) reported maximum population during July. Maximum temperature showed a strong positive correlation for both *A. rugioperculatus* (r=0.495**) and *P. bondari* (r=0.774**) population. Maximum *A. rugioperculatus* population was noticed during first week of April, 2022 when the maximum temperature prevailed at 36.2°C whereas *P. bondari* was highest

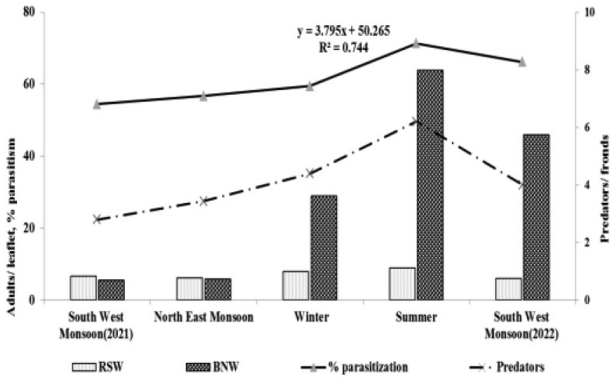


Fig. 1. Population dynamics of *A. rugioperculatus* and *P. bondari* and its natural enemies

Table 1. Popultion dynamics of *A. rugioperculatus and P. bondari*

		Adult	Parasitoid	Chrysopid	Coccinellids/ Cybocephalids	Spiders	Max. temp.	Min. temp	Min. RH	Max.RH	Rainfall
		Y	X1	X2	X3	X4	X5	X6	X7	X8	X9
		Biotic Factors						Abiotic factors			
		A. rugioperculatus									
Y	Adult	1	0.328*	0.699**	0.162	0.437**	0.495**	0.010	-0.508**	-0.518**	-0.394**
X1	Parasitoid		1	0.442**	0.285*	0.249	0.385**	0.230	-0.382**	-0.418**	-0.101
X2	Chrysopid			1	0.372**	0.182	0.571**	0.202	-0.623**	-0.614**	-0.364**
X3	Coccinellids/ Cybocephalids				1	-0.036	0.298*	0.157	-0.347*	-0.315*	-0.314*
X4	Spiders					1	0.144	-0.142	-0.089	-0.166	0.051
X5	Max. temp.						1	0.724**	-0.833**	-0.904**	-0.287*
X6	Min. Temp.							1	-0.364**	-0.546**	0.172
X7	Min. RH								1	0.940**	0.542**
X8	Max. RH									1	0.379**
X9	Rainfall										1
		P. bondari									
		Adult	Chrysopid	Coccinellids/ Cybocephalids	Spiders	Max. temp.	Min. temp	Min. RH	Max. RH	Rainfall	
			X1	X2	X3	X4	X5	X6	X7	X8	
		Biotic factors						Abiotic factors			
Y	Adult	1	0.759**	0.366**	0.246^{NS}	0.774**	0.458**	-0.785**	-0.825**	-0.362**	
X2	Chrysopid		1	0.372**	0.182^{NS}	0.558**	0.202^{NS}	-0.623**	-0.614**	-0.364**	
X3	Coccinellids/ Cybocephalids			1	-0.036^{NS}	0.293*	0.157^{NS}	-0.347*	-0.315*	-0.314*	
X4	Spiders				1	0.144	-0.142^{NS}	-0.089^{NS}	-0.166^{NS}	0.051	
X5	Max. temp					1	0.724**	-0.830**	-0.901**	-0.281*	
X6	Min. temp						1	-0.364**	-0.546**	0. 172	
X7	Min. RH							1	0.940**	0.542**	
X8	Max. RH								1	0.379**	
X9	Rainfall									1	

*significant at p=0.05; **at p=0.01

during third week of March when the temperature was 36.0°C on 11th SMW. With regard to the minimum temperature, *A. rugioperculatus* population had a non-significant correlation (r=0.010), whereas it was positively correlated (r = 0.458**) for *P. bondari* (Table 1). Mane (2019) revealed a positive correlation between *A. rugioperculatus* and maximum temperature. Similarly, maximum temperature and relative humidity had positive correlation for cotton whitefly (Ahmad et al., 2018).

Both *A. rugioperculatus* and *P. bondari* population with maximum relative humidity had a highly significant negative correlation of r = -0.518**** and r = -0.825** respectively. Similarly, Elango and Jeyarajan Nelson (2020) reported the negative correlation between relative humidity and whitefly populations. Rainfall had a negative correlation (r = -0.394**) with *A. rugioperculatus* population (Table 1), whereas during northeast monsoon period the population of *P. bondari* was very low (Fig. 2), 1.50 adults/leaflet (46th SMW, fourth week of November, 2021). According to Ranjith et al. (1996), in Kerala *A. dispersus* rebounded throughout the summer and dropped off after severe pre-monsoon showers. Among the abiotic factors, rainfall was the predominant factor which helped in the reduction of *A. rugioperculatus* population.

The aphelinid parasitoid *Encarsia guadeloupae* had a positive correlation (r = 0.328*) with *A.rugioperculatus* population which helped in regulating the population of *A. rugioperculatus* (Table 1). The activity of *E. guadeloupae* coincided with the occurrence of *A. rugioperculatus* all through and the highest parasitism of 84% on nymphal stage was recorded in 24th SMW of June, 2022 and the lowest parasitism of 40% was recorded during 33rd SMW of August, 2021. Mani and Krishnamoorthy (2000) reported nearly 95.68% parasitism by *E. guadeloupae on Aleurodicus dispersus* which is in line with our study, thus it could be a probable reason for low population density of *A. rugioperculatus* compared to *P. bondari.* Among the chrysopids, *Mallada* sp. was found throughout the study period feeding on the immature stages of *A. rugioperculatus* as well as *P. bondari* and its population ranged from 1 to 4 grubs/ frond. Chrysopid population had a highly significant positive correlation with both the whiteflies population i.e., *A. rugioperculatus* (r = 0.699**) and *P. bondari* (r = 0.759**) (Table 1). The *P. bondari* population alone had positive correlation (r=0.366**) with predatory colepterans (Table 1). The ladybird beetles observed were *Cryptoleamus montrouzieri* and *Scymnus coccivora* along with predatory cybocephalid beetles. Ballal et al. (2021) reported a positive link of coccinellid predator larvae with adults and the immature stages of *Aleurotrachelus trachoides* (Back). Furthermore, spider population had a positive correlation (r = 0.437**) with *A. rugioperculatus* population besides it was not significant for *P. bondari* population (Table 1). Naik et al. (2009) stated a major positive connection between whitefly, coccinellids and spiders populations in brinjal crop and alike to our findings.The coefficient of determination (R^2) between *A. rugioperculatus* and the parasitoid was found to be 74.4% of total variation (Fig. 1), whereas for the abiotic factors it was found as 0.02. Thus, it is clear that *A. rugioperculatus* population was suppressed only by *E. guadeloupae.* In case of *P. bondari* the total variation was 74.73 for abiotic factors in population regulation (Fig. 2).

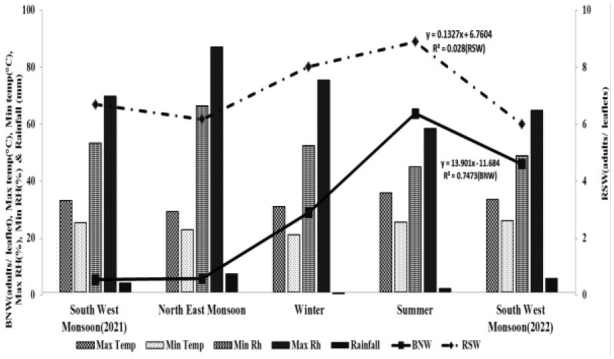


Fig. 2. Seasonal incidence *A. rugioperculatus* and *P. bondari*

The fixed plot analysis on the exotic coconut whiteflies revealed that *A. rugioperculatus* was very minimal throughout the study when compared to Bondars nesting whitefly, due to the higher rates of parasitism by *Encarsia guadeloupae*. High temperature favoured whitefly outbreak as observed in high numbers during the summer months. Rainfall drastically reduced the population of both the whiteflies. Thus, weather factors and parasitism by *E. guadeloupae* played a critical role in the population regulation of *A. rugioperculatus* in the surveyed area. Innundative release of *Apertochrysa* sp. is advised in coconut plantations to suppress pest outbreak. The farmers are advised a pesticide holiday approach as well as to release the predators during the months of April and May so as to reduce the flaring whitefly population. Adoption of conservation biological control program helps in reducing the cost of plant protection for the coconut growers and safeguard the environment as well.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

P.L: For collection, analysis, interpretation of data and drafting the manuscript. R. N: Advisor for the research work and for drafting the manuscript. A. J: Advisor for the research work and for drafting the manuscript P.C: Advisor for the research work. M.L.M: Advisor for the research. R.D: Advisor for the research. M.M: Advisor for the research. K.S: Advisor for the research.

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RESIDUAL TOXICITY OF INSECTICIDES TO EGG PARASITOIDS OF *SPODOPTERA FRUGIPERDA* (J E SMITH)

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ABSTRACT

A laboratory study was carried out to assess the residual toxicity of certain insecticides to egg parasitoids of *Spodoptera frugiperda (*J E Smith), *Trichogramma chilonis* Ishii, *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae), and *Telenomus remus* Nixon (Hymenoptera: Scelioniadae). The median lethal concentrations were recorded at 24 and 48 hr after exposure period to adults of egg parasitoids to nine insecticides. Among the tested spinetoram was found to be "dangerous", however, chlorantraniliprole, chlorantraniliprole + lambdacyhalothrin, chlorpyriphos + cypermethrin, indoxacarb, cyantraniliprole, emamectin benzoate, thiamethoxam + lambdacyhalothrin and azadirachtin were found to be "slightly to moderately toxic" to *T. chilonis* and *T. pretiosum*. Chlorantraniliprole, emamectin benzoate, indoxacarb and spinetoram were found to be "dangerous", however, chlorpyriphos + cypermethrin, thiamethoxam + lambdacyhalothrin, chlorantraniliprole + lambdacyhalothrin, cyantraniliprole and azadirachtin were "slightly to moderately toxic" to *T. remus.*

Key words: *Trichogramma chilonis*, *T. pretiosum*, *T. remus*, LC_{50}, insecticides, residual toxicity, moderately toxic, slightly toxic, dangerous, egg parasitoids, invasive pest, *Spodoptera frugiperda*, *Corcyra cephalonica*, risk quotients

The fall army worm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), is an important invasive pest originating from America. In India, the pest was first observed on maize crop Shivamogga and neighboring districts of Karnataka (Sharanabasappa et al., 2018). Since then, several synthetic chemical insecticides have been recommended and used as emergency responses to check the pest's distribution and minimize its damage (Bharadwaj et al., 2020). Globally, a large amount of money is annually spent in attempts to control this pest (Bueno et al., 2010; Cruz, 1996). In many cases, the application of insecticides does not solve the problem but cause a negative impact, such as contamination of the environment, risk to human health and other non-target organisms and cause resistance (Beserra and Parra, 2004; Agboyi et al., 2020). For the effective and sustainable management of this invasive pest, an excellent alternative is the development and dissemination of ecologically and environmentally friendly crop protection methods, such as biological control (Bueno et al., 2010; Agboyi et al., 2020). The natural enemies are greatly reduced due to the injudicious application of nonselective insecticides and their repeated spraying. Thus, assessment of safety of insecticides to natural enemies is thus, essential. Parasitic Hymenoptera are often more susceptible to insecticides than their hosts. The objective of this study was to determine the residual toxicity of nine insecticides, commonly used for suppression of *S. frugiperda* assessing the adult mortality of egg parasitoids.

MATERIALS AND METHODS

The egg parasitoids such as *Trichogramma chilonis*, *T. pretiosum*, and *T. remus* maintained at the Bio-Control Laboratory, Department of Agril. Entomology, College of Agriculture Latur, during 2019-2020 were used. The insecticides recommended for the control of *S. frugiperda* on maize viz., are azadirachtin 0.15%EC (Coromandal International Ltd.), chlorantraniliprole 18.5%SC (DuPont India Pvt. Ltd.), chlorantraniliprole 9.3% + lambdacyhalothrin 4.6%ZC (Syngenta Group of Companies Pvt. Ltd.), chlorpyriphos 50% + cypermethrin 5%EC (TATA chemicals Ltd.), cyantraniliprole 10.26%OD (DuPont India Pvt. Ltd.), emamectin benzoate 5%SG (Syngenta Group of Companies Pvt. Ltd.), indoxacarb 15.8%EC (Gharda Chemicals Ltd.), spinetoram 11.7%SC (T Stanes and Co. Pvt. Ltd.) and thiamethoxam 12.6 + lambda-cyhalothrin 9.5%ZC (Syngenta Group of Companies Pvt. Ltd.) were assessed.

Insecticides were prepared in distilled water with three concentration of each insecticide (one lower than recommended (half dose of recommended), one recommended (full dose of recommended) and one higher than recommended (double dose of recommended). Cleaned glass test tubes (50 ml capacity with an internal surface area of 50 cm^2) were rinsed with distilled water and air dried for 4 hr, coated evenly with 0.5 ml of each concentration dried thoroughly and used to assess the residual toxicity of insecticides to parasitoids as per the standard procedure proposed by Hassan et al. (1998) with slight modification. Preliminary range-finding tests were carried out to fix the test concentrations that caused 10-90% mortality of the parasitoid. As a control, distilled water was used. Ten newly emerged adults of respective egg parasitoids were released in the treated test tube, and mortality was recorded at 24 and 48 hr after treatment. These experiments were carried out in a completely randomized design (CRD) with five replications.

Corrections for natural mortality in the control were made as per Abbott (1925) for the *T. chilonis*, *T. pretiosum*, and *T. remus* bioassays. The data were subjected to probit analysis by Finney (1971) using Polo Plus 1.0 (LeOra software) to determine the median lethal concentration (LC50). The risk quotient was calculated by a simple, screening-level methodology identifying high- or low-risk situations. In this method, the estimated environmental concentration (EEC) is compared to an effect level, such as an LC_{50} (EPA, 2016). The insecticides were classified into various categories based on risk quotient values such as < 50, which means harmless, a value between 50 and 2500, which means slightly to moderately toxic, and > 2501 which means dangerous and classified as 'class 1', 'class 2' and 'class 3' numbers respectively (Preetha et al., 2009; Cheng et al., 2018).

RESULTS AND DISCUSSION

Mortality were recorded at 24 and 48 hr after the exposure period to adults of *T. chilonis*, *T. pretiosum* and *T. remus* to different insecticides and presented in Tables 1 and 2, respectively.

Trichogramma chilonis: The data revealed that among the insecticides tested in dry film test tube residue bioassay, chlorantraniliprole exhibited higher toxicity to *T. chilonis* adults followed by chlorantraniliprole + lambda cyhalothrin, spinetoram, indoxacarb, thiamethoxam + lambda-cyhalothrin, emamectin benzoate, chlorpyriphos + cypermethrin, cyantraniliprole and azadirachtin was found to be less toxic to the adults of *T. chilonis* after 24 and 48 hr, respectively (Table 1). According to Khan (2020) spinetoram induced mortality was > 95% in four residual-age treatments of *T. chilonis* and was found to be the most harmful for both emergence and parasitism. The combination product of lambdacyhalothrin + thiamethoxam recorded least emergence, indicating its detrimental effects on *T. chilonis* (Sant et al., 2019). Chlorantraniliprole and indoxacarb had a lesser effect on adult emergence and were categorized as harmless (< 30% mortality), by Duraimurugan and Lakshminarayana (2018), while emamectin benzoate was rated as slightly harmful (30-79% mortality) to *T. Chilonis* (Wang et al., 2012). The data on the risk quotient to *T. chilonis* adults at 24 and 48 hr interval of exposure are presented in Table 2. The results of present study are similar to those of Khan (2020) who found that spinetoram was most harmful to *T. chilonis*. Sharanabasappa et al. (2018) shows that azadirachtin was slightly harmful, and chlorantraniliprole was moderately harmful to *T. chilonis.* Based on risk quotient values Chunke (2017) illustrated that, chlorantraniliprole and indoxacarb were moderately toxic, and cyantraniliprole and emamectin benzoate were slightly toxic to *T. chilonis*.

Trichogramma pretiosum: The data revealed that among the insecticides tested in dry film test tube residue bioassay, chlorantraniliprole + lambdacyhalothrin exhibited maximum toxicity to *T. pretiosum* adults followed by chlorantraniliprole, indoxacarb, spinetoram, thiamethoxam + lambda cyhalothrin, chlorpyriphos + cypermethrin, emamectin benzoate, and cyantraniliprole and azadirachtin was found to be least toxic to the adults of *T. pretiosum* after 24 and 48 hr intervals of exposure (Table 1). The findings of the current study are consistent with those of Grande et al. (2018) that spinetoram adversely affected immature and pre-emergent adult *T. pretiosum*. Singh et al. (2016) found indoxacarb harmless (>30% mortality) against adults of *T. pretiosum* while spinetoram induced nearly 100% adult mortality, however <10% mortality was in chlorantraniliprole (Khan et al., 2015).

The data on risk quotient to *T. pretiosum* adults at 24 and 48 hr intervals of exposure are presented in Table 2. These results are similar with those of Grande et al. (2018) on chlorantraniliprole and classified as harmless/ slightly harmful to *T. pretiosum*. Chlorantraniliprole + lambdacyhalothrin at highest rate at 25 gm a.i./ ha was classified as moderately harmful to *T. pretiosum*. Chlorantraniliprole and azadirachtin

Table 1. Toxicity of insecticides to *T. chilonis*, *T. pretiosum* and *T. remus*

Egg parasitoids		*T. chilonis*				*T. pretiosum*				*T. remus*			
Insecticides	Period (hr)	LC_{50} (mg a.i./ ℓ)	90% fiducial limits of LC_{50}		χ^2 Value	LC_{50} (mg a.i./ *l*)	90% fiducial limits of LC_{50}		χ^2 Value	LC_{50} (mg a.i./ ℓ)	90% fiducial limits of LC_{50}		χ^2 Value
			Lower limit	Upper limit			Lower limit	Upper limit			Lower limit	Upper limit	
Azadirachtin 0.15%EC	24	12.7625	0.7034	231.54	0.0109	8.9369	0.5786	138.05	0.0023	6.5610	1.7168	25.0735	0.2168
	48	13.0672	0.7271	234.82	0.0122	9.0629	0.5902	139.14	0.0015	6.5032	1.7775	23.7929	0.2072
Chlorantraniliprole 18.5%SC	24	0.0818	0.0267	0.2500	0.1068	0.1095	0.0435	0.2752	0.0538	0.0576	0.0157	0.2109	0.2072
	48	0.0823	0.0275	0.2463	0.1073	0.1099	0.0439	0.2749	0.0630	0.0571	0.0149	0.2184	0.2168
Chlorantraniliprole 9.3% + Lambdacyhalothrin 9.5%ZC	24	0.1399	0.0705	0.2778	0.0058	0.1006	0.0156	0.6464	0.0003	0.4217	0.1234	1.4409	0.0449
	48	0.1406	0.0712	0.2775	0.0060	0.1017	0.0159	0.6486	0.0001	0.4245	0.1231	1.4634	0.0403
Chlorpyriphos 50% + cypermethrin 5%EC	24	0.5182	0.2388	1.1247	0.0482	0.4711	0.1366	1.6240	0.0403	0.7301	0.2905	1.8350	0.0538
	48	0.5212	0.2409	1.1278	0.0522	0.4741	0.1387	1.6199	0.0449	0.7329	0.2931	1.8329	0.0630
Cyantraniliprole 10.26%OD	24	1.1509	0.5319	2.4902	0.0522	1.0932	0.3653	3.2714	0.1073	2.1450	0.1388	33.1340	0.0023
	48	1.2653	0.3703	4.3229	0.0449	1.0996	0.3599	3.3593	0.1068	2.1751	0.1416	33.3947	0.0015
Emamectin benzoate 5%SG	24	0.3929	0.0616	2.5035	0.0001	0.7150	0.0462	11.0446	0.0015	0.0697	0.0184	0.2635	0.0115
	48	0.3972	0.0618	2.5506	0.0003	0.7250	0.0472	11.1315	0.0023	0.0696	0.0179	0.2704	0.0189
Indoxacarb 15.8%EC	24	0.1754	0.0556	0.5529	0.1188	0.1800	0.0589	0.5500	0.1068	0.1268	0.0346	0.4641	0.2072
	48	0.1765	0.0572	0.5446	0.1193	0.1811	0.0605	0.5419	0.1073	0.1257	0.0329	0.4805	0.2168
Spinetoram 11.7%SC	24	0.1729	0.0472	0.6328	0.2072	0.1529	0.0395	0.5913	0.0085	0.1681	0.0446	0.6334	0.2127
	48	0.1714	0.0448	0.6552	0.2168	0.1527	0.0384	0.6068	0.0151	0.1677	00437	0.6436	0.2345
Thiamethoxam 12.6 + Lambdacyhalothrin 9.5%ZC	24	0.2624	0.0686	1.0029	0.2168	0.3575	0.0231	5.5223	0.0023	0.0545	0.0178	0.1666	0.1068
	48	0.2601	0.0711	0.9517	0.2072	0.3625	0.0236	5.5657	0.0015	0.0548	0.0183	0.1642	0.1073

Table 2. Toxicity and risk quotient of insecticides to *T. chilonis*, *T. pretiosum* and *T. remus*

Insecticides	Recommended Dose (mg a.i./ ha)	Period (hr)	*T. chilonis*		*T. pretiosum*		*T. remus*	
			Risk quotient	Category	Risk quotient	Category	Risk quotient	Category
Azadirachtin 1500 ppm	2500	24	195.88	2	279.71	2	381.03	2
		48	191.31	2	275.84	2	384.42	2
Chlorantraniliprole 18.5%SC	150	24	1833.74	2	1369.86	2	2604.16	3
		48	1822.60	2	1364.87	2	2626.97	3
Chlorantraniliprole 9.3%+ Lambdacyhalothrin 9.5%ZC	200	24	1429.59	2	1988.07	2	474.27	2
		48	1591.08	2	1966.56	2	471.14	2
Chlorpyrifos 50% + cypermethrin 5%EC	1000	24	1929.75	2	2122.69	2	1369.67	2
		48	1918.64	2	2109.25	2	1364.44	2
Cyantraniliprole 10.26%OD	600	24	381.99	2	474.19	2	279.72	2
		48	384.44	2	471.14	2	275.84	2
Emamectin benzoate 5%SG	200	24	509.03	2	279.72	2	509.03	3
		48	503.52	2	275.86	2	503.52	3
Indoxacarb 15.8%EC	330	24	1881.41	2	1833.33	2	1881.41	3
		48	1869.68	2	1822.19	2	1869.68	3
Spinetoram 11.7%SC	450	24	2602.66	3	2943.10	3	2602.66	3
		48	2625.43	3	2946.95	3	2625.43	3
Thiamethoxam 12.6 + lambdacyhalothrin 9.5%ZC	125	24	379.82	2	276.06	2	379.82	2
		48	380.98	2	272.27	2	380.98	2

were slightly and moderately harmful when irradiated *Corcyra* eggs were exposed to *T. pretiosum*, while chlorantraniliprole and azadirachtin found slightly harmful when unirradiated *Corcyra* eggs were exposed to *T. pretiosum* (Sharanabasappa et al., 2018).

Telenomus remus: The data revealed that among the insecticides tested in dry film test tube residue bioassay, chlorantraniliprole exhibited greater toxicity to *T. remus* adults followed by thiamethoxam + lambda-cyhalothrin, emamectin benzoate, indoxacarb, spinetoram, chlorantraniliprole + lambda-cyhalothrin, chlorpyrifos + cypermethrin, and cyantraniliprole. Azadirachtin was found to be lower toxic to the adults of *T. remus* (Table 2). Present results are the accordance to Pazini et al. (2016) who states that chlorantraniliprole, lambdacyhalothrin, lambdacyhalothrin + thiamethoxam reduced parasitism by 10, 97 and 100% when adults of *Telenomus sp.* were exposed to dry residues of pesticides. Liu et al., (2016) showed that emamectin benzoate, indoxacarb and chlorantraniliprole were dangerously toxic to *T. remus* adults.

The data on risk quotient of insecticides to *T. remus* adults at 24 and 48 hr interval of exposure are presented in Table 2. Based on risk quotient values Pazini et al. (2019) classified the thiamethoxam + lambdacyhalothrin as slightly to moderately toxic to *Telenomus sp.* with RQ values ranging from 79.55 and 1646.67. Feltrin-Campos et al. (2018) shows that chlorantraniliprole + lambdacyhalothrin mixture at its highest dose was classified as noxious. Liu et al. (2016) shows that emamectin benzoate and indoxacarb were dangerously toxic to *T. remus* adults at 24 hr post-treatment. According to Silva et al. (2022) thiamethoxam + lambdacyhalothrin sprayed on pupae significant reduction of adult emergence of *Telenomus* sp. compared with the control and it was classified as slightly harmful (class 2) for *Telenomus* sp.

Based on risk quotient values, among the insecticides, spinetoram was found to be highly dangerous to the adults of *T. chilonis* and *T. pretiosum.* Chlorantraniliprole, emamectin benzoate, indoxacarb, and spinetoram were found to be highly dangerous to the adults of *T. remus*. Azadirachtin was found to be least toxic to the adults of all egg parasitoids. However, under field condition, the insecticide may be performing differently with all three parasitoids. Since, all three natural enemies can take advantage of natural shelter, avoiding treated areas and other means.

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AUTHOR CONTRIBUTION STATEMENT

R S Mahajan and V K Bhamare conceptualized and designed the study, R S Mahajan conducted the study, analyzed the data, and authored the report under the supervision of V K Bhamare.

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No conflict of interest.

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FIELD RESISTANCE TO CYANTRANILIPROLE IN *PLUTELLA XYLOSTELLA*

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ABSTRACT

Cyantraniliprole belongs to new chemical class of diamides and used *Plutella xylostella* one of the highly devastating lepidopteran insect pests of cole crops has been found to develop resistance to cyantraniliprole worldwide including India. However their mechanism of resistance is not examined in Indian scenario. Hence a survey was conducted to analyse the current resistance status of field population of *P. xylostella* to cyantraniliprole. The maximum lethal concentration expressed by field population in Tamil Nadu was 19.81 ppm which had higher ratio (2828.57) of resistance to that of susceptible population. The enzyme analysis revealed the over expression of GST up to at 24 hr after exposure (HAE) and MFO up to 48 hr upon exposure. Further the synergistic study stated that PBO and DEM could increase the toxicity of cyantraniliprole to some extent.

Key words: Cyantraniliprole, MFO, GST, CarE, *Plutella xylostella*, $LC_{50,}$ synergist, cole crops, resistance, toxicity, ratio of resistance, enzyme

India ranks second in cabbage and cauliflower production worldwide and these are affected by number of pests. The diamondback moth (DBM) *Plutella xylostella*, (Lepidoptera : Plutellidae) causes 50-80% yield loss (Uthamasamy et al., 2011). The larvae of DBM is oligophagous and are destructive feeders of brassica plants. The DBM challenge is often worst in tropics and subtropics, where round the year cultivation of brassica crops is in vogue (Grzywacz et al., 2010). Farmers use intensive applications of insecticides to check DBM menace, even about 14-16 sprays (Biradar, 2020), often accounting for one spray for each week (Abhijith et al., 2019). However due to selection pressure (Hawkins et al., 2019), DBM had resisted several insecticides, by evolving resistance mechanisms. In addition, since DBM has a shorter life cycle with 25 generations annually, a stronger selection pressure is getting experienced. The high fecundity rates in DBM also support evolution of resistance (Wright, 2004). To date a variety of field-applied insecticides have become ineffective (Kim et al., 2011). Diamides with two distinct subclasses viz., phthalic (flubendiamide) and anthranilic (chlorantraniliprole and cyantraniliprole) are one among the newer type of insecticides which inhibit further muscular contraction and results in paralysis and finally death among targeted insects (Sattelle et al., 2008). Lepidopterans are the primary targets of diamides but these failed to manage DBM (Troczka et al., 2012) and later on, diamide resistance was noticed in several other lepidopteran insects (Yao et al., 2017; Huang et al., 2021). In India, flubendiamide, chlorantraniliprole and cyantraniliprole are the three commercial formulations of diamides approved for use since 2007. Diamides are the major used insecticides in the fields of cabbage and cauliflower in India (Biradar, 2020; Anjali et al., 2018). Field populations of DBM were found to exhibit resistance to these (Shanmugapriya et al., 2019). Several reports worldwide emanated adjudging insecticide resistance mechanisms in DBM (Jiang et al., 2015; Banazeer et al., 2021). But, in India the mechanism mediating such resistance against diamides in DBM is unexplored, and hence the present study with a survey on the susceptibility of *P. xylostella* to cyantraniliprole in the major cabbage and cauliflower growing zones of Tamil Nadu. This study was done also to identify the enzymatic mechanisms that rule the cyantraniliprole resistance.

MATERIALS AND METHODS

The field population of *P. xylostella* were collected from cauliflower in Coimbatore in 2020 and was maintained in the laboratory to develop the susceptible population. The laboratory reared larvae were fed with insecticide free leaves of cauliflower (*Brassica oleracea var. botrytis*, cv. Snow White). The larvae upon pupation, were collected separately and placed safely in the fine meshed cage (45x45x45 cm). The emerged adults were supplemented with 10% sugar solution. The adults were allowed to lay eggs on the

young (4-5 days old) mustard (*Brassica nigra,* hybrid: Pioneer 45s46) seedlings and left uninterrupted until egg larval hatch. Then, the neonates were transferred in to contamination- free cauliflower leaves. The whole rearing was performed under a controlled condition (27±2°C; 50-70% RH; 16:8 L:D). Field populations of *P. xylostella* were collected for resistance assessment in the major cole crops cultivating areas of Tamil Nadu (covering three agroclimatic zones of seven districts) during 2021-2022. Field-collected populations were kept in cages apart from susceptible cultures under controlled environment, as explained for susceptible cultures.

Leaf dip bioassay was conducted based on IRAC (Insecticide Resistance Action Committee) susceptibility test method no: 018 using the commercially available cyantraniliprole. To conduct bioassay, cauliflower leaf discs (4.5 cm) were dipped in different concentrations of insecticide solutions and finally added with Triton X-100 (1 g/ ℓ) as a surfactant. The control was prepared by treating leaf discs with distilled water containing Triton X-100 (1 g/ ℓ) and each treatment including control was replicated thrice. About 10 uniform sized late second instar larvae were selected, starved and released in each replication. Then the larval mortality was assessed after 72 hr. The impact of synthetic synergists (PBO, DEM and TPP) and non-edible oils (rosemary oil (RO) and thyme oil (TY)) on the toxicity of cyantraniliprole to two field populations and lab maintained susceptible population were also assessed using the common leaf disc bioassay as described above. The synergist (100 mg/ ℓ) was then added to each concentration of cyantraniliprole as they exhibited no toxicity effects when tested alone and the toxicity was assayed as mentioned in bioassay described above.

The activity of detoxifying enzymes such as mixed function oxidase (MFO), glutathione S-transferase (GST) and carboxyl esterase (CarE) were assessed in both susceptible and the most resistant populations (NIL and KRI) of *P. xylostella* at different hours after exposure (HAE) (viz., 3, 24 and 48 HAE) to cyantraniliprole at sublethal (LC_{25}) quantity. Alive, healthy, late second instar larvae after insecticide exposure were weighed and homogenized under ice cold conditions using pestle and mortar. The following buffers : 0.1M Tris buffer, 10 mM glutathione reduced, pH 8.0) for GST; 50 mM ice cold tris buffer, 1.15% KCl, and 1.0 mM EDTA, pH 7.5 for MFO; and 0.1M phosphate buffer, pH 7.5, containing 0.1% Triton X-100 for CarE were used. Then the extracted contents were centrifuged at 10000 rpm, at 4°C for 12 min and the supernatant was stored at -20°C to quantify respective enzymes. For protein assay bovine serum albumin (BSA) was used as a protein standard, the Bradford method was used to quantify the total protein content (Bradford, 1976). Using *p*-nitroanisole as the substrate, MFO was measured in accordance with Yasoob et al. (2018) with a few changes. One 100 µl of the enzyme source was mixed with 1 ml of 50 Mm *p*-nitroanisole (in ethanol) and 200 µl of 10.0 mM NADPH and incubated in darkness for 3 min at room temperature. Using BioSpectrometer®, the absorbance of the end product, *p*-nitrophenol, was measured at 405 nm for 3 min. at the interval of 30 sec. Using the extinction coefficient of 3.32 $mM^{-1}cm^{-1}$, the activity of MFO was estimated (Sharma, 2017). Quantification of GST was done according to Kao et al. (1989) using CDNB (0.1M) as a substrate. 0.1M tris buffer was added to one hundred µl of enzyme source followed by adding 0.1 M CDNB (20 µl). Then enzyme activity was quantified using spectrophotometer (BioSpectrometer®) at the wavelength of 340 nm for 3 min at 30 sec. interval. The extinction coefficient of CDNB - 9.6 mM^{-1} cm^{-1} was used to estimate the activity of GST. Quantification of CarE was carried out using the substrate 1-naphthyl acetate as described by Gong et al. (2013) with slight changes. Chilled phosphate buffer 0.04 M (450 µl) and 1-naphthyl acetate 0.3mM (1.80 ml) was added to one hundred µl of enzyme source and incubated at room temperature for 30 min. Two parts of fast blue RR salt and five parts of sodium dodecyl sulphate was added to the reaction mixture and absorbance was taken after 15 min at wavelength of 600nm. To calculate the specific activity of CarE, the extinction coefficient of 1-naphthyl acetate (2.22 $mM^{-1}cm^{-1}$) was used (He, 2003).

The obtained control mortality was >10 % and was corrected using Abbott's formula (Abbott, 1925). Further the mortality data was subjected to Probit analysis to obtain $LC_{50,}$ LC_{95}, their respective fiducial limits, chi square (χ^2) and slope. Resistance ratio (RR) was calculated by dividing the LC_{50} values of field population with the LC_{50} of susceptible population. The insect population was categorised as susceptible, decreased susceptible, low resistance, moderate resistance, high resistance and very high resistance according the values of resistance ratio RR (<3.0, 3.1-5, 5.1-10.0, 10.1-40.0 40.1-160.0 and >160.0, respectively). Likewise the synergist ratio (SR) was calculated using LC_{50} values of field population with synergist divided by LC_{50} values alone. With IBM SPSS® Statistics 22.0, the data relevant to

the measurement of various enzyme activities were analysed using one-way ANOVA, ($p < 0.05$), followed by the Tukey's test (Tukey, 1977) for mean comparison.

RESULTS AND DISCUSSION

The concentration-mortality responses of the *P. xylostella* to cyantraniliprole were extremely a good fit by the probit model. The lab maintained population though not a true susceptible population exhibited significantly low LC_{50} (1.50 ppm) to cyantraniliprole at its 27th generation than any other field population. Among the field collected *P. xylostella* population, NIL that belonged to hilly and high altitude zone of TN exhibited significantly highest LC_{50} (19.81 ppm) than any other field population (Table 1). This was followed by KRI (9.51 ppm) and CBE population (8.53 ppm) belonging to North Western zone and Western zone respectively however, CBE population exhibit faster rate of resistance development than other field population as their fiducial limits were on par with the limits of NIL population while the rate of resistance development in other field populations (KRI, DIN, DHA, TIR and THE) were similar**.** When calculated using LC_{50} of susceptible population engaged in the present study, the RR was found very low (3.44-13.20) calculated for cyantraniliprole among all field population studied and were grouped in to decreased susceptible to moderate resistance categories, however at the same time on comparison to that of the susceptible DBM population used in Shanmugapriya et al. (2019) utilized in earlier work from same TNAU Entomology laboratory provided RR adjudged as extremely high (RR=950-2829) and pointed that the these populations studied were categorised in to very high resistance group (Table 1).

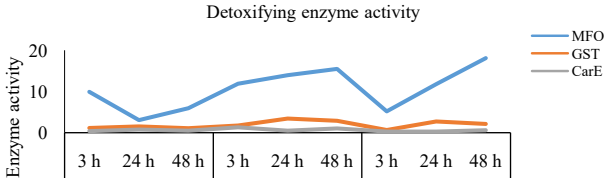
High resistance (RR=2024) to cyantraniliprole was also reported in the field population of *P. xylostella* from Brazil by Ribeiro et al., (2017) and from many parts of the world (Dunn et al., 2022; Pasupathi et al., 2022). There were also reports of cross resistance in *P. xylostella* and other insects to diamide insecticides as they possess similar mode of action (Bolzan et al., 2019; Liu et al., 2015). Thus, field population studied in the present study too found to have a high ratio of resistance to cyantraniliprole though the fields sprayed with other diamide like flubendiamide and chlorantraniliprole (Anjali et al., 2018) suggesting the occurrence of cross resistance to cyantraniliprole. Thus, the present findings are similar with those of Liu et al. (2015), who encountered the occurrence of cross resistance in *P. xylostella* within diamide insecticide group.

Table 1. Toxicity of cyantraniliprole to field population of *P. xylostella* from different agroclimatic zones of Tamil Nadu, India

Agro climatic zones	Location	Population	n	Slope± E	χ^2(df)	LC_{50} (ppm) (95% FL)	LC_{95} (ppm) (95% FL)	RR_1	RR_2
	Susceptible	-	180	1.31± 0.33	2.22(3)	1.50 (0.95-2.09)	14.38 (6.60-127.67)	-	-
Western zone	Coimbatore	CBE	180	1.47± 0.49	2.58(3)	8.53 (6.06-25.12)	63.79 (22.92-7894.58)	5.68	1218.57
	Theni	THE	180	1.22± 0.34	0.12(3)	5.20 (2.23-7.83)	58.51 (28.39 -570.10)	3.44	742.86
	Tiruppur	TIR	180	0.90± 0.25	1.09(3)	6.65 (3.26-11.79)	175.65 (53.84-8430.54)	4.43	950.00
	Dindigul	DIN	180	2.37± 0.41	0.69(3)	6.83 (5.32-8.46)	23.74 (16.97-44.08)	4.55	975.71
Hilly and high altitude zone	Nilgiris	NIL	180	2.40± 0.77	0.92(3)	19.81 (16.22-32.25)	67.91 (37.94-806.68)	13.20	2828.57
North western zone	Dharmapuri	DHA	180	2.35±0.41	3.98(3)	6.98 (3.76-10.93)	24.45 (14.20-187.13)	4.65	997.14
	Krishnagiri	KRI	180	1.33±0.31	2.66(3)	9.51 (6.41-14.56)	88.20 (40.46-643.18)	6.34	1358.57

Where; n: No. of larvae tested; Df: degrees of freedom; FL: Fiducial limits; RR_1, Resistance Ratio - the LC_{50} of laboratory maintained susceptible population was utilized as factor divisor. RR_2: Based on LC_{50} (0.007 ppm) of previous susceptible lab. Population was utilized as factor divisor

In the resistance populations (NIL, KRI) of *P. xylostella,* the activity of MFO was found to increase at steady rates significantly ($P < 0.05$) from 3 to 48 HAE to cyantraniliprole (Fig. 1). The activity of MFO in laboratory maintained susceptible was found significantly at decreased level at 24 HAE, however there was an increase in the level noticed at 48 HAE, indicating the involvement of MFO to some extent. In addition, the activity of GST was found to increase significantly up to 24 HAE in both laboratory maintained susceptible and resistance populations (NIL and KRI), with maximum level (3.48 (µmol/min/mg of protein) registered in NIL at 24 HAE. Thus, our results depicted the association of MFO and GST involvement to some extent in resistance development aspect of *P. xylostella* to cyantraniliprole. While the activity of CarE was found to be either increased or decreased significantly irrespective of the time of insecticide exposure in all the tested insect populations. Further, lower activities of CarE in resistant populations (NIL, KRI) than laboratory reared susceptible population were observed, which had pointed out the limited role of CarE activity in the resistance development contribution prospects of *P. xylostella* against cyantraniliprole.



MFO, Mixed function oxidase (expressed as nmol/min/mg of protein); GST, Glutathion S transferase (expressed as µmol/min/mg of protein); CarE, Carboxyl esterase (expressed as µmol/min/mg of protein)

Fig. 1. Activities of MFO, GST and CarE detoxifying enzymes in the laboratory maintained susceptible and two most resistant field population of *P. xylostella* hours after exposure to cyantraniliprole

Insecticide resistance in insects follow several known and well established routes besides yet to be explained unknown strategies too. However, broadly studied mechanisms are based on either increase or decrease in expression detoxifying enzymes or by mutations happening in the target sites on their combinations (Feyereisen, 1995; Ffrench-Constant, 1999). According to Arthropod Pesticide Resistance Database (APRD), *P. xylostella,* a widely studied insect around the globe is found to resist about 101 insecticidal compounds including diamides with various resistance mechanisms viz., decreased cuticular penetration, increased activity of detoxifying enzymes and changes in target-sites that crop up through genetical routes such as point mutations, amino acid substitutions, and reduced or no binding to target-sites (Banazeer et al., 2021). Thus, in line to previous reports, in current study, it was observed that the resistant population of *P. xylostella* was tracked to express higher activity of MFO (3.03 fold) and GST (2.25 fold) at 48 and 24 HAE, respectively than susceptible population. The higher activity of GST and MFO was found in resistance than susceptible populations when exposed to chlorantraniliprole (Kang et al., 2017), a similar chemistry to cyantraniliprole. Likewise, Hu et al. (2014) suggested the stronger involvement of GST mediated resistance to chlorantraniliprole, while MFO and CarE were involved to some extent only. Since cross resistance reports between chlorantraniliprole and cyantraniliprole among DBM were previously documented (Liu et al., 2015; Zhang et al., 2016;), exposure to any one of them itself is enough it seems for the resistance expression against them and it may be construed that the mechanism(s) of resistance might be more or less similar between these two insecticide compounds. Anyhow, in yet another insect whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), with a different mode of feeding, Zang et al. (2022) reported the involvement of cytochrome P450 (CYP) genes in the resistance against cyantraniliprole. But, there are reports of target site mutations to diamides including cyantraniliprole (Dunn et al., 2022).

The effect of synthetic synergists viz., PBO, DEM and TPP and non-edible essential oils (RO and TY) were shown in Table 2. When *P. xylostella* was treated along with synergist, the LC_{50} of cyantraniliprole was found to decrease in all the populations viz, laboratory maintained susceptible (1.33 to 0.29 ppm), NIL (16.81-6.91 ppm) and KRI (8.99-2.85 ppm). The SR of PBO were found to be 2.08, 2.87 and 1.99 and for DEM, the SR registered were 5.17, 2.76 and 3.34, in susceptible, NIL and KRI populations, respectively. But, the SR registered for remaining synergists, TPP, TY or RO (1.06-1.85) were very lower in all the three population studied. A similar reduction of LC_{50} to cyantraniliprole by use of PBO and DEM was previously also documented (Liu et al., 2015). But, synergists like TPP, TY or RO were not effectively participated in breaking the resistance of *P. xylostella* to cyantraniliprole. But still there are several research reports that these synergists increases the toxicities of insecticides and acaricides (Wu et al., 2007; Zhang et al., 2014; Tak et al., 2017; Raghavendra et al., 2017).

Thus, the results of present study provides documentation of the build up diamide resistance in field population of *P. xylostella* in cole crops growing tracts of Tamil Nadu.

Table 2. Effect of different synthetic and natural synergists on the toxicity of cyantraniliprole to laboratory selected susceptible and highly resistant field populations of *P. xylostella*

Population	Insecticide	n	Slope±SE	χ^2(df)	LC_{50} (ppm) (95% FL)	SR
NIL	Cyantranililprole	180	2.395± 0.767	0.92(3)	19.81 (16.22-32.25)	-
	Cyantranililprole + DEM	180	3.088± 0.641	0.38 (3)	7.19 (5.25- 8.60)	2.76
	Cyantranililprole + PBO	180	2.91± 0.72	0.51 (3)	6.91 (4.25-8..55	2.87
	Cyantranililprole + TPP	180	8.16± 2.04	0.95 (3)	16.81 (14.04-18.16)	1.18
	Cyantranililprole + TY	180	5.95± 1.40	0.29 (3)	14.34 (11.39-15.95)	1.38
	Cyantranililprole + RO	180	4.82± 1.19	1.75 (3)	12.33 (9.11-14.04)	1.61
KRI	Cyantranililprole	180	1.325± 0.309	2.66(3)	9.51 (6.41-14.56)	-
	Cyantranililprole + DEM	180	2.60± 0.47	3.04 (3)	2.85 (1.29-4.05)	3.34
	Cyantranililprole + PBO	180	1.92± 0.46	1.49 (3)	4.79 (2.94-6.24)	1.99
	Cyantranililprole + TPP	180	2.32± 0.51	0.08 (3)	8.33 (6.10-10.28)	1.14
	Cyantranililprole + TY	180	2.58± 0.57	0.56 (3)	8.99 (7.22-10.87)	1.06
	Cyantranililprole + RO	180	2.38± 0.52	0.10 (3)	8.60 (6.32-10.56)	1.10
Susceptible	Cyantranililprole	180	1.31± 0.33	2.22(3)	1.50 (0.95-2.23)	-
	Cyantranililprole + DEM	180	0.71± 0.17	1.83 (3)	0.29 (0.13-0.60)	5.17
	Cyantranililprole + PBO	180	1.13± 0.20	2.74 (3)	0.72 (0.45-1.15)	2.08
	Cyantranililprole + TPP	180	2.35± 0.41	3.01 (3)	1.05 (0.54- 1.50)	1.42
	Cyantranililprole + TY	180	3.28± 0.577	2.22 (3)	1.33 (1.07-1.56)	1.13
	Cyantranililprole + RO	180	1.77± 0.31	4.03 (3)	0.81 (0.24-1.40)	1.85

n, No of larvae tested, Df, degrees of freedom; FL, Fiducial limits; SR, Synergist Ratio - LC_{50} of cyantraniliprole without synergist/ LC_{50} along with synergist.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

K. E: For collection, analysis and interpretation of data; drafting the manuscript. M. M: Advisor for the research work and for drafting the manuscript. S. V. K: Advisor for the research work and providing research facilities. N. S: Advisor for the research work. D. V: Advisor for the research.

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EFFECT OF MICRONUTRIENT SUPPLEMENTED MULBERRY LEAVES ON LARVAE OF SILK WORM *BOMBYX MORI*

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ABSTRACT

The present study explores the effect of micronutrient supplemented mulberry leaves on PM×CSR$_2$ hybrid variety of *Bombyx mori* L. larvae. The field experiment with twelve treatments including a control (T0 to T11), replicated thrice, was supplemented with the desired quantity of the respective micronutrient in individual or in combination. Twenty early *B. mori* fourth and fifth instar larvae/ replication were fed with the 5-6 fully mature mulberry leaves (T0 to T11) twice a day (morning and evening). The results showed that food consumption increased to a maximum of 31.56 and 22.98%, and assimilation increased by 51.38 and 34.56% over control (4th and 5th instar, respectively) with treatment T9 ($CuSO_4$ 15 kg/ ha + $ZnSO_4$ 15 kg/ ha + $FeSO_4$ 30 kg/ ha). With the same treatment, total growth increased by 94.95 and 65.67%, and approximate digestibility by 15.14 and 9.33%; tissue growth efficiency also increased by 65.35 and 49.61% with this treatment, while maximum ecological growth efficiency increased by 49.58% and 52.0% over control in T11 ($CuSO_4$ 25 kg/ ha + $ZnSO_4$ 25 kg/ ha + $FeSO_4$ 25 kg/ ha). Larval duration was maximum with T1 ($FeSO_4$ 10 kg/ ha alone) (7.63± 19.16 days) as against when fed with the control (T0). Larval length increased by 18.0 and 18.51% in T9, with the larval width increasing by 50.0% in more than one treatment. Maximum chawki larval weight and larval weight increased by 16.78 and 29.44%, while maximum silk gland weight was 2.14± 0.05g in T9.

Key words: *Bombyx mori*, micronutrients, mulberry, $CuSO_4$, $ZnSO_4$ food consumption, larval assimilation, total growth, digestibility, tissue growth efficiency, morphometrics, characteristics

Nutritional quality in mulberry leaves helps to accelerate the growth and metamorphosis in *B. mori* which are the physiological foundations for sericulture. The mulberry silkworm, *B. mori* feeds exclusively on the leaves of mulberry (*Morus alba* L). During the last two instars (i.e. 4th and 5th instars) of the silkworm life cycle, 97% of the total food intake and 80-85% of the feed utilization in the fifth instar larva of the total leaves consumed were metabolically active (Rahamathulla et al., 2003; Rahamathulla and Suresh, 2012). It is therefore essential to improve either food quality or appetite (or both) of larval instars of silkworm for better performance in silk production. Nutritional qualities of food, biochemical status of nutrients in the food, hormonal level in the body and environmental conditions all play a role in the growth, development and eventually physiology of insect bodies (Murugan and George, 1992; Singh et al., 2021). The silkworms respond in variety of way when fed with the mulberry leaves of different nutritional indicating efficient food utilization and conversion into silk substances (Yamamoto and Fujimaki, 1982). Furthermore, the rate of food consumption and leaf quality influence significantly on larval growth and weight. Micronutrients are useful in enhancing the energetic efficiency of the silkworm, as they are cofactors in the activity of several enzymes, increasing energy levels, which in turn reflects on the silk production in silkworms (Chamundeswari and Radhakrishnaiah, 1994). Additionally, foliar micronutrient supplementation has been shown to improve the yield and quality of mulberry leaves, and subsequently the healthy growth of silkworms, resulting in increased cocoon yield and quality (Geetha et al., 2016). The effect of micronutrients supplementation on the biochemical characteristics of *B. mori* larvae was recently reported by the present authors (Marin et al., 2021). Therefore, analysing the nutritional indices of the larvae as well as, morphometrics and their characteristics would be useful in understanding the larval parameters of the silkworm. In view the above facts, an attempt was made to determine the impact soil application of micronutrients in mulberry plants fed to *B. mori*.

MATERIALS AND METHODS

The field experiment was conducted on a three years old mulberry garden at Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E) at an elevation/ altitude of 29 m above sea level. The experimental plot was free from other plants and received direct sunlight exposure with proper irrigation. For the experiments, MR2 (Mildew Resistant Variety –2) mulberry plant (*M. alba*) developed by the Sericulture Department, Govt. of Tamil Nadu experimental station, Coonoor, Tamil Nadu, India was selected and the spacing between the plants was 90x60cm. Prior to the commencement of the experiment, mulberry plants were pruned in June, followed by ploughing, and farm yard manure was applied at the rate of 20 t/ ha/ year, and single dose of nitrogen, phosphorous and potash at 120:120:60 kg/ ha/ year was hoed in the soil uniformly. Depending upon the climatic conditions, irrigation was provided every five days of interval and micronutrients were added to the soil after twenty days of pruning. The experimental plot was protected from plant pests, and the diseased/ affected parts of the plant were removed periodically. The field experiment was laid out in a randomized block design with twelve treatments, each treatments were replicated thrice. Each treatment was supplemented with the required amount of each micronutrient, either individual or in combination as follows. T0 - Control (mulberry plants which did not receive micronutrients supplementation); T1 - $FeSO_4$ 10 kg/ ha; T2 - Zn SO_4 5 kg/ ha; T3 - Cu SO_4 5 kg/ ha; T4 - $CuSO_4$ 5 kg/ ha + $ZnSO_4$ 5 kg/ ha; T5 - $CuSO_4$ 5 kg/ ha + $FeSO_4$ 10kg/ ha; T6 - $FeSO_4$ 10 kg/ ha + $ZnSO_4$ 5 kg/ ha; T7 - $CuSO_4$ 5 kg/ ha + $ZnSO_4$ 5 kg/ ha + $FeSO_4$ 10 kg/ ha; T8 - $CuSO_4$ 10 kg/ ha + $ZnSO_4$ 10 kg/ ha + $FeSO_4$ 20 kg/ ha; T9 - $CuSO_4$ 15 kg/ ha + $ZnSO_4$ 15 kg/ ha + $FeSO_4$ 30 kg/ ha; T10 - $CuSO_4$ 20 kg/ ha + $ZnSO_4$ 20 kg/ ha + $FeSO_4$ 40 kg/ ha; and T11 - $CuSO_4$ 25 kg/ ha + $ZnSO_4$ 25 kg/ ha + $FeSO_4$ 25 kg/ ha. Twenty early *B. mori* fourth and fifth instar larvae were used as a replication, and were fed with the 5-6 fully grown mature mulberry leaves (T0 to T11) twice a day (morning and evening).

The $PMxCSR_2$ hybrid variety of *B. mori* used in the present study was procured from Government Sericulture Training Centre, Konam, Nagercoil, Kanyakumari, Tamil Nadu, India. Silkworm rearing began when the mulberry plants were 45 days old. Since the experiments required continuous maintenance of the test species, the silkworms were reared in a rearing room in accordance with the procedure of Krishnaswami (1978). *B. mori* larvae were fed with mulberry leaves and the remaining leaves and litter were weighed and recorded on a daily basis, and also the weight of the larvae was recorded in each treatment. The remaining leaves and the excreta were dried in a hot air oven at 70°C till constant weight and the values were recorded. To determine the larval growth, initial and final weights of the larvae as suggested by Waldbauer (1968) and Kaushal et al. (1988) food consumption, assimilation, total growth, approximate digestibility, tissue and ecological growth efficiency were calculated, besides other larval parameters. All data were affirmed as mean ± standard deviation (S.D.), and were subjected to Student's 't' test to find out the significant difference between control and treatment groups.

RESULTS AND DISCUSSION

On fourth and fifth instar *B. mori* larvae, several treatments had different effects. Among the treatments, T9 had a higher value in both the fourth and fifth instar. Highest food consumption by fourth and fifth instar was observed in T9 (1032.8± 48.3 and 2080.4± 164.6 mg/ larva/ day), with an increase of 31.56% and 22.98% over control, was observed in T9, and T1 had the lowest food consumption (848.3± 26.5 and 1819.2± 113.3 mg/ larva/ day) with an increase of 8.14% and 7.54% over control respectively. T9 (730.3± 21.4 and 1694.7± 110.7 mg/ larva/ day) had highest assimilation which increased by 51.38% and 34.56% over control, while T11 (504.3± 26.9 mg/ larva/ day) which increased by 4.50% over control and T1 (1291.8± 104.3 mg/ larva/ day) which increased by 3.41% over control had lowest. The maximum total growth of the fourth instar was recorded in T9 (522.1± 4.7 mg/ larva/ day) which increased by 94.95% over control, and minimum in T1 (296.3± 3.4 mg/ larva/ day) which increased by 10.64% over control. For fifth instar, it was also observed in T9 (954.8± 14.7 mg/ larva/ day) and T1 (612.7± 8.5 mg/ larva/ day) with an increase of 65.67% and 6.31% over control respectively (Table 1; Fig. 1). Food consumption has direct influence on larvae weight, and also depends on the types of nutrition (Shivakumar, 1995) as well as silkworm breeds (Remadevi et al., 1992). T9 showed considerably greater rate of food consumption (31.56% and 22.98%) in the fourth and fifth instar over control, which may be assumed due to their palatability, nutritional superiority and water retention capacity of leaves for longer duration. Further, the rate of food consumption would have also been influenced by the physical and chemical nature of food and also the physiological state of the insect too (Waldbauer, 1968).

Table 1. Effect of treatments on the nutritional characteristics and morphometrics of *B. mori* larvae

Treat-ment	Food consumption (mg/ larva/ day)		Larval assimilation (mg/ larva/ day)		Total growth (mg/ larva/ day)		Approximate digestibility (%)		Tissue growth efficiency (%)		Ecological growth efficiency (%)	
	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar
T0	784.4± 31.4	1691.6± 123.5	482.4± 25.3	1259.4± 93.8	267.8± 3.6	576.3± 4.7	61.49± 6.2	74.45± 8.6	55.5± 6.8	45.75± 7.9	34.14± 4.1	32.17± 4.3
T1	848.3± 26.5	1819.2± 113.3	546.8± 18.4*	1291.8± 104.3	296.3± 3.4*	612.7± 8.5	64.45± 3.7	70.97± 7.9	54.18± 4.6	47.45± 6.4	34.93± 5.2	33.67± 2.6
T2	862.4± 49.2*	1830.4± 122.7	510.9± 19.4	1320.6± 95.3	323.7± 7.5*	631.0± 6.4*	59.24± 4.5	72.13± 6.4	63.35± 5.2*	47.80± 4.2	37.53± 3.7*	34.47± 2.3
T3	868.5± 21.6*	1834.6± 142.9	516.3± 22.4	1334.1± 104.8	327.8± 9.8*	842.3± 7.5*	59.44± 3.1	72.74± 5.7	63.49± 4.4*	63.14± 3.7*	37.74± 3.6*	45.91± 3.2*
T4	875.6± 14.3*	1954.7± 124.7*	543.2± 38.6*	1402.7± 129.5*	390.4± 5.9*	897.2± 8.2*	62.03± 3.6	71.75± 8.3	71.87± 5.1*	63.96± 3.2*	44.58± 3.4*	45.89± 5.2*
T5	858.7± 48.8*	1974.3± 134.7*	546.2± 24.3*	1474.8± 113.4*	411.8± 3.4*	884.1± 10.4*	63.60± 4.1	74.71± 8.9	75.42± 5.7*	59.94± 3.6*	47.95± 3.2*	44.78± 2.2*
T6	870.3± 32.4*	1998.5± 152.6*	568.1± 31.2*	1518.2± 108.5*	426.9± 6.4*	879.4± 7.2*	65.28± 4.7	75.97± 5.6	75.14± 5.6*	57.92± 3.8*	49.05± 3.6*	44.0± 2.3*
T7	840.4± 24.5	1875.4± 117.8*	518.7± 21.6	1465.1± 104.6*	457.8± 4.1*	911.3± 12.4*	61.72± 5.8	78.13± 7.8	88.25± 3.4*	62.20± 3.1*	54.47± 3.3*	48.59± 2.7*
T8	1023.3± 31.6*	2067.9± 103.7*	721.9± 33.7*	1637.6± 112.5*	515.5± 9.5*	926.7± 13.4*	70.48± 2.9*	79.19± 6.3	71.40± 3.2*	56.58± 5.7*	50.37± 4.1*	44.81± 3.4*
T9	1032.8± 48.3*	2080.4± 164.6*	730.3± 21.4*	1694.7± 110.7*	522.1± 4.7*	954.8± 14.7*	70.74± 4.9*	81.44± 6.7*	71.49± 4.1*	56.34± 4.4*	50.55± 4.2*	45.89± 3.8*
T10	964.2± 36.1*	2064.7± 142.5*	662.5± 25.2*	1594.9± 97.9*	487.2± 3.6*	912.4± 10.3*	68.65± 6.4*	77.24± 7.8	73.53± 5.3*	57.20± 5.1*	50.52± 3.7*	44.19± 4.1*
T11	906.1± 46.8*	1862.5± 136.3*	504.3± 26.9	1302.4± 97.2	462.8± 4.6*	891.3± 12.6*	55.56± 5.8	71.48± 6.5	91.77± 4.2*	68.45± 4.6*	51.07± 4.2*	48.90± 1.5*

Morphometrics

Treat-ment	Larval duration (days)		Larval length (cm)		Larval width (cm)		Chawki larval weight (g/ 10)	Mature larval weight (g/ 10)	Silk gland weight (g)
	Fifth instar	Total	Fourth instar	Fifth instar	Fourth instar	Fifth instar			
T0	7.79± 17.12	25.22± 55.11	5.0± 0.12	5.4± 0.17	0.4± 0.9	1.0± 0.22	1.43± 0.59	33.89± 4.8	1.44± 0.02
T1	7.63± 19.16	25.08± 56.44	5.6± 0.15*	6.1± 0.26*	0.6± 0.3*	1.4± 0.3	1.50± 0.87	36.86± 5.2	1.58± 0.05
T2	7.51± 23.22	25.06± 58.14	5.4± 0.17	6.0± 0.19*	0.5± 0.1*	1.4± 0.14	1.49± 0.51	37.07± 3.9*	1.53± 0.1
T3	7.46± 34.45	25.06± 59.17	5.2± 0.14	5.8± 0.19	0.5± 0.2*	1.4± 0.3	1.43± 0.47	38.83± 1.6*	1.67± 0.03
T4	7.22± 34.23	24.46± 61.23	5.1± 0.15	5.5± 0.18	0.5± 0.4*	1.5± 0.23*	1.43± 0.83	38.67± 2.9*	1.62± 0.01
T5	7.20± 25.45	24.87± 63.23	5.2± 0.11	5.6± 0.11	0.5± 2.0*	1.5± 0.21	1.51± 1.20	40.05± 4.22*	1.83± 0.02
T6	7.20± 36.21	24.73± 63.34	5.3± 0.19	5.7± 0.08	0.5± 2.0*	1.4± 0.26	1.48± 1.04	40.72± 3.54*	1.87± 0.04
T7	7.01± 39.26	24.68± 67.56	5.4± 0.21	5.7± 0.23	0.5± 0.5*	1.5± 0.11*	1.56± 0.73*	42.11± 1.11*	1.71± 0.07
T8	6.86± 43.35	23.81± 69.12	5.7± 0.10*	6.2± 0.08*	0.6± 0.8*	1.6± 0.10*	1.62± 1.42*	42.87± 5.30*	2.00± 0.04
T9	6.94± 42.21	23.96± 70.13	5.9± 0.13*	6.4± 0.10*	0.6± 0.1*	1.6± 0.43*	1.67± 1.52*	43.87± 3.29*	2.14± 0.05
T10	7.14± 35.45	23.03± 70.16	5.6± 0.16*	6.2± 0.15*	0.6± 0.1*	1.6± 0.13*	1.58± 0.94*	42.45± 3.69*	1.98± 0.02
T11	7.29± 45.32	24.04± 73.18	5.5± 0.14*	5.9± 0.12*	0.6± 0.6*	1.4± 0.19	1.56± 0.39*	41.97± 4.62*	1.84± 0.01

Values mean± S.D. *Significant @ P≤0.05 (t-test)

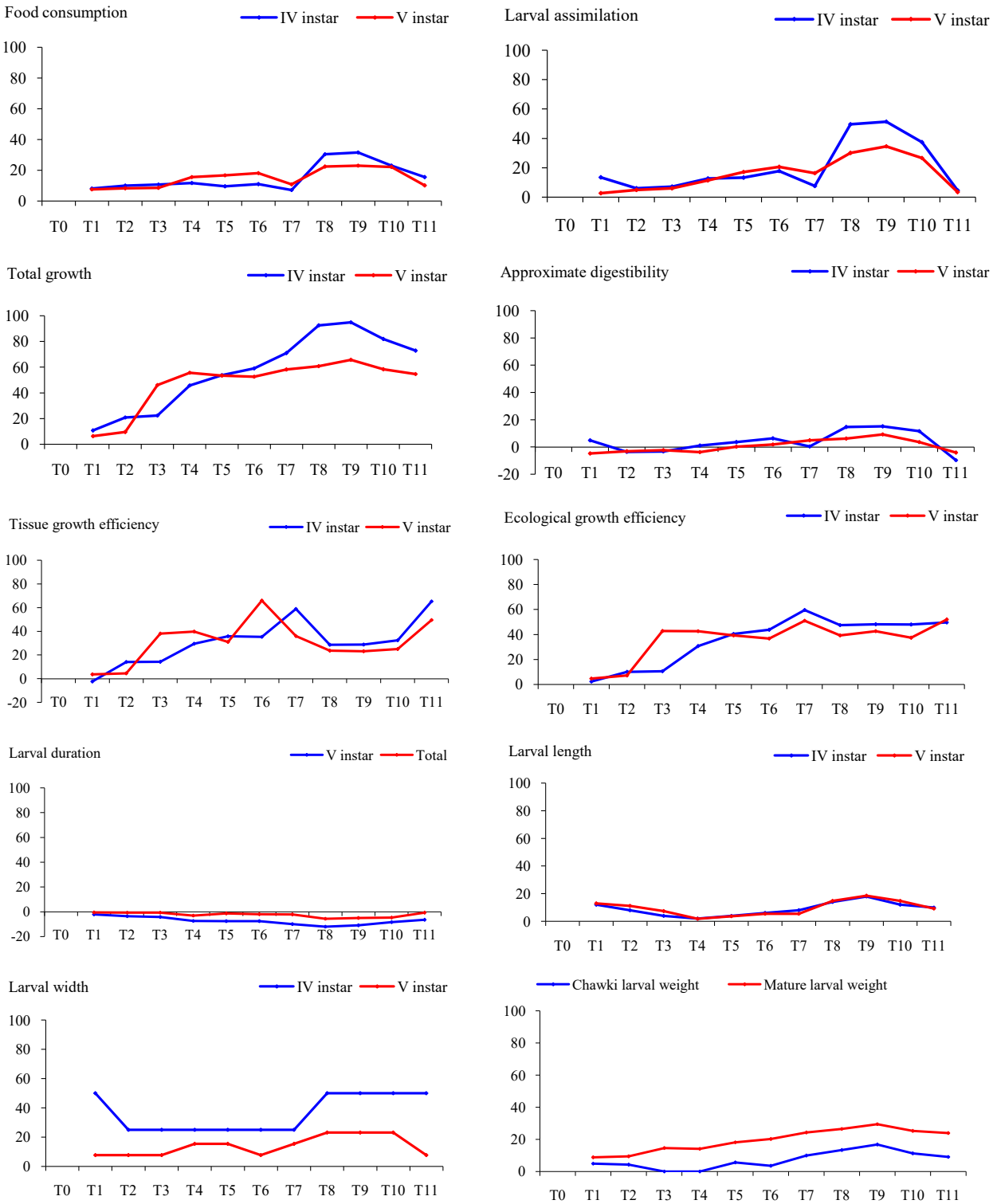


Fig. 1. *B. mori* larval parameters - effects of treatments

Similar to food consumption, assimilation of fourth and fifth instar was also significantly higher in all the treatments when compared to control. Increased digestion and assimilation results in improved assimilation rate efficiency (Thrived et al., 2003), and the larval performance in the present study was evident by the digestion and assimilation of the nutritional materials present in mulberry leaves as reported by Lalfelpuii et al. (2014 a,b). Food assimilation depends on various factors like ingested food and leaf moisture content, and enhanced assimilation of silkworm may be due to easy digestibility, and higher enzyme synthesis corresponding to enhanced food intake. The larvae growth is determined by the amount of food they consume and how well they utilised it. Nearly 80% of the food is consumed in its late stage of life cycle. Micronutrients have been shown to accelerate larval growth (Ito and Niminura, 1966; Horie et al., 1967), and this was observed in the present findings too, wherein, total larval growth was found to be higher in T9, due to the increase in food intake, and the ability of the larva to convert the food into biomass, besides, rate of metabolism, and hormonal mechanisms which directly/ indirectly favours growth.

Maximum approximate digestibility was noticed in T9 for fourth (70.74± 4.9%) and fifth (81.44± 6.7%) instars with an increase of 15.14% and 9.33% over control, while minimum approximate digestibility was observed in T11 (55.56± 5.8%) and T1 (70.97± 7.9%) with a decrease of -9.60% and -4.70% over control respectively. The fourth and fifth instars showed maximum tissue growth efficiency in T11, with values of 91.77± 4.2% and 68.45± 4.6% which increased by 65.35% and 49.61% over control, while their minimum values were 54.18± 4.6% and 45.45± 6.4% in T1, which decreased and increased by -2.37% and 3.71% over control respectively. For ecological growth efficiency, the fourth instar larvae registered its maximum and minimum value in T11 (51.07± 3.3%) and T1 (34.93± 5.2%) with an increase of 49.58% and 2.31% over control respectively, and the respective values for fifth instar were 48.9± 1.5% and 33.67± 2.6% which was also observed in T11 and T1 with an increase of 52.0% and 4.66% over control (Table 1; Fig. 1). Approximate digestibility is the ratio of the amount of food digested to the amount of food ingested in percentage. The variation among the different treatments in this study was due to the general difference in the leaf quality. The passage of food through gut facilitates increased digestion and assimilation which ultimately result in improved approximate digestibility. According to Muniandy et al. (1995), multivitamins and mineral compounds increase food intake, growth and conversion efficiency of silkworm. The present findings clearly indicated that the leaves supplemented with micronutrients showed high conversion efficiencies which may reduce the larval life span. The amount of digested food metabolized for energy decreases tissue growth efficiency but increases the maintenance of physical activities (Waldbauer, 1968), and this study correlated with the above facts. The decreasing trend of tissue growth efficiency and ecological growth efficiency with the increasing age of larvae was attributed to their differential response of the physical and chemical constitution of food (Delvi and Pandian, 1972; Mehrotra et al., 1972).

T1 had a long larval duration (in days) (7.63± 19.16), and total duration of 25.08± 56.44. This was reported to be short when compared to the control group (T0). On the other hand, the shortest duration was noticed in T8 (6.86± 42.21) with total duration of 23.81± 69.12. The maximum length of fourth instar was reported in T9 (5.9± 0.13 cm), and its minimum value was reported in T4 (5.1± 0.15 cm) which increased by 18.0% and 2.0% over control. For the fifth instar it was 6.4± 0.10 cm in T9 and 5.5± 0.18 cm in T4 which increased by 18.51% and 1.85% over control respectively. With respect to the larval width, significantly maximum values were recorded in T8 (0.6± 0.8 cm), followed by T11 (0.6± 0.6 cm), T1 (0.6± 0.3 cm), T10 (0.6± 0.14 cm) and T9 (0.6± 0.1 cm) all of which increased by 50.0% over control, while the remaining treatments (i.e. T2, T3, T4, T5, T6, T7 and T8) reported significantly minimum values of 0.5 cm. In the case of fifth instar, maximum width was observed in T9 (1.6± 0.43 cm), T10 (1.6± 0.13 cm) and T8 (1.6± 0.10 cm) which increased by 23.07% over control, while a minimum value of 1.0cm was observed in the other treatments (Table 2; Fig. 1). Maximum chawki larval weight was recorded in T9 (1.67± 1.52 g/ 10), with an increase of 16.78% over control, and minimum value in T3 and T4 (1.43± 0.47 g/ 10 and 1.43± 0.83 g/ 10) which was similar with the control (1.43± 0.59 g/ 10). For mature larval weight, maximum and minimum values were 43.87± 5.30 g/ 10 in T9 and 36.86± 5.2 g/ 10 in T1 which increased by 29.44% and 8.76% over control. In the case of silk gland weight, the maximum value was noted in T9 (2.14± 0.05 g), and minimum in T2 (1.53± 0.1 g) (Table 2; Fig. 1).

The morphometrics of fourth and fifth instar larvae significantly increased in the present study. Increased larval weight could be attributed to high quality of leaves containing micronutrients, which makes the

larvae healthier with better utilization and assimilation of micronutrients (Bose et al., 1994; Ramarethinam and Chandra, 2007), and other larval characteristics (Sarker et al., 1995; Nirwani and Kaliwal, 1995, 1996; Etebari et al., 2004; Balasundaram et al., 2008). The higher larval weights lead to higher silk gland weight. Silk gland attain maximum growth towards the end of the fifth instar owing to fibroin synthesis, and it is clear that the silk gland weight is one of the vital parameters for assessing the silk production potential of the larvae (Legay, 1958). Micronutrients show positive growth trends with reference to the growth of silk gland and larval body (Chakraborti and Medda, 1978; Bhattacharya and Kaliwal, 2005a,b), and the same was reported in the present study too. Amongst all treatments, T9 showed the maximum value as compared to other treatments. The aadministration of zinc to silkworm larvae through mulberry leaves significantly increase larval weight (Chamundeswari and Radhakrishnaiah, 1994; Balamani et al., 1995; Hugar and Kaliwal, 1999, 2002; Ashfaq et al., 2010). Further, zinc has a stimulating effect on the growth of silk gland and larval body, and with this effect, it shifts the balance towards higher gland-body ratio during fifth instar development (Kavitha et al., 2012).

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TWO NEW RECORDS OF LEAFHOPPERS OF THE GENUS *OPSIUS* FIEBER FROM INDIA

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ABSTRACT

Present study records two *Opsius* leafhopper species, *Opsius richteri* (Dlabola) and *Opsius stactogallus* Fieber from Delhi, India for first time. Earlier *Opsius richteri* had been described from Iran and *Opsius stactogallus* is distributed worldwide and now reported from India. This study reveals the new geographic distribution, and the species are redescribed and illustrated herein.

Key words: Auchenorrhyncha, Deltocephalinae, Cicadellidae, Opsiina, leafhopper, Opsiini, *Opsius*, new records, morphology, taxonomy, Hemiptera, redescription, distribution, *Opsius stactogallus*, *O. ritcheri*

Leafhopper family Cicadellidae, is one among the largest insect families with over 22,000 described species and many more awaiting description (Oman et al., 1990; Dietrich et al., 2001; Dietrich and Rakitov, 2002). Among Cicadellidae, Deltocephalinae is diverse, largest and most economically important subfamily with 38 tribes, 923 genera and >6700 valid species (Dai et al. 2017; Zahniser and Dietrich, 2013). The tribe Opsiini is distinguished from other tribes by the face oblique, not strongly depressed, not concave in profile; anterior margin of the head without carinae; antennal bases near middle or posteroventral (lower) corner of eyes; gena not extended on to dorsum behind eyes; the stem of connective longer and bifurcated aedeagus with two gonopores (Zahniser and Dietrich, 2013). This tribe is divided into four subtribes with >300 species, of which the subtribe Opsiina has been added with more genera (El-Sonbati et al., 2016, 2017; Sunil et al., 2020) and species (El-Sonbati et al., 2018, 2019; Sunil et al., 2020). The genus *Opsius* was established by Fieber (1866) with type species *Opsius stactogalus*. This genus is distinguished by its anterior margin of head never with carinae or ridge, face convex, and neither horizontal nor concave, aedeagal socle swollen and bulbous, with or without a pair of ventral processes. From India two species have been reported viz., *Opsius smaragdinus* (Distant, 1908) and *Opsius versicolor* (Distant, 1908). This genus was revised by El-Sonbati (2020) from Kingdom of Saudi Arabia dealing with seven species including *Opsius wilsoni* El-Sonbati,, and all these species are restricted to moist habitats and feed on *Tamarix* spp. (Newete et al., 2019), and *Opsius* leafhoppers are well known honey dew producers on *Tamarix* spp. (Wiesenborn 2004; Virla et al. 2010; Siemion and Stevens 2015). *Opsius* contains thus 20 species worldwide, including four species from India so far. In this study first record of *Opsius stactogalus* and *Opsius ritchteri* Dlabola are documented from India.

MATERIALS AND METHODS

The specimens studied at the National Pusa Collection (NPC) insect museum, Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. The terminology followed is after Zahniser and Ditriech (2013) and El- Sonbati (2020). Male genitalia dissections were carried out as described by Oman (1949) and Knight (1965), and it is as follows- the abdomen was removed by inserting a sharp pin between the abdomen and thorax with gentle piercing. The abdomen was treated in 10% KOH for 2~4 hr to remove unsclerotized material by gently prodding the abdomen with the head of a pin. Afterwards, the abdomen was rinsed thoroughly in water. The internal structures were then removed by a hooked pin, before being stored in glycerol vials for study. Photographs were taken with a Leica DFC 425C digital camera on the Leica M205FA stereozoom automontage microscope.

RESULTS AND DISCUSSION

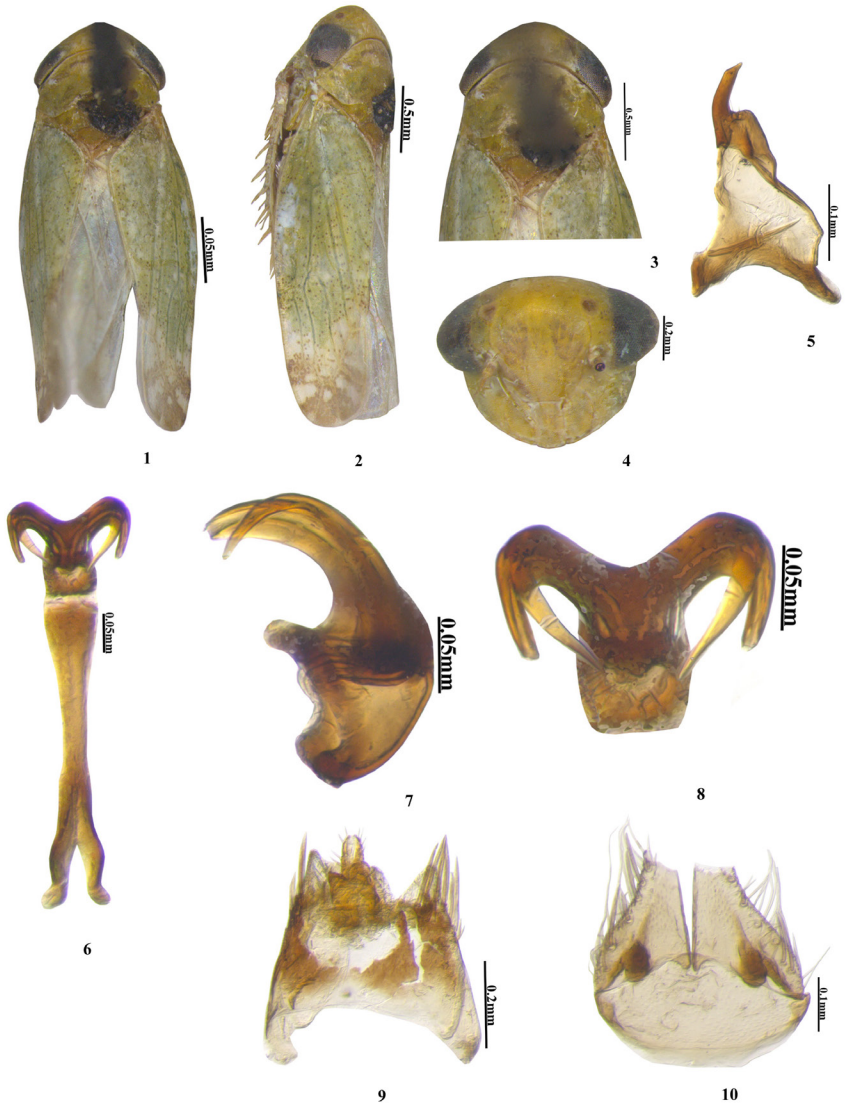
***Opsius richteri* Dlabola** (Figs. 1-10; 11-19)

***Opsius richteri* Dlabola 1960: 15**

Redescription

Colour of head, pronotum and scutellum greenish yellow. Face yellow, genal margin greenish; ocelli reddish, eyes dark red (Figs. 1, 11). Forewings greenish yellow with small brown spots scattered throughout wings; antepical posterior region and apical cell region dusty whitish with large groups of brown spots (Figs. 2, 12). Head including eye 1.06x about width of pronotum. Anterior margin of vertex slightly produced; length of vertex half as long as it's wide in between eyes or 0.6x as width of eyes (Figs. 4,14). Pronotum length 0.49x as short as its width; anterior margin convex, posterior margin concave. Scutellum length 0.65x as short as its width; scutoscutellar suture well developed (Figs. 3, 13).

Male genitalia with pygofer in dorsal view, 0.54x as long as wide; group of macrosetae confined to posterior half (Fig. 9). Valve broadly rounded at base, anterior margin of valve at middle greatly produced; middle length of valve 0.49x as short as its basal width (Fig. 10). Subgenital plate broadly triangular, uniseriate rows of macrosetae along with long hair like confined to outer lateral margin; tip of subgenital plate with rounded apex (in our specimen it's been broken) (Fig. 10). Style broadly bifid at base, wider at median region with well developed preapical lobe, apophysis short, fingerlike; length of style 0.36x as long as it's wide



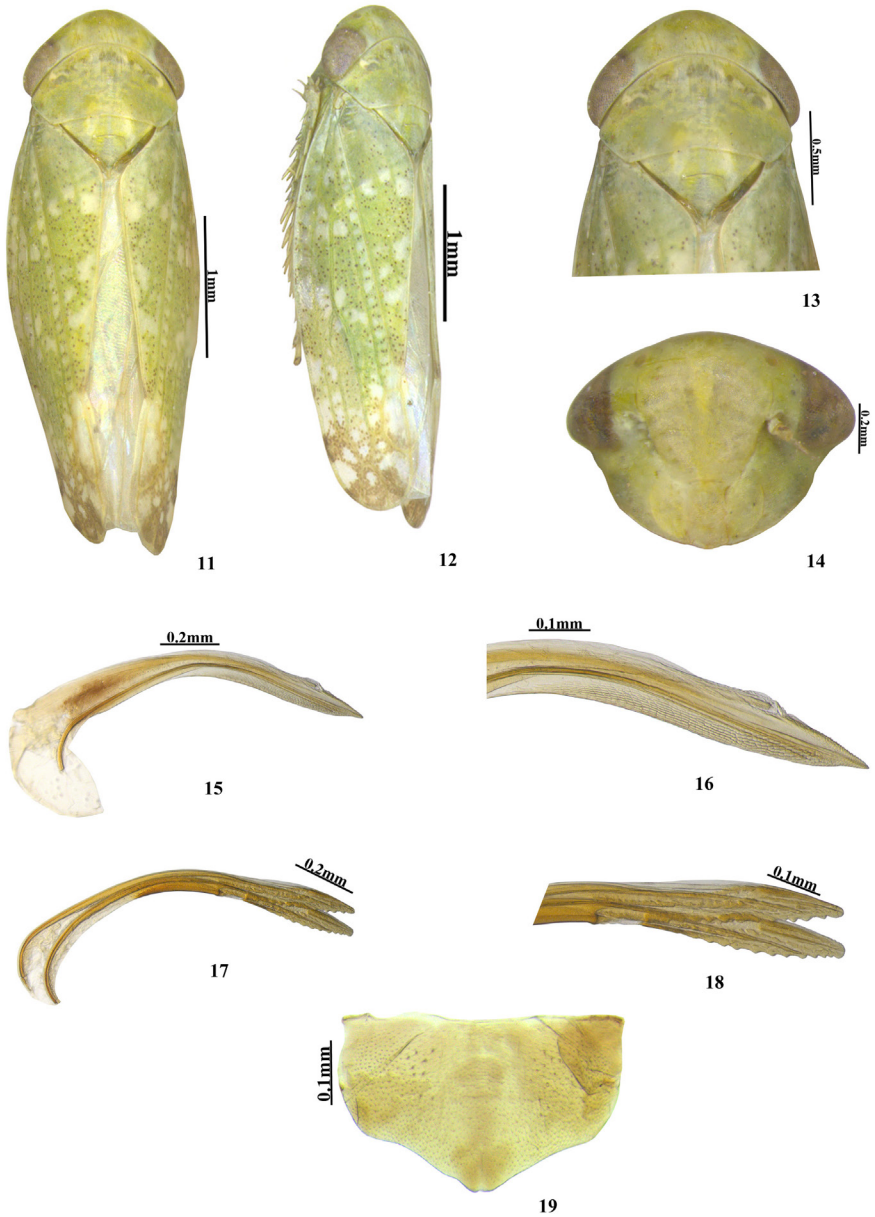
Figs. 1-10. *Opsius richteri* Male: 1. Dorsal habitus; 2. Lateral habitus; 3. Pronotum; 4. Face; 5. Style;6. Aedeagus with connective; 7. Lateral aedeagus; 8. Dorsal aedeagus; 9. Pygofer; 10. Subgenital plate with valve

(Fig. 5). Connective inverted Y-shape, length of stem 3.1x as long as its arms (Fig. 6). Aedeagus in lateral view, well developed dorsal apodeme, aedeagus shaft and process C-shaped (Fig. 7). Aedeagus in dorsal view with only dorsal process, both distinctively curved or bent inward at base, aedeagal shafts with diverging branches bent inward at base, shorter than dorsal process. Gopopore opens apical (Fig. 8). Female genitalia with VIIth sternite 3x as broad at base as long medially, posterior margin concave with V-shape notch at middle, posterolateral angles acutely rounded (Fig. 19). First valvula lightly curved at middle, subapical dorsal region maculose (Figs. 15-16). Second valvula slender throughout their length, small sparsely serrated teeth on subapical dorsal region (Figs. 17-18).

Measurements (mm): Male: Total body length 3.19; wide across eyes1.037♂, Female: Total boby length 3.16; wide across eyes 1.01

Material examined: 2♂, 11♀ India: IARI, New Delhi, 28.6139° N, 77.2090° E, light trap, 15.iv.1965, Subba Rao and Ramakrishnan (NPC).

Remarks: Earlier this species was reported from

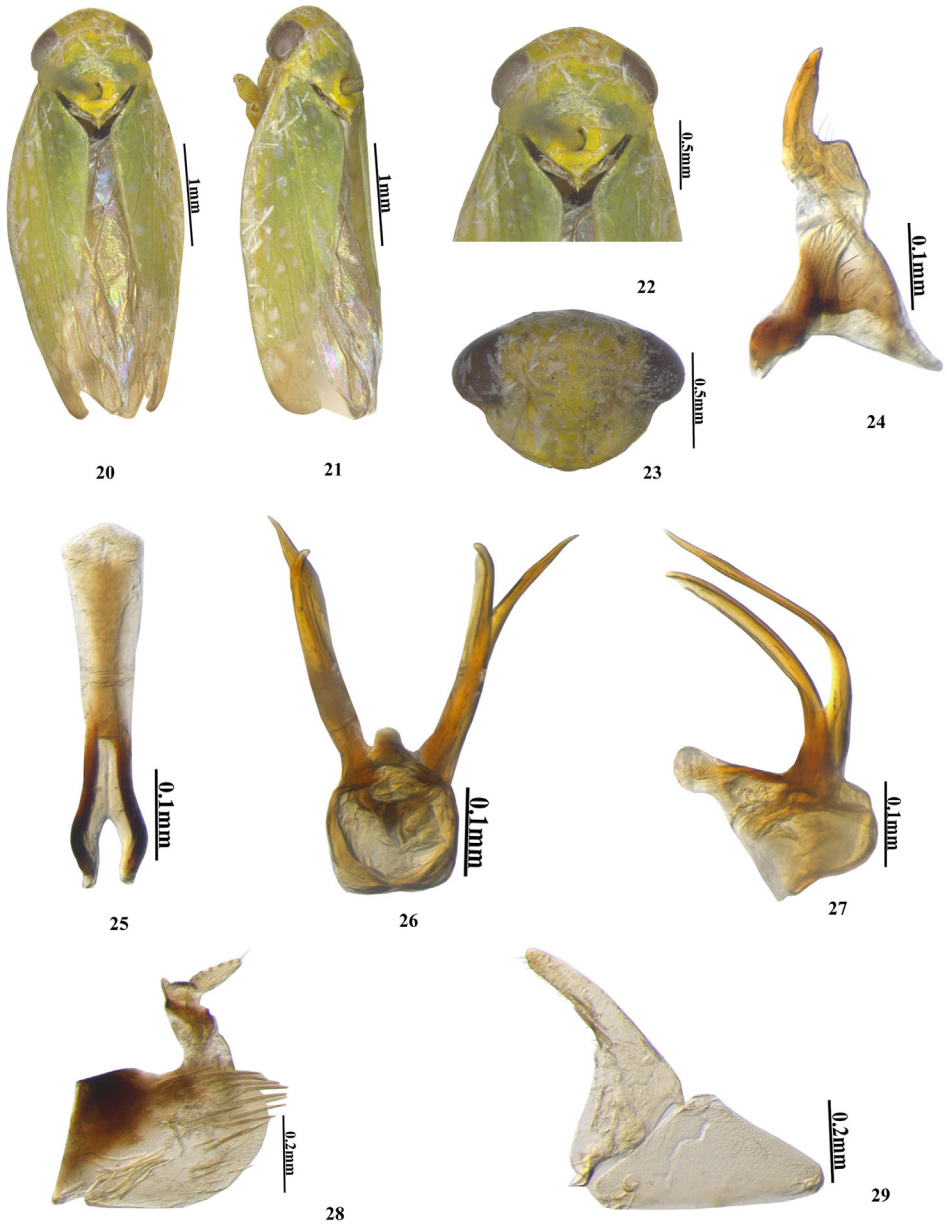


Figs. 11-19. *Opsius richteri* Female: 11. Dorsal habitus; 12. Lateral habitus; 13. Pronotum; 14. Face; 15. Valvula I; 16. Valvula I apical; 17. Valvula II; 18. Valvula II apical; 19. VII sternite

Iran (Dlabola, 1960); Oman by El. Sonbati et al. (2020), now it is first time reported from India. This species similar to *O. scutellaris* with the aedeagal shaft branches diverging but differ by produced vertex, aedeagal shafts and dorsal process distinctively curved or bent inward at base, and length of aedeagal shaft 2x as short as dorsal process.

***Opsius stactogallus* Fieber** (Figs. 20-29)

Stactogala Amyot, 1847:217 (Official Index of Rejected and Invalid Works in Zoological Nomenclature, ICZN Op. 2165) (Non binom.); *Iassus stactogala* Walker, F., 1851:894 (Nom.nud.); *Typhlocyba stactogala* Perris, 1857:176 (Nom. nud.); *Opsius stactogalus* Fieber, 1866:505; *Jassus (Thamnotettix) tamaricis* Kirschbaum, 1868:90 (Prim. hom.: *Jassus tamaricis* Becker, 1865); *Athysanus stactogalus* Fieber, 1872:11; *Athysanus heydeni* Fieber, 1872:11 (Nom.nud.); *Athysanus (Opsius) stactogalus* Fieber, 1872:31; *Athysanus (Opsius) heydeni* Fieber, 1872:31 (Nom.nud.); *Athysanus heydeni* Puton, 1875:138 (Nom.nud.); *Athysanus stactogala* Ferrari,



Figs. 20-29. *Opsius stactogallus* Male: 20. Dorsal habitus; 21. Lateral habitus; 22. Pronotum; 23. Face; 24. Style; 25. Connective; 26. Dorsal aedeagus; 27. Lateral aedeagus; 28. Pygofer; 29. Subgenital plate with valve

1882:129 (Missp.); *Athysanus stractogala* Fokker, 1900:33 (Missp.); *Limotettix stactogala* Butler, E.A., 1902:248; *Athysanus stactogallus* Graeffe, 1903:49 (Missp.); *Eutettix osborni* Ball, 1907:39; *Euscelis stactogalus* Olsen, 1921:33; *Limotettix stactogalus* Singh-Pruthi, 1925:208; *Eutettix stactogala* Osborn, 1928:301; *Eutettix stactogalia* Osborn, 1928:371 (Missp.); *Opsius stactogalis* Knowlton, 1935:111 (Missp.); *Opsius stagtogalus* Lindberg, 1948:160 (Missp.); *Opsius stetogalus* Stroud, 1950:666 (Missp.); *Opsius stactagallus* Hopkins, L. & Carruth, 1954:1128 (Missp.); *Opsius stactogalus* Evans, 1977:118; *Opsius spinulosus* Giustina, 1981:106 tamarisk leafhopper (Common name); *Opsius tamaracis* (Missp.)

Redescription

Colour of head, pronotum and scutellum lemon yellow. Vertex pale yellow; face greenish yellow with yellowish transverse lines on frons. Eyes and ocelli dark brown (Fig. 20). Pronotum anterior half-light yellowish and posterior half-light green. Scutellum yellowish, anterolateral triangles green (Fig. 22). Forewing yellowish green with brown spot irregularly placed, posterior subapical and apical cells pale brown (Fig. 21).

Head including eyes 1x as width of pronotum. Anterior margin of vertex rounded, parallel to posterior margin, length of vertex 0.63x as short as its half width between eyes (Fig. 23). Length of pronotum 0.47x as short as its width, anterior margin convex, not produced beyond eyes and posterior margin slightly straight. Length of scutellum 0.65x as short as its width, scutoscutellar suture well developed (Fig. 22).

Male genitalia with pygofer in lateral view, 0.65x as long as its width, group of macrosetae confined to posterior half, posterior dorsal margin conically rounded (Fig. 28). Valve broadly triangular, 0.56x longer than its median length (Fig. 29). Subgenital plate triangular with digited apex, uniseriate macrosetae confined to outer sublateral margin (Fig. 29). Style bifid, broad at base, preapical lobe well developed with few setae, apophysis beak shape, directed posteriorly (Fig. 24). Connective inverted Y-shape, stem 3.57x as long as arms (Fig. 25). Aedeagus in lateral view, well developed dorsal apodeme, aedeagus shaft slightly curved in middle and dorsal process bent medially, directed anteriorly (Fig. 27). In dorsal view, a pair of dorsal process present close to aedeagal shaft, shaft and process almost similar in length, projected anterior. Gonopore opens apical (Fig. 26).

Measurements (mm): Male: Total body length 4.05; wide across eyes 1.12

Material examined: 1♂, India: Delhi, 28.6139° N, 77.2090° E, light trap, 2.iii.1940, W. Kerr (NPC).

Remarks: *Opsius stactogallus* is a cosmopolitan species, though it was first time reported from India. This species closely resembles to *O. heydeny* (Lethiery) but differentiated by style with broad base, well developed preapical lobe, subgenital plate with digitated apically. Aedeagus with dorsal process bent medially, directed anteriorly.

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AUTHOR CONTRIBUTION STATEMENT

SS conceived and designed the experiment, performed the experiment, prepared figures, and approved final draft. NMM conceived and designed the experiment, authorized or reviewed draft of the paper, and approved the final draft. PT performed the experiment, reviewed draft of the paper, and approved the final draft.

CONFLICT OF INTEREST

No conflict of interest.

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DIFFERENTIAL APYRENE SPERM ACTIVATION ELICITED BY VARIOUS REGIONS OF MALE REPRODUCTIVE SYSTEM OF *SPODOPTERA LITURA* (F)

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ABSTRACT

In this study, the sperm activation potential of various regions of male reproductive system of *Spodoptera litura* (F.) has been evaluated by in-vitro bioassay. This has been done using their respective secretions as sperm activator for the sperm derived from the duplex of 2-3 days old virgin male moths. The results revealed that the prostatic part elicited a significantly pronounced sperm activity profile (in terms of proportion of active sperm and their activity intensity), as compared to the subregions of the prostatic part. The sperm activity profile by the prostatic part secretions was further observed to be reinforced by the addition of secretions of accessary glands. The sperm activity peak plateau reached around 30-90 min during the sperm activation bioassay. Further studies are in progress towards identification and characterization of potential biomolecule(s) responsible for sperm activity in the secretions of male prostatic part of the ejaculatory duct. This study has pragmatic application in exploring the anti-reproductive potential of various biorationals and optimization of radiogenetic IPM methods that are based on sperm dynamics and mating competitiveness.

Key words: *Spodoptera litura*, sperm activity, ductus ejaculatorius duplex, male reproductive system, noctuid moth, prostatic part, in vitro bioassay, biorational molecules, IPM

Spodoptera litura (F.) is a serious polyphagous pest that causes significant damage to many crops like soya bean, cotton, tobacco and vegetables and has attained global importance. Development of resistance to various major classes of insecticides i.e., organophosphates, organochlorines and synthetic pyrethroid has been reported against *S. litura* in many parts of the world (Ramakrishnan et al.*,*1984; Saleem et al., 2008; Tong et al., 2013; Gandhi et al., 2016). Moreover, the injudicious and indiscriminate use of pesticides has worsened the situation causing environmental pollution, elimination of natural controlling agents and outbreak of secondary pests (Vreysen et al., 2006; Seth et al., 2016b). Hence, a considerable emphasis is being laid to tackle such serious pests by evolving some biorational tactics that would be ecofriendly. Activity of sperm is a crucial parameter for success of reproduction in any species and sperm behaviour might be an important target for achieving success in lepidopteran pest control strategies. Sperm dimorphism in lepidopterans provides a wide window to develop methods that might influence the sperm dynamics. In lepidopterans, the males produce two distinct types of sperm- nucleated eupyrene and non-nucleated apyrene sperms. *Spodoptera litura* is a noctuid moth and is reported to exhibit sperm polymorphism (Seth et al., 2016a). The apyrene sperm of *S. litura* generally comprise about 75% of the sperm in the ejaculate (Seth et al., 2016b). Research has been conducted to find out proteins responsible for sperm activation in *Bombyx mori*. Sperm plays an important role in reproduction and can be targeted in either male (before copulation) or female (after copulation in spermatophore). The sperm passage from male testes to female eggs till fertilization undergoes a variety of modifications and is affected by many factors like pH, enzymes, gland secretions etc. (Werner and Simmons, 2008). The present study ascertains the differential potential of secretions of different parts of the male reproductive tract in eliciting activation of apyrene sperm derived from ductus ejaculatorius duplex (herein after referred to as 'duplex') of *S. litura* so that an appropriate region in male reproductive tract could be identified to extend the research on identification of biomolecule(s) responsible for sperm activation.

MATERIALS AND METHODS

The culture of *S. litura* (F.) was maintained in the laboratory (27± 1°C, 75± 5% RH, and a 12:12 hr L:D photoperiod with lights on at 06.00 hr and lights off at 18.00 hr) on a chickpea based semisynthetic diet (as described by Seth and Sharma, 2001). Proper care was

taken to avoid microbial infection in the culture. The freshly emerged adults in 4-5 pairs were transferred to the perspex cages (20x20x20cm) with 10% honey solution to feed on and castor leaf to act as ovipositional trap. The eggs were collected daily and incubated for hatching in to the first instar larvae (L1) that moulted five times during larval phase, followed by larval-pupal moult and adult eclosion. For invitro sperm activation assay, 2-3days old virgin adult male of *S. litura* was dissected, and reproductive tract was taken out on a glass slide in Belar's saline (0.6g NaCl, 0.2g KCl, 0.2g $CaCl_2$, 0.2g Na_2CO_3 and water to make volume upto 1l). The duplex, prostatic part and accessory glands were dissected out separately. The prostatic part was further divided into upper and lower prostatic parts for sperm bioassay. The duplex was minced in 50µl of 0.3 M of HEPES- KOH buffer at pH 7.0 containing 20mg/ ml bovine serum albumin to extract sperm. The sperm solution was kept in parafilm container placed on moist tissue paper at room temperature.

The prostatic part whole, its different regions (upper and lower) and accessory glands were separately minced in 40µl of 0.03 M ammonium bicarbonate buffer with a pH of 7.0. The extracts were then transferred in to 1.5ml micro centrifuge tube and centrifuged at 6000 rpm for 10 min at 4°C. The supernatants of the prostatic region, its subregions and accessory glands were collected to act as an individual activator in invitro sperm activation assay. Then, 5µl of a specific supernatant (activator) was mixed with 5µl of sperm solution from the duplex. The solution was put on a glass slide, covered with coverslip and observed under microscope at 400x magnification. Two parameters related to sperm motility were observed and recorded upto 135 min (with a frequency of 15-30 min). These parameters included- (i) % sperm active (proportion of sperm that elicited activity out of the total number of sperm observed) and (ii) sperm activation intensity (number of to and fro motions or undulations of activated sperm/ sec). Each data point comprised of '2' phases: (i) computing the number undulations/ sec and (ii) transforming the number of undulations into a scale from 0-4 ('0'= no undulation, '1'=1-5 undulations/ sec, '2'= 6-10 undulations/sec, '3'= 11-15 undulations/ sec, '4'= > 15 undulations/sec).Ten replicates were performed for assessing each parameter and each replicate comprised an average of 10-15 readings from different microscopic views pertaining to one insect. The data were replicated ten times, and subjected to one way ANOVA; % data was transformed using arcsine √x value before ANOVA, but data shown in tables and graphs are back transformed. The significance level was set at $p \leq 0.05$, and LSD test was performed to determine significant differences among the treatments (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Invitro bioassay was conducted to study the sperm activation potential of various regions of male reproductive system of *S. litura* using the secretions of different parts of reproductive system as sperm activator for eliciting the activation in the sperm derived from the duplex of 2-3days old virgin male moth. The secretions from the prostatic part elicited a significantly pronounced sperm activity profile assessed as proportion of active sperm, as compared to the secretions from different sub-regions of the prostatic part. The sperm activity was substantially enhanced at 15 min during the assay and the peak plateau of the sperm activity reached around 30-90 min. with >70% of the sperm eliciting activity, during the sperm activation bioassay. During sperm activation bioassay, significantly enhanced sperm activity was exhibited by whole prostatic part as compared to the lower and upper prostatic parts or accessory gland, although lower prostatic part elicited a better response. Further, the sperm activity by the prostatic part was found to be reinforced due to addition of the accessory gland secretions, that caused instant sperm activity due to addition of activator, with activity profile reaching the plateau faster than the activity profile induced by prostatic part alone. Similar pattern of sperm undulation intensity during sperm activation bioassay was noticed vis-à-vis the profile of % active sperm (Fig. 1; Table 1). The lower prostatic part seemed to elicit the sperm undulation intensity better than the upper part, and the sperm activation intensity elicited by prostatic part was further pronounced by addition of secretions from the accessory glands. The peak plateau of sperm activity intensity due to prostatic secretions ranged from 3-4 scale, amounting to 11-15 undulations/ sec or more.

Various regions of the male reproductive tract eliciting a varying degree of sperm activity, can be arranged in descending order as prostatic part (as a whole) > lower prostatic part > accessory gland > upper prostatic part (Table 1; Fig. 1).The involvement of various regions of male reproductive system was ascertained towards apyrene sperm activation in *S. litura* by the sperm activation bioassay (as per method described by Seth et al., 2016a). The presence of sperm activator biomolecules in the prostatic part of *S. litura* has been reported earlier by Seth et al. (2016a), whereas in the present study the male accessory glands secretions

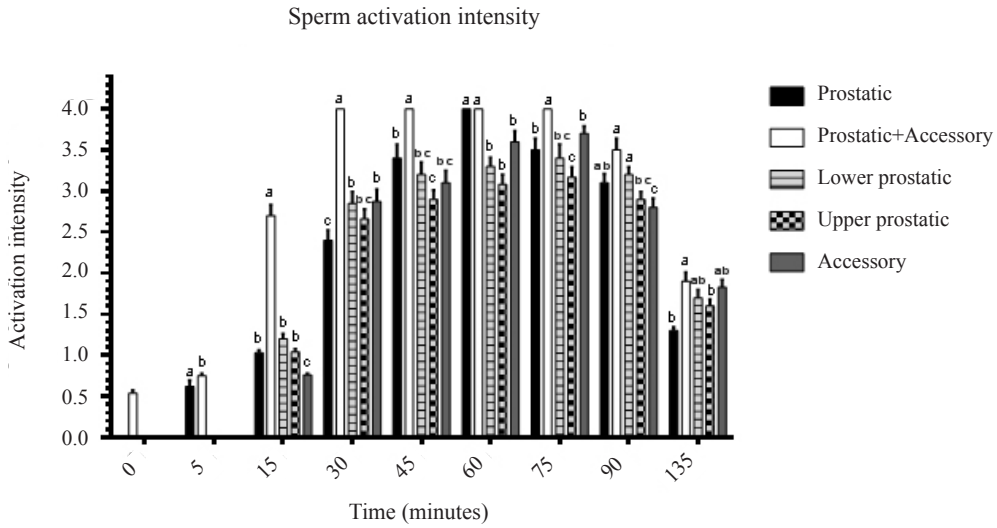


Fig. 1. Sperm activity was determined as sperm activation intensity (in-vitro assay) in sperm from duplex mixed with activator from different regions of male reproductive system; Means followed by the same alphabets among various regions with respect to each specific time during the assay are not significantly different at P<0.05 level (ANOVA followed by LSD post-test). Activity intensity was assessed by transforming the number of undulations into a scale from 0-4 ('0'= no undulation, '1'=1-5 undulations/ sec, '2'= 6-10 undulations/sec, '3'= 11-15 undulations/sec, '4'= > 15 undulations/sec).

Table 1. Sperm activity (in vitro assay) elicited by different regions of male reproductive system

	0	5	15	30	45	60	75	90	135
Prostatic	0	19.98a± 2.6	66.59a± 2.89	89.41a± 3.23	86.21ab± 4.26	84.46ab± 3.04	83.11ab± 3.5	81.55ab± 4.17	30.77ab ± 2.92
prostatic+ accessory	16.53± 2.68	27.83b± 2.46	73.83a± 4.32	92.16a± 2.81	91.62a± 2.56	87.16a± 3.34	86.20a± 3.55	84.06a± 2.08	36.16a± 3.18
Lower prostatic	0	0	52.16b± 2.02	76.83b± 2.07	85.54ab± 2.76	82.12ab± 3.43	81.23ab± 2.7	80.80ab± 2.69	28.83ab± 2.39
Upper prostatic	0	0	46.16b± 2.89	72.38b± 2.72	79.90b± 2.92	77.08b± 3.56	75.97b ± 2.9	74.40b± 2.78	26.82b± 2.42
Accessory	0	0	49.11b± 3.22	73.04b± 4.83	82.77b± 3.50	83.90ab± 3.54	84.57ab± 3.41	74.76b± 3.1	32.12ab± 2.55
F value		18.180	66.315	22.293	6.354	2.52	2.71	2.93	11.583

Sperm activity was determined as % sperm eliciting activity (in-vitro assay) in sperm from duplex mixed with activator from different regions of male reproductive system; Means± SE followed by the same alphabets within a column are not significantly different at *P*<0.05 level (ANOVA followed by LSD post-test). % data was transformed for ANOVA and data in the table is untransformed.

were also found to reinforce the sperm activity. Stephens et al. (2018) reported the presence of trypsin like protease in the male accessory gland of *Culex pipiens* and *Culex quinquefasciatus*. Their further studies suggested the likely involvement of accessory gland serine proteases in activating *Culex* sperm motility during or after transfer to the female reproductive tract (Stephens et al., 2018).

Sperms are non- motile in the testis and become motile only during copulation or after deposition in the female as spermatophore (Davis, 1965; Hughes and Davey, 1969). The process of spermatogenesis in males differs between eupyrene and apyrene sperms as reported by Friedländer, 1997. The apyrene sperm must have a specific function in the reproductive process (Silberglied et al.,1984).The apyrene sperm may have multiple functions, such as regulating female receptivity (Cook and Wedell, 1999; Swallow and Wilkinson, 2002), assisting eupyrene sperm for fertilization (Till-Bottraud et al., 2005), assisting the migration of eupyrene sperm to the spermatheca (Holt and North, 1970), as carriers of nutrition for eupyrene sperm (Boggs, 1995), etc. During mating in *S. litura*, the eupyrene sperm (as bundles) and apyrene sperm (as individual, dissociated from bundles) are transferred within a spermatophore to the bursa copulatrix of female. Eventually, movement of dissociated apyrene

as well as eupyrene sperm occurs into the spermatheca (Seth et al., 2002).

The present study revealed the presence of apyrene sperm activator molecules in accessory gland and different regions prostatic part with a pronounced effect due to the secretion from the prostatic part. The combination of secretions from prostatic part and accessory gland showed an additive effect. It was noticed that the initiation of sperm activity was instantaneous due to combined secretions of prostatic part and accessary glands, while there was a little latency of 2-5 min in the initiation of sperm activity when individually secretions of prostatic part or accessory gland were used as sperm activator in separate bioassays. This infers that presumably increase in concentration of sperm activator molecules might induce faster sperm activation. Both % active sperm and sperm intensity data analysis showed similar profile of sperm activity elicited by different specific regions of the male tract. With the progression of time after initiation of sperm activation bioassay, the number of activated apyrene sperm increased in the present study, indicating that activator molecule might be a self-catalytic molecule, as suggested by Nagaoka et al.(2012). A sharp increase in activation intensity in apyrene sperm was noticed from 15 min onwards showing a peak plateau at 30-90 min. (Fig. 1). Sperm activator molecules were found to be predominantly present in the prostatic part.

Sperm activity is a promising domain for devising novel biorational IPM strategies to target male reproductive competence. Sperm viability matters in insect sperm competition under different stress conditions, and sperm competition can be a potent selective force acting on an array of male reproductive traits. The frequency of successful insemination can be considered an important indicator of competitiveness, especially for lepidopteran insects (LaChance et al., 1978). Sperm viability is a major male fitness component, as increased sperm viability would be associated with enhanced sperm competitiveness (Tourmente et al. 2019). Pest control can be exercised in many ways by using radiation (Seth et al., 2016), using protein inhibitors (Zhao et al., 2019; Singh et al., 2020), gene mutation (Zhang et al., 2019; Xu et al., 2020) and RNAi technology (Jayachandran et al., 2013; Singh et al., 2020). Radiation induced F1 sterility technique has been proposed for *S. litura* (Seth and Sharma, 2001; Seth et al., 2016a, b). Formulating pest control methods to target sperm activation molecules would be ecofriendly in nature as it might not affect non targeted organisms and be compatible with other pest management tactics to be used in IPM. The role of accessory gland secretions in activation of moth sperm has been evaluated in the present study. Further studies are in progress for identification and characterization of potential biomolecule(s) in the male prostatic part of the ejaculatory duct. This study has practical implications in terms of (i) assessing and establishing the antireproductive or sterilant role of various pesticides, xenobiotics, other stresses (viz., radiation, temperature, environmental pollution) (ii) devising novel biorational pest control tactics, using sperm activity as target through biochemical inhibitors(to check sperm activation) and RNAi technology, that might be coupled effectively with radiation mediated genetic control methods against lepidopteran pests.

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AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST

No conflict of interest.

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INSECTICIDE SPRAY REGIMES TO MANAGE *HELICOVERPA ARMIGERA* (HUBNER) AND THEIR IMPACT ON NATURAL ENEMIES IN COTTON UNDER HIGH DENSITY PLANTING SYSTEM

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ABSTRACT

This study evaluated some new class insecticides viz., chlorantraniliprole 18.5SC, flubendiamide 480SC, spinosad 45%SC, indoxacarb 14.5SC and emamectin benzoate 5%SG in cotton under high density planting system (HDPS). These were evaluated against *Helicoverpa armigera* (Hubner) and their impact on natural enemies were also observed. Experiments were conducted at the ICAR-Central Institute for Cotton Research, Nagpur during 2013-14. Each of the new molecules were sprayed in eight different windows 60 days after sowing (DAS), 60 and 80 DAS, 60, 80 and 100 DAS, 60, 80 100 and120 DAS, 100 and 120 DAS, 80,100 and 120 DAS, 120 DAS and unsprayed control along with three replicates each were analysed in randomized complete block design. The damage by *H. armigera* was observed by counting healthy and damaged squares and bolls, and % worked out. The whole plant incidence was recorded in case of major predators sampled including spiders and coccinellids. The spray regime 2 (60 and 80 DAS), regime 3 (60, 80 and 100 DAS), regime 4 (60, 80, 100 and 120 DAS) and regime 6 (80, 100 and 120 DAS) were found superior in managing bollworms with by recording least square damage. The results on natural enemies' populations (spiders and coccinellids) clearly revealed that, all spray regimes were more or less safe to the natural enemies. However, spray regime 1 (spray at 60 DAS), regime 2 (spray at 60 and 80 DAS) and regime 3 (spray at 60, 80 and 100 DAS) recorded maximum natural enemies. Maximum natural enemies was reported at 80 DAS in all the insecticidal spray regimes. Chlorantraniliprole, flubendiamide, spinosad, indoxacarb and emamectin benzoate through the spray regime 2 (spray at 60 and 80 DAS) was observed to be the best option against *H. armigera* by controlling it in early stage itself and to sustain natural enemies in the cotton ecosystem.

Key words: Bollworms, *Helicoverpa armigera,* cotton, high density planting system, insecticides, natural enemies, spray regimes, Chlorantraniliprole, flubendiamide, spinosad, indoxacarb and emamectin benzoate

Diligent production and economic strategies are important for cotton growing farmers due to of expanding cost of cultivation and stagnating productivity. Adoption of high-density planting system (HDPS) and newly released desi cotton varieties offer an alternative way to the sustainable production and decreased production cost (Kumar et al., 2017). In India, cotton is grown in 12.23 million ha with productivity of 524 kg lint/ ha (Anonymous, 2017), and productivity has not shown any remarkable improvement in the last 10 years. Bollworms (BW) especially, American bollworm *Helicoverpa armigera* (Hubner) and pink bollworm, *Pectinophora gossypiella* (Saunders) cause considerable damage (Deore et al., 2010). Theoretically, higher planting density ensures earlier crop canopy cover, higher sunlight interception leading to higher and earlier yields at reduced cost. The obvious advantage of this system is earliness (Rossi et al., 2004) since high density planting needs less bolls/plant to achieve the same yield as compared to conventional cotton and the crop does not have to maintain the late formed bolls to mature. In general, it was observed that lower plant densities produce high values of growth and yield attributes/ plant, but yield/ unit area was higher with higher plant densities (Namdeo et al., 1991; Dhoble et al., 1992; Sharma et al., 2001). Changes in plant density modify the microclimate and this may alter the incidence of pests and diseases (Venugopalan et al., 2013). Early cotton genotypes early in duration (<150 days), tolerant to sucking pests having compact plant architecture ideally suited for HDPS and mechanised harvesting. These genotypes can provide higher cotton yields under HDPS and they require very less of inputs, management and time thus, providing better economic returns to the cotton growers (Parihar et al., 2018).

Chemical control is still the most important method for managing pests (Korkor et al., 1995) and the

synthetic insecticides are often a part of management programs to control lepidopterous pests (Aydin and Gurken, 2006). The success of bollworms complex control program relies on use of insecticides belonging to different chemical groups in certain sequences, application time and spray intervals (Watson et al., 1986; El-Feel et al., 1991; Abd El-Mageed et al., 2007). Conventional insecticides have not provided a long-term solution to the bollworms problem, moreover as a result of continued massive use of certain synthetic insecticides against the cotton pests has resulted in development of tolerant and resistant strains (Schmutterer, 1985). In addition to this, the toxicity of conventional insecticides to the natural enemies present in various agroecosystems has been demonstrated in laboratory tests and most of them were found harmful to the different parasites and predators (Balakrishnan et al., 2009; Sahito et al., 2011; Sabry and El-Sayed, 2011). Novel substances with different biochemical targets are effective at low doses and have less exposure in the environment. This study focuses on some new insecticides viz*.,* chlorantraniliprole, flubendiamide, emamectin benzoate indoxacarb and spinosad (with new mode of actions, low dose and environmentally safe) by evaluating different spray regimes against cotton bollworms and its impact on natural enemies.

MATERIALS AND METHODS

A field experiment were conducted to study the effect of different insecticide spray regimes on cotton (*Gossypium hirsutum*) for the management of bollworms and its impact on under HDPS. Sowing was done with Suraj (non Bt) variety with spacing of 45x 10 cm during 2013-2014 under rainfed condition in a deep black soil and followed all recommended agronomic practices to raise crop (Venugopalan et al., 2013). The five insecticides viz*.,* chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 45 SC, indoxacarb 14.5 SC and emamectin benzoate 5 SG at 27.75, 600, 56.25, 36.25 and 10 g.ai/ha, respectively were used at 60 DAS, 80 DAS, 100 DAS and 120 DAS in 8 treatments (spray regimes) including control in 3 replicates each were arranged in randomized complete block design. Details of different spray regimes are given in Table 1.

Treatment-wise application of insecticides was given as per the regimes by using high volume knapsack sprayer with required concentration. Control plots were kept without spray throughout the season for comparison. The incidence of *H. armigera* on squares and bolls damage were observed in six randomly selected plants/ plot. Number of spiders and coccinellids/ plant was also observed. In each plot, data was recorded from six pre-tagged plants. The observation was recorded at 60, 80, 100, 120 days after sowing by counting the number of insects prior to and after each spray applications i.e., 3 and 7 DAT. The data obtained for square damage and the data on natural enemies were subjected to arcsine and square root transformation respectively, and were subjected to ANOVA in randomized complete block design (RBD, p=0.05) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The data on square damage as influenced with chlorantraniliprole in different spray regimes during 2013-14 and comparison of different spray regime are given in Table 2. The results showed that square damage at 80 DAS was nil in the regime 4 and which was at par with regime 2, 3, 6 and 7, recording 0.78 to 4.20% damage; maximum damage was noticed in the regime 1 (6.20%); similarly, at 100 DAS, results were non-significant among all treatments, but at 120 DAS regime 4 was the best (1.04%) followed by regime 5 and 6 (1.65 and 2.40%). Similarly, for emamectin benzoate, data at 80 DAS showed there was no damage was observed in regime 4 followed by regime 3 (0.37%) and all remaining regimes found at par with this and at 100 DAS lowest damage was noticed in the regime 3 (0.57%) and regime 4 (1.12%). Likewise, at 120 DAS

Table 1. Regime wise insecticidal treatment sprays at different days after sowing (2013-14)

Spray regime	Spraying (DAS)			
Regime 1	60 DAS	–	–	–
Regime 2	60 DAS	80 DAS	–	–
Regime 3	60 DAS	80 DAS	100 DAS	–
Regime 4	60 DAS	80 DAS	100 DAS	120 DAS
Regime 5	–	–	100 DAS	120 DAS
Regime 6	–	80 DAS	100 DAS	120 DAS
Regime 7	–	–	–	120 DAS
Regime 8 (control)	–	–	–	–

Table 2. Effect of insecticides spray regimes on bollworms and yield of cotton under HDPS (2013-14)

Treatment	Square damage (%)														
	Clorantraniliprole			Emamectin benzoate			Flubendiamide			Indoxacarb			Spinosad		
	80DAS	100DAS	120DAS	80DAS	100DAS	120DAS	80DAS	100DAS	120DAS	80DAS	100DAS	120DAS	80DAS	100DAS	120DAS
Regime 1	6.20 (14.25)	31.18 (30.17)	6.30 (14.46)	3.78 (11.06)	7.43 (12.77)	3.42 (8.57)	3.84 (11.15)	2.22 (4.99)	2.19 (6.89)	7.03 (15.07)	14.38 (21.77)	1.71 (6.14)	5.56 (10.85)	5.53 (13.47)	2.78 (5.59)
Regime 2	0.78 (2.94)	6.68 (14.91)	5.29 (12.86)	1.15 (4.99)	3.69 (9.02)	5.08 (10.57)	1.42 (5.51)	3.29 (8.45)	3.19 (10.13)	0.99 (4.65)	8.68 (17.03)	0.00 (0.00)	2.17 (4.93)	0.00 (0.00)	3.13 (8.32)
Regime 3	0.84 (4.30)	2.56 (7.38)	5.21 (13.10)	0.37 (2.01)	0.57 (2.52)	5.56 (8.03)	0.28 (1.76)	0.71 (2.80)	4.85 (12.44)	0.82 (4.24)	2.33 (5.11)	0.00 (0.00)	2.96 (9.67)	4.13 (11.16)	2.21 (6.88)
Regime 4	0.00 (0.00)	5.18 (10.79)	1.04 (3.39)	0.00 (0.00)	1.12 (4.97)	0.00 (0.00)	0.29 (1.79)	2.45 (5.24)	0.00 (0.00)	2.52 (8.71)	10.61 (18.32)	0.00 (0.00)	1.74 (6.19)	1.60 (5.87)	1.47 (5.68)
Regime 5	4.42 (11.91)	4.63 (12.04)	1.65 (6.00)	6.41 (13.72)	3.69 (8.66)	4.55 (9.96)	2.98 (9.77)	0.93 (3.20)	2.15 (6.89)	10.39 (18.69)	2.73 (9.49)	0.00 (0.00)	12.65 (20.68)	3.24 (10.19)	3.29 (10.39)
Regime 6	1.75 (6.07)	3.53 (10.80)	2.40 (7.26)	1.87 (7.67)	1.82 (6.28)	1.26 (3.73)	0.45 (2.23)	3.44 (8.34)	3.00 (9.95)	1.11 (3.51)	4.24 (11.50)	2.35 (7.12)	4.12 (9.59)	2.01 (6.51)	0.69 (2.77)
Regime 7	4.20 (11.82)	15.61 (22.81)	1.74 (6.15)	5.71 (13.08)	10.35 (18.49)	0.78 (2.92)	3.70 (10.97)	6.64 (14.73)	1.87 (6.41)	6.54 (14.72)	16.26 (23.72)	0.79 (2.96)	13.51 (21.37)	7.38 (15.72)	2.22 (6.83)
Untreated	4.72 (12.46)	14.42 (21.98)	7.06 (15.14)	16.13 (23.17)	18.26 (25.03)	5.88 (13.99)	3.63 (10.91)	12.21 (20.34)	4.92 (12.72)	15.23 (22.48)	14.27 (21.90)	4.72 (12.41)	11.02 (18.93)	10.87 (19.12)	7.14 (15.41)
SEm	1.87	7.29	2.73	2.33	3.42	4.86	1.70	3.82	2.30	2.00	2.93	2.04	3.12	2.17	3.16
CD (p = 0.05)	5.68	NS	8.27	7.07	10.37	NS	5.15	NS	6.98	6.08	8.89	6.19	9.47	6.58	NS

Treatment	Seed cotton yield (kg/ ha)				
	Chlorantraniliprole	Emamectin benzoate	Flubendiamide	Indoxacarb	Spinosad
Regime 1	1324	1590	1859	1513	1708
Regime 2	1715	1977	1974	2021	2036
Regime 3	1828	2054	2018	2235	1952
Regime 4	1973	2220	2159	1832	2120
Regime 5	1754	1824	2061	1877	1627
Regime 6	1893	2132	1870	2156	1766
Regime 7	1403	1374	1626	1457	1982
Regime 8 (Control)	1348	1262	1517	1332	1418
SEm	1.87	1.82	2.32	2.34	2.52
CD (p = 0.05)	NS	5.53	NS	NS	NS

Figures in parentheses are arcsine transformed values; NS = Non-significant

non-significant results were observed among all regimes. However, no damage was observed in regime 4. The plots sprayed with flubendiamide showed that regime 3 (0.28%) was found superior at 80 DAS, followed by regime 4. Similarly, at 100 DAS non-significant results were observed, while at 120 DAS the damage was nil in regime 4. About indoxacarb, the results revealed less damage in spray regimes 2 and 3 (0.99 and 0.82%); at 100 DAS, the significantly superior ones were regime 3 and 5 (2.33 and 2.73%, respectively); maximum damage was in regime 7 (16.26%); and the damage was nil in regimes 2, 3, 4 and 5 at 120 DAS. Spinosad treated plots exhibited a minimum damage in regime 4 and 2 (1.74 and 2.17%) and these were at par with regime 1, 3 and 6; there was no damage was noticed in the regime 2 and it was followed by regime 4 (1.60%) at 100 DAS; at 120 DAS the results were found non-significant; spray regime 6 and 4 were found superior (0.69 and 1.47%, respectively (Table 2).

The boll damage was found negligible and below economic threshold level in all treatments except in

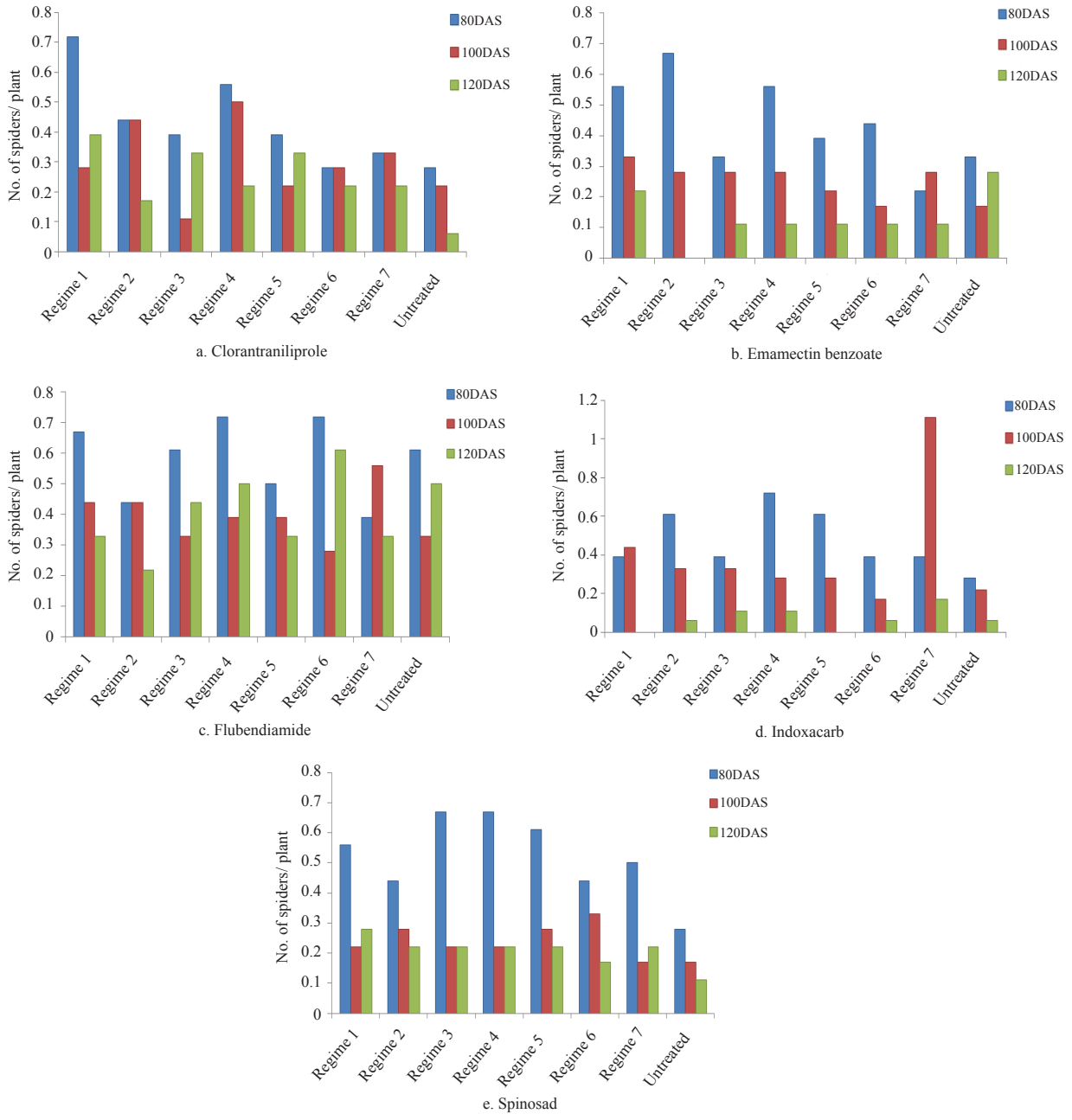


Fig. 1. Impact of different insecticides spray regimes on spider population on cotton under HDPS (2013-14)

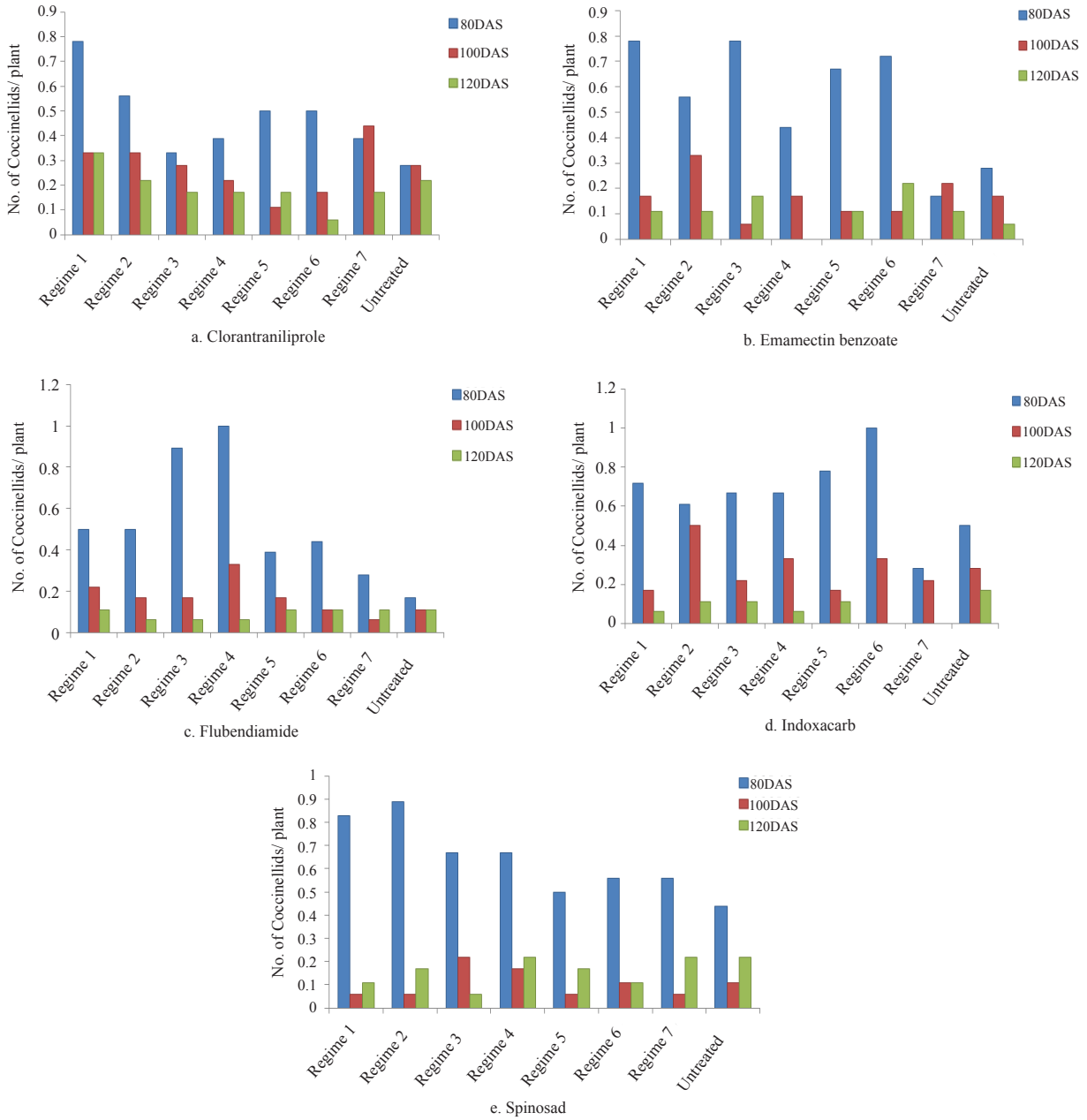


Fig. 2. Impact of different insecticides spray regimes on coccinellid population on cotton under HDPS (2013-14)

untreated plots; this might be attributed to effective control of pest in square and flowering stage itself; and control plot was showing damage above ETL, indicating that insecticides were effective against bollworm on cotton under HDPS. The counts on spiders and coccinellids during 2013-14 in different spray regimes presented in (Fig. 1, 2) reveal the variations in their occurrence was non-significant among spray regimes including control; all the insecticidal treatments were observed equally safe; however, spray regime 1 (spray at 60 days after sowing) and 2 (spray at 60 and 80 days after sowing) recorded maximum counts. The data on seed cotton yield from insecticide spray regimes during 2013-14 given in Table 3 reveal that differences are statistically non-significant; however, regime 4 led to maximum (1973 kg/ ha); in case of emamectin benzoate it was 2220 kg/ ha.

The present study was carried out to evaluate spray regime windows to find the most effective spray window against bollworms and to find spray interval that pose minimal impact on natural enemies.

Insecticide resistance rendered insecticides ineffective, thus increasing the need for repeated applications, wastage of resources and consequent environmental pollution (Kranthi, 2007). Resistance of key insect pests to insecticides continues to be a significant problem (Cook et al., 2005; Dhingra et al., 1988; McCaffery et al., 1989; Armes et al., 1992 & 1994; Kranthi et al., 2001). In India, the first case of control failure after spraying synthetic pyrethroids from suspected insecticide resistance in *H. armigera* (Hubner) was from Guntur in Andhra Pradesh (Reddy, 1990). The success of bollworm control programs relies mainly on the spraying those insecticides belonging to novel modes of action in a particular time interval. Chemicals such as spinosad, indoxacarb, emamectin benzoate, novaluron and lufenuron ensured effective control and less toxic to beneficial insects (Kranthi, 2007). The most probable reason for increased use of new chemistry insecticides is resistance (Razaq, 2006). There is a need to develop alternative insecticides/ techniques, allowing rational use of pesticides (Barrania et al., 2016). Sharah and Ali (2008) concluded that it is very important to determine which of the available insecticides are most effective and economical at particular stage of crop phenology, doses and intervals should the spray be done to achieve the best result. Spray regimes 2 and 3 was effective in taking care of bollworms in the initial stage itself by minimising square damage in early growth stages. Earlier reports of (Donnelly and Adeyemi, 1966; Akinlosotu, 1969; Fadare, 1984) clearly revealed that early chemical intervention reduced the damage levels of flowers.

In the present study, flubendiamide, emamectin benzoate and spinosad were found relatively superior. Flubendiamide 60 g a.i. ha^{-1} showed marked reduction in the larvae and damage (Thilagam et al., 2010); Tomar et al., 2005) also obtained similar results with square damage caused by bollworms. Saleh et al. (2013) observed that emamectin benzoate achieved high efficacy against pink and spotted bollworms; Gupta et al. (2005) and Sontakke et al. (2007) found this as the most potent. Johnson et al. (1997) and Dandale et al. (2000) observed that spinosad was quite effective. Ghure et al. (2008) observed that indoxacarb and spinosad were effective. The present results agree with those of Johnson et al. (2000), Haidar et al. (2002); Omar et al. (2005), Ghure et al. (2008) and Gosalwad et al. (2009). Chlorantraniliprole also proved equally effective under high density planting system in the present study and this is in accordance with Dhengre et al. (2017); Ma et al. (2000) reported that chlorantraniliprole was found to be the most effective against *H. armigera* in cotton. These pesticides were effective against targeted arthropod pests but relatively non-harmful to natural enemies (De Clercq, 1995; Charleston, 2005).

Insecticides evaluated in present investigation not only effective for controlling bollworms but were relatively safe on spiders and coccinellids. Ruberson et al. (1998) and Lacey et al. (1997) assumed the compatibility of natural enemies with pesticides depends on a range of factors; Tohnishi et al. (2005) and Kubendran et al. (2006) found flubendiamide to be least toxic against beneficial arthropods. Chlorantraniliprole at doses ranging from 20- 50 g a.i./ ha was safe to natural enemies (Misra, 2011; Yang et al., 2012). These diamides are relatively safe to natural enemies (Brugger et al., 2010); spinosad was found to be harmless to *Coccinella septempunctata* grubs (Olszak and Sekrecka, 2008). Spray regime 1 (spray at 60 days after sowing) and 2 (spray at 60 and 80 days after sowing) recorded maximum counts of spiders and coccinellids. Interestingly, in all insecticidal spray regimes maximum natural enemy population was noticed 80 DAS. The insecticides, flubendiamide, emamectin benzoate, spinosad and chlorantraniliprole are thus superior as spray regimes against bollworms; in particular, spray regime 2 (spray at 60 and 80 DAS) was the best and can be recommended under HDPS system in cotton.

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EFFECT OF TEMPERATURE ON BIOLOGY OF *BREVICORYNE BRASSICAE* L. AND *MYZUS PERSICAE* SULZER OF CABBAGE

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ABSTRACT

Knowing the effect of temperatures on growth and development of crop pests is essential. In this study, effect of six temperature regimes on the cabbage aphids *Brevicoryne brassicae* L., and *Myzus persicae* Sulzer was studied in Manipur, India. For both the species, 25˚C was found to be the optimal in relation to the most of the parameters studied; *B. brassicae* could not tolerate the extreme temperatures and died in its 2nd nymphal stage. A decrease in reproductive periods with increase in temperature may accommodate 1-2 additional brood(s) a year, consequently will lead to increased damage to cabbage, more particularly, under staggered transplanting.

Key words: *Brevicoryne brassicae*, *Myzus persicae*, temperature, aphid, cabbage, Allen's rule, Bergmann's rule, morphometrics, thermotolerance, global warming, Manipur

Cabbage aphid *Brevicoryne brassicae* L. and green peach aphid *Myzus persicae* Sulzer are the most damaging pests on cabbage (Theunissen, 1989). Temperature greatly affects the development, reproduction, survival and other behaviour of insect pests (Bursell, 1974; Summers et al*.,* 1984; Bale et al., 2007). Temperature related studies have been used extensively to predict population dynamics and developmental rate of aphids (De Loach, 1974; Hayakawa et al., 1990; Soh et al*.,* 2018); however, geographically separated populations of aphids may differ in their response to temperature (Campbell et al*.,* 1974). Studies on the effect of temperatures on the life parameters of *B. brassicae* and *M. persicae* have not been done in the northeast region of India. A basic understanding of the insect's development in relation to temperature variation is essential for planning IPM strategies. This is particularly required for the temperature regimes predicted to occur in a place due to changing climate. The temperature in the state of Manipur fluctuates within 21.5-29.4°C in summer and within 4.3-21.7°C during winter (https://www.weather-ind.com/en/india/imphal-climate#temperature). If no measures are taken average temperatures in India are predicted to increase by 1.5 to 3°C (Mani et al*.,* 2018). Thus, Manipur may experience the highest maximum temperature of 24.7°C during winter and 32.4°C in summer. No comparative study has been done on biology of aphids under different temperatures in the northeast. Hence, this study to know the effect of temperatures on the biology of *B. brassicae* and *M. persicae.*

MATERIALS AND METHODS

The study was conducted in the Entomology Laboratory of the College of Agriculture, Central Agricultural University Imphal, Manipur (24°48'52.4"N, 93°53'25.2"E). *Brevicoryne brassicae* and *M. persicae* were subjected to six constant temperature regimes viz*,* 10˚, 15˚, 18˚, 20˚, 25˚, and 30˚C, with a 12 hr. photoperiod and a RH of 70± 10%. Rearing methodology was adopted as described by Ramalho et al. (2015). Colonies of *B. brassicae* and *M. persicae* were raised separately from the field populations collected from the experimental farm of the University. Colonies were maintained on cabbage seedlings raised in plastic pots of 10 cm dia containing sterile potting mix and covered with glass chimney (10 cm dia and 170 cm in height). The upper portions of the chimney were covered with muslin cloth held tight by the rubber band. Fifty pots were maintained in the laboratory in order to get sufficient number of newly laid nymph every day. Randomly selected apterous females from the stock culture were transferred in sufficient numbers to the excised cabbage leaf disc placed upside down in petridishes. Offspring born within 24 hr were confined individually on cabbage leaf discs in petri dishes. Such individuals of both *B. brassicae* and *M. persicae* under observations

were transferred separately everyday to fresh leaf disc. Immature stages and adults were observed and their survivorship recorded. Developmental periods were recorded separately. Differences in parameters were tested using ANOVA, with significant differences ones compared using LSD tests. Error graphs and trend lines are also drawn.

RESULTS AND DISCUSSION

Period of life stages: *B. brassicae* could not tolerate the extreme temperatures (10°C and 30°C) and died in the 2nd nymphal stage. Similar to the present result, the highest mortality of *B. brassicae* was also recorded at 30°C by Pal et al. (2008); likewise, 10, 30, and 35°C have been reported to be more lethal to *B. brassicae* than 15, 20, and 25°C (Soh et al., 2018). Except for few occasions, the pre-reproductive, reproductive, and post-reproductive periods of both the aphid species decreased significantly with the increase of temperature (Table 1). Such a decrease in reproductive periods may accommodate 1-2 additional brood(s) in a crop season which may lead to potential increase in population size of aphid and more damage to cabbage crop. If it happens, the additional brood is likely to cause more damage, since the transplanting of cabbage is staggered on account of small land-holdings of poor/ marginal farmers of Manipur and their inadequate knowledge on resource allocation in relation to pest management. Such an increase in brood number has also been reported from other countries too, eg., in the UK, an increase in temperature of only 2ºC would allow the number of generations/ years to increase from 18 to 23°C with a potentially huge increase in population size (Harrington et al., 1999). The pre-reproductive, reproductive and post-reproductive periods of both *B. brassicae* and *M. persicae* were the shortest at 25°C; similar results were reported by Campbell and Mackauer (1977) in pea aphid *Acyrthosiphon pisum*.

The adult longevity varied at different temperature regimes (Table 1); longevity of *M/ persicae* was more than *B. brassicae* at each temperature regime by about 4-6 days. Adult longevity differed significantly in temperature regimes in *B. brassicae* also; however, it was at par at 18 and 30°C in case of *M. persicae* (Table 1). It was the longest at 15°C for *B. brassicae* and at 10°C for *M. persicae.* Under the predicted elevated temperature on account of global warming, *M. persicae* is likely to pose more threat to cabbage in the state of Manipur. Higher surviving ability with the longest adult longevity of *M. persicae* at low temperature of 10°C might help it establish and intensify the damage on cabbage in hilly tracts that usually experience lower minimum temperature than in plains. One study carried out by Zhou et al. (1995) reveals that temperature, especially the winter temperature, is the dominant factor affecting phenology for five species of aphids, including *M. persicae,* and for 1°C increase in average winter temperature advanced the migration phenology by 4-19 days depending on species. Temperature has also been reported to be one of most influential weather factors on mustard aphid in some recent studies under Indian condition (Baviasa et al. 2018; Das et. al., 2019).

Morphometrics: The nymphal body length, body width, antennal length and cornicle length of both the aphid species were positively related with temperature (Table 2-5) up to 25°C. At 30°C, these parameters showed an abrupt decline in *M. persicae.* Even though 30°C is not lethal to *M. persicae,* as in case of *B. brassicae*, it may affect the vigour of former species. The maximum body length, width, antennal and cornicle length of both the aphid species were observed at 25°C; however, minimum lengths of these parameters were observed for *B. brassicae* at 15°C and for *M. persicae* at 10°C. At 30°C, the tested morphometric parameters declined abruptly in *M. persicae.* The morphological data are in general agreement with the Allen's rule that "protruding body parts" are relatively shorter in cooler temperatures. The data are also in agreement to a great extent with Bergmann's Rule which states that individual body size tends to be less in the warmer parts of a species' range. Bergmann's Rule came in to effect in the present study in five out of six temperatures studied.

Temperature affects the size of aphids and they develop smaller at higher temperature (Wool, 1977; Nealls et al., 1984; Rao et al., 2016; Tiwari and Singh, 2018). The adult weight of aphids, *Acyrthosiphon pisum* on peas (Lamb et al., 1987); and *Macrosiphum euphorbiae* on *Solanum dulcamara* decrease with increase in temperatures (Flynn et al., 2006). In Taiwan, global warming is likely to affect life history traits and demographic parameters of *Aphis craccivora*-Chen et al. (2013). Murdie (1969) also indicated a relative shortening of legs and antennae of pea aphid at higher temperatures. One might also expect to be smaller below the optimum temperature (Blackman and Spence, 1994). In the present study, 25°C has emerged as the optimum temperature for both the aphid species; however, further investigation with due consideration of sets with narrower temperature range within 20-30°C would find the optimum temperature

Table 1. Reproductive periods and adult duration of *B. brassicae* and *M. persicae* at different temperatures regimes

Temperature (^{0}C)	Duration in days (Mean± S.D.)							
	B. brassicae				*M. persicae*			
	Pre- reproductive period	Reproductive period	Post- reproductive period	Adult longevity	Pre-reproductive period	Reproductive period	Post- reproductive period	Adult longevity
10	(-)	(-)	(-)	(-)	2.97± 0.02^{a}	31.8± 0.65^{a}	2.03± 0.03^{a}	36.80± 0.64^{a}
15	1.95± 0.01^{a}	21.0± 0.5^{a}	2.99± 0.01^{a}	25.95± 0.48^{a}	1.94± 0.03^{b}	27.2± 0.41^{c}	1.87± 0.01^{bc}	31.02± 0.44^{b}
18	1.86± 0.02^{b}	18.0± 0.35^{b}	1.87± 0.01^{b}	21.73± 0.35^{b}	1.83± 0.02^{c}	24.8± 0.41^{c}	1.92± 0.05^{ab}	28.42± 0.43^{c}
20	1.34± 0.02^{c}	14.8± 0.65^{c}	1.62± 0.02^{c}	17.76± 0.64^{c}	1.73± 0.01^{d}	21± 0.50^{d}	1.78± 0.09^{cd}	24.42± 0.48^{d}
25	0.97± 0.01^{d}	13.6± 0.6^{c}	0.99± 0.01^{d}	15.56± 0.56^{d}	1.04± 0.03^{e}	17.6± 0.44^{e}	1.06± 0.02^{e}	19.71± 0.41^{e}
30	(-)	(-)	(-)	(-)	1.92± 0.01^{b}	25.4± 0.56^{c}	1.85± 0.01^{bed}	29.19± 0.56^{c}
SE (d)	0.22	0.55	0.02	0.54	0.02	0.66	0.06	0.66
CD (P=0.05)	0.04*	1.2*	0.04*	1.19*	0.05*	1.39*	0.13*	1.38*

Table 2. Nymphal body length (in mm; Mean± S.D.) of instars of *B. brassicae* and *Myzus persicae* at different temperature regimes

Temperature (^{0}C)	*B. brassicae*				*M. persicae*			
	Instar I	Instar II	Instar III	Instar IV	Instar I	Instar II	Instar III	Instar IV
10	0.62± 0.001^{de}	(-)	(-)	(-)	0.629± 0.003^{e}	0.66± 0.009^{f}	0.69± 0.005^{e}	0.74± 0.016^{e}
15	0.63± 0.004^{cd}	0.74± 0.005^{c}	0.86± 0.004^{c}	1.02± 0.001^{c}	0.64± 0.002^{d}	0.75± 0.009^{d}	0.86± 0.01^{c}	1.1± 0.003^{d}
18	0.64± 0.01^{c}	0.76± 0.01^{c}	0.87± 0.001^{c}	1.03± 0.001^{c}	0.66± 0.01^{bc}	0.78± 0.003^{c}	0.89± 0.006^{c}	1.07± 0.007^{c}
20	0.67± 0.005^{b}	0.83± 0.04^{b}	1.14± 0.03^{ab}	1.34± 0.01^{b}	0.67± 0.004^{b}	0.88±0.006^{b}	1.12± 0.002^{b}	1.37± 0.007^{b}
25	0.71± 0.01^{a}	0.94± 0.005^{a}	1.15± 0.02^{a}	1.41± 0.02^{a}	0.73± 0.01^{a}	0.96± 0.007^{a}	1.22± 0.016^{a}	1.53± 0.01^{a}
30	0.63± 0.001^{cde}	(-)	(-)	(-)	0.63± 0.003^{de}	0.68± 0.004^{e}	0.73± 0.01^{d}	1.04± 0.01^{d}
SE (d)	0.007	0.02	0.02	0.01	0.008	0.009	0.01	0.01
CD (P=0.05)	0.01*	0.05*	0.04*	0.03*	0.01*	0.01*	0.03*	0.02*

Each of the tabulated data in Table 1 & 2 is mean value of 5 replications. (-) Life cycle not completed. Mean values in columns superscripted by the same letter are statistically *at par*.

Table 3. Nymphal body width in mm (Mean± S.D.) of *B. brassicae* and *M. persicae* at different instars at different temperature regimes

Temperature (^{0}C)	*B. brassicae*				*M. persicae*			
	Instar I	Instar II	Instar III	Instar IV	Instar I	Instar II	Instar III	Instar IV
10	0.26± 0.05^{de}	(-)	(-)	(-)	0.25± 0.01^{e}	0.28± 0.007^{e}	0.31± 0.006^{a}	0.4± 0.013^{e}
15	0.27± 0.001^{cd}	0.36± 0.005^{c}	0.48± 0.02^{c}	0.60± 0.005^{c}	0.27± 0.003^{cde}	0.36± 0.004^{c}	0.5± 0.006^{c}	0.6± 0.005^{c}
18	0.29± 0.01^{abc}	0.37± 0.01^{c}	0.52± 0.03^{bc}	0.61± 0.01^{c}	0.29± 0.01^{bc}	0.37± 0.008^{c}	0.53± 0.01 ^{b}	0.62± 0.007^{c}
20	0.30± 0.004^{ab}	0.40± 0.01^{ab}	0.54± 0.01^{b}	0.66± 0.01^{b}	0.3± 0.01^{ab}	0.39± 0.01^{b}	0.55± 0.008^{b}	0.65± 0.008^{b}
25	0.31± 0.01^{a}	0.42± 0.001^{a}	0.62± 0.01^{a}	0.88± 0.01^{a}	0.32± 0.01^{a}	0.41± 0.004^{a}	0.63± 0.01^{a}	0.88± 0.007^{a}
30	0.27± 0.01^{cde}	(-)	(-)	(-)	0.28± 0.01 ^{bcd}	0.3± 0.005^{d}	0.33± 0.008^{de}	0.46± 0.006 ^{d}
SE (d)	0.012	0.008	0.024	0.008	0.011	0.008	0.013	0.011
CD (P=0.05)	0.025*	0.017*	0.054*	0.017*	0.024*	0.017*	0.028*	0.023*

Table 4. Nymphal antennal length in mm (Mean± S.D.) of *B. brassicae* and *M. persicae* at different instars at different temperature regimes

Temperature (^{0}C)	*B. brassicae*				*M. persicae*			
	Instar I	Instar II	Instar III	Instar IV	Instar I	Instar II	Instar III	Instar IV
10	0.22± 0.03^{de}	(-)	(-)	(-)	0.23± 0.01^{de}	0.27± 0.008^{e}	0.31± 0.006^{e}	0.51± 0.004^{b}
15	0.24± 0.005^{cd}	0.34± 0.01^{c}	0.54± 0.02^{bc}	0.61± 0.005^{d}	0.25± 0.005^{cd}	0.36± 0.007^{c}	0.4± 0.006^{d}	0.61± 0.004^{d}
18	0.25± 0.004^{c}	0.36± 0.007^{b}	0.58± 0.06^{bc}	0.63± 0.07^{c}	0.28± 0.007^{b}	0.38± 0.006^{b}	0.56± 0.05^{c}	0.63± 0.005^{c}
20	0.29± 0.006^{ab}	0.37± 0.001^{ab}	0.60± 0.005^{b}	0.87± 0.001^{b}	0.31± 0.005^{a}	0.39± 0.003^{a}	0.63± 0.02^{b}	0.87± 0.005^{b}
25	0.30± 0.006^{a}	0.38± 0.002^{a}	0.80± 0.01^{a}	1.06± 0.006^{a}	0.32± 0.009^{a}	0.4± 0.006^{a}	0.74± 0.009^{a}	1.07± 0.006^{a}
30	0.23± 0.007^{cde}	(-)	(-)	(-)	0.24± 0.009^{de}	0.3± 0.004^{d}	0.38± 0.009^{de}	0.55± 0.007^{e}
SE (d)	0.01	0.009	0.041	0.007	0.01	0.007	0.033	0.007
CD (P=0.05)	0.021*	0.019*	0.089*	0.015*	0.022*	0.016*	0.070*	0.016*

Table 5. Nymphal cornicle length in mm (Mean± S.D.) of *B. brassicae* and *M. persicae* at different temperature regimes.

Temperature (^{0}C)	*B. brassicae*				*M. persicae*			
	Instar I	Instar II	Instar III	Instar IV	Instar I	Instar II	Instar III	Instar IV
10	0.021±0.001^{e}	(-)	(-)	(-)	0.024± 0.001^{de}	0.036± 0.0008^{de}	0.04± 0.006^{de}	0.047± 0.0007^{de}
15	0.04± 0.001^{cd}	0.04± 0.001^{c}	0.05± 0.04^{c}	0.05± 0.001^{c}	0.049± 0.001^{cd}	0.05± 0.0005^{cd}	0.053± 0.01^{d}	0.056± 0.001^{cd}
18	0.06±0.001^{c}	0.06± 0.001^{c}	0.07± 0.001^{c}	0.07± 0.001^{c}	0.064± 0.001^{c}	0.064± 0.01^{c}	0.075± 0.0008^{c}	0.077± 0.0009^{c}
20	0.087± 0.001^{b}	0.16± 0.03^{b}	0.22± 0.011^{b}	0.28± 0.01^{b}	0.09± 0.0007^{b}	0.17± 0.02^{b}	0.23± 0.01^{b}	0.308± 0.006^{b}
25	0.11± 0.02^{a}	0.24± 0.02^{a}	0.31± 0.02^{a}	0.39± 0.01^{a}	0.13± 0.02^{a}	0.26± 0.01^{a}	0.33± 0.01^{a}	0.45± 0.01^{a}
30	0.03± 0.0005^{de}	(-)	(-)	(-)	0.032± 0.01^{de}	0.044± 0.002^{cde}	0.045± 0.0008^{de}	0.051± 0.001^{cde}
SE (d)	0.01	0.02	0.02	0.12	0.014	0.013	0.0086	0.01
CD (P=0.05)	0.02*	0.06*	0.06*	0.027*	0.029*	0.027*	0.018*	0.022*

Each of the tabulated data in Table 3, 4 & 5 is mean value of 5 replications. * Life cycle not completed. Mean values in columns superscripted by the same letter are statistically *at par.*

more precisely; *M. persicae* could thrive well in all the temperature regimes studied, but, *B. brassicae* died in both the extreme temperatures. Thus, *M. persicae* has shown thermotolerance capacity which may magnify its damage to cabbage under changing climatic conditions.

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EVALUATION OF SOME BOTANICALS, THERMAL TREATMENT AND PACKING MATERIALS AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* L. IN CHICKPEA

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ABSTRACT

The pulse beetle *Callosobruchus chinensis* (L.) is a major pest of stored chickpea. In the present study, efficacy of neem (0.5 and 1.0%) and turmeric powders (0.5 and 1.0%) has been evaluated against this pest, in combination with mustard oil (0.3%), thermal treatment @ 60°C for 20 min and packing materials including open, PP (polypropylene) and LD (low density polyethylene). The results revealed increased efficacy with concentration of neem and turmeric powder. Use of neem oil @ 1.0%+ mustard oil @ 0.3% kept in open packing, proved to be significantly superior at four months of storage. Turmeric powder @ 0.5%+ mustard oil @ 0.3% treated grains showed a significantly better germination. Moisture content differed significantly with treated samples. The colour change and myroflora incidence were comparatively minimum with neem leaf powder @ 0.5%+ mustard oil @ 0.3% treated samples. Thermal treated grains proved superior compared to botanicals, whereas, colour change led to significantly lesser effect. Among the packings, PP and LD packed samples significantly influenced the infestation and germination irrespective of the botanicals.

Key words: Chickpea, *Callosobruchus chinensis*, neem, turmeric, thermal treatment, packing materials, mycoflora incidence, moisture content, colour change, germination, protein content

The pulse beetle *Callosobruchus chinensis* (L.) is a major storage pest of chickpea causing 32-64% loss in Asia and Africa (Demnayk et al., 2007; Ketoh et al., 2005). Globally pest control measures in stored grains rely on insecticides and fumigants (Shaheen and Khaliq, 2005; Sharma et al., 2007). These pesticides are hazardous to man and the environment, also residues may remain on the treated grains. To reduce the use of insecticides, there is a need for alternatives such as biopesticides (Yusuf et al., 2011; Ashok et al., 2020; Singh et al., 2021), hot and cold treatment (Alice et al., 2013), and high-density polyethylene bags (Sanon et al., 2011) for the control of *Callosobruchus* spp. All these measures have different mode of action, but comprehensive knowledge that is essential on the use of combination of such methods against *Callosobruchus* spp. is lacking. The present study is an attempt to evaluate few such alternatives against *C. chinensis*.

MATERIALS AND METHODS

The study was done at the laboratory of Department of Processing and Food Engineering, Punjab Agricultural University (PAU), Ludhiana. Freshly harvested grains of chickpea var. GPF 2 were procured from the PAU, Ludhiana Research Farms. The whole lot was subjected to phosphine fumigation (one tablet of 3 gm of aluminium phosphide/ t and exposed for 7 days) to eliminate hidden infestation (Anonymous, 2020-21). The rearing was done by releasing five pairs of *C. chinensis*/ kg grains in an incubator (29± 2ºC, 70± 5% RH); and 10 pairs of *C. chinensis* were released in 10 kg grains and kept for one month for uniform infestation. Then infested grains (500 gm) were treated with- T_1 (neem leaf powder @ 0.5% + mustard oil @ 0.3%), T_2 (neem leaf powder @ 1.0 % + mustard oil @ 0.3%), T_3 (turmeric powder @ 0.5% + mustard oil @ 0.3%), T_4 (turmeric powder @ 1.0% + mustard oil @ 0.3%), T_5 (thermal treatment @ 60°C for 20 min) and T_6 (untreated control). The treated grains were stored in three types of packings- open type (where plastic jars covered with muslin cloth fastened with a rubber band); LD (low density polyethylene); and PP (polypropylene) at ambient conditions and left for four months. Monthly observations on insect infestation (%), germination (%), and colour change were recorded at regular intervals. To record observations on total infestation (%), both the damaged grains (%) and grains with eggs were sorted out and weighed separately. The infestation method given by Indian Standards: 7715 (1975) was followed. Further, to determine the moisture content in grains- hot air oven method (Association of Official Analytical Collaboration, 1984); for

germination test- blotting paper method (International Seed Testing Association,1985); and colour change measurement done by Colour Reader CR-10 (Konica Minolta Sensing Inc.) with the equation given by Gnanasekharan et al. (1992). Whole experiment was replicated thrice using completely randomized design (CRD), and the observations/ data obtained were statistically analysed.

RESULTS AND DISCUSSION

Table 1 depicts the efficacy of botanical treatments packed in three kinds of packings against infestation by *C. chinensis*; these reveal that the infestation decreased with the increase in concentration of neem as well as turmeric powder; and there was significant difference at different doses along with mustard oil in the study conducted in open packings (Table 1); and T_2 (neem oil @ 1.0% + mustard oil @ 0.3%) significantly superior against infestation and mycoflora incidence (%); insect infestation (%) started decreasing after 2 months in all the botanicals, and with mycoflora incidence, T_1 and T_2 showed more effectiveness at one month and later started decreasing. Verma and Anandhi (2010), Nisha and Asaf (2019) reported that neem leaf powder gave maximum mortality of *C. chinensis* in stored mung bean; turmeric and ginger were the least effective. Thakur and Pathania (2013) revealed that mustard oil, powder of black pepper, neem oil proved promising in black gram seeds up to five months. Adarkwah et al. (2017) showed that the combined mixture of plant powders and diatomaceous earth controlled the beetles faster in case of *Acanthoscelides obtectus*, *Sitophilus granarius* and *Tribolium castaneum* in stored grain cereals.

Results on the mycoflora incidence in the present study agree with those of Bhardwaj et al. (2013) in pea

Table 1. Effect of treatments/ packing materials on *C. chinensis* and mycoflora incidence on chickpea grains

Treatments (A)	Packing material (B)	Storage period (months) (C)							
		*Insect infestation (%)				*Mycoflora incidence (%)			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th
T_1	Open	5.33	5.66	3.00	2.00	43.30	40.00	36.70	36.70
	PP	2.66	5.66	2.33	1.16	40.00	36.70	33.30	30.00
	LD	2.33	4.66	2.33	1.93	36.70	36.70	33.30	30.00
T_2	Open	3.00	3.33	1.66	1.26	43.30	36.70	33.30	30.00
	PP	2.00	3.66	1.33	1.00	36.60	30.00	26.60	30.00
	LD	1.66	2.66	1.66	1.43	33.30	26.70	20.00	26.60
T_3	Open	2.00	5.76	3.60	2.20	56.70	60.00	60.00	60.00
	PP	1.66	5.30	3.00	2.20	53.30	56.70	56.60	50.00
	LD	2.16	4.50	2.33	2.46	50.00	56.70	50.00	53.30
T_4	Open	1.66	2.66	1.66	2.70	53.30	56.60	60.00	63.30
	PP	2.00	2.66	2.33	2.33	50.00	50.00	53.30	56.70
	LD	2.33	3.00	1.66	1.00	46.70	43.30	46.70	50.00
T_5	Open	6.33	6.33	5.33	5.33	53.33	56.67	63.33	66.67
	PP	4.66	3.33	3.50	2.33	53.33	53.33	56.67	60.00
	LD	5.00	2.33	2.00	2.33	50.00	50.00	53.33	56.67
T_6	Open	11.66	30.30	30.30	98.66	70.00	83.30	90.00	100.0
	PP	5.66	5.66	5.66	19.00	66.70	80.00	93.30	96.70
	LD	5.66	6.33	6.33	4.33	66.70	76.70	90.00	93.30
CD (p=0.05)		A= 0.48; B= 0.34; C= 0.39; AB= 0.83; AC= 0.96; BC= 0.68; ABC= 1.60				A= 2.77; B= 1.96; C= NS; AB= NS; AC= NS; BC= NS; ABC= 1.60			

T_1: Neem 0.5% + mustard oil 0.3%; T_2= Neem 1.0% + mustard oil 0.3%; T_3= Turmeric powder 0.5% +mustard oil 0.3%; T_4: Turmeric powder 1.0% +mustard oil 0.3%; T_5: Thermal 60^{o}C/20 min; T_6: untreated control; *Means of 3 replications; PP= Polypropylene; LD= Low density polyethylene, initial mycoflora incidence: 42.5%

treated with neem oil. The moisture content in T_2 was significantly high, but may be strong effect of neem powder, the insect and mycoflora incidence was low; also increased significantly with increase in storage period up to first three months and then decreased further (Table 2). Similar results were obtained by Beedi et al. (2018) in gram; and Rathinavel and Raja (2007) in cotton. The botanicals influenced the grain colour significantly; it was observed more at Ist month of storage but gradually decreased. It may be due to the colour of botanicals attached to grains initially, but later the grains reverted back to their original colour. The results of the present study are contradictory to those of Ogendo et al. (2004) which found that the grain colour and odour were unaffected by the botanicals. The effect of botanicals on the germination of chickpea grains was insignificant with open packings (Table 2); it was above 95% in general and it corroborates with the study conducted by Bajiya (2009) on mung bean. Rahman and Talukder (2009) also showed that oil treatment had no adverse effects on germination even after three months of treatment in black gram. Increase in germination of seed was also observed in botanical treated grains in later months in PP and LD packed grains Similar results were observed by Nazareth et al. (2018) who found that the neem and tulsi leaf powders increased seed germination and emergence with increased shoot and root length of oilseeds.

Botanical treated grains showed insignificant differences in protein content (%); in botanical treated grains it was significantly less (18.30 in T_2) as compared to control sample (21.10%) at four months of storage (Table 2). The increase in crude protein content in control samples was directly correlated to the increase grain damage, insect excreta being uric acid as a main constituent and presence of eggs contributed a major part of N_2 estimated in the damaged grains and then converted into crude proteins. It was further observed there was little increase of true constituents in the treated samples also, which may be due to hidden infestation. A similar trend was observed by Hamdi et al. (2017), Bamaiyi et al. (2006) and Mbah and Silas (2007) that protein, moisture and ash contents increased in infested seeds of cowpea. Thermal treatment (T_5) i.e. exposure of grains at 60^{o}C for 20 min showed that the infestation was significantly high in thermal treated grains compared to botanical treated grains in open packings (Table 1). The insect infestation was high in initial months as compared to later months which may be due to the reason that thermal treated grains take time to kill the hidden infestation. Alice et al. (2013) showed the effect of exposure to sunlight on the mung bean grains having eggs of *C. maculatus* and found highest number of eggs laid/ seed was observed in seeds exposed to 4 hr (0.65) while it was the least when exposed to 24 hr (0.02). Renault et al. (2004) and Colinet et al. (2011) found the injurious effect of chill injuries in adult *Alphitobius diaperinus* exposed to low temperature. The incidence of mycoflora was less at Ist month and later started increasing which may be due that effect of thermal treatment reduced in later stages as moisture content also started increasing (Table 2). Increase in moisture content is directly related to fungi incidence (Mohapatra et al., 2017). Germination of thermal treated grains differed insignificantly in open packing and with botanicals. Germination of seeds was not affected by the thermal treatment (Kariluoto et al., 2006). Significantly less colour change was observed in thermal treated grains as compared to botanical treated ones. Hou et al. (2015) also studied effect of thermal treatment on colour change of chestnuts and suggested that the yellowness (b*) was more sensitive to temperature changes than the lightness (L*). Protein content in thermal treated grains were more as compared to other treatments in open packings which may be because roasting saves and improves protein parameters. The similar results were obtained by Jurkovic and Colic (1993) in wheat grains.

About the packings it was observed that infestation increased up to two months and then started decreasing; LD packing was significantly superior compared to that of PP and open packings in 2nd, 3rd and 4th months, showing the least infestation (Table 1). These results are similar to those of Gomaa and Salem (2018) and Sanon et al. (2011). Qasim et al. (2013) studied the penetration ability of the beetle, *Tribolium castaneum* for three packaging types includes polyethylene (PE), polypropylene (PP) and polyvinylchloride (PVC) and found PE as a susceptible medium, while LD packing was more effective against fungi. The moisture content increased in all the treatments with the increase in storage time. The results are similar to those of Yeole et al. (2018) found that air tight packing materials like polypropylene bags and hermetic bag had higher microbial load because the environment inside these bags has high relative humidity with warm temperature. The colour of grains is used as an indicator for the quality of food products. study, significant change in colour of moong grains was observed from 1-4 months of storage period in all the tested packings (Table 2). Grains packed in LD packings showed less change in colour, while maximum colour change found in PP bags. Similar results were found by Yeole et al. (2018). The

Table 2. Effect of treatments/ packing materials on quality parameters of stored chickpea grains

Treatments (A)	Packing material (B)	Storage period (months) (C)															
		*Moisture content (%)				*Colour Change				* Germination (%)				*Protein content (%)			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
T_1	Open	11.00	13.05	16.46	15.06	6.59	5.92	4.44	2.20	95.33	95.67	95.00	95.67	18.30	18.50	18.48	18.40
	PP	8.37	9.65	14.12	13.56	6.31	6.09	3.72	2.55	98.00	97.00	97.00	97.67	18.10	18.50	18.45	18.40
	LD	9.49	9.66	14.27	13.78	5.10	3.02	2.57	2.41	98.00	96.67	96.67	97.67	18.14	18.50	18.42	18.39
T_2	Open	10.35	13.81	17.38	15.28	9.34	9.15	3.68	2.52	96.00	95.67	95.67	95.67	18.40	18.45	18.34	18.30
	PP	7.50	10.21	14.69	14.52	6.54	5.59	5.15	2.66	97.33	97.00	97.67	97.67	18.30	18.40	18.44	18.40
	LD	7.21	10.28	14.64	14.27	3.74	3.04	3.13	4.17	98.00	97.33	97.33	98.00	18.30	18.38	18.40	18.40
T_3	Open	9.36	13.10	16.21	15.22	7.07	5.03	4.72	4.39	96.33	95.67	96.00	96.33	18.20	18.75	18.80	18.40
	PP	9.06	9.35	14.71	14.35	6.35	6.19	6.10	6.08	97.00	98.00	98.00	97.67	18.10	18.64	18.60	18.58
	LD	9.26	9.76	14.96	14.77	6.51	5.47	4.72	4.50	97.67	98.00	98.00	98.00	18.25	18.52	18.50	18.49
T_4	Open	9.88	13.48	16.72	15.67	7.07	5.74	5.47	5.35	96.00	96.33	96.00	96.00	18.24	18.44	18.50	18.46
	PP	8.96	9.85	15.11	14.06	6.31	7.82	7.63	6.21	97.67	98.00	98.00	97.33	18.30	18.40	18.40	18.46
	LD	9.12	10.17	13.70	12.79	5.30	10.85	7.61	3.61	97.33	98.33	98.00	97.33	18.30	18.44	18.40	18.36
T_5	Open	9.40	13.52	14.85	17.67	0.49	0.90	1.99	2.69	95.33	95.33	95.33	95.00	18.50	18.56	18.50	18.50
	PP	9.36	11.88	14.03	14.35	1.80	2.91	4.27	6.66	96.33	97.67	97.00	97.67	18.35	18.30	18.42	18.42
	LD	7.77	10.70	13.88	15.04	1.36	1.45	3.48	4.11	97.00	97.67	97.33	97.33	18.40	18.42	18.40	18.45
T_6	Open	11.43	15.04	20.09	19.22	1.14	3.11	3.42	3.66	79.33	66.00	66.00	14.33	18.60	19.60	20.80	21.10
	PP	9.09	11.65	15.23	14.84	2.86	3.28	3.42	7.27	85.00	73.33	73.33	52.67	18.40	18.50	19.20	19.60
	LD	9.78	11.39	15.86	15.03	1.39	1.90	2.18	2.85	88.67	88.67	88.67	86.00	18.40	18.60	18.70	18.70
CD (p=0.05)		A=0.23; B=0.16; C=0.4; AB=0.41; AC=0.53; BC=0.37; ABC=0.91;				A=0.32; B=0.23; C=0.26; AB= 0.56; AC=0.65; BC=0.46; ABC=0.11				A=0.40; B=0.28; C=0.36; AB=0.69; AC=0.89; BC=0.63; ABC=1.55				A=0.13; B=0.95; C=0.12; AB=0.23; AC=0.30; BC=0.21; ABC=0.52.			

T_1: Neem 0.5% + mustard oil 0.3%; T_2= Neem 1.0% + mustard oil 0.3%; T_3= Turmeric powder 0.5% +mustard oil 0.3%; T_4: Turmeric powder 1.0% +mustard oil 0.3%; T_5: Thermal 60^{o}C/20 min; T_6: untreated control;*Means of 3 replications; PP= Polypropylene; LD= Low Density Polyethylene, Initial moisture content: 8.73%, Initial protein content: 17.9%, Initial germination=98.0%

protein content (%) in grains packed in various type of packings differed non-significantly. Also, there is insignificant effect of PP and LD packing on protein content. The results are similar to the studies conducted by Mbah and Silas (2007) which reported an increase in protein contents with severity of infestation.

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RESISTANCE IN CERTAIN CANTALOUPE *CUCUMIS MELO* L. CULTIVARS TO SOME SAP SUCKING PESTS IN EGYPT

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ABSTRACT

Field and laboratory studies were oriented to evaluate the relative resistance of three cantaloupe cultivars; Tenorio RZ, 62, and Ideal to some sap sucking pest; whitefly, *Bemisia tabaci* (Genn), onion thrips, *Thrips tabaci* L, aphid, *Aphis gossypii* (Glover) and two spotted spider mite *Tetranychus urticae* Koch. The results showed that the total number of pests were the highest on Tenorio RZ cultivar and the least on Ideal during two seasons. Ideal cultivar had the highest levels of alkaloids and phenolics compound, while terpene compounds was undetectable. Also, the results showed that Tenorio RZ cultivar was having the lowest least levels of secondary metabolites. During summer, numbers of sucking pests were the least on Ideal. Results showed also that the levels of plant secondary metabolites tend to decrease pest population levels.

Key words: Cantaloupe cultivars, sap sucking pests, secondary metabolites, *Bemisia tabaci, Thrips tabaci*, *Aphis gossypii*, *Tetranychus urticae*, alkaloids, phenolics, terpenes

Cantaloupe *Cucumis melo* L. (Fam. Cucurbitacae) is one of the world's most important vegetable cash crops (Numfez-Palenius et al., 2008). In Upper Egypt cantaloupe plants are commonly infested with several types of pests that endanger yields, namely: the cotton aphid *Aphis gossypii* (Glover), onion thrips *Thrips tabaci* L., whitefly *Bemisia tabaci* (Gen.) as well as the two-spotted spider mite *Tetranychus urticae* Koch. (Abdel- Rahman et al., 2016). Antibiosis-resistant plants may result in decreased insect survival, delayed insect growth time, decreased insect size, and decreased fitness of new generation adults (Sarfraz et al., 2006, 2007; Gogi et al., 2010). Host plant resistance is often based on a strong natural chemical defense that negatively influences the fitness and performance of herbivores. It can also act as a feeding deterrent (Hopkins et al., 2009). The present study was carried out to evaluate relative susceptibility of some cantaloupe cultivars to certain sap sucking pests. In addition, little is known about the secondary metabolites of cantaloupe crop.

MATERIALS AND METHODS

The present studies were conducted at the farmer fields in Dirout city, Assiut governorate, Upper Egypt during summer and Nile (fall) plantations of 2021. Field and laboratory studies were oriented with an area of about quarter feddan (1050 m^2) divided into 60 plots. Three varieties were sown (Tenorio, 62 and Ideal). All the recommended agricultural practices were performed. Weeds were removed by hand. Plants were sown in two plantations of 2021. In summer plantations the seeds were sown on 31st of April, whereas Nile (fall) plantations were sown on 14th of August. Sap sucking pests were examined and counted on leaves. Weekly 14th samples of ten plant leaves were collected and numbers of pest species counted and recorded. Analysis of plant secondary metabolites was carried out at the analytical chemistry unit (ACAL) of the Chemistry Department at Assiut University, Egypt. Secondary metabolites were extracted from cantaloupe leaves from each cultivar, after one month planting and five leaves of each cultivar were collected. The most juicy and healthy leaves were selected and were washed well prior to extraction. One gram of leaves from each cultivar was ground well, and then added 2.5 ml of chloroform and 0.5 ml of phosphoric acid to the ground leaves. The mixture was sonicated (the mixture injected in ultra sonic 104x instrument) for 15 min, and then centrifuged at 8,000 r.p.m. for 15 min. The clear chloroform layer was collected and injected in a gas chromatography instrument (GC/Ms) (7890A-5975B) in USA. Dominance (%) degrees of the identified species were calculated according to the formula of Facylate (1971). Susceptibility degrees of the cultivars were evaluated according to the Semeadah (1985) and Nosser

(1996). The cultivars could be categorized as follows:

1. Cultivars with higher infestation than (MN+UC) belong to the highly susceptible group (HS);
2. Cultivars with infestations ranging from MN to (MN+UC) belong to the susceptible group (S);
3. The relative resistant group (RR) consists of cultivars infestation less than MN to (MN-UC);
4. The moderately resistant (MR) cultivars: Cultivars with infestation levels ranging from (MN-UC) to (MN- 2UC); and 5. The resistant group (R) consists of cultivars with a low infestation rate (less than (MN-2UC). Data obtained were statistically analyzed by ANOVA conducted by F-test and means were compared according to Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Four species of sap-sucking pests were found infesting cantaloupe plants. These species were: *B. tabaci*, *A. gossypii*, *T. tabaci,* and *T. urticae*. Table 1 showed that during summer regardless of the cultivars, the whitefly, *B. tabaci* was dominant (94.08%). On the other hand, *A. gossypii* and *T. urticae* was dominant degrees (1.91% and 2.92%) suggesting that these species could be economically valuable if environmental conditions switched in their benefit. Furthermore, *T. tabaci* (1.09%) may have a little role as a pest in cantaloupe during summer plantations. In the Nile (fall) plantations, data showed that *B. tabaci* is the most important (88.44%). The relatively high dominance of *A. gossypii* and *T. urticae* (1.89% and 6.55%) suggests that if environmental conditions changed in their favour, these could become economically important. On the other hand, *T. tabaci*, is of minimal importance due to its low dominance and abundance (3.12%). In general, regardless of two plantations (summer and Nile), whitefly, *B. tabaci* is the most important pest.

In summer plantations, maximum population level of the pests (503 individuals/ 10 leaves) was recorded on the cultivar of Tenorio RZ followed by 62 (357 individuals/ 10 leaves) wherase, cultivar Ideal had the lowest number (247 individuals/ 10 leaves). In the Nile plantations, cultivars of Tenorio RZ and 62 6 harbored the highest number of the pests (1703 and 1197 individuals/ 10 leaves, respectively). Ideal harbored the lowest number of the pests (658 individuals/ 10 leaves). Thus, Tenorio RZ was highly susceptible (HS) and 62 as well as Ideal Relative resistance (RR) of the remain pests during the two plantations. The mean numbers of the pests ten leaves during the growing seasons were subjected to evaluate and arrange the infestation rank. ANOVA of susceptibility of the cultivars to pest infestation revealed highly significant differences among the cultivars. In conclusion, resistant cultivars sometimes provide a vital management component in the cantaloupe plants. Resistance in a plant may be due to antixenosis, antibiosis and tolerance, or a combination of these components (Painter, 1951). The resistance or susceptibility of plants to insect attack is the result of their having inherited qualities that determine the extent of insect damage.

The analysis of cantaloupe leaves revealed that the leaves contain three groups of secondary metabolites: alkaloids, terpenes and phenolic compounds (Table 3). Except Ideal cultivar, all the cultivars contained terpenes. The highest concentration of alkaloids was detected in Ideal, followed by 62, and lastly by Tenorio RZ (which contained the lowest levels). The highest levels of phenolic compound was found in Ideal (2.63%), followed by 62; and no phenolic compounds were detected in Tenorio RZ cultivar.

The current study revealed that four species of pests attacked cantaloupe plants; *B. tabaci*, *A. gossypii*, *T. tabaci*, and *T. urticae*. (El-Dabi, 1999; Amro, 2008;

Table 1. Pests infesting cantaloupe cultivars (summer and Nile plantations, Assiut, Egypt, 2021)-Numbers/ 10 leaves and dominance degrees (%)

Pests	Growing plantations							
	Summer				Nile			
	Tenorio RZ	62	Ideal	Total	Tenorio RZ	62	Ideal	Total
A. gosypii	16b	9b	6b	21b	114b	60b	0d	174b
T. tabaci	4b	4b	4b	12b	63	46b	2c	111b
B. tabaci	469a	331a	232a	1032a	1406a	1031a	603a	3040a
T. urticae	14b	13b	5b	32b	120b	60b	53b	233b
Total	503	357	247	1097	1703	1197	658	3558

*Numbers followed by the same letter vertically not significantly different (p=0.05)

Table 2. Susceptibility Index (S. I) of cantaloupe cultivars to sap sucking pests (summer and Nile plantations, Assiut, Egypt, 2021)

Pests	Growing plantations					
	Summer			Nile		
	Tenorio RZ	62	Ideal	Tenorio RZ	62	Ideal
A. gosypii	RR	RR	RR	RR	RR	RR
T. tabaci	RR	RR	RR	RR	RR	RR
B. tabaci	HS	HS	HS	HS	HS	HS
T. urticae	RR	RR	RR	RR	RR	RR

HS= Highly susceptible S= Susceptible RR= Relative resistant

Table 3. Alkaloid compounds in cantaloupe cultivars (leaves)

Cultivars	Alkaloid compounds					
	Alkaloids	Value (%)	Terpene	Value (%)	Phenolic	Value (%)
Tenorio RZ	2,6-dimethylmorpholine	1.52	Squalene	4.68	Non	----
	3-(perhydro-2,5-dioxoimidazo[4,5-d] imidazole-1-yl)-propanoic acid	0.54				
62	hydroxy-2-(methylamino)ethyl]1.2-benzenediol l	0.219	Farnesol	1.056	4-(2-Amino-1-hydroxypropyl)phenol	1.45
	Isobutyl urea	0.338	Squalene	4.68		
Ideal	Cyanoacetyl urea	1.86				
	Cyanoacetyl urea	2.24			2[(Dimethylamino)methyl] -4-methoxy-phenol	2.63
	1-Hydroxy-2-o-fluorophenyl-4-nitroimidazole-3-oxide	3.07				
	1-(1-Oxooctadecyl)-1H-imidazole	1.10				
	2-(4-fluoro-1H-imidazole-5-yl) ethylamine	0.18				
	Alpha-methyl-beta-oxo-2-pyridinepropanoic acid ethyl ester	2.41				

Metwally et al., 2013; Abou-El-Saad, 2015). Three groups of secondary metabolites were investigated: alkaloids, terpenoid, phenolic compounds. These metabolites are non-nutritive chemical compounds whose distributions are restricted to the plant species (Shyaula and Manandhar, 2021). Secondary metabolites are molecules produced by plants that serve as defense agents against herbivores and microbes; and types such as alkaloids, terpenoids, phenolics, quinones, polyacetylenes, and peptides are very structurally diverse (Wink, 2003). The same results have been observed by other researchers who reported that secondary metabolites such as alkaloids, phenolics, and terpenoids aid plants in defending against herbivores and disease-causing microorganisms (Tang et al., 1995; Meijden, 1996). The composition of secondary metabolites is not fixed; rather, they vary in both composition and concentration (Wink, 2003). The present results are consistent with this view, i.e., different concentrations of secondary metabolites were found among three different cultivars of cantaloupe.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

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EFFICACY OF NATIVE, COMMERCIAL *BACILLUS THURINGIENSIS* AND FUNGAL FORMULATIONS AGAINST OKRA SHOOT AND FRUIT BORER

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ABSTRACT

Okra shoot and fruit borer commonly known as spotted bollworm is a major pest of the economically important crops, viz., cotton and okra. Field experiment was conducted with 13 treatments including seven native *Bt* isolate treatments viz., 49, 51, 52, 55, 16, HD1 and 493 along with commercial *Bt* treatment (Dipel), three Entomopathogenic fungal treatments viz., *Metarhizium anisopliae, Beauveria bassiana, Metarhizium rileyi,* one chemical check viz., chlorantraniliprole and untreated control. The cumulative efficacy of treatments tested for the management *E. vitella* on okra during kharif 2021-2022 revealed that all the treatments were found effective over untreated control (20.08%). Among the treatments, Chlorantraniliprole 18.5% SC @ 0.27 ml l^{-1} was found superior with lowest mean fruit infestation (8.05%) and it was on par with *M. rileyi* (1x10^{8} CFU g^{-1}) @ 5 g l^{-1} (9.70%), isolate 493 (1x10^{10} CFU g^{-1}) @ 3 g l^{-1} (9.17%), *M. anisopliae* (1x10^{8} CFU g^{-1}) @ 5 g l^{-1} (9.90%) and isolate 16 (1x10^{10} CFU g^{-1}) @ 3 g l^{-1} (9.87%).

Key words: *Earias vittella*, okra, chlorantraniliprole, *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis*, management, cumulative efficacy.

Earias vittella commonly known as spotted bollworm or okra fruit and shoot borer is a major pest of the economically important crops, viz*.,* cotton and okra (Memon et al., 2004). Since the introduction of *Bt* cotton, the insecticidal usage was drastically reduced for the management of *E. vittella* in cotton, at the same time there are many reports on increased infestation of *E. vittella* in okra crop (Suman et al., 1984). The okra cultivators frequently spray chemical insecticides, to kill the larvae before they enter into the shoots or fruits. The indiscriminate use of insecticides creates problems like insecticide resistance, pest resurgence, adverse effect on the non target species, environmental pollution. Pesticide residues in harvested okra fruits are hazardous to the consumers (Jat and Pareek, 2003). The current tendency in pest management is to use chemical pesticides judiciously, particularly on vegetables, not only to save money but also to reduce pollution, residue-free crop produce and to prevent the development of insecticide resistance in insect pests. According to estimates, *Earias* sp. can cause a 36-90% loss in okra production (Misra et al., 2002). The toxin from *B. thuringiensis* enters the insect via ingestion and binds to glycoprotein receptors on the targeted insect's midgut epithelium, disrupting the cytoplasmic membrane and causing cell lysis (Hilder and Boulter, 1999). The integration of all control measures is highly valued in today's era, the era of IPM. Hence, there is renewed interest in search for new biopesticides, and the present study evaluates some of the native *Bt* isolates and commercial entomopathogenic fungus.

MATERIALS AND METHODS

The field experiment on the evaluation of efficacy of insecticides was carried out at the Agricultural College Farm in Bapatla, Guntur district, Andhra Pradesh during kharif 2021-2022. The experiment was laid out using a Randomized Block Design (RBD) with thirteen treatments replicated twice. Each plot was 20 m^2 (5x4 m) in size, with a row to row and plant to plant spacing of 60x30 cm. *B. thuringiensis* treatments: Six native *Bt* isolates as well as the reference *Bt* strain (HD 1) and the commercial *Bt* formulation Dipel were included in the field experiment. Entomopathogenic Fungal treatments (EPF): The EPFs used in this study were commercial formulations namely *M. anisopliae*, *B. bassiana*, and *M. rileyi*. These were applied as a foliar spray on the crop using pre-calibrated knapsack sprayer when the pest incidence reach above ETL. Second spray was repeated after 15 days of the first spray. For the observations, ten plants were selected at random from each treatment. Border rows were not included in the observations. The observations, which included the total number of fruits and the number of damaged fruits per ten plants per plot were recorded one day before each spray as

pre-treatment data and three, five, seven, and ten days later as post-treatment data (Malik et al., 2013).

The incremental cost-benefit ratio was calculated by dividing the additional benefit gained from increased yield by the additional cost incurred for each treatment. Total cost included the cost of insecticides as well as labour charges for spraying insecticides. To determine the most cost-effective management method for spotted bollworm in okra, the incremental cost-benefit ratio of each treatment was calculated. Randomized block design (RBD) was used to statistically analyse the data collected from field studies. Data was subjected to ANOVA after arc sine transformation, and treatment means were compared by LSD (Least significant difference), using ADEL-R (Analysis and design of experiments with R-3.2.0 for Windows) version 2.0 (Angela et al., 2017) software.

RESULTS AND DISCUSSION

The cumulative efficacy of different treatments tested for the management *E. vittella* on okra during kharif 2021 revealed that (Table 1) all the treatments were found effective over untreated control (20.08%). Among the treatments, chlorantraniliprole 18.5% SC @ 0.27 ml l^{-1} was found superior with lowest mean fruit infestation (8.05%) and it was on par with *M. rileyi* [1×10^8 Colony Forming Units (CFU) g^{-1}] @ 5 g l^{-1} (9.70%), Isolate 493 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} (9.17%), *M. anisopliae* (1×10^8 CFU g^{-1}) @ 5 g l^{-1} (9.90%) and Isolate 16 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} (9.87%). However Isolate 52 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1}, Isolate 49 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1}, Isolate 55 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1}, Isolate 51 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1}, and *B. bassiana* (1×10^8 CFU g^{-1}) @ 5 g l^{-1} were least effective treatments with 11.89, 12.78, 13.40, 13.53 and 13.95 % respectively, which were on par with each other.

Highest mean % population reduction over untreated control was recorded 59.90 in case of chlorantraniliprole 18.5% SC @ 0.27 ml l^{-1}. The next best was Isolate 493 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} which recorded 54.34 mean % population reduction over untreated control. Remaining treatments showed 51.69 to 30.50 mean % population reduction over untreated control. The present results are in accordance with Hosamani et al. (2011) and Venkanna et al. (2015) where the fruit yield of 87.72 t ha^{-1} and 88.36 t ha^{-1} respectively were recorded in plants protected with the spray treatment of rynaxypyr 20 SC @ 0.4 ml l^{-1}. Whereas, Biswas et al. (2009) and Bansode et al. (2015) also reported the highest yield obtained in plants protected with spray treatment of flubendiamide 480 SC @ 0.25 ml l^{-1}.

From the data presented in the Table 1, revealed that all the treatments recorded better fruit yield over untreated control (9.02 t ha^{-1}). Among the treatments chlorantraniliprole 18.5% SC @ 0.27 ml l^{-1} recorded highest fruit yield i.e. 11.23 t ha^{-1} with incremental cost benefit ratio 1:6.56 followed by Dipel ES (5×10^9 CFU ml^{-1}) @ 3 ml l^{-1} (1:4.031), HD1 Strain ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} (1:3.41), *M. rileyi* (1×10^8 CFU g^{-1}) @ 5 g l^{-1} (1:3.16), *M. anisopliae* (1×10^8 CFU g^{-1}) @ 5 g l^{-1} (1:3.07) and Isolate 493 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} (1:2.97). Least ICBR recorded was 1:0.19 in Isolate 51 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} treatment. Results pertaining to ICBR were supported by Gurve et al. (2016) who reported on economics of spray treatments, additional income over control obtained in rynaxypyr 20 SC @ 0.4 ml l^{-1} was highest (Rs. 11200/- ha^{-1}) followed by flubendiamide 48 SC @ 0.25 ml l^{-1} (Rs. 98800/- ha^{-1}). The incremental cost benefit ratio (ICBR) was 1:10.0 and 1:10.3 in spray treatment of rynaxypyr 20 SC @ 0.4 ml l^{-1} and 174 flubendiamide 48 SC @ 0.25 ml l^{-1} respectively. Damage caused to fruits directly affected the yield of marketable quality of okra fruits. In case of untreated okra plants the fruit infestation was 35.55% limiting the fruit yield of 6.75 t ha^{-1}. Yields of fruits were significantly more in respect all evaluated spray treatments, except azadirachtin 10,000 ppm (8.42 t ha^{-1}) and NSKE 5% (7.89 t ha^{-1}). The experiment results indicated that chlorantraniliprole 18.5% SC @ 0.27 ml l^{-1} recorded the best control measure with highest fruit yield followed by native *Bt* Isolate 493 ($1x10^{10}$ CFU g^{-1}) @ 3 g l^{-1} and *M. rileyi* (1×108 CFU g-1) @ 5 g l^{-1} treatment.

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AUTHOR CONTRIBUTION STATEMENT

Author 1: Collected data, performed the statistical analysis, wrote the paper; Author 2: Designed the experiment, Monitored during the experiment, Contributed data and analysis and corrected the manuscript; Author 3, 4 & 5: Monitored during the experiment and corrected the manuscript.

Table 1. Efficacy of bioinsecticides against *E. vitella* on okra (kharif 2021-2022)

Tr. No.	Treatments	Dose	% infestation (No. basis)				Yield of okra t/ ha	ICBR
			First spray	Second spray	Third spray	Mean		
T_1	Isolate 16 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	11.04 (19.41)^{abc}	10.17 (18.59)^{a}	8.40 (16.85)^{bc}	9.87 (18.31)^{abc}	10.28	2.73
T_2	Isolate 49 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	13.98 (21.95)^{bcd}	13.69 (21.72)^{c}	10.66 (19.05)^{cd}	12.78 (20.94)^{de}	10.23	2.59
T_3	Isolate 51 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	14.13 (22.08)^{bcd}	14.74 (22.57)^{d}	11.74 (20.04)^{de}	13.53 (21.58)^{e}	9.42	0.19
T_4	Isolate 52 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	14.58 (22.44)^{d}	11.72 (20.02)^{b}	9.39 (17.85)^{bcd}	11.89 (20.17)^{cde}	9.48	0.36
T_5	Isolate 55 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	14.40 (22.30)^{cd}	13.94 (21.93)^{d}	11.86 (20.14)^{de}	13.40 (21.47)^{e}	9.86	1.49
T_6	Isolate 493 ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	10.51 (18.92)^{a}	9.39 (17.85)^{ab}	7.59 (16.00)^{ab}	9.17 (17.62)^{ab}	10.36	2.97
T_7	HD-1 Strain ($1x10^{10}$ CFU g^{-1})	3 g l^{-1}	12.83 (20.99)^{abcd}	10.09 (18.52)^{ab}	8.45 (16.90)^{bc}	10.46 (18.87)^{bcd}	10.51	3.41
T_8	Dipel ES (5×10^{9} CFU ml^{-1})	3 ml l^{-1}	13.10 (21.22)^{abcd}	10.34 (18.76)^{abc}	7.63 (16.03)^{ab}	10.36 (18.77)^{abcd}	10.58	4.03
T_9	*M. anisopliae* (1×10^{8} CFU g^{-1})	5 g l^{-1}	10.88 (19.26)^{ab}	9.90 (18.34)^{ab}	8.93 (17.38)^{bc}	9.90 (18.34)^{abc}	10.27	3.07
T_{10}	*B. bassiana* (1×10^{8} CFU g^{-1})	5 g l^{-1}	16.30 (23.81)^{de}	11.39 (19.72)^{bcd}	14.17 (22.11)^{e}	13.95 (21.93)^{e}	10.01	2.22
T_{11}	*M. rileyi* (1×10^{8} CFU g^{-1})	5 g l^{-1}	10.38 (18.79)^{a}	8.96 (17.42)^{ab}	9.76 (18.20)^{bcd}	9.70 (18.15)^{abc}	10.3	3.16
T_{12}	Chlorantraniliprole 18.5% SC	0.27 ml l^{-1}	10.15 (18.58)^{a}	7.98 (16.41)^{a}	6.02 (14.20)^{a}	8.05 (16.48)^{a}	11.23	6.56
T_{13}	Untreated Control	--	18.87 (25.74)^{e}	20.36 (26.82)^{e}	21.00 (27.27)^{f}	20.08 (26.62)^{f}	9.02	-
SE (m)±		--	0.953	0.965	0.831	0.773	0.526	-
CD ≤ 0.05		--	2.935	2.974	2.561	2.380	1.619	-
CV (%)		--	6.362	6.867	6.319	5.480	7.337	-

*Values in parentheses are arc sine transformed values; In a column means followed by same letters do not differ significantly by LSD (P = 0.05).

CONFLICT OF INTEREST

No conflict of interest.

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SPECIES COMPLEX OF LEAF ROLLER INFESTING LITCHI (*LITCHI CHINENSIS* SONN.) IN WEST BENGAL WITH THEIR SEASONAL INCIDENCE

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ABSTRACT

An experiment was conducted at Fruit Research Station, Mandauri, Bidhan Chandra Krishi Viswavidyalaya with a view to rule out the leaf roller species complex associated with litchi (*Litchi chinensis*) in West Bengal. The result revealed that three species namely litchi leaf roller, *Gatesclarkeana* sp. (Lepidoptera: Tortricidae); leaf webber, *Dudua aprobola* Meyrick (Lepidoptera: Tortricidae); litchi leaf roller, *Statherotis leucaspis* Meyrick (Lepidoptera: Tortricidae) were associated with the crop and hence their seasonal incidence and interaction with abiotic factors have also been studied. Among the species *Gatesclarkeana* sp. was found least abundant and occasionally observed during fruiting season while *Dudua aprobola* observed only in copper leaf stage or in soft tender leaves. *Statherotis leucaspis* was observed as most dominant species in basin of West Bengal which has not yet been reported from the state. The pest remained very active from 26th standard meteorological week and continued upto 47th standard week. Interestingly the same pest was observed during panicle initiation stage to flowering stage, attacking the panicle (maximum 23.75%) which led direct yield loss.

Key words: *Litchi chinensis*, Tortricidae, leaf roller, *Gatesclarkeana* sp., *Dudua aprobola*, *Statherotis leucaspis*

Litchi (*Litchi chinensis* Sonn) is an important subtropical evergreen fruit crop belonging to family Sapindaceae. India secured second position in litchi production after China, with an area and production of approx. 95,000 ha and 7, 27,000 MT respectively during 2018-19 (NHB database, 2018). Bihar, Jharkhand, West Bengal and Tripura are the major litchi growing states in India and contribute almost 85% of the total litchi production in the country. Like other crops, the biotic and abiotic stress are the limiting factors in litchi production where insect pests are the major biotic constraints (Mathur and Tandon, 1974; Butani, 1979) reported more than 40 insect and mite species used to attack litchi at different stages of plant growth and fruiting. However, from last 20 years litchi fruit borer (*Conopomorpha* sp.) and litchi leaf roller (*Dudua aprobola*) have acquired the status of major pests of litchi in some states of India (Kumar et al., 2011; Choudhary et al., 2013; Kumar et al., 2013).

The status of litchi in West Bengal depicted that litchi is preferred by the people of West Bengal because of their attractive colour, flavour and juicy nature. There is no doubt that the litchi fruit borer (*Conopomorpha* sp.) is a major limiting factor of litchi production in West Bengal but apart from this pest, a number of insects were observed causing direct or indirect crop loss. There are very limited literature available on status of insect pests of litchi in West Bengal where only the fruit borer *Conopomorpha* sp was mentioned. During the present survey it was observed that a number of species were associated with litchi leaves and causing symptom of leaf roll or fold or web. These species directly or indirectly cause economic loss to the crop and remain active almost throughout the year and keeping these information in mind the present research work was formulated with a target to best bit the insect species causing leaf roll or fold or web in litchi of west Bengal with their seasonal incidence and relationship with abiotic factors. This research findings will enrich the present status of litchi insect pests in West Bengal.

MATERIALS AND METHODS

An experiment was undertaken in experimental field of litchi under All India Coordinated Research Project on Fruits at Mandauri, Mohanpur, Nadia during 2020 and 2021 to study the seasonal occurrence and impact of weather parameters with litchi leaf roller species. The experimental field location was 22.56˚ N and 88.32˚E. Ten well managed litchi trees, of 7-8 years old were selected for the experiment purpose where all the trees were kept unsprayed condition to provide better environment for the pest multiplication. 10 twigs from

each side i.e. east, west north and south were tagged for recording the data. A total 40 twigs were selected and out of which 10 leaves/ twig were considered for recording data. The data were taken at weekly interval to rule out the incidence of insect species causing leaf roll, fold and web. The percent leaf infestation was calculated by using formula (Charak et al., 2020). In order to study the effect of weather parameters on pests incidence, the data of physical factors of environment viz rainfall (RF), wind speed (WS), maximum (MaxT) and minimum (MinT) temperature, relative humidity (RH) were correlated. Standard Week-wise data on various parameters were recorded from the portal Crop Weather Outlook, All India Coordinated Research Project on Agrometeorology (AICRPAM). The periodic mean incidence of the pests were worked out and the values of individual year were pooled and used for discussing seasonal occurrence of insect pests. Simple correlation and multiple regression analysis were worked out between various leaf roller species using their weekly mean damage and data analysis was done using software SPSS version 21.

RESULTS AND DISCUSSION

The experimental result revealed that three species of tortrix leaf roller or folder or webber causing damage to the crop namely *Gatesclarkeana* sp. (Lepidoptera: Tortricidae); Leaf Webber, *Dudua aprobola* Meyrick (Lepidoptera: Tortricidae); Litchi Leaf Roller, *Statherotis leucaspis* Meyrick (Lepidoptera: Tortricidae). Among them *Statherotis leucaspis* was found most dominant species during present investigation and mentioned for the first time as pest from West Bengal. However, Chakraborty and Samanta (2005) reported *D. aprobola* as major pest of litchi in West Bengal. So, it may be replacement of the species *D. aprobola* by similar species *Statherotis leucaspi* belonging to same family (Tortricidae).

Gatesclarkeana sp. also commonly named as tortrix leaf roller and found associated with litchi leaf at copper leaf stage. The species was observed active from 25th standard week to 39th standard week (Fig. 1). This species was observed least abundant species among all the leaf roller species recorded. 1.5% of leaf sample was found infested during 25th standard meteorological week while in 26th standard week incidence increased to 1.75% and there after the population was declined and observed occasionally after 39th standard meteorological week. The forewing of adult moth was dark brown, rugose with few micro spots while the hind wing was transparent.

The abiotic variables or weather parameters showed positive and negative correlation with the damage of *Gatsclarkeana* sp. The experimental result revealed non-significant relationship with all the weather parameters except evening relative humidity which showed significant positive correlation (r= 0.404**) (Table 1) with the pest damage. The equation of multiple regression indicated that increase in one unit of maximum temperature, minimum temperature, morning relative humidity, evening relative humidity and rainfall resulted with increase of pest damage by 0.012, 0.012, 0.011, 0.011, 0.003 while with the increase of one unit

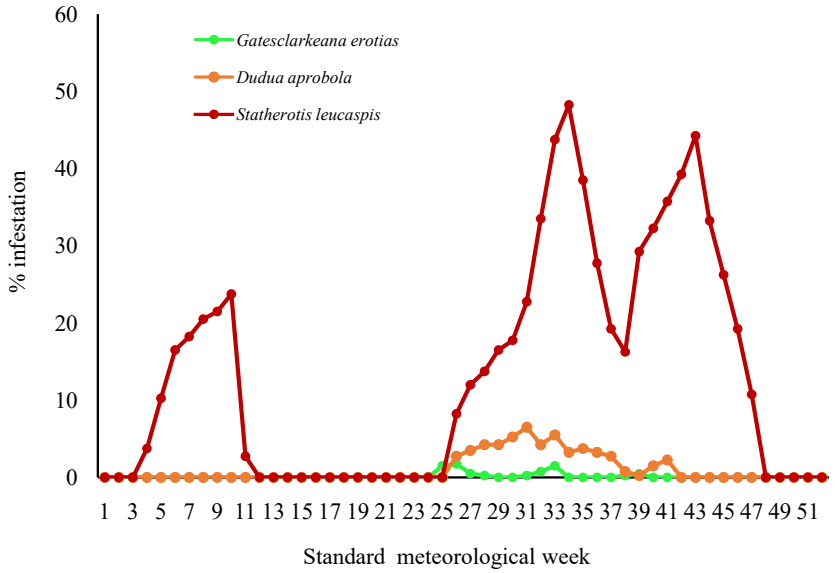


Fig. 1. Seasonal incidence of leaf roller species of litchi in West Bengal

Table 1. Correlation coefficient (r) between weather parameters and leaf roller species of litchi in West Bengal

Variables	Temperature		Relative humidity		Rainfall	Sunshine	Wind speed
	Max	Min	Morning	Evening			
Gatesclarkeana sp.	0.125^{NS}	0.182^{NS}	0.248^{NS}	0.404^{**}	0.073^{NS}	0.056^{NS}	-0.071^{NS}
Dudua aprobola	0.336^{*}	0.296^{*}	0.498^{**}	0.363^{**}	0.468^{**}	0.598^{**}	-0.053^{NS}
Statherotis leucaspis	0.247^{NS}	-0.002^{NS}	0.611^{**}	0.194^{NS}	0.493^{**}	0.658^{**}	-0.155^{NS}

Table 2. Multiple regression equation of weather parameters and leaf roller species of litchi in West Bengal

Variable (Y)	Multiple regression analysis	R^2 value
Gatesclarkeana sp.	$Y = -2.034 + 0.012 (X_1) + 0.012 (X_2) + 0.011 (X_3) + 0.011 (X_4) + 0.003 (X_5) – 0.002 (X_6) -0.014 (X_7)$	0.237
Dudua aprobola	$Y = -8.094 + 0.010 (X_1) + 0.062 (X_2) + 0.050 (X_3) + 0.027 (X_4) + 0.002 (X_5) + 0.110 (X_6) + 0.013 (X_7)$	0.514
Statherotis leucaspis	$Y = -123.280 -0.127 (X_1) + 0.113 (X_2) + 1.623 (X_3) – 0.301 (X_4) + 0.043 (X_5) + 0.688 (X_6) -0.963 (X_7)$	0.585

of other abiotic factors such as sunshine hours and wind speed resulted with decrease of pest damage by 0.002 and 0.014 respectively (Table 2).

D. aprobola which is commonly known as litchi leaf webber was observed at copper leaf stage and the larvae concealed themselves in between folded leaves. The forewing of adult moth with colour ranging from an overall brownish shade to dark greyish-blue with blackish spots while the hind wing is greyish. The species was found restricted to copper leaves only and never observed at fruiting stage. The incidence of pest was started in 26th standard week and remain active up to 41st standard week with peak incidence during 31st standard week (6.5%) to 33rd standard week (5.5%) and there after incidence decreased. Ray and Mukherjee (2013) observed maximum population during July-February while in present study maximum population observed during July- August and remain upto October which is in support to the previous investigators; Chakraborty and Samanta (2005) also reported that the litchi leaf roller, *D. aprobola* Meyrick is one of the economically important pests causes severe damage to litchi foliage in Bihar, Uttar Pradesh and West Bengal which contradict with the present finding where *S. leucaspis* was recorded as major pest causing economic damage.

The result of influence of abiotic factors on pest incidence depicted that all the abiotic factors such as maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, rainfall showed significant positive relationship (r = 0.336, 0.296, 0.498, 0.363, 0.468, 0.598, respectively) (Table 1) with the pest damage except wind speed which showed non-significant negative relationship (-0.053). The result of regression equation represented that with increase of one unit of maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, rainfall, wind speed the pest damage incidence increased by 0.010, 0.062, 0.050, 0.027, 0.002, 0.110, 0.013 (Table 2).

S. leucaspis which is commonly known as litchi leaf roller become a threat to litchi production in West Bengal. During the study period, it was observed that this species *S. leucaspis* was the most dominant species in fruit research station, Mandauri, Nadia, and West Bengal. The adult moth with black coloured forewing decorated with a greyish yellow spot while the hind wing is greyish. The pest incidence was observed in 26th standard week and continued up to 47th standard week with peak incidence during 33rd and 34th standard week (43.75 and 48.25, respectively). After 47th standard week the pest was not observed in the field, however, another peak incidence was observed at panicle initiation stage (Fig. 1). More than 20% panicles were found infested with this peat from 8th to 10th standard week (20.5, 21.5, and 23.75% respectively) and thereafter very interestingly the pest was almost disappeared from the field. Randhawa et al., (2015) reported 81 per cent damage to the new growth/ flush in District Gurdaspur and later on in 2016 Singh and Kaur again recorded its severity during June-September 2015 at FRS, Gangian and Mukerian in District Hoshiarpur. However, this insect was never mentioned as a pest of litchi from West Bengal and in this paper for the first time the pest is mentioned and observed that it could become a threat to litchi production in West Bengal

the pest not only causing damage to new flush but also cause a severe damage to panicle.

S. leucaspis or litchi leaf roller showed significant positive relationship with the morning relative humidity, rainfall and sunshine hours (r= 0.611, 0.493, 0.658, respectively) while maximum temperature, evening relative humidity showed non-significant positive correlation with pest incidence, however minimum temperature and wind seed showed non-significant negative relation (Table 1). The equation of regression analysis indicated that with increase of one unit of minimum temperature, morning relative humidity, rainfall, sunshine hours the pest damage increased by 0.113, 1.623, 0.043, 0.688 while with increase of one unit of maximum temperature, evening relative humidity and wind speed the pest damage incidence decreased by 0.127, 0.301, 0.963 (Table 2).

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AUTHOR CONTRIBUTION STATEMENT

The senior author planed the experiment, taken data, analysed and prepared the manuscript while D. Majhi, K. Chakraborti and D. Misra helped inexecution of work, data collection and preparation of manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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FIRST RECORD OF *MEGASELIA SCALARIS* (DIPTERA: PHORIDAE) AS A PARASITOID OF *THYAS CORONATA* (LEPIDOPTERA: EREBIDAE)

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ABSTRACT

***Thyas coronota* (Fabricius) (Lepidoptera: Erebidea) is one of the most significant fruit-piercing moths, particularly on citrus fruits, d as a result of feeding, it causes significant injury. The parasitoid, *Megaselia scalaris* was observed on the exterior surface of last instar larva of *T. coronata*. The fly laid egg on the exterior surface of the host larva, the incubation period was three to four days. The fly went through larval instars, each lasting three to four days, then pupated for seven to eight days. Adults liked for five to eight days. This study first time reports the parasitisation of dipteran scavenger fly *Megaselia scalaris* on the serious fruit piercing moth *Thyas coronota* from India.**

Key words: *Thyas coronota*, *Megaselia scalaris*, humpbacked fly, Diptera, Lepidoptera, Erebidae, fruit piercing, parasitoid, Kerala, India, new record

Megaselia scalaris (Loew), known as the scuttle fly or humpbacked fly, is a widely dispersed insect with forensic significance (Disney 1994, Campobasso et al. 2004, Reibe and Madea ,2010). These flies have a broad range of biological and environmental niches that they can explore (Disney, 2008). It was also shown that this species parasitized and preyed upon a variety of arthropods (Costa et al., 2007; Koch et al., 2013).

According to field reports, this fly can parasivise a wide range of living arthropods, including species from the Orthoptera, Diptera, Lepidoptera, Coleoptera, Hymenoptera, Ixodida, and Araneae-some of which are essential for agriculture and the environment—as well as the orders Orthoptera, Diptera, Lepidoptera, and Coleoptera. Due to its remarkable ecological adaptability, *M.scalaris* has also gained a reputation as a laboratory pest for infesting invertebrate cultures. Adult *Thyas coronata* are well known fruit piercing moths belonging to the family Erebidae recognised as a serious pest of several synthetic and wild fruits. Their sclerotized proboscises are used to penetrate ripening fruit, and they macerate the fruit to extract the juice. Adults presumably preferred to consume slices of citrus, pineapple, banana, and papaya fruits, according to study on eating preferences and phototaxis in adults (Suthapradit et al. 2006). Adult posess two white bands on the black head, body brown to blackish, marked with black spots and red longitudinal streaks. The pupa is black-brown. The adult moth has rufous and fuscous forewings tinged with a black spot in the middle. The hind wings are bright yellow with a dark band at the anterior and the posterior borders. Time required for egg to adult development is 40.35± 0.59 days (mean±SEM. As a result, citrus fruits suffer considerable harm. For the first time in India, the humpbacked fly *Megaselia scalaris* (Loew) (Diptera: Phoridae) was identified as a parasitoid of *Thyas coronata* and it is studied herein.

MATERIALS AND METHODS

The parasitoid flies were collected from the Banasura forest (75.93467811^{o}N,11.67662398^{o}E), Wayanad district of Kerala, India.The flies were identified as *Megaselia scalaris* through published literature (Deshmukh et al., 2021). During the time of survey five flies were seen to be attacking the larvae of *Thyas coronata*. Together with the larva the flies were collected and reared under laboratory conditions. The rearing of maggots was carried out as per methodology adopted by Deshmukh et al. (2021). The experiment was repeated to confirm the results. The adults emerged from the pupae of the reared maggots were transferred to containers which contained the 5th instar larva of *Thyas coronata*. The adult parasitoid flies were fed with 10% honey solution.

RESULTS AND DISCUSSION

***Thyas Coronata* (Fabricius, 1775)** (Fig. 1,2)

Noctua coronata Fabricius, 1775, Syst. Ent.: 596. *Noctua leonina* Fabricius, 1775, Syst. Ent.: 596. *Noctua ancilla* Fabricius, 1794, Ent. Syst. *III*, 2: 17. *Corycia magica* Hübner, 1827, Zuträge



Fig. 1. *Thyas coronata* last instar larva parasitoid by *Megaselia scalaris*

Samml. exot. Schmett., 3: 32. *Ophiodes ponderosa* Mabille, 1879, Annls Soc. ent. Fr., (5) 9: 346. *Anua coronata* Fabricius; Holloway, 1976: 2. *Ophiusa coronata* Fabricius; Kobes, 1985: 36.



Fig. 2. Adult *Thyas coronata*

Larva: Bell (MS), Gardner (1941; 1947), Sevastopulo (1939a), and Bigger (DE) (1988) described and depicted the larva.The immature larvae are slender and green with black markings. The adult larva also has the usual ophiusine morphology of a thin body with a prognathous head. The head and body are longitudinally striped. The stripes are divided by thin darker brown lines with alternate colours (lighter fawn and deeper purple fawn). Three of the darker stripes appear in a row on each side, and the dorsal, thinner stripe is enlarged into ellipses on A5 and A6. On A8, the dorsolateral stripes include a pair of tiny, light yellow tubercles. The prolegs of A4 are short compared to third abdominal segment (Fig. 1).

Megaselia scalaris Loew, 1866– biology and taxonomy

Along with the sixth instar larva flies attached on it were collected in the closed containers. After two days white long oval eggs were observed on the body of the larva. After four days a few maggots (Ist Instar) hatched and were visible feeding the larva. The first instar larva devoleped into second instar within two days. The third instar was developed after 3 days and matured to fourth instar larva in next two days. This fourth instar larva pupated within two days and the adult of *M. scalaris* emerged after three to four days. The laboratory experiment was carried out to validate the results. The flies laid white oval eggs on the host larval's surface after two days (Fig. 3). After two to three days, the eggs hatched, and milky white maggots bursted forth and started feeding the host larvae. After five to six days, the mature maggots developed. Typically, the larvae are relatively little, measuring about 1 and 6 mm in length. They pupated within two days (Fig. 4), and adult flies emerged from the pupae after seven to eight days (Fig. 5). It was observed that the male *Megaselia scalaris* fly pupated two days earlier than the female.



Fig. 3. Larva with *Megaselia scalaris* infestation. a-egg, b-larva



Fig. 4. Pupae of *Megaselia scalaris*



Fig. 5. Emptied pupae after the emergence of fly

The mature fly had equidistant supra-antennal bristles and was yellowish brown in colour. The thorax was brownish dorsally, the prothoracic pleura had several little hairs and 2-4 long ventral bristles, the mesopleuron lacked distinguishing bristles, and the scutum had a pair of long bristles at either end. Legs were whiter; the femur was larger; it was darker apically with dark brown tiny hairs; halters were creamy yellowish; wings had half of the costal border densely fringed; and the abdomen was a deeper brown. The scutellum had two pairs of bristles (Fig. 6).



Fig. 6. *Megaselia scalaris* emerged from pupal stage as adult

This is the first report of parasitisation of the fruit piercing moth *T. coronata* by the dipteran scavenger fly *M. scalaris*. The species *M. scalaris* that infest *T.coronata* have been documented for the first time in India. Many studies have been carried out on the dipteran flies which have high forensic significance (Disney, 1994; Campobasso et al. 2004; Reibe and Madea, 2010). These flies have a broad range of biological and environmental niches that they can explore (Disney, 2008). It has also been noted that this species parasitized and preyed upon a variety of arthropods (Zwart et al., 2005; Costa et al. 2007; Koch et al. 2013). Due to its remarkable ecological adaptability, *M. scalaris* is considered as a laboratory pest for infesting invertebrate cultures. It was also reported as the laboratory parasitoid of *Spodoptera frugiperda* in India and China (Deshmukh et al., 2021; Tang et al., 2021).

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AUTHOR CONTRIBUTION STATEMENT

RR AND RSM S: data collection. RR and RSM S: original draft writing. The manuscript's published version has been reviewed and approved by all authors.

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No conflict of interest.

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NEW RECORD OF A MOSQUITO *URANOTAENIA LOWII* (THEOBALD, 1901) FROM INDIA

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ABSTRACT

This study reports *Uranotaenia lowii* (Theobald, 1901) as a new record from India. It was collected from Berhampur University Campus, District Ganjam, Odisha, India. This species is not considered to have medical importance, as it does not bite mammals and feeds upon the blood of reptiles and amphibians.

Key words: *Uranotaenia lowii*, Culicidae, pale-footed, acoustic sensitivity, a new record, Odisha, India, diversity, zoogeography, vector, medical importance

Mosquitoes, among the wide range of vectors, play an influential role in the transmission of different kinds of diseases to a variety of animals and primarily to human beings by feeding on blood. Malaria, filariasis, dengue fever, Japanese encephalitis, Chikungunya, and various other viral diseases are transmitted by mosquitos. In other words, they are disease agents or carriers. More than 17% of death (near about 7 lakhs) occurs due to the infection caused by vector-borne diseases out of all infectious diseases (WHO, 2020). The mosquito is the most dangerous of all the insects that spread diseases. It continues to be humanity's most persistent foe, despite costly efforts to eliminate or even control it. The global mosquito fauna consisted of 3541 species belonging to 50 genera, two subfamilies, and 12 tribes (Tyagi et al., 2015). More than 404 species and subspecies of mosquitoes are available in India, accounting for more than 12% of the global mosquito fauna (Tyagi et al., 2015).

Uranotaenia lowii is a tiny mosquito found throughout the Western Hemisphere, including the Gulf states of the United States. It breeds in small ponds and grassy lake edges and is also known as the pale-footed *Uranotaenia*. Larvae can be seen all year in Florida, but they are only found in the summer and early fall (Carpenter and Lacasse, 1974), in 1945, Remington conducted extensive research on this species feeding habits in Louisiana. The females did not feed on humans or reptiles but fed upon toads, frogs, and salamanders (Matheson, 1966). The pale-footed *Uranotaenia*, like its larger relative, exhibits little interest in people. Because of its lack of attention and small size, the species sometimes goes overlooked in mosquito management and surveillance operations. These small mosquitos feed on reptiles and amphibians, particularly frogs while avoiding humans and other living organisms. Amphibian populations are rapidly falling worldwide, and many scientists believe the key culprits are habitat degradation and climate change. The impact of mosquitos on non-human species is little understood (VDCI, 2016). Most mosquitoes track carbon dioxide plumes and thermal taxis to discover blood sources. *Uranotaenia lowii's* host preference shows that their natural habitat consists of grasslands, lake edges and shallow ponds with moderate sunlight. Females of this tiny species, according to studies conducted in Costa Rica, have auditory sensitivity that other mosquitos may not have. According to some studies, they may even have extraordinary sensory abilities. While other Male mosquitos are attracted to the sounds made by their female counterparts. The only known species in which females exploit auditory sensitivity to locate an amphibian bloodmeal is *Uranotaenia lowii.*

Uranotaenia lowii is not known to be a vector of parasites, as very little work has been done on this species considering the non-medical significance. The lack of scientific literature on *Uranotaenia* raises more issues regarding the species potential ecological role in the declining populations of their amphibian victims. The present finding of the mosquito from the Berhampur University campus, Ganjam, Odisha, is the first material evidence of this mosquito from India. The current record will help as the baseline information for understanding the distribution and zoogeography.

MATERIALS AND METHODS

Mosquitoes were collected from the campus area of Berhampur University, Odisha, India (19.2977358°N84.8781602°E). The collection was carried out from January 2018 to December 2019 using an insect battery-operated mechanical aspirator (Pooter) and torchlight. The collected mosquitoes were then transferred to a test tube, covered with a loose cotton plug, and examined in the laboratory for identification. Identification of these mosquitoes was made with the help of a 10x fabric lens, and simultaneously the photographs were taken with the help of a mobile camera mounted with a 10x macro lens and L.E.D. Identification of the collected mosquitoes was based on adult characters using standard taxonomic keys and catalogues of Mosquitoes Identification key of Christophers (1933), Barraud (1934), and online identification keys from Species Identification Table Adult, Florida Medical Entomology Laboratory - University of Florida, Institute of Food and Agricultural Sciences; fmel.ifas.ufl.edu. Retrieved April 15, 2022, from https://fmel.ifas.ufl.edu/mosquito-guide/species-identification-table/species-identification-table-adult/#Uranotaenia. The identity of the mosquito was confirmed with the Indian Council of Medical Research-Regional Medical Research Centre, Bhubaneshwar. These mosquitoes were deposited and registered in the National Repository of Estuarine Biology Regional Centre, Zoological Survey of India, Gopalpur-on-Sea, Odisha, India.

RESULTS AND DISCUSSION

The taxonomic account of the species is provided below:

Uranotaenia lowii (Theobald, 1901) (Fig. 1)



Fig. 1. *Uranotaenia lowii*

Materials examined: 18 examples, Registration Number: EBRC/ZSI/In-12262 A-R, collected by: Santhosh Goud, Adults: ♀ small-sized; body length 0.25 to 0.3 cm.

Female: A small mosquito that is dark greyish-brownish in appearance. Palps are dark and approximately 1/5 the length of the proboscis. The proboscis is dark and fat at the tip. Dark greyish brown scutum. Thorax is pale in colour, with a black patch and a few pale blue scales. Dark abdomen with pale iridescent scale patterns on the sides. On the stem of the 5th vein, the wings have a short stripe of pale blue scales. White booties, dark hind legs, and pale knee patches.

Remarks: The species *Uranotaenia lowii* was first described by Theobald in 1901, and bionomics, distribution and larval forms were documented by Remington in 1945 and Carpenter and Lacasse in 1974. This species looks similar to *Culex mimeticus* in terms of size and appearance; however, it can be distinguished from the *Culex mimeticus* by the absence of pair of dark spots on the wings and white patches on the head. The presence of the mosquito *Uranotaenia lowii* from Odisha constitutes the first material evidence of this mosquito from the Indian subcontinent. The current record will help as the baseline information for understanding its distribution, ecology, and zoogeography.

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AUTHOR CONTRIBUTION STATEMENT

S Goud conducted the survey, collected the specimen, taken the photograph of the specimen. Identified by S Goud and J K Seth, I Biswal, P Dash, B B Panda, S Pattnaik, and R K Hazra prepared the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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GROWTH AND DEVELOPMENT OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* ON CEREALS

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ABSTRACT

This laboratory study was conducted to evaluate the growth and development of fall army worm *Spodoptera frugiperda* (J E Smith) on four cereal host plants. It was observed that the significantly least total lifecycle duration of male was on maize (27.61± 0.60 days) followed by pearl millet (30.85± 0.46 days), sorghum (31.31± 0.77 days). It was maximum on sugarcane (34.36± 0.80 days). As regards female *S. frugiperda*, the least was on maize (29.78± 0.66 days) followed by sorghum (32.52± 0.44 days), pearl millet (33.34± 0.52 days) and maximum on sugarcane (36.04± 0.51 days). Likewise maximum fecundity was observed on maize (436.44± 22.44) followed by sugarcane (248.08± 15.54), pearl millet (139.30± 13.02) and the least was on sorghum (106.44± 11.18). Male to female sex ratio was highest in sorghum (1:1.31) followed by pear millet (1:1.26), maize (1:1.20) and the least on sugarcane (1:1.19).

Key words: *Spodoptera frugiperda*, maize, pearl millet, sorghum, sugarcane, fecundity, egg hatching, larval instars, larval, pupal, adult periods, growth index, pupation, longevity

Fall army worm, *Spodoptera frugiperda* (J E. Smith) (Lepidoptera: Noctuidae) is a dreaded insect pest native to tropical and subtropical America (Luginbill, 1928; Sparks, 1979) spread all over the globe and assumed the position of level A1 threat. Short development cycle (Sharanabasappa et al., 2018), wide host range (EPPO, 2019), high prolificacy (Sparks, 1979) and high dispersal ability (Westbrook et al., 2016) make it a potentially dangerous. In India, the invasion of *S. frugiperda* was reported first in May 2018 on maize from Shivamogga, Karnataka (Sharanabasappa and Kalleshwaraswamy, 2018). It has been known to be polyphagous, gregarious and destructive and attacks 353 plant species from 76 families, principally of Poaceae (106), Asteraceae (31) and Fabaceae (31) (Montezano et al., 2018). The growth, development and reproduction of insects are directly influenced by the quality of the host plant. The responses of insect herbivores to changes in host plant quality vary within and between feeding guilds (Awmack and Leather, 2002). Hence, the biological studies of insect pest on different food sources are important for addressing the effect of the nutritional composition on growth and development of *S. frugiperda*. Hence the present study on growth and development of *S. frugiperda* on different cereals.

MATERIALS AND METHODS

The study on the growth and development of *S. frugiperda* was carried under laboratory conditions at a constant temperature and humidity. Four cereal host plants viz., maize (*Zea mays* L.) variety Narendra (M909), sorghum (*Sorghum bicolor* (L.) Moench) variety Parbhani Shakti (ICSR 14001), pearl millet (*Pennisetum glaucum* (L.) R. Br. (1810)) variety ABPC-4-3 and sugarcane (*Saccharum officinarum* L.) variety Nira (Co 86032) were included. These cereal host plants were grown by an adopting recommended package of practices as per V N M K V, Parbhani (Anonymous, 2016), except plant protection on the Research Farm of Department of Agricultural Entomology, College of Agriculture, Latur, during kharif 2019.

The initial culture of *S. frugiperda* was developed by collecting large sized larvae from maize crops cultivated on the research farm. The collected larvae were reared individually in clean round plastic vials (3.5 cm dia x 4 cm). Daily larvae were fed on unsprayed field collected leaves, stems and whorls of respective host plants during the morning hours till pupation. After pupation, the sexes of pupae were also determined on the basis of the distance between the two apertures. In males, the distance between genital and anal aperture was less, while it was more in females (Luginbill, 1928). The adults that emerged on the same day were sexed. Then one male and one female adult were paired together in an oviposition cage for copulation and egg laying. A cotton swab dipped in 50% honey solution was provided as food to the adults in the oviposition cage. The fresh leaves of respective host plants were also placed as

an oviposition substrate. After every 24 hr, the leaves were examined for presence of eggs or egg masses and replaced with fresh ones. Thus, the freshly laid eggs were used for the study.

One hundred freshly laid eggs in a group of 20/ replication were kept in the petridish for recording observations in respect of incubation period and % egg hatching. The newly hatched larvae were reared individually on leaves and slices of the stem of respective host plants in round plastic vials (3.5 cm dia x 4 cm). While rearing due care was taken to transfer the larvae into a clean plastic vial. Fresh food was provided daily and old food was removed. The observations on the larval duration, % pupation, growth index, pupal duration, total development period (egg to adult emergence), % adult emergence, adult longevity and total lifecycle duration were recorded on respective host plants. The adults that emerged on the same day were sexed and one male and female were paired together for copulation and egg laying in an oviposition cage. A cotton swab dipped in 50 % honey solution was provided as food to the adults. The fresh and tender leaves of respective host plants were provided as a substrate for egg laying in each oviposition cage. The number of eggs laid by each female was counted daily till the death of the female moth. The observations on initiation of oviposition, last oviposition and death of female were recorded. From this, preoviposition, oviposition and post-oviposition periods were worked out on respective host plants. The data collected were analysed using descriptive statistics. The growth index was calculated by using Howe's (1953)- Growth Index = % larvae pupated/ Mean larval duration (days).

RESULTS AND DISCUSSION

The data on the growth and development of *S. frugiperda* on cereals are presented in Table 1. The fecundity was observed to vary significantly- maximum of 436.44 eggs/ female was observed on maize followed by sugarcane (248.08 eggs/ female). These observations agree with those of Maruthadurai and Ramesh (2020) on fodder maize. Hutasoit et al. (2020) and Sharanabasappa et al. (2018) observed the fecundity as 1064- 1662 eggs/ female on maize. The incubation period varied significantly with cereal hosts, minimum (2.00 days) was on maize and pearl millet. Guo et al., (2020) observed this as two days on maize, potato and tobacco, while Maruthadurai and Ramesh (2020) observed it as 2.20, 2.80, 2.80, and 2.40 days on fodder maize, Guinea grass, para grass and green amaranth, respectively. Egg hatchability was significantly maximum of 94%, on maize and sugarcane, while Montezano et al. (2019) noticed this as 97.40 % on an artificial diet; and Sharanabasappa et al. (2018) as 96.6 % on maize. Total larval duration ranged 12.58 to 19.17 days in different cereals, with shortest being on maize (12.58 days) followed by sorghum (15.93 days). Plessis et al. (2020) observed the mean developmental period on sweet corn as 10.45 to 34.39 days at varying temperatures; Maruthadurai and Ramesh (2020) observed this as 13.80, 17.30, 18.60 and 17.10 days on fodder maize, Guinea grass, para grass and green amaranth, respectively; and Kalyan et al. (2020) as 16.97 days, Tendeng et al. (2019) as 14 days and Sharanabasappa et al. (2018) as 15.9 days.

There were six larval instars irrespective of the host plants, with a duration from 2.46 to 3.32, 2.05 to 3.06, 2.01 to 3.07, 2.00 to 3.20, 2.02 to 3.29 and 2.04 to 3.23 days, respectively on different cereal host plants; significantly minimum and maximum I, II, III, IV, V and VI instar larval duration were to the extent of 2.46, 2.05, 2.01, 2.00, 2.02 and 2.04 days and; 3.32, 3.06, 3.07, 3.20, 3.29 and 3.23 days was recorded on maize and sugarcane, respectively. Guo et al. (2020) observed the developmental time for I, II, III, IV, V, VI larval instars as 2.62, 2.94, 3.06, 3.23, 3.28 and 2.88 days, respectively on maize; Sharanabasappa et al. (2018) found these as 2.60, 2.20, 2.00, 2.00, 2.40 and 4.50 days, respectively. Pupation was significantly more with sorghum (91%) followed by maize (88 %). Barros et al. (2010) observed this as 16.9, 33.8, 18.0 and 32.5% on cotton, millet, corn and soybean, respectively. Significantly maximum growth index was in the case of larvae fed on maize (6.99) followed by sorghum (5.71), and it was maximum with sugarcane; while the pupal duration was significantly minimum on maize (6.74 days). Kalyan et al., (2020) observed this as 8.96 days on maize, and Hutasoit et al., (2020) as 6.31and 6.76 days for male and female, respectively on maize. The total developmental period thus was significantly lowest on maize (21.32 days), and maximum on sugarcane (30.18 days). Montezano et al. (2019) and Sharanabasappa et al. (2018) observed this to be 27.09 and 28.9 days on artificial diet and on maize, respectively.

Adult emergence was again significantly more as 92% with maize, and the least with pearl millet (86 %) as has been shown by Barros et al. (2010) as 77.0, 80.0, 71.0 and 72.0% on cotton, millet, corn and soybean, respectively. The preoviposition was significantly the least on sugarcane (3.24 days) followed by pearl

Table 1. Growth and development of *S. podoptera frugiperda* on cereal hosts

S. No.	Parameters	Host plants			
		Maize (Mean± SD)	Sorghum (Mean± SD)	Pearl millet (Mean± SD)	Sugarcane (Mean± SD)
1.	Fecundity (no/female)	436.44± 22.44	106.44± 11.18	139.30± 13.02	248.08± 15.54
2.	Incubation period (days)	2± 0.33	2.26± 0.21	2± 0.43	2.52± 0.30
3.	Egg hatchability (%)	94± 1.05	89± 1.82	84± 1.33	94± 1.56
4.	Larval period (days)	12.58± 0.75	15.93± 0.94	16.93± 0.61	19.17± 0.51
5.	Instar period (days)				
	I	2.46± 0.06	3.17± 0.05	3.11± 0.03	3.32± 0.06
	II	2.05± 0.03	2.31± 0.03	2.60± 0.13	3.06± 0.06
	III	2.01± 0.04	2.46± 0.02	2.67± 0.03	3.07± 0.02
	IV	2.00± 0.03	2.62± 0.02	2.81± 0.05	3.20± 0.04
	V	2.02± 0.03	2.69± 0.02	2.88± 0.04	3.29± 0.04
	VI	2.04± 0.02	2.68± 0.03	2.86± 0.05	3.23± 0.03
6.	Pupation (%)	88± 0.84	91± 1.17	71± 1.06	85± 0.74
7.	Growth ratio (days)	6.99± 0.26	5.71± 0.22	4.19± 0.27	4.43± 0.25
8.	Pupal period (days)	6.74± 0.44	7.99± 0.24	7.61± 0.38	8.49± 0.42
9.	Total developmental period (days)	21.32± 0.07	26.18± 0.05	26.54± 0.06	30.18± 0.05
10.	Adult emergence (%)	92± 0.49	91± 0.69	86± 0.50	87± 0.37
11.	Pre-oviposition period (days)	4.22± 0.17	3.80± 0.29	3.76± 0.88	3.24± 0.35
12.	Oviposition period (days)	1.88± 0.04	1.14± 0.05	1.20± 0.03	1.36± 0.04
13.	Post-oviposition period (days)	2.36± 0.02	1.40± 0.04	1.84± 0.04	1.26± 0.03
14.	Adult longevity (days)				
	Male	6.29± 0.09	5.13± 0.07	4.31± 0.05	4.18± 0.09
	Female	8.46± 0.03	6.34± 0.03	6.80± 0.06	5.86± 0.04
15.	Total life cycle duration (days)				
	Male	27.61± 0.60	31.31± 0.77	30.85± 0.46	34.36± 0.80
	Female	29.78± 0.66	32.52± 0.44	33.34± 0.52	36.04± 0.51
16.	Male: Female ratio	1:1.20	1:1.31	1:1.26	1:1.19

Mean± SD (n=100/ host); SD - Standard deviation

millet (3.76 days), and maximum with maize (4.22 days). Kalyan et al. (2020), Hutasoit et al. (2020) and Sharanabasappa et al. (2018) observed this as 3.33 to 3.6 days on maize; and the oviposition period was significantly more on maize (1.88 days) and the least on sorghum (1.14 days). Kalyan et al., (2020) and Sharanabasappa et al., (2018) found this as 2.96 and 2.8 days on maize; and Marua et al. (2008) as 5.31 to 7.44, days on various hosts. The post oviposition period was again more with maize (2.36 days) and lowest on sugarcane (1.26 days), agreeing with Kalyan et al. (2020) and Sharanabasappa et al., (2018) as regards maize. Maximum adult longevity of male was noticed on maize (6.29 days) and the least on sugarcane (4.18 days); and with female also it was on maize (8.46 days); females lived longer irrespective of host plants. Lekha et al. (2020) observed an adult male and female longevity of 4.50-8.00 and 7.00-10.33 days on different hosts; while Kalyan et al. (2020) observed this as 10.67-13.00 days. The lifecycle occupied the least duration with maize (27.61 days) followed by pearl millet (30.85 days), sorghum (31.31 days) and maximum being with sugarcane (34.36 days). Similar was the trend with female indicating that total lifecycle of male and female got shortened on maize and extended on sugarcane. Females lived longer than males irrespective of host plants. Plessis et al. (2020) observed a developmental time for the egg to adult as varying between 71.44 and 20.27 days at varying temperature. Maruthadurai and Ramesh (2020) observed a lifecycle duration of 24.60, 29.70, 30.60 and 30.20 days on fodder maize, Guinea grass, para grass and green amaranth, respectively. Kalyan et al. (2020) observed this to be 37.68 days on maize.

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EFFICACY OF *PIPER NIGRUM* AND *CUMINUM CYMINUM* SEED POWDERS AGAINST *CALLOSOBRUCHUS CHINENSIS* L.

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ABSTRACT

Evaluation of efficacy of black pepper (*Piper nigrum* L.) and cumin (*Cuminum cyminum* L.) seed powder against *Callosobruchus chinensis* L. infesting green gram revealed a dose-dependent adult mortality and a positive correlation with period of exposure. A complete adult mortality was observed with *P. nigrum* seed powder @ 1 g/ 100 g seed as against 60% adult mortality with *C. cyminum* at 72 hr after treatment (HAT). The LC_{50} values of *P. nigrum* and *C. cyminum* seed powders were observed as 0.16 and 1.47 g/ 100 g of seed at 48 HAT. Both *P. nigrum* and *C. cyminum* seed powders showed strong ovicidal properties, and affecting the adult emergence to an extent of 0 and 6% when used @ 10 g/ 100 g seed as against 90-91% in untreated control.

Key words: *Callosobruchus chinensis,* green gram, *Piper nigrum, Cuminum cyminum,* seed powders, efficacy, oviposition deterrent, ovicidal, adult mortality, bioactive compounds, LC_{50}

Stored products of agricultural and animal origin are globally attacked by several biotic stresses, out of which insect pests account 2.0 - 4.2% (Kumar and Kalita, 2017). About 14 mt of food grains is lost during storage annually in India (Banga et al., 2020), where insect pests alone cause a loss of nearly 20-25% (Rajashekar et al., 2012). Over 600 species of beetles and 70 species of moths cause quantitative and qualitative losses in stored products (Rajendran, 2002), to a tune of 20-30% in the tropical and 5-10% in the temperate countries (Talukder, 2006; Tadesse and Ali, 2021). Deteriorations of grain chemical composition due to insect infestations aggravate the situation (Grish et al., 1975; Pushpamma and Reddy, 1979). Serious damage to the stored pulses including cowpea, pigeonpea, chickpea, soya bean, black-eyed beans and others, is caused by the pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) (Srivastava and Dhaliwal, 2010; Ghosh et al., 2007; Appleby and Credland, 2004). Minimizing post-harvest loss could be a viable and sustainable option to assure food security. Protection of stored pulses from insect-pest attack has been a major challenge in recent past as use of insecticides lead to many hazards (Pavela, 2008; Metcalf, 1975). This has increased thrust on use of natural plant products for storage insect pest management (Pirali- Kheirabadi and da Silva 2010), which led to identification of plant products (Akinneye et al., 2006; Emeasor et al., 2005; Nadra, 2006). This study evaluates seed powders of some spices against *C. chinensis* attacking stored green gram.

MATERIALS AND METHODS

The experiment was conducted at the Post Graduate Research Laboratory, Department of Entomology, Assam Agricultural University, Jorhat (94°22′E, 26°75′N, 91 masl) during 2018-19. Based on previous research and available information, spices like black pepper *Piper nigrum* L. (Family: Piperaceae) and cumin, *Cuminum cyminum* L. (Family: Apiaceae) were selected. Seeds were collected from local market, dried under shade and ground to finer particles by sieving with 150 μm mesh size, and stored in airtight glass containers under refrigeration until their use in subsequent experiments. Mass culturing of *C. chinensis* was carried out on green gram seeds, *Vigna radiata*, with 1 kg seeds taken in 5 l plastic container; 10 pairs of adults (1: 1 sex ratio) were released for egg-laying in these. Insects were removed after 48 hr of release and containers were put into the BOD incubator at 30ºC and 80-85% RH for the emergence of adults (Kalita and Hazarika, 2020). The efficacy test was conducted with 100 g of uninfested green gram seeds in 200 ml plastic containers with open tops covered with muslin cloth. Plant products were admixed with the green gram seeds on a weight by weight (w/w) basis as per standard procedures to attain the dosage of 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, and 10.0 g/ 100 g of seed. Later, 20 neonate adults (1: 1 sex ratio)

Table 1. Effect of *Piper nigrum* and *Cuminum cyminum* seed powders on adult mortality of *C. chinensis*

Dosage (g / 100 g seeds)	Adult mortality (%) at different time interval													
	Piper nigrum							*Cuminum cyminum*						
	6HAT	12HAT	24HAT	48HAT	72HAT	96HAT	120HAT	6HAT	12HAT	24HAT	48HAT	72HAT	96HAT	120HAT
10.0	50.00 (50.60)	71.67 (63.17)	98.33 (98.25)	100 (90.99)	100 (90.99)	100 (90.99)	100 (90.99)	45.00 (47.79)	51.67 (51.69)	78.33 (67.23)	85.00 (71.69)	91.67 (78.74)	100 (90.99)	100 (90.99)
7.5	35.00 (40.05)	66.67 (58.03)	93.33 (92.98)	100 (90.99)	100 (90.99)	100 (90.99)	100 (90.99)	41.67 (44.98)	45.00 (47.56)	66.67 (57.42)	81.67 (70.58)	88.33 (74.89)	95.00 (83.25)	100 (90.99)
5.0	46.67 (47.93)	63.33 (44.43)	95.00 (94.74)	100 (90.99)	100 (90.99)	100 (90.99)	100 (90.99)	30.00 (38.09)	33.33 (39.48)	60.00 (54.06)	71.67 (61.93)	80.00 (67.83)	95.00 (83.25)	100 (90.99)
4.0	30.00 (36.83)	55.00 (50.10)	96.67 (96.49)	100 (90.99)	100 (90.99)	100 (90.99)	100 (90.99)	30.00 (38.09)	33.33 (39.42)	50.00 (50.60)	65.00 (56.86)	78.33 (67.23)	91.67 (78.74)	100 (90.99)
3.0	30.00 (36.83)	41.67 (45.12)	96.67 (96.49)	100 (90.99)	100 (90.99)	100 (90.99)	100 (90.99)	23.33 (32.43)	26.67 (34.00)	46.67 (47.95)	65.00 (56.86)	76.67 (65.05)	90.00 (77.38)	100 (90.99)
2.0	25.00 (33.43)	40.00 (43.16)	90.00 (89.47)	98.33 (89.66)	100 (90.99)	100 (90.99)	100 (90.99)	21.67 (31.69)	23.33 (32.43)	43.33 (45.57)	60.00 (54.06)	70.00 (59.19)	88.33 (74.99)	88.33 (74.99)
1.0	20.00 (29.68)	38.33 (42.65)	81.67 (80.70)	96.67 (86.81)	100 (90.99)	100 (90.99)	100 (90.99)	13.33 (24.10)	16.67 (26.31)	40.00 (43.16)	46.67 (47.95)	60.00 (54.06)	61.67 (54.43)	88.33 (74.99)
Control	3.33 (0.33)	3.33 (0.33)	5.00 (0.33)	5.00 (0.33)	5.00 (0.33)	5.00 (0.33)	5.00 (0.33)	1.67 (0.33)	3.33 (0.33)	5.00 (0.33)	5.00 (0.33)	5.00 (0.33)	5.00 (0.33)	6.67 (0.33)
S.Ed. (±)	1.02**	2.01**	1.78**	0.76**	NS	NS	NS	1.42**	1.44**	1.07**	1.31**	1.07**	0.93**	0.89**
CD (p=0.05)	2.16	4.25	3.78	1.61	-	-	-	3.01	3.06	2.28	2.79	2.28	1.97	1.88
CD (p=0.01)	2.98	5.86	5.20	2.22	-	-	-	4.15	4.21	3.14	3.84	3.14	2.72	2.59
LC_{50} values	-	-	-	0.16	-	-	-	-	-	-	1.47	0.77	0.65	-
df	-	-	-	19	-	-	-	-	-	-	19	19	19	-
Reg. equation (y=a+bx)	-	-	-	3.943+ 2.199x	-	-	-	-	-	-	0.925+ 1.112x	1.242+ 1.084x	2.181+ 1.837x	-
Chi square (X^2)	-	-	-	32.810	-	-	-	-	-	-	12.114	10.075	28.624	-
Slope ± S Em	-	-	-	2.199± 0.492	-	-	-	-	-	-	1.112±0.092	1.084±0.098	1.837±0.128	-

Data within parentheses mean of the Abbott's corrected angular transformed values; HAT- Hours after treatment; y= probit kill, x=log dose

were released in each of the plastic containers and the data on adult mortality (%) was recorded at 6, 12, 24, 48, 72, and 96 hr after treatment (HAT). Each treatment was replicated thrice along with a control. To test the ovicidal properties, 20 neonate adults at 1:1 sex ratio were released into plastic containers containing 100 g seed for egg laying and insects were removed after 12 hr of release. Seeds were then treated with the edible plant powders @ 1, 5, and 10 g/ 100 g of seed and numbers of marked egg hatched was recorded when more than 90.0% adults emerged in the control. The data on adult mortality was recorded at different time intervals and the mortality was considered when the beetle did not respond to gentle touch. The data on % adult mortality were subjected to Abbott's correction, and subjected to angular transformation before ANOVA, p=0.05 under a completely randomized block design. The data on adult mortality were also subjected to probit analysis to calculate LC_{50} values using SPSS computer software (Version 12.0).

RESULTS AND DISCUSSION

The results revealed that treatments with *P. nigrum* seed powder were superior in controlling *C. chinensis*, with 100% adult mortality @ 1 g/ 100 g of seed at 96 HAT onwards as compared to 20%, 38.33%, 81.67% and 96.67% mortality at 6, 12, 24, 48, and 72, respectively; while treatments with 10 g/ 100g of seed recorded 100% mortality at 48 HAT followed by 98%, 71.67% and 50% adult mortality at 24, 12 and 6 HAT. Black pepper powder gave 100% adult mortality at the lowest dosage of 3.0 g/ 100g of seed after 48 HAT; while *C. cyminum* seed powder led to 100% mortality at 10 g/ 100g seeds after 96 and 120 HAT; least mortality of 13.33% was observed with 1 g/ 100 g of *C. cyminum* powder after 6 HAT. The adult mortality ranged from 88.33- 100% at 120 HAT as against 6.67% in untreated control (Table 1). Awoyinka et al. (2006) and Scott et al. (2005) revealed the insecticidal property of seed extract of black pepper against the *C. chinensis*. The toxicity of *P. nigrum* seed powder against *C. chinensis* could be attributed to chavin, piperine, and unsaturated amides (Lale, 1992). Mortality of *C. chinensis* increased with dosage and exposure period of the *C. cyminum* seed powder; and this can be attributed to the bioactive compounds like cymene, γ-terpinene, cuminaldehyde and (−) β-pinene (Srivastava and Dhaliwal, 2010).

The LC_{50} value of *P. nigrum* seed powder was found to be 0.16 g/ 100 g of seed at 48 HAT, while the LC_{50} values for *C. cyminum* seed powder against *C. chinensis*

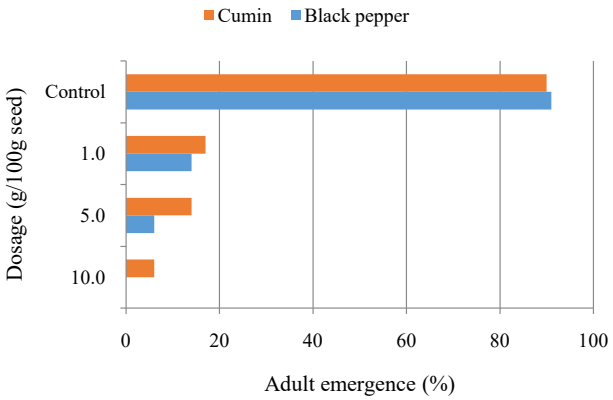


Fig. 1. Ovicidal effect of seed powders against *C. chinensis* (23 days after infestation)

was 1.47, 0.77 and 0.65 g/ 100g seeds at 48, 72 and 96 HAT, respectively (Table 1). The data on the effect of *P. nigrum* seed powder on ovicidal properties at 23 days of exposure revealed a complete inhibition of adult emergence @ 10 g/ 100g seed, while 6.00% adult emergence was observed with 5 g/ 100g seeds (Fig. 1); maximum emergence (14.00%) was observed with 1 g/ 100g seeds as compared to 91% in untreated control. With *C. cyminum* seed powder, the least emergence (6%) was observed @ 10 g/ 100g seeds followed by 14% @ 5 g/ 100g seeds; while maximum of 17% was observed with 1 g/ 100 g seeds as compared to 90% in the untreated control. The ovicidal effect of *P. nigrum* on *C. chinensis* might be attributed due to the progressive accumulation of the bioactive compounds on the treated seeds. It can be concluded that the dry seed powder of *P. nigrum* and *C. cyminum* could successfully be utilized against *C. chinensis* infesting green gram under storage.

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DIVERSITY OF NATURAL ENEMIES ASSOCIATED WITH LAC INSECT *KERRIA LACCA* (KERR) IN PUNJAB

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ABSTRACT

A survey was conducted to record the diversity of lac insect *Kerria lacca* (Kerr) associated natural enemies (predators/ parasitoids) during 2019-2020. In katki crop, the abundance of natural enemies ranged from 1.08 to 44.09%. There were three parasitoids viz, *Parechthrodryinus clavicornis* (Cameron) (1.08%), *Aprostocteus purpureus* (Cameron) (26.88%), *Tachardiaephagus tachardiae somervilli* (2.15%); and two predators; *Chrysoperla zastrowi sillemi* (5.38%), *Eublemma amabilis* (44.09%) and only one hyperparasitoid viz; *Bracon greeni* (Ashm.) (20.43%). Shannon-Weiner diversity index, evenness index and dominance index for natural enemies was found to be 0.67, 0.80 and 0.20. In baisakhi crop, the abundance ranged from 6.56 to 45.90% with maximum abundance of predator *E. amabilis* (45.90%) and the least of parasitoid *T. tachardiae* How. (6.56%); and the Shannon-Weiner diversity index, evenness index and dominance index was 0.65, 0.77 and 0.23, respectively.

Key words: *Kerria lacca*, baisakhi, katki, parasitoids, predators, diversity, abundance, evenness, dominance, species diversity, parasitism

Kerria lacca (Kerr) is a beneficial soft-bodied lac insect, known to produce lac of recoverable and commercial value (Kumar and Bhatnagar, 2013), and India is the largest producer with 21,008 tons of annual production Yogi et al., 2015). It is exported earning annually $ 16 to 22 million as foreign exchange (Prasad et al., 2004; Shah et al., 2015). Lac cultivation plays a major role in the up-liftment of economic status of tribal people (Kumar, 2002), giving employment to marginal, small as well as big farmers (Pal, 2009). Lac insect is prone to natural enemies (insect parasitoids and predators), causing considerable loss to the lac yield (Singh et al., 2011a); and 22 species of lac insect predators, 30 species of primary parasitoids, 45 species of secondary parasitoids are known (Das, 1990) and several fungal pathogens of lac insects as well as lac hosts (Sharma et al., 2006). Among predators, *Chrysopa* spp. from order Neuroptera (Singh et al., 2011b), and *Eublemma amabilis* (Moore) and *Pseudohypatopa pulverea* (Meyr) from order Lepidoptera (Jaiswal et al., 2008 and Singh et al., 2009) are the most potential. Parasitoids belonging to family Braconidae, Aphelinidae, Encyrtidae, Eulophidae, Eupelmidae and Pteromalidae parasitise lac insects (Sharma et al., 1997); and parasitization varies between 15.5% in summer (baisakhi crop) to 18.6% in rainy season crop (katki crop) of rangeeni strain; it is 19.04% for kusumi strain in winter crop (aghani crop) and 22.8% in summer crop (jethwi crop) (Kumari et al., 2012). Earlier, Punjab contributed significantly in lac cultivation, but later it was abandoned (Sharma et al., 2006); and scope of lac cultivation in Punjab has been indicated by Sangha et al. (2019). However, information on the diversity of lac insect associated parasitoids and predators from the state is lacking. Therefore, the present study to collect the information regarding lac insects and diversity of its natural enemies from different agroclimatic zones of Punjab.

MATERIALS AND METHODS

Studies on the insect fauna associated with lac insect were carried out during katki and baisakhi crop during 2019 and 2020. The samples (brood sticks and lac sticks) were collected from the three agroclimatic zones: 1) Central zone: Amritsar, Taran Taran, Kapurthala, Jalandhar, Ludhiana, Fatehgarh Sahib, Patiala and Moga; 2) Submontaneous zone: Roop Nagar, Hoshiarpur, Pathankot, Shaheed Bhagat Singh Nagar, Gurdaspur, SAS Nagar and Chandigarh; 3) Arid Irrigated zone: Bathinda, Mansa, Sri Muktsar Sahib, Faridkot, Ferozepur and Sangrur. These regions were surveyed to record the population status of lac insect and their host plants. GPS coordinates of each location were also recorded and data on insect predators on insect infested plants by traditional methods viz., hand net, hand picking, stem beating etc.

The lac insect infested branches (brood sticks) of the plants were collected randomly, in five replications, and these samples were kept in specially designed bioagent collection cages (20x 20x 30 cm) fitted with glass tubes (ICAR- IINRG). These cages were monitored twice a week to record the emergence of parasitoids and predators from broodlac sticks. The samples of parasitoids and predators were preserved in ethyl alcohol, before getting identified from ICAR- IINRG, Ranchi, India. The lac encrusted sticks were observed visually for the presence of predators and numbers recorded. To check the diversity or dominance of individual parasitoids and predators collected, the data on number of individuals were subjected to computation of Shannon and Weiner diversity index (1963), Pielou evenness index (1966) and Southwood dominance index (1978).

RESULTS AND DISCUSSION

Number of insect parasitoids and predators belonging to order Hymenoptera, Lepidoptera and Neuroptera were observed during the study. The numerical abundance of parasitoids and predators belonging to these was: Hymenoptera (50.54%)> Lepidoptera (44.09%) > Neuroptera (5.38%). In katki crop, the abundance of three parasitoids namely *Aprostocteus purpureus* (Cam.) (Hymenoptera: Eulophidae) (26.88%), *Tachardiaephagus tachardiae somervilli* (Mahd) (Hymenoptera: Encyrtidae) (2.15%), and *Parechthrodryinus clavicornis* (Cam.) (Hymenoptera: Encyrtidae) (1.08%) was observed. One species of hyperparasitoid, namely *Bracon greeni* (Ashm.) (Hymenoptera: Braconidae) (20.43%) was also seen. Among the predators, two species, namely *Eublemma amabilis* Moore (44.09%), *Chrysoperla zastrowi sillemi* (5.38%) belonging to order Lepidoptera (Family: Noctuidae) and Neuroptera (Family: Chrysopidae), respectively was recorded. The abundance of these ranged from 1.08 to 44.09%, of which the maximum abundance was of *E. amabilis* (44.09%) and the least of *P. clavicornis* (1.08%) (Table 1). In baisakhi crop, maximum number of natural enemies belonged to Hymenoptera (54.10%), followed by Lepidoptera (45.90%); there were three parasitoids- *T. tachardiae* How. (Hymenoptera: Encyrtidae) (6.56%), *P. clavicornis* Alam. (8.20%) and *Aprostocetus tachardiae* Cam. (8.20%); one species of hyperparasitoid *B. greeni* (Hymenoptera: Braconidae) (31.15%); one species of predator *E. amabilis* (Lepidoptera: Noctuidae) (45.90%) were also observed. The abundance of these ranged from 3.08 to 43.08%, with maximum being of *E. amabilis* (45.90%) and the least of *T. tachardiae* (6.56%) (Table 1).

The data related to diversity indices shown in Table 1 revealed even distribution of all species of natural enemies. The distribution and dominance observed with Shannon Weiners index (H'), evenness index (J) and dominance index (D) with katki (0.58, 0.74, and 0.26, respectively) and baisakhi crops (0.65, 0.77 and 0.23) confirmed these. Lac insect is immobile and after settlement it becomes

Table 1. Natural enemies associated with lac insect and their diversity in Punjab

S. No.	Name	Predator/ parasitoid	Family	Order	% abundance
		Katki (2019)			
1.	*Eublemma amabilis* (Moore)	Predator	Noctuidae	Lepidoptera	44.09
2.	*Aprostocteus purpureus* (Cam.)	Parasitoid	Eulophidae	Hymenoptera	26.88
3.	*Bracon greeni* (Ashm.)	Hyper parasitoid	Braconidae	Hymenoptera	20.43
4.	*Chrysopa zastrowi sillemi* (Esben-peterson)	Predator	Chrysopidae	Neuropteran	5.38
5.	*Tachardiaephagus tachardiae somervilli* (How.)	Parasitoid	Encyrtidae	Hymenoptera	2.15
6.	*Parechthrodryinus clavicornis* (Cam.)	Parasitoid	Encyrtidae	Hymenoptera	1.08
		Baisakhi (2019-20)			
7.	*Eublemma amabilis* (Moore)	Predator	Noctuidae	Lepidoptera	45.90
8.	*Bracon greeni* (Ashm.)	Hyper parasitoid	Braconidae	Hymenoptera	31.15
9.	*Tyndarichus clavicornis* (Alam)	Parasitoid	Encyrtidae	Hymenoptera	8.20
10.	*Aprostocetus tachardiae* (Cam.)	Parasitoid	Eulophidae	Hymenoptera	8.20
11.	*Tachardiaephagus tachardiae somervilli* (How.)	Parasitoid	Encyrtidae	Hymenoptera	6.56

Diversity indices

Crop	Shannon Weiners index (H')	Pielou evenness index (J)	Southwood dominance index (D)
Katki	0.58	0.74	0.26
Baisakhi	0.65	0.77	0.23
Overall	0.60	0.77	0.23

susceptible to many natural enemies, and about 35-40% damage is usually caused. Many reports document the natural enemies observed now. Sharma et al. (1997) reported 14 species of parasitoids, out of which *A. purpureus* (55.82%) and *T. tachardiae* (28.37%) are the most abundant. Among beneficial fauna, 5.37% relative abundance of *B. greeni* as hyperparasitoid had been reported. Jaiswal and Saha (1995) documented predator- *E. amabilis*; parasitoid- *T. tachardiae*, *Eoccophagus tschirchii* Mahd. and *P. clavicornis* and hyperparasitoid- *B. greeni* and *Elasmus claripennis* Cam. as being dominant in harvested lac. Bhattacharya and Yogi (2015) reviewed 72 insect parasitoids and predators, and according to them, *E. amabilis*, *T. tachardiae* and *A. purpureus* are the most abundant.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization and designing of the research work by R Kaur, Shera P S and S Sharma, Execution of field/lab experiments and data collection, analysis of data and preparation of manuscript carried out by A Singh, A Thakur and H K Mangat. The identification of natural enemies had been carried out by A Mohansundaram. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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A RAPID AND EASY BIOASSAY METHOD FOR STINGLESS BEES *TETRAGONULA TRAVANCORICA* SHANAS AND FASEEH

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ABSTRACT

An attempt was made to develop an effective bioassay method for toxicological studies for the stingless bee, *Tetragonula travancorica* Shanas and Faseeh. The developed bioassay employed stingless bees sampled from the forest area with no history of past insecticide exposure and vegetable ecosystem having frequent insecticide exposure with insecticides viz., chlorantraniliprole and thiamethoxam. A residual film bioassay using pre-treated conical flasks and transferring the bees to the treated flasks in the field itself was easy and less time-consuming. Stingless bees survived for 12 hours in the control flasks with the bioassay method III. Whereas mortality was significantly low in bioassay method I and II within 3h and 6h, respectively. Chlorantraniliprole was less toxic (LC_{50} – 10.98 ppm to 17.30 ppm) to stingless bees than thiamethoxam (LC_{50} – 0.30 ppm to 0.78 ppm). Thiamethoxam was highly toxic (LC_{50} – 0.30 ppm to 0.37 ppm) to bees from the forest ecosystem, whereas bees in the vegetable ecosystem were more tolerant (LC_{50} – 0.67 ppm to 0.78 ppm). The findings may help carry out ecotoxicology and risk assessment studies in stingless bees, a key pollinator of many crops, more rapidly and easily.

Key words: *Tetragonula travancorica*, chlorantraniliprole, thiamethoxam, bioassay, residue film, vegetables, forest, toxicity, stingless bee, ecotoxicology, risk assessment

Plants need pollination to reproduce, and many cross-pollinated crops rely on bees as pollinators (Potts et al., 2010). Bees account for one out of every three bites of food we consume (MSU Extension, 2021). Bees visit >90% of the world's most significant crop types, and pollinate around 70% of all cultivated plant species (Ricketts et al., 2008; Slaa et al., 2006). Stingless bees (Meliponini) are increasingly being recognised as viable commercial pollinators with their remarked biological traits that make them suitable for supervised pollination. The genus *Trigona* with more than 130 species, is the largest and most widespread among stingless bees (Michener, 2013). Chlorantraniliprole and thiamethoxam are the widely used new-generation insecticides in vegetables against lepidopteran and sucking pests, respectively. Chlorantraniliprole is a novel anthranilic diamide insecticide that acts as a selective ryanodine receptor agonist and thiamethoxam is a neonicotinoid that acts on postsynaptic nicotinic acetylcholine receptors (nAChRs) (Ihara and Matsuda, 2018). The recent decline in pollinator diversity and abundance due to unsustainable agricultural landscapes and excessive pesticide usage has prompted concerns about pollination services' long-term viability (Dively and Kamel, 2012). So diversifying crop pollinators with stingless bees can improve pollination services. Despite this, research in ecotoxicology and risk assessment of insecticides, stingless bees receive little consideration (Tomé et al., 2015). There is a scarcity of information on toxicological guidelines for stingless bees, thus research to establish the effects of insecticides on these bees is clearly needed (Boyle et al., 2019; Padilha et al., 2020). In this context, the impact of two insecticides, chlorantraniliprole, and thiamethoxam, was tested on the stingless bee, *Tetragonula travancorica* Shanas and Faseeh, collected from the forest and vegetable ecosystem using a simple and quick residual film bioassay method.

MATERIALS AND METHODS

The study for comparing insecticide tolerance of stingless bees on exposure was carried out in the College of Agriculture, Kerala Agricultural University, Vellanikkara during 2018 to 2021. Three bioassay methods were carried out with stingless bees collected from a feral colony in Kerala Agricultural University campus (N 10°32'.49.7544''; E 76°16'57.20412''), with no history of past insecticide exposure and a domesticated bee hive maintained at the vegetable ecosystem of Krishi Vigyan Kendra, Thrissur, (N 10°32'.48.79428''; E 76°16'4.70064'') with frequent insecticide exposure.

Bees collected were identified morphologically using taxonomic keys as *Teragonula trvancorica* followed by barcoding and sequences deposition in GenBank with submission ID - SUB10957255 and accession number OM293512. Technical grade analytical standards of chlorantraniliprole and thiamethoxam from Sigma-Aldrich were used to prepare the stock solution with acetone as solvent. For the initial broad range bioassay, concentrations viz., 10, 5, 1 ppm chlorantraniliprole and 50, 10, 5, 1 ppm for thiamethoxam were taken from the stock. From the wide range bioassay, the insecticide concentrations for a narrow range were selected. Three different bioassay methods were compared to find out the most effective one in a completely randomized design of six replications in 250 ml conical flasks. The residue film method was adopted for determining contact toxicity in stingless bees. From each insecticide concentration, 2 ml was taken using a micropipette and transferred to conical flasks followed by rotating the flasks so as to evenly coat the insecticide as a thin film without a runoff on the walls of flasks. The flasks were then allowed to air dry for two hours and the mouth of conical flasks was covered using a white muslin cloth. Acetone (2 ml) was used as a control in all bioassay methods.

In the first method, stingless bees collected in plastic bottles from the feral colony and vegetable ecosystem colony were brought to the lab and transferred to each conical flask of various concentrations with six replications two hours after impregnating insecticides. The second bioassay method was according to Botina et al. (2020), with slight modifications in which stingless bee workers collected at the hive entrance using glass bottles were brought to the laboratory immediately and anaesthetized in a -20 ˚C deep freezer for 10 minutes. The duration of ten minutes in the deep freezer was standardized after exposing the bees for 1, 3, 5, 7, and 10 minutes. Ten minutes was the appropriate time where the bees got anaesthetized completely. Five anaesthetized bees were transferred to each conical flask using a camel hairbrush. In the third bioassay method, pre-treated 250 ml conical flasks were carried to the field, and bees were collected directly from the hive entrance to the treated flasks at a rate of 5 bees per flask two hours after treatment and bees were then returned to the laboratory. In all three bioassay methods, a cotton ball dipped in 10 % honey solution was hung in the conical flask as food for the bees. Mortality of bees was recorded at 3, 6, 9 and 12h after insecticide exposure and mortality data were analysed using POLO PLUS software. The survival % of bees in the control was observed for all three methods after 3, 6, 9 and 12h to assess their efficiency. The data on the survival of bees was analysed as a Completely Randomized Block design using GRAPES software (Gopinath et al., 2020).

RESULTS AND DISCUSSION

The % survival of stingless bees *T. travancorica* in control was only 13.33% after 3 hr in bioassay method I, and complete mortality was observed subsequently in both feral and vegetable ecosystem-based colonies. In the bioassay method II, the % survival of bees for the feral colony was 90 % after 3 hr which gradually decreased to 46.67, 26.67, 6.67 % by 6, 9, 12 hr, respectively; for those bees from the vegetable ecosystem, it was 86.67% which decreased to 10.00% by 12 hr. The bees from both the colonies survived in bioassay method III for 12 hr, and remained active even for 48 hr in the flasks (Table 1). On comparing the three bioassay methods, method I was significantly inferior. The bees carried to the laboratory in plastic bottles from the feral and vegetable ecosystem could not live longer. Moreover, transferring active bees from collected plastic bottles to each treated flask @ 5 bees/ flask was difficult, time-consuming, and damaged the delicate bees. Oliveira et al. (2012) also recorded low activity followed by the death of stingless bees in plastic containers because of excessive moisture inside.

Table 1. Comparison of % survival of stingless bees in control under three bioassay methods

Method	3 h		6 h		9 h		12 h	
	Feral	Vegetable ecosystem	Feral	Vegetable ecosystem	Feral	Vegetable ecosystem	Feral	Vegetable ecosystem
Bioassay I	13.33^{b} (21.85)	13.33^{b} (21.85)	0.00^{c} (12.92)	0.00^{c} (12.92)	0.00^{c} (12.92)	0.00^{c} (12.92)	0.00^{c} (12.92)	0.00^{c} (12.92)
Bioassay II	90.00^{a} (70.26)	86.67^{a} (67.98)	46.67^{b} (42.73)	33.33^{b} (34.82)	26.67^{b} (30.62)	13.33^{b} (21.85)	6.67^{b} (17.31)	10.00^{b} (19.58)
Bioassay III	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)	100.00^{a} (77.08)

*Figures in parentheses denote arc sign transformed values

Bioassay method II and III did not differ significantly for the first three hours in both feral and vegetable ecosystem-based colonies. However, after 6, 9 and 12 hr, bioassay method III was significantly superior over method II, which caused freezing injury to the bees while exposing the bees at -20° C for 10 min before transferring the collected bees to pretreated conical flasks. Botina et al. (2020) used plastic cups for bioassay, whereas this study used glass bottles. The higher exposure of 10 min used now was due to the use of glass bottles for the bioassay, as the glass bottles took more time to cool than plastic ones. Once anaesthetized, transferring the bees to the insecticide coated flasks damaged the bees as they were sticking to the camel hairbrush. As % of bees survived in the control flask after 3 hr decreased drastically in bioassay II, it could be utilized only for the bioassay of those insecticides with very high contact toxicity. Therefore, bioassay method III was the most appropriate, as errors were negligible. The survival of bees for 48 hr would enable bioassay for insecticides with weak contact toxicity. Moreover, the chances of injury during the transfer of bees to insecticide coated flasks could be avoided in this method.

After 3 hr of exposure, the LC_{50} values of chlorantraniliprole and thiamethoxam in bioassay methods II and III showed that the LC_{50} of chlorantraniliprole was 10.98 and 12.53 ppm, respectively, with bioassay methods II and III for the feral colony (Table 2). The LC_{50} values of vegetable ecosystem-based colony with chlorantraniliprole was 13.30 and 17.30 ppm, respectively. The fiducial limits for the bioassay methods II and III overlap for the feral colony and vegetable ecosystem-based colony, indicating the comparative efficacy of bioassay method III with the already reported bioassay method II. With the neonicotinoid thiamethoxam, the LC_{50} values were 0.30 and 0.37 ppm with the feral colony and 0.67 and 0.78 with the vegetable ecosystem-based colony in bioassay methods II and III, respectively. As with chlorantraniliprole, the bioassay methods had comparable fiducial limits with thiamethoxam too, with bees from both the colonies. In the vegetable ecosystem, two to three sprays of chlorantraniliprole and thiamethoxam were given. The susceptibility of stingless bees from feral and vegetable ecosystem colonies to chlorantraniliprole were comparable. In contrast, bees from the feral colony were more susceptible to thiamethoxam than the bees from the vegetable ecosystem- based colony; and frequent exposure of these to thiamethoxam could be the reason for their lower susceptibility. This indicates that stingless bees exhibited tolerance to thiamethoxam even with two to three sprays.

With the bioassay method III and at a concentration of 3 ppm, the mortality of bees from vegetable ecosystem colony was 96.67% with thiamethoxam, and it was only 10% with chlorantraniliprole. The exposure of bees from feral colony to 1 ppm thiamethoxam resulted in complete mortality. But, with chlorantraniliprole, the mortality was only 33.33% at 10 ppm, and at 30 ppm, the mortality was 96.67%. Thus, chlorantraniliprole is less toxic to *T. tranvancorica* compared to thiamethoxam. Tomé et al. (2015) also reported a low risk of chlorantraniliprole with insignificant mortality to stingless bees compared to neonicotinoids. According to Williams et al. (2020), 72 hr of exposure of *Apis mellifera* to chlorantraniliprole was not acutely hazardous to honey bees. Thiamethoxam is reported to be highly toxic to various other stingless bees such as

Table 2. Susceptibility of *T. travancorica* to chlorantraniliprole and thiamethoxam (bioassay method II and III)

Colony type	Treatment	Slope		Chi- square		LC_{50} (ppm)		95 % Fiducial limit	
		Bioassay method II	Bioassay method III	Bioassay method II	Bioassay method III	Bioassay method II	Bioassay method III	Bioassay method II	Bioassay method III
Feral	Chlorantraniliprole	4.53	4.83	2.81	0.13	10.98	12.53	9.16-13.48	10.95-15.14
	Thiamethoxam	3.88	3.06	3.72	3.90	0.30	0.37	0.09-0.42	0.23-0.53
Vegetable ecosystem	Chlorantraniliprole	2.29	1.61	0.58	0.35	13.30	17.30	9.6-50.53	11.07-63.31
	Thiamethoxam	3.12	3.06	1.92	0.49	0.67	0.78	0.48-0.84	0.64-0.92

*Based on 3 hr exposure

Scaptotrigona bipunctata (Moreira et al., 2018) and *T. angustula* (Jacob et al., 2019). The sensitivity ratio, R (LC_{50}a/LC_{50}s) between *A. mellifera* (a) and stingless bee species (s) calculated by Arena and Sgolastra (2014) compared the sensitivity of the two bee species to pesticides. Stingless bees had a sensitivity ratio >1, indicating the higher sensitivity of stingless bees to thiamethoxam than *A. mellifera*. The acute oral LC_{50} for thiamethoxam was 0.227 ng a.i./ µl (ppm) in laboratory tests with female workers of *A. mellifera* and 0.0543 ng a.i./ µl (ppm) with stingless bee, *Melipona scutellaris* at 24 hr (Miotelo et al., 2021). These findings support the assertion that stingless bees are more sensitive to thiamethoxam than honey bees. Bee depletion is causing increasing public and political worry around the world. The loss of habitat and food supplies, pesticide exposure and climate change contribute to bee decline. The newly standardised bioassay method can help carry out ecotoxicology assays of *T. travancorica* more easily and effectively.

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EFFICACY OF INSECTICIDE BASED SEED TREATMENTS WITH NEEM SEED EXTRACT SPRAYS AGAINST SORGHUM SHOOT FLY *ATHERIGONA SOCCATA* IN SORGHUM

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ABSTRACT

This study was carried out under field conditions at the Sorghum Research Unit, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola during kharif 2020 to ascertain the effect of neem seed extract sprays along with seed treatments against sorghum shoot fly *Atherigona soccata* (Rondani). The counts of deadhearts in % at 21 and 28 days after emergence (DAE) varied significantly with the treatment schedule- least values of 13.15 and 15.22%, respectively were observed in seed treatment with imidacloprid 48FS @ 12 ml/ kg seed followed by quinalphos 25EC @ 2 ml/ l water spray 15 DAE. The next best treatments were viz., seed treatment with imidacloprid 30FS @ 12 ml/ kg seed with 13.68 and 16.29% deadhearts, respectively; it was followed by seed treatment with thiamethoxam 48FS @ 12 ml/ kg seed. Maximum grain yield (32.33 q/ ha) was observed with seed treatment with imidacloprid 48FS @ 12 ml/ kg followed by quinalphos 25EC @ 2 ml/ l water spray at 15 DAE. Maximum incremental cost and benefit ratio (ICBR) was obtained with seed treatment of thiamethoxam 30FS @ 12 ml/ kg with 1:96.68. Neem seed extract-based treatment schedules viz., NSE 5% spray 7 DAE, treatment schedule NSE 5% spray 7 and 11 DAE, treatment schedule NSE 5% spray 7, 11 and 14 DAE and treatment schedule NSE 5% spray 7 and 18 DAE gave less ICBR values.

Key words: Sorghum, *Atherigona soccata*, seed treatment, foliar spray, neem, azadirachtin, imidacloprid 48FS, thiamethoxam 30FS, quinalphos 25EC, grain yield, cost benefits

Sorghum (*Sorghum bicolour* (L) Monesh) is an important cereal crop (Anonymous, 2014), and in India, its productivity is 1235 kg/ ha (Anonymous, 2020). Maharashtra is one of the major sorghum growing state with a productivity of 1941 kg/ ha (Anonymous, 2018). The sorghum production is hampered due to many factors, of insect pests is one of the major factors lowering productivity of sorghum. In India >150 insect pests are known (Sheshureddy, 1983), and in Maharashtra about 18 important insect pests are reported. Some of these are shoot fly *Atherigona soccata* (Rondani), stem borer *Chillo partellus* and midge fly *Contarina sorghicola.* It is essential to reduce the pesticide use, and use ecofriendly ones like neem seed extract. NSKE @ 5% cow urine-NSKE, imidacloprid 70 WS-NSKE and thiamethoxam 70 WS-NSKE have been reported significantly effective (Kumar et al., 2017). Similarly, NSKE @ 5% and azadirachtin @ 1% both in kharif and rabi were found useful (Sathish et al., 2017). Such organic amendments and botanicals conserved more coccinellids compared to insecticides. This study evaluated neem seed extract in schedule form in kharif sorghum against *A. soccata*.

MATERIALS AND METHODS

The field experiment was carried out at the Sorghum Research Unit, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during kharif 2020, laid out in randomized block design with eight treatments replicated thrice. The plot was size 2.25 x 4.05 m, with seeds of var. PDKV-Kalani (AKSV-181) sown on July 23rd. 5% neem seed kernel extract was prepared following standard procedure. Eight plants/ plot in eight rows each were observed at 12 days after seedling emergence as suggested by Sharma et al. (1997). The deadhearts caused by shoot fly were recorded at 14th, 21st and 28th day after emergence (DAE), and % deadhearts computed. Grain yield/ plot obtained along with fodder yield. The data obtained were converted to appropriate transformations and were subjected to statistical analysis to test the level of significance (Gomez and Gomez, 1983).

RESULTS AND DISCUSSION

Results revealed that treatment schedule of seed treatment, along with first spray at 7 and second at 11,

third at 14 and fourth at 18 DAE was the most effective. Significantly least number of deadhearts (10.74%) were observed with the schedule T5 (imidacloprid 48FS @12 ml/ kg seed), schedule T7 (NSE 5% spray 7 and 18 DAE), T3 (NSE 5% spray 7, 11 and 14 DAE), T1-NSE 5% spray 7 DAE; and T2 (NSE 5% spray 7 and 11 DAE) was at par with untreated control (T8) and thus the least effective. The least deadhearts i.e.13.15% were observed in schedule T4, seed treatment with imidacloprid 48FS @ 12 ml/ kg seed followed with quinalphos 25EC @ 2 ml/ l spray 15 DAE. Similar results were obtained at 28 DAE with T4 (Fig. 1). Thus, seed treatment-based schedules were observed to be the most effective. These results corroborate with those of Aghav et al. (2007), Yadav et al. (2017), Kumar et al. (2018) and Sonalkar et al. (2018). Maximum grain yield i.e. 32.33 q/ ha was obtained with schedule T4 (seed treatment with imidacloprid 48FS @ 12 ml/ kg seed followed by quinalphos 25EC @ 20 ml/ 10l spray 15 DAE. Results with fodder yield gave similar trend (Table 1).

Satish et al. (2017) revealed that higher grain yield was noted with imidacloprid seed treatment. Yadav et al. (2017) and Karabhantanal et al. (2018) also recorded higher grain yield with seed treatment of imidacloprid. Costs of treatment and incremental benefit revealed minimum cost of Rs. 560/ ha in the treatment schedule T5 (imidacloprid 48FS @ 12 ml/ kg seed); and Rs. 36660/ ha was obtained with treatment schedule T4 (seed treatment with imidacloprid 48FS @ 12 ml/ kg seed followed by quinalphos 25EC @ 2 ml/ l spray at

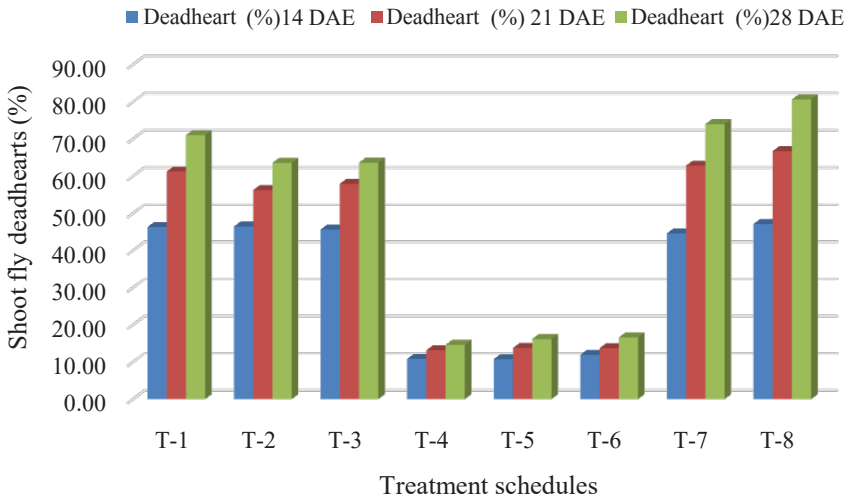


Fig. 1. Effect of treatments on *A. soccata* deadhearts

Table 1. Economics of the treatments and incremental cost benefit ratio (ICBR)

Treatment	Grain Yield increase over control (q/ ha	Fodder yield increase over control (q/ ha)	Income from increased grain yield (Rs/ ha)A	Income from increased fodder yield (Rs/ ha) B	Total income from increased yield (Rs/ ha)	Total cost of the treatment (Rs./ ha) (A+B+C+D+E)	ICBR (I/F)
						F	J
T1	3.41	13.56	5115.00	2712.00	7827.00	2059.00	1:3.80
T2	6.18	23.60	9270.00	4720.00	13990.00	4207.00	1:3.33
T3	6.00	24.08	9000.00	4816.00	13816.00	6429.00	1:2.15
T4	24.44	91.79	36660.00	18358.00	55018.00	1234.00	1:44.26
T5	24.00	90.41	36000.00	18082.00	54082.00	560.00	1:96.58
T6	24.04	90.41	36060.00	18082.00	54142.00	600.00	1:96.68
T7	2.15	9.49	3225.00	1898.00	5123.00	4398.00	1:1.16
T8	0	0	0.00	0	0	0	

Rate of grain sorghum Rs. 1500/ q, rate of fodder sorghum Rs. 200/ q. *NSE 5 kg neem seed for 100 l water + 200 g detergent therefore for 463 l water neem seed required 21.85 kg; cost of kg neem powder Rs. 50/ kg- 21.85 kg = 1092.50; detergent 926 g/ ha @ Rs 150/ kg. Neem powder Rs.50/ kg.

15 DAE). Similar was the case with fodder yield. Thus, gross income from grain and fodder was maximum (Rs. 55018/ ha) with treatment schedule T4 giving more incremental income (yield and fodder) (Table 1). Satish et al. (2017) revealed that higher grain yield was obtained with imidacloprid seed treatment. Yadav et al. (2017) and Karabhantanal et al. (2018) also observed similar increase in grain yield with seed treatment of imidacloprid 70 WS @ 5 gm/ kg.

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EFFICACY OF CERTAIN INSECTICIDES AGAINST SUCKING PESTS AND PHYLLODY IN SESAMUM

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ABSTRCT

A field experiment was conducted at the Agricultural college, Naira during rabi, 2020-21 on the control of major insect pests on sesamum viz., leafhopper *Hishimonus phycitis* (Distant) and whitefly *Bemisia tabaci* (Gennadius) and phyllody disease. The results revealed that *thiamethoxam* 25WG @ 0.25 g/ l was very effective against both the pests with 72.2 and 50.5% reduction in incidence, respectively. Thiamethoxam was also effective against phyllody (PDI of 5.72), and gave a maximum seed yield of 6.50 q/ ha and B:C ratio (1:4.7). Seed treatment with imidacloprid 17.5SL @ 5 ml/ kg seed stood as the next best with B:C ratio of 1:3.7.

Key words: Sesamum, whitefly, leafhopper, phyllody, seed treatment, thiamethoxam, imidacloprid, yield, cost benefits

The sesamum *Sesamum indicum* L. is an oilseed crop and India ranks third in the world with 19.47 lakh ha with a productivity of 470 kg/ ha. (Directorate of Economics and Statistics, Government of India, 2019-2020). The crop is attacked by 38 insect pests (Rai, 1976), among which leafhopper *Hishimonus phycitis* (Distant) and whitefly *Bemisia tabaci* (Gennadius) are the major sucking pests. Both nymphs and adults of these suck the cell sap from leaves, flowers and pods. Due to this curling of leaf margins, stunted growth of the plants occur. Leafhopper is also responsible to transmit phyllody diseases in sesamum (Ahirwar et al., 2010). There is scope of utilizing the certain new insecticide molecules effective in small quantity against these. Keeping this in view, this study evaluates the efficacy of certain insecticides against these.

MATERIALS AND METHODS

The field experiment was conducted at the Agricultural college, Naira (83.56^{0}E, 18.23^{0}N, 27 masl) in the north coastal zone of Andhra Pradesh during rabi, 2020-2021. The experiment was laid out in randomized block design with ten treatments including untreated check, and replicated thrice. Plot size was 20 m^2 with spacing of 30x 10 cm with the variety YLM- 66. The treatments include T_1: Seed treatment with imidacloprid 17.5 SL @ 5ml/ kg seed, T_2: thiamethoxam 25WG @ 0.25g/l, T_3: diafenthiuron 50WP @ 1.6g/l, T_4: pymetrozine 50WG @ 0.6g/ l, T_5: spinosad 45SC @ 0.33 g/ l, T_6: novaluron 10EC @ 1.0 ml/ l, T_7: chlorantraniliprole 18.5SC @ 0.3 ml/ l, T_8: chlorantraniliprole 9.3% + λ cyhalothrin 4.6% @ 0.5ml/ l, T_9: acephate 75 WP @ 1.5 g/ l, T_{10}: untreated check. When the pest reached its respective ETLs level, first spraying was done at 35 days after sowing. Precount of insect pests were recorded one day prior to the spraying, with post-treatment counts on 5th, 10th, 15 days after spraying. Counts on number of pests/ plant were recorded from randomly selected 10 plants from each treatment by excluding border rows. The data on PDI of phyllody was taken 10 days interval after spraying. The data on number of leafhoppers and whiteflies/ plant were subjected to ANOVA. The % reduction in incidence was calculated using modified Abbots formula (Flemming and Ratnakaran 1985,). These data were subjected to ANOVA with arc sine value transformation (Gomez and Gomez, 1984) using OPSTAT 2021 software, V 11.9.08. The PDI and benefit cost ratio were computed using standard methodology.

RESULTS AND DISCUSSSION

The data from Table 1 reveals that the *H. phycitis*

Table 1. Efficacy of insecticides against *H.phycitis* and *B. tabaci* in sesamum (rabi, 2020-21)

S. No	Treatments	Dose (ml or g/l)	No. of leafhoppers/3 leaves/plant					No. of whitefly/3 leaves/plant					
			1 DBS	5 DAS	10 DAS	15 DAS	Overall mean	Reduction Over Control (%)	1 DBS	5 DAS	10 DAS	15 DAS	Overall mean	Reduction over control (%)
1	Seed treatment with Imdiacloprid 17.5SL	5ml / kg seed	1.31 (1.13)	0.98 (0.99)^{bcd}	1.53 (1.24)^{bcdef}	1.68 (1.25)^{b}	1.36 (1.16)^{bd}	52.7%	10.10 (3.17)	10.60 (3.25)^{bcde}	14.50 (3.81)^{defg}	11.33 (3.36)^{b}	13.24 (3.63)^{bc}	20.48%
2	Thiamethoxam 25WG	0.25g/l	1.67 (1.29)	0.43 (0.65)^{a}	0.90 (0.95)^{a}	0.80 (0.89)^{a}	0.80 (0.89)^{a}	72.2%	10.77 (3.28)	5.70 (2.38)^{a}	7.03 (2.65)^{a}	5.80 (2.23)^{a}	8.23 (2.87)^{a}	50.5%
3	Diafenthiuron 50WP	1.6g/l	1.36 (1.16)	1.00 (0.99)^{bcde}	1.26 (1.12)^{abc}	2.20 (1.48)^{be}	1.34 (1.15)^{bc}	53.4%	10.57 (3.24)	12.33 (3.50) ^{bcdefgh}	14.47 (3.80)^{def}	14.00 (3.73)^{bcde}	11.60 (3.40)^{b}	30.3%
4	Pymetrozine 50WG	0.6g/l	1.47 (1.20)	0.97 (0.98)^{b}	1.19 (1.06)^{ab}	1.90 (1.37)^{bc}	1.28 (1.13)^{b}	55.5%	7.27 (2.44)	11.30 (3.32)^{bcde}	12.30 (3.50)^{bcde}	12.93 (3.59)^{bcd}	14.67 (3.82)^{bcdef}	11.8%
5	Spinosad 45SC	0.33g/l	1.37 (1.16)	1.13 (1.05)^{bcdefg}	1.45 (1.20)^{bcd}	1.97 (1.38)^{bcd}	1.51 (1.23)^{bce}	47.5%	10.63 (3.26)	9.43 (3.05)^{bc}	11.07 (3.30)^{bcd}	12.33 (3.51)^{bc}	15.67 (3.96)^{cdefgh}	6%
6	Novoluron 10EC	1.0ml/l	1.33 (1.15)	1.37 (1.15)^{bcdefg}	1.60 (1.24)^{bcdef}	2.63 (1.61)^{cdef}	1.67 (1.29)^{bcdefg}	42.1%	10.50 (3.24)	11.67 (3.40)^{bcdefg}	11.47 (3.37)^{bcd}	15.67 (3.96)^{bcdef}	13.33 (3.64)^{bcd}	20%
7	Chlorantraniliprole 18.5SC	0.3ml/l	1.77 (1.33)	1.17 (1.07) ^{bcdefgh}	1.85 (1.34)^{cdefh}	2.27 (1.50)^{bcdef}	1.93 (1.39)^{defghi}	32.9%	10.37 (3.22)	11.37 (3.33)^{bcdef}	12.03 (3.25)^{b}	17.33 (4.16)^{cdefghij}	13.83 (3.72)^{bcde}	16.9%
8	Chlorantraniliprole 9.3% + λ Cyhalothrin 4.6%	0.5ml /l	1.36 (1.1.7)	1.25 (1.17) ^{bcdefghi}	1.70 (1.30)^{bcdefg}	2.87 (1.65)^{cdefg}	1.78 (1.34) ^{bcdefgh}	38%	11.03 (3.22)	9.27 (3.02)^{b}	10.83 (3.27)^{bc}	16.23 (4.03)^{bcdefgh}	14.67 (3.83)^{bcdefg}	11.8%
9	Acephate 75 WP (standard check)	1.5g/l	1.39 (1.18)	1.03 (1.01)^{bcdef}	1.87 (1.37)^{defghi}	3.07 (1.75)^{efgh}	1.59 (1.26)^{bcdef}	44.7%	11.77 (3.42)	13.67 (3.69)^{defghi}	11.37 (3.36)^{bcd}	16.00 (4.00)^{bcdefg}	14.33 (3.77)^{bcdef}	13.9%
10	Untreated check		1.72 (1.31)	2.80 (1.67)^{j}	3.35 (1.83)^{j}	3.80 (1.95)^{gh}	2.88 (1.68)^{j}		12.50 (3.54)	14.37 (3.77)^{defghij}	15.63 (3.94)^{efgh}	17.07 (4.13)^{cdefghi}	16.65 (4.88)^{i}	
	F test		NS	Sig	Sig.	Sig.	Sig.		NS	Sig	Sig	Sig	Sig	
	SEm±		0.05	0.08	0.08	0.11	0.07		0.27	0.19	0.17	0.25	0.15	
	CD (p= 0.05)		-	0.23	0.24	0.33	0.21		-	0.57	0.51	0.75	0.44	
	CV		7.52	12.42	11.09	12.77	9.17		14.34	10.14	8.67	11.57	6.94	

DBS – Day before spray DAS –Days after spraying; NS- Non significant Sig- Significant,*Values in parentheses square root transformed values; Values with same alphabet in each column do not vary significantly from each other.

Table 2. Efficacy of insecticides against sesamum phyllody and benefit cost ratio rabi, 2020-21

S. No.	Treatments	Mean % Disease Index (PDI)		Yield (q/ha)	Increase in yield over untreated check (q/ha)	Cost of insecticides (Rs/ha)	Total cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C Ratio
		Pre spraying	10 days after 1st spray							
1	Seed treatment with Imdiacloprid 17.5SL 5ml / kg seed	15.07 (22.82)	36.90 (37.4)^{hi}	4.80	1.30	350	7850	36000	28150	1:3.7
2	Thiamethoxam 25WG 0.25g/ l	20.73 (27.65)	5.72 (13.82)^{a}	6.50	3.00	1010	8510	48750	40240	1:4.7
3	Diafenthiuron 50WP 1.6g/ l	18.67 (25.57)	34.67 (36.04)^{h}	4.35	0.85	2500	10000	32625	22625	1:2.2
4	Pymetrozine 50WG 0.6g/ l	17.80 (24.90)	10.60 (18.92)^{b}	5.50	2.00	1500	9000	41250	32250	1:3.5
5	Spinosad 45SC 0.33g/ l	16.50 (23.92)	14.70 (22.47)^{bcde}	5.20	1.70	6500	14000	39000	25000	1:1.7
6	Novoluron 10EC 1.0ml/ l	16.60 (23.94)	10.73 (19.07)^{bcd}	4.90	1.40	1100	8600	36750	28150	1:3.2
7	Chlorantraniliprole 18.5SC 0.3ml/ l	15.47 (23.00)	11.60 (19.06)^{bc}	5.70	2.20	4500	12000	42750	30750	1:2.5
8	Chlorantraniliprole 9.3%+λ Cyhalothrin 4.6% 0.5ml / l	13.87 (21.77)	19.63 (26.30)^{efg}	4.29	0.79	1940	9440	32175	22735	1:2.4
9	Acephate 75 WP (standard check) 1.5g/ l	15.80 (23.55)	18.83 (25.33)^{ef}	4.18	0.68	600	8100	31350	23250	1:2.8
10	Untreated check	20.17 (26.55)	44.23 (41.08)^{i}	3.50		-	7000	26,250	19,250	1:3.0
	F test	NS	Sig.							
	SEm±	1.27	1.37							
	CD (p= 0.05)	-	4.10							
	CV%	9.07	9.06							

NS- Non-significant, Figures in parentheses are angular or arc-sine transformed values; Means followed by same letter do not differ significantly following DMRT
Minimum Support Prize (MSP) for Sesamum @ 75/ kg.

incidence ranged from 1.31 to 1.72/ plant before treatment and the difference between treatments were non-significant. After the first spray all the treatments were significantly superior over untreated check, of which thiamethoxam 25WG @ 0.25g/ l resulted in significantly less incidence (0.80/ plant) with 72.2% reduction. Pymetrozine 50WG @ 0.6ml/ l, diafenthiuron 50WP @ 1.6 g/l and seed treatment with imidacloprid 17.5SL @ 5ml/ kg seed were the next best and on par with each other. With *B. tabaci* incidence before treatment counts ranged from 10.10 to 12.50/ plant with insignificant variations. But with *thiamethoxam* 25WG @ 0.25 g/ l the incidence reduced to 8.23/ plant, with 50.5% reduction followed by diafenthiuron 50WP @ 1.6 g/ l (11.60/ plant) with 30.3% reduction. The next best treatments were seed treatment with imidacloprid 17.5SL @ 5ml/ kg seed, novaluron 10EC @ 1.0ml/ l, chlorantraniliprole 18.5 SC @ 0.3 ml/ l. These observations on the efficacy of insecticides against *H. phycitis* corroborates with those of Saradava (2004) on sucking pests infesting groundnut under dry farming conditions. Imidacloprid 0.005% and thiamethoxam 0.05 % were the most effective against sucking pests like leafhoppers, aphids and whiteflies of okra. Sharma and Lal (2002) observed that thiamethoxam @ 25 g a.i./ ha is effective against whiteflies in brinjal. Mhaske and Mote (2005) also found imidacloprid 22.5 g/ ha and thiamethoxam 50 g/ ha as effective against whiteflies in brinjal. Naik et al. (2009) also found that thiamethoxam @ 0.005% was the most effective and significantly superior in brinjal. As regards phyllody incidence, it ranged from 15.07 to 20.17 before treatment, and the mean PDI values at 10 days after 1st spray was from 5.72 to 44.23; thiamethoxam 25WG @ 0.25 g/ l (5.72) resulted in 87% reduction in PDI, and pymetrozine 50WG @ 0.6 ml/ l and novaluron 10EC @ 1.0ml/ l were the next best (Table 2). Maximum B:C ratio of 1: 4.7 was obtained with thiamethoxam 25WG @ 0.25 g/ l with maximum net returns of Rs 40240/ ha (Table 2). Seed treatment with imidacloprid 17.5SL @ 5ml/ kg seed, pymetrozine 50WG @ 0.6ml/l and novaluron 10EC @ 1.0ml/l were the next best.

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AUTHOR CONTRIBUTION STATEMENT

Divya conducted the original experiment and wrote the original manuscript and done the data analysis. Chalm and Govindarao contributed the editing, conceptualization, visualization and supervision during the writing of manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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BIONOMICS OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J E SMITH) ON MAIZE

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ABSTRACT

The fall army worm *Spodoptera frugiperda* (J E Smith) is an invasive pest in India on maize crop, and noticed to be polyphagous. Its biology was studied under laboratory conditions at the Department of Agricultural Entomology, College of Agriculture, GKVK, Bengaluru, Karnataka, India. The incubation, total larval and pupal period were observed to be of 2.80, 15.70 and 10.04 days, respectively. The total lifecycle of male and female took 35.05 and 38.30 days, respectively. Preoviposition, oviposition and post-oviposition period were 2.95, 3.10 and 4.50 days, respectively.

Key words: *Spodoptera frugiperda*, bionomics, invasive, incubation period, larval period, pupal period, longevity, total life cycle, instars, oviposition period, post-oviposition period

The fall army worm *Spodoptera frugiperda* (J. E. Smith) (Noctuidae: Lepidoptera), is a new invasive species in India and known for polyphagous behaviour (Montezano et al., 2018). It is native to the Americas and found in most parts of the Western Hemisphere. Of late, the pest was noticed in West and East Africa. Currently, this pest is damaging crops in over 20 African countries (Goergen et al., 2016; Abrahams et al., 2017; Cock et al., 2017). In Brazil and United States, the pest has been reported to causes 32-60% reduction in yield (Hruska and Glandstone, 1988; Wiseman and Isenhour, 1993; Lima et al., 2010). In India, a quick roving survey at Karnataka suggested that pest can cause the damage ranging from 9 to 62.5% on maize (Ganiger et al., 2018; Shylesha et al., 2018). It has been reported for the first time in Karnataka state, India on maize (Ganiger et al., 2018; Sharanabasappa et al., 2018). Subsequently, it has been reported from other maize growing states viz., Tamil Nadu, Andhra Pradesh, Telangana, Maharashtra, Gujrat and northeastern states (Swamy et al., 2018; Srikanth et al., 2018; Chormule et al., 2019). This pest is occurring in serious proportions, causing significant damage to maize. It is important to know the biology and feeding behaviour of this. The earlier reports on the bionomics on artificial diet include Nagamandla and Uma (2019). In the present study, the bionomics are studied on its natural host maize under laboratory conditions.

MATERIALS AND METHODS

The initial culture of *S. frugiperda* was collected from the maize fields near College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka. The field collected larvae were reared on maize leaves placed in plastic rearing boxes and fresh maize leaf bits were provided every day till the end of larval stage in laboratory (27±2ºC, 60-75%RH, L12:D12 photoperiod). The grown-up larvae were transferred to the cages containing a layer of coco-peat as substrate for pupation. The pupae were collected and placed in an oviposition cage for adult emergence. The moths on emergence were provided with young maize seedlings as substrate for the oviposition and cotton soaked in 10% sugar solution as their food. Ten pairs of adults were maintained separately in ovipositional cages to record the fecundity. Egg masses were collected every day and individual larval rearing (n=25) was continued using maize leaf bits as feeding substrate till pupation. The observations on life stages viz., egg, larva, pupa and adults were recorded daily. The parameters like preoviposition period, oviposition period, post-oviposition period, egg period, larval period (instars-wise), pupal period, adult longevity (Male and Female) and total length of life cycle in days were observed.

RESULTS AND DISCUSSION

Egg: The mated females laid eggs in masses of 80 to 250 on both surfaces of leaves, whorls and at the base of the young seedlings (Fig. 1). Freshly laid egg masses were light green in colour covered with greyish scales. A single gravid female was noticed to lay 3 to 4

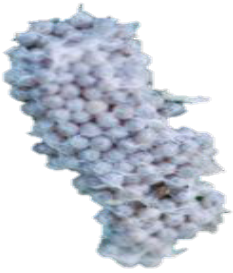


Fig. 1. Egg mass



Fig. 2. 1st Instar larva



Fig. 3. 2nd Instar larva



Fig. 4. 3rd Instar larva



Fig. 5. 4th Instar larva



Fig. 6. 5th Instar larva



Fig. 7. 6th Instar larva



Fig. 8. Pupae



Fig. 9. Male Adult



Fig. 10. Female Adult

egg masses with overall fecundity of 780 to 1200 eggs per female. A mean incubation period of 2.80 days was observed. The observations are similar as reported earlier (Luginbill, 1928; Sparks, 1979; Sharanabasappa et al., 2018). Larva: There were six instars in one life cycle. First instars were greenish in colour with black head and seen in congregation when they hatched (Fig. 2). The second instar larvae were turned to light brownish colour with series of black spots on the body (Fig. 3). Third instar larvae were brown in colour with well-developed head capsule and developing hair like setae all over the body (Fig. 4). Fourth instar larvae were greenish brown with prominent black spots and well-developed cuticular setae all over the body (Fig. 5). Later instars were dark brown with three whitish dorsal lines. Head with well-developed inverted-Y suture was noticed. On eight abdominal segments the black spots were arranged in square manner, but were arranged in trapezoidal manner on remaining abdominal segments (Fig. 6, 7). Larval period was completed on an average of 15.70 days and same was observed to differ depending on the rearing temperature. The similar morphological observations and larval durations were recorded in the earlier reports (Sharanabasappa et al., 2018; Ganiger et al., 2018; Deole and Paul, 2018).

Pupa: Before going for pupation larvae stops feeding. Initially pupae were light green in colour, later turned to dark brown colour with well-developed cremaster at anal end (Fig. 8). The mean pupal period of 10.04 days were recorded. Similar pupal characters with pupal period ranging from 6 to 14 days were recorded (Deole and Paul, 2018; FAO, 2018). Contrarily, varying pupal period of 8.54 to 9.70 days was observed depending on host plant larvae fed (Silva et al., 2017). Adult: The adults of *S. frugiperda* are varying in their morphology. Male adult moths with greyish brown body, light brown coloured fore wing with whitish triangular patch at apical margin and distinct circular spot at the centre (Fig. 9). Adult female with uniform greyish brown forewing without any distinct markings on wing (Fig. 10). Hind wings of both the sexes were whitish with brown border. The longevity of male adult was 7.90 days with a total length of life cycle 35.05 days. But female adults were survived longer compared to male adults with longevity of 11.20 days and total length of life cycle was 38.30 days. The pre-oviposition, oviposition and post-oviposition periods of 2.95, 3.10 and 4.50 days were observed. The similar observations on adult male and female morphology were made by Ganiger et al. (2018) during their report of occurrence of fall armyworm in Karnataka, India. Adult insect were recorded to have longevity of 2 to 3 weeks (FAO, 2018). Contrarily, Deole and Paul (2018) reported adult longevity of 5 to 7 days, the variations may be attributed to feeding of adult insects with sugar solution, where it enhances longevity of adults.

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BIOLOGY OF LEAF EATING CATERPILLAR *TRILOCHA VARIANS* (WALKER) ON *FICUS MICROCARPA*

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ABSTRACT

The biology of *Trilocha varians* (Walker) was studied on *Ficus microcarpa* L.f. under laboratory conditions in Jodhpur, Rajasthan. The fecundity ranged from 140-200 and incubation period was 3-5 days. It has five larval instars, with total larval and pupal period being 16-22 and 10-15 days, respectively. The adult male and female longevity were 4-7 and 6-12 days, respectively.

Key words: *Trilocha varians*, Bombycidae, Lepidoptera, *Ficus microcarpa*, pest, leaf eating caterpillar, biology, measurements, life stages, fecundity

Leaf eating caterpillar *Trilocha varians* (Bombycidae: Lepidoptera) is one of the major pest of *Ficus* spp. (Naeem-Ullah et al., 2020) and well distributed in Nepal, India, Pakistan, Sri Lanka, Thailand, Taiwan, Java, Malaysia, Southern China, Japan, Philippines and Indonesia (Zolotuhin and Witt, 2009; Gurule, 2013; Chuenban et al., 2017). The major hosts of *T. varians* are *Ficus religiosa*, *F. infectoria*, *F. elastica* and *F. bengalensis* (Kedar et al., 2014). Other host plants are *Artocarpus heterophyllus*, *A. communis*, *A. kamansi*, *F. nitida*, *F. septica*, *F. mclelandi* and *F. caraica* (Navasero et al., 2013; Chu and Wang, 1993). The young larvae feed on leaves, twigs and tender shoots, due to their feeding the leaves appear transparent while the late instars cause complete defoliation of the plants. A review of literature revealed that this is the first severe incidence of *T. varians* feeding on *F. microcarpa* in Rajasthan. Earlier, *T. varians* infesting several species of *Ficus,* has been reported in Haryana, Punjab, parts of Maharashtra, Assam, Karnataka, Tamil Nadu (Kedar et al., 2014, Singh et al., 2017, Gurule and Nikam, 2013; Pathre et al., 2019; Subhasish and Tariang, 2018; Udayagiri, 1988; Rajavel and Shanthi, 2007). Various species of *Ficus* viz., *F. benjamina*, *F. neuda*, *F. panda* and *F. microcarpa,* etc. are planted to enhance the aesthetic value and for landscaping purpose, mainly planted alongside the roads, in many countries. However, maintaining healthy *Ficus* flora has great challenge to gardeners due to sudden outbreak of pests including defoliating insect pests. The present study has been conducted to evaluate the biological parameters of *T. varians* under laboratory conditions.

MATERIALS AND METHODS

Study on biology of leaf eating caterpillar *T. varians* on *Ficus* was conducted during 2020 in the Laboratory of Department of Entomology, Agricultural Research Station, Mandor- Jodhpur, Rajasthan. The fourth and fifth instar larvae were collected from the infested *Ficus* trees planted in the University campus and brought to laboratory for culturing of the pest for further studies. The new and fresh *Ficus* leaves provided as feed and placed into containers containing *T. varians* larvae. On daily basis, left over leaves were changed and replenished with new leaves for larvae feeding. Final instars larvae transformed into pupae and emerged as adults. A pair of adults was placed into rearing cage for egg laying. The eggs were laid by female moth on jar wall and on *Ficus* leaves in the jar. Number of eggs was counted on daily basis and observations were recorded on their colour, size, shape and incubation period. Newly hatched larvae were released singly in petriplates for studying duration of each instar and pupal period. The pre-oviposition, oviposition and post-oviposition periods, fecundity and longevity were studied by keeping the paired moths in glass jars. Measurement of various stages viz., eggs, larval instars, pupae and adults were recorded with the help of digital caliper.

RESULTS AND DISCUSSION

The maximum infestation (10-15%) of *T. varians* was observed from second fortnight of September to first fortnight of November. Early instar larvae feed in cluster on under surface of leaves resulting in white and

papery patches on upper surface of leaves. Later instar larvae got dispersed and fed irregular portions from the leaf margins towards the midribs consuming all the leaves of a branch. When most of the leaves were eaten up, twigs died back and in extreme cases, the plants succumbed to death. Otherwise, the plants recovered and produced new flush. The biological parameters of *T. varians* were studied under laboratory conditions. The results indicated that adult female laid 163.00± 22.63 eggs in long rows touching each other and round flat in shape. The freshly laid eggs were pale yellow in colour while before hatching changed into black colour. These findings are in line with Rajavel and Shanthi (2007) and Daimon et al. (2012). The incubation period of eggs was 4.30± 0.67 days (Table 1). Similarly, Singh and Brar (2016) also observed that eggs hatched within 3-8 days. Egg width and thickness were measured 0.82± 0.18 and 0.49± 0.11 mm, respectively.

There were five larval instars and newly hatched larvae were brown in colour. After 24 hr, the colour of larvae changed to greyish white and remained same in colour up to fourth instar. The fifth instar larvae were dull brown with greyish bands. A caudal horn was present on 8th abdominal segment of each larva. Similar findings were recorded by Daimon et al. (2012) and Singh and Brar (2016). Total larval duration was 18.30± 2.00 days. The body length of 1st, 2nd, 3rd, 4th and 5th larval instars was 2.45± 0.60, 4.63± 0.73, 13.30± 2.36, 16.70± 3.43 and 30.60± 4.01 mm, respectively (Table 2). The length and width of cocoon were 9.82± 1.66 and 4.96± 0.56, respectively. The male pupa had an average length of 7.99± 0.91 and width of 2.86± 0.27 mm while the female pupa had length of 9.10± 1.29 and width of 3.83± 0.45 mm, respectively. Pupation took place in boat shaped silken cocoon. The colour of newly pupated larva was whitish yellow and changed into dark brown prior to adult emergence.

The present findings are in line with Rajavel and Shanthi (2007) and Daimon et al. (2012). Pupal stage lasted for 11.70± 1.57 days (Table 1). The head, thorax and abdomen of adults were dark reddish brown. The forewings were pale reddish brown having curved waved line. The hind wings were greyish with reddish brown outer margins. Our findings are in line with the

Table 1. Duration and morphometrics of life stages of *T. varians* on *F. microcarpa*

Parameter	Mean*± SD	Parameter	Mean*± SD
Egg		Pupa	
Incubation period (days)	4.30± 0.67	Pupal period (days)	11.70± 1.57
		Adult	
		Pre- oviposition period (days)	2.30± 4.80
Hatching (%)	68.00± 10.33	Oviposition period (days)	3.00± 0.67
Larva		Post-oviposition period (days)	1.60± 0.70
I instar (days)	2.50± 0.53	Longevity	
II instar (days)	3.30± 0.82	Male (days)	5.50± 0.85
III instar (days)	3.20± 0.79	Female (days)	9.30± 2.00
IV instar (days)	4.30± 0.67	Fecundity per female	163.00± 22.63
V instar (days)	5.00± 0.67	Total life cycle	
Total larval period (days)	18.30± 2.00	Male (days)	35.50± 2.95
		Female (days)	39.30± 3.97

Parameter (mm)	Larva					Adult	
	First	Second	Third	Fourth	Fifth	Male	Female
	Mean*± SD						
Body length	2.45± 0.60	4.63± 0.73	13.30± 2.36	16.70± 3.43	30.60± 4.01	7.22± 0.54	8.88± 0.65
Head capsule length	0.32± 0.02	0.48± 0.14	1.00± 0.42	1.62± 0.28	2.38± 0.29	-	-
Head capsule width	0.34± 0.06	0.42± 0.06	1.00± 0.17	1.51± 0.06	2.12± 0.13	-	-
Caudal horn	0.45± 0.08	1.20± 0.32	1.45± 0.14	2.20± 0.13	1.46± 0.23	-	-
Antennal length	-	-	-	-	-	3.26± 0.31	2.53± 0.57
Forewing length	-	-	-	-	-	6.80± 1.23	10.00± 1.33
Forewing width	-	-	-	-	-	4.50± 0.85	5.10± 0.88
Hindwing length	-	-	-	-	-	7.00± 1.05	7.40± 0.52
Hindwing width	-	-	-	-	-	4.40± 0.70	5.60± 0.52
Wing span	-	-	-	-	-	15.00± 1.76	23.00± 1.94

* n=10

findings of Singh and Brar (2016). Body length and wing span of male were 7.22± 0.54 and 15.00± 1.76, respectively while 8.88± 0.65 mm and 23.00± 1.94 mm, respectively in the female. The female was bigger in size than male. The female adult lived long as compared to male. Total life cycle of male and female was 35.50± 2.95 and 39.30± 3.97 days, respectively (Table 1). Jia and Jinxin, 1997 reported that the egg, larva, pupa and adult stages lasted for 4-8, 11-15, 3-17 and 5-16 days, respectively. The sex could be determined based on the presence and absence of a suture on the ninth abdominal segment in female and male, respectively.

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EVALUATION OF ADJUVANTS ON GROWTH AND VIRULENCE OF *METARHIZIUM RILEYI* AGAINST *SPODOPTERA LITURA* (F.)

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ABSTRACT

This study evaluated the effect of adjuvants viz. glycerol, boric acid, carboxy methyl cellulose (CMC) and Tween-80 on the growth of *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber isolates. Their virulence against *Spodoptera litura* (F.) larvae has also been assessed. The isolates were observed to exhibit maximum surface area coverage and biomass with glycerol @ 5% and CMC @ 0.5%. Further, NIPHM isolate of *M. rileyi* supplemented with mixture of adjuvants- glycerol @ 5% and CMC @ 0.5% when evaluated against *S. litura* larvae gave maximum mortality (63.33%) and increase over control (without adjuvants- 2.69%). This study demonstrates the possible role of adjuvants in enhancing the growth of *M. rileyi* and its virulence against *S. litura.*

Key words: *Spodoptera litura*, *Metarhizium rileyi*, adjuvants, carboxy methyl cellulose, glycerol, boric acid, Tween-80, mixtures, virulence, surface coverage, biomass, mortality

Entomopathogenic biocontrol agents are ecofriendly alternatives to pesticides for managing crop pests. With the increasing emphasis on environmental protection and sustainable agriculture, biopesticides have become essential alternatives (Dhakal and Singh 2019). Entomopathogenic fungi for biological plant protection has an important role in sustainable IPM (Sinha et al., 2016). The fungus *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler et al., 2014) is an entomopathogenic fungus that infects several important pests of crops including many noctuids. However, microbes lose their viability under unfavourable conditions like temperature, humidity and ultraviolet radiations (Raypuria et al., 2019). The shelf-life and performance of formulations can be improved by adding suitable adjuvants that may act as UV protectants, wetting agents, adhesives or nutrients which can be beneficial for the growth and viability of the fungus (Patil and Jadhav, 2016). The application of adjuvants can provide protection and stimulate the establishment of antagonist on the host surface (Guijarro et al., 2018). There is a need for basic research on the standardization of suitable adjuvants for the development of myco-formulations (Ritika et al., 2019). *Metarhizium rileyi* has a lot of potential as a promising entomopathogen. There is a need for detailed studies to understand and develop economic production and formulation techniques to elevate the status of this fungus in pest control (Fronza et al., 2017). Hence, the present study to evaluate the effect of few adjuvants on the growth of *M. rileyi* and its efficacy against *S. litura*.

MATERIALS AND METHODS

Two *M. rileyi* isolates viz. MTCC4254 and MTCC10395 procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India and one isolate procured from National Institute of Plant Health Management, Hyderabad, India (NIPHM isolate) were used. These isolates were grown and maintained on Sabouraud maltose agar with yeast extract (SMAY) (mycological peptone 1%, maltose 4%, agar 2%, yeast extract 1% and chloramphenicol 0.5%), and refrigerated till further use. The conidial suspensions were prepared by scraping the fungal mat and suspending it in 100 ml distilled water. The conidial suspension was vortexed for 5 min to produce a uniform suspension and filtered through a muslin cloth. The fungal suspension was quantified to 10^8 conidia/ml using a haemocytometer. Adjuvants at concentrations viz. glycerol (1%, 2%, 5%), carboxymethyl cellulose (CMC) (0.1%, 0.2%, 0.5%), tween-80 (0.1%, 0.2%, 0.5%) and boric acid (1%, 2%, 5%) were sterilized in hot air oven at 160°C for 1 hr and added individually to optimum concentration (1x 10^8 cfu/ ml) of *M. rileyi* and incubated at 25± 2°C. One ml of this formulated suspension was then added to 100 ml Sabouraud maltose broth (SMB) and incubated

at 25± 2°C for 10 days following the methodology of Patil and Jadhav (2016). The surface area coverage was recorded according to Ritika et al. (2019) on 7th day after inoculation (DAI) and the fungal biomass was recorded 10 days after inoculation. There were ten treatments with three replicates each.

For bioassay studies, *Spodoptera litura* larvae and eggs were collected from cabbage and reared in the laboratory on cabbage leaves placed in glass jars covered with muslin cloth at ambient environmental conditions (25± 2°C). The food was changed daily. Conidial suspension was prepared by individually supplementing three *M. rileyi* formulations @ 1x 10^8 cfu/ ml with a mixture of adjuvants (glycerol @5% + CMC @0.5%). Laboratory bioefficacy study was carried out against 2nd instar larvae of *S. litura* by leaf-dip method according to Devi et al. (2003). Leaves were dipped in this supplemented conidial suspension, and larvae were released on to the treated leaves kept in plastic containers. Each replication consisted of 20 larvae, and there were six replications. Fresh untreated leaves were provided daily. The control larvae were kept on leaves treated with *M. rileyi* alone (without adjuvants). The cumulative % mortality and increase in mortality over control (*M. rileyi* without adjuvants) was recorded 5, 7 and 10 days after treatment (DAT). The dead larvae were transferred to petri plates containing moist paper to confirm the cause of death. The data were subjected to one-way ANOVA in SPSS 16.0. Means were compared by Tukey's post-hoc test (p=0.05).

RESULTS AND DISCUSSION

Growth of *M. rileyi* isolates on media supplemented individually with adjuvants was recorded as surface area covered and biomass produced; surface area covered was recorded at 7th day after inoculation (Table 1). In *M. rileyi* (NIPHM isolate), growth in media supplemented with adjuvants ranged from 35.0 to 98.33%, and surface area covered were statistically significant over control (without adjuvants) except for treatment supplemented with boric acid; in this isolate, glycerol @ 5% (98.33%) gave maximum surface growth and at par with glycerol @ 2% (95.0%); glycerol and CMC supported the maximum growth on all days of observation. Biomass observed after 10 days of treatment revealed that *M. rileyi* NIPHM isolate produced maximum biomass (1.50 g/ 100ml) in media supplemented with glycerol @5%; this was followed by CMC @ 0.5% (0.95 g/ 100ml); and the least (0.48 g/100ml) was in media supplemented with boric acid @ 5%. All isolates of *M. rileyi* recorded maximum surface area and biomass in media supplemented with a higher concentration of glycerol (5%) and CMC (0.5%). These results are similar to those obtained by Patil and Jadhav (2016) who evaluated various chemicals and oils, and combinations thereof, as adjuvants with *N. rileyi*; maximum biomass was on CMC @ 0.5% followed by glycerol @2%. The maximum surface area and biomass of *M. anisopliae* were observed with glycerol @ 10% by Boruah et al. (2015); Raypuria et al., (2019) studied its compatibility with various adjuvants and observed minimum growth

Table 1. Effect of adjuvants on growth of *M. rileyi* isolates

Treatments	Concentration (%)	NIPHM		MTCC 4254		MTCC 10395	
		Surface area at 7 DAI	Biomass at 10 DAI	Surface area at 7 DAI	Biomass at 10 DAI	Surface area at 7 DAI	Biomass at 10 DAI
M. rileyi+ Glycerol	1	93.33^{ba}	0.78^{dcb}	75.00^{ba}	0.79^{cb}	86.67^{a}	0.70^{ba}
	2	95.00^{a}	0.90^{cb}	88.33^{a}	0.81^{cb}	91.67^{a}	0.77^{a}
	5	98.33^{a}	1.50^{a}	93.33^{a}	0.99^{a}	95.00^{a}	0.82^{a}
M. rileyi+ CMC	0.1	80.00^{cba}	0.68^{ed}	86.67^{a}	0.70^{dc}	88.33^{a}	0.67^{ba}
	0.2	90.00^{ba}	0.77^{dcb}	91.67^{a}	0.72^{dcb}	90.00^{a}	0.71^{ba}
	0.5	93.33^{ba}	0.95^{b}	93.33^{a}	0.88^{ba}	93.33^{a}	0.76^{a}
M. rileyi+ Tween 80	0.1	51.67^{ed}	0.57^{fe}	50.00^{dcb}	0.56^{ed}	31.67^{b}	0.37^{d}
	0.2	66.67^{dc}	0.64^{fed}	60.00^{cb}	0.60^{ed}	33.33^{b}	0.55^{cb}
	0.5	75.00^{cb}	0.71^{ed}	75.00^{ba}	0.65^{dc}	45.00^{b}	0.70^{ba}
M. rileyi+ Boric Acid	1	50.00^{ed}	0.74^{edc}	50.00^{dcb}	0.69^{dc}	41.67^{b}	0.67^{ba}
	2	40.00^{e}	0.69^{ed}	43.33^{dc}	0.64^{dc}	31.67^{b}	0.66^{ba}
	5	35.00^{e}	0.48^{f}	38.33^{dc}	0.55^{ed}	30.00^{b}	0.46^{dc}
M. rileyi (Control*)	-	46.67^{e}	0.47^{f}	30.00^{d}	0.45^{e}	40.00^{b}	0.44^{dc}

**M. rileyi* without adjuvant; DAI= Days after inoculation; Mean value followed by same letter (a, b, c, d, e) in vertical column not significantly different (Tukey's post hoc test, p=0.05)

inhibition with CMC, also a higher concentration of CMC (0.5%) supported maximum radial growth and highest spore load. The growth of the fungus on higher concentration of CMC was attributed to the possible release of cellulolytic enzymes by *M. anisopliae* on CMC, which are necessary in the natural biodegradation to carbon and are therefore, essential for growth of microorganisms (Betty et al., 2013). The properties of CMC as an adjuvant include sticker, binder and stabilizer, while glycerol acts as humectant, carrier and osmotic protectant (Raypuria et al., 2019).

Babu et al. (2014) conducted compatibility studies of *M. anisopliae* with pesticides, insecticides and botanicals and reported that boric acid had a detrimental effect on growth of all fungal isolates. Boric acid inhibited fungal growth in *P. variotii* and *Trichoderma* strains (Ang et al., 2011). Surfactants reduce the surface tension of aqueous solutions by wetting of surfaces. However, the antifungal activity of surfactants depends on the length of the alkyl chain (Jackson et al., 2009). Various types of adjuvants that can improve spore adhesion or provide protection from adverse environmental conditions are often necessary to improve the performance of a biocontrol agent. However, some adjuvants can also be toxic to certain fungi (Wyss et al., 2004).

Against 2nd instar larvae of *S. litura,* after 5 days of treatment (DAT), the cumulative % mortality was not significantly different among isolates (Table 2); after 7 DAT, maximum mortality was observed with *M. rileyi* NIPHM (46.67%) followed by MTCC 4254 (40.00%); and maximum mortality (63.33%) was in *M. rileyi* NIPHM followed by MTCC 4254 (58.33%) at 10 DAT; though not statistically significant, an increase in mortality over control (*M. rileyi* without adjuvants) was observed in all isolates. The increase in mortality over control in *M. rileyi* NIPHM, MTCC 4254 and MTCC 10395 isolates was observed to be 3.71%, 4.35% and 5.01% at 7 DAT and 2.69%, 2.94% and 3.85% at 10 DAT respectively. Boruah et al. (2015), evaluated the efficacy of bioformulation of *M. anisopliae* against cowpea aphid under pot and field conditions. They observed mortality of 30.16% when supplemented with glycerol @ 10%; 50.00% when supplemented with sunflower oil @ 0.5 %; and up to 80.00% when supplemented with both glycerol @ 10% + sunflower oil @ 0.5% after thirty days of spraying. It was suggested that the addition of adjuvants and oils reduced conidial desiccation and increased the efficacy. Williams et al. (2000) studied the potential of different adjuvants for increasing the virulence of *Verticillium lecanii* and observed an additive improvement in infection with glycerol. Glycerol is a polyhydric alcohol that plays an important role in fungal growth when humidity fluctuates (Jennings 1995).

The production of hydrolytic enzymes by the microorganism is one of the mechanisms involved in biocontrol. The type of nutrients and their amount in the formulation must allow sufficient production of these hydrolytic enzymes (Stack et al., 1988). The increase in mortality observed in present study when supplemented with adjuvants, though not statistically significant, might be due to an increased level of hydrolytic enzymes secreted by the fungus in presence of these adjuvants that act as nutrients, adhesives, etc. Kim et al. (2010) described the role of certain adjuvants in promoting the aphicidal activity of *Beauveria bassiana* enzyme precipitate. It is concluded from the present study that media supplemented with glycerol (@ 5%) and CMC (@ 0.5%) improved growth of *M.*

Table 2. Mortality of *S. litura* larvae by *M. rileyi* supplemented with adjuvants

Treatments	% mortality								
	5 DAT			7 DAT			10 DAT		
	Control*	With Adjuvants**	% increase over control	Control*	With Adjuvants**	% increase over control	Control*	With Adjuvants**	% increase over control
M. rileyi NIPHM	30.00 (33.02)	31.66	5.53	45.00 (42.10)	46.67	3.71	61.67 (51.78)	63.33 (52.77)	2.69
M. rileyi MTCC 4254	26.67 (31.05)	28.33	6.22	38.33 (38.17)	40.00	4.35	56.67 (48.84)	58.33 (49.81)	2.94
M. rileyi MTCC 10395	26.67 (30.98)	26.67	0	33.33 (35.20)	35.00	5.01	43.33 (41.14)	45.00 (42.10)	3.85
CD (5%)	8.96	NS		9.69	NS		6.90	7.26	

Critical difference (CD), p= 0.05; DAT= days after treatment; **M. rileyi* formulation without adjuvants; ***M. rileyi* supplemented with CMC @ 0.5% + glycerol @ 5%; Values in parenthesis arc sine transformed values.

rileyi. Further field studies in understanding the role of adjuvants in virulence can lead to development of newer methods of production and application of mycoformulations.

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BIOLOGY AND LIFETABLE OF WHITEBACKED PLANTHOPPER *SOGATELLA FURCIFERA* (HORVATH) ON RICE

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ABSTRACT

Whitebacked planthopper *Sogatella furcifera* (Horvath) is an important insect pest causing economic damage to rice. Studies on its biology in West Bengal revealed that the fecundity was 197± 13.37 eggs/ female. The incubation period was 7.6± 0.69 days in October, with nymphal duration being 16.2± 0.85 days; and longevity of male macropterous ones was 9.5± 2.06, while for brachypterous it was 7.3± 1.33 days; and female brachypterous lived for 13.50± 1.08 days. The net fecundity rate, intrinsic rate of increase, gross reproductive rate, generation time, doubling time, finite rate of increase and net reproductive rate were 31.30, 0.122, 108.60, 29.29, 5.68 days, 1.129 and 64.91, respectively. The seasonal abundance when studied on rice in rainy season revealed that slight incidence occurred at 60 days, and its peak was at 32.00 and 34.20/ hill at 120 DAT during second week of November. Then it disappeared from the third week of November with the onset of winter during 2019 and 2020.

Key words: *Sogatella furcifera*, bionomics, population dynamics, temperature, humidity, seasonal incidence, peak, winter, macropterous, brachypterous, fecundity, longevity

Whitebacked planthopper *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae) is a major insect pest of rice in eastern India. It was first reported from Japan in1899 and from India in 1903 (Chaudhury et al., 1968; Dale, 1994). Its outbreak was also reported in Bangladesh, Indonesia, Malaysia, Nepal, Pakistan and Vietnam (Mochida et al., 1979). Later *S. furcifera* gradually spread to other countries like Myanmar, China, Hong Kong, Japan, Kampuchea, Laos, Philippines, Ryukyu Islands, Taiwan, Thailand, USSR, Australasia and Pacific Islands, Australia, Caroline Islands, Fiji, Irian Jaya, Marianas Island and Marshal Island (Dale, 1994). Khan and Misra (2003) reported that its population usually peaked in October in Uttar Pradesh, while Vijaykumar and Patil (2004) reported that it appeared from September to December in Karnataka. Katti et al. (2005) recorded it during rainy and summer seasons in Andhra Pradesh. The maximum occurrence of *S. furcifera* was observed during 2nd week of November and 3rd week of September in Odisha (Sarkar et al., 2018). Kumar et al. (2015) studied the biology of *S. furcifera* on basmati rice under agroclimatic conditions of Haryana and found that its fecundity was 132.8 eggs/ female in a cluster of 5 to 30 eggs. The effect of temperatures on its biology was studied in Punjab (Sandhu and Suri, 2018). To evaluate natality and reproduction of an insect population the analysis of lifetables is the most suitable method (Southwood, 1966; Price, 1997). Chi (1988) prepared the lifetable of brown planthopper *Nilaparvata lugens* (Stal.). The fecundity of *S. furcifera* gained prominence in Asia over the last few decades due to high levels of adoption by Asian farmers of hybrid rice varieties, particularly those with a cytoplasmic male sterile lineage, that are highly susceptible. It is also possible that gradual changes in global temperatures/ climate might have contributed to this (Horgan et al., 2016). There is still lack of information on the lifetable of *S. furcifera* on rice crop in eastern India, and hence, the present study.

MATERIALS AND METHODS

A field experiment was conducted for two wet seasons during July to October of 2019 and 2020 at the Regional Research Station, Chakdaha, Bidhan Chandra Krishi Viswavidyalaya (20.50^0- 24.50^0N, 86.00- 89.00^oE, 9.75 masl). Long duration pest susceptible variety MTU 7029 was transplanted in 0.40 ha on 3rd July, with 20x15cm spacing following normal agronomic practices. Incidence of *S. furcifera* was recorded at weekly intervals starting from 15 days after transplanting. It was done by trapping both nymph and adult from 30 randomly selected plants using 30x 20 cm enamel tray containing small quantity of soap

water. Data were also recorded on *S. furcifera* at 3 days interval using sweeping nets and light traps; 10 m^2 areas were selected at random and *S. furcifera* were collected by sweeping five times during the morning hours. The pooled data were retransformed before subjecting to statistical analysis. Simultaneously in another study, *S. furcifera* were reared in the laboratory on the potted rice plant cultivar MTU7029 at the research station RRSS, Chakdaha. Initially heavily infested plants were grown inside a cage to maintain a mass culture containing freshly emerged last instars nymph. After one or two days the mated females were transferred to another set of 10 potted plants covered with glass chimney. The potted plant was changed daily morning till the adults died. The experiment was repeated five times by introducing female of each cohort in the laboratory (25 to 30º, 80 to 85% RH). Daily observation was taken to study the biology till the start of subsequent generation. Observations were recorded on preoviposition and oviposition periods, period from emergence to death and the fecundity of female. After emergence of first instar nymphs the plants were cut longitudinally and total number of hatched egg and unhatched eggs were counted under microscope. Data were subjected to computation of lifetable as per Southwood (1966)

RESULTS AND DISCUSSION

Sogatella furcifera a was observed passing egg, nymph and adult stages, with both adult male and female observed immediately after emergence remaining together for at least 3 hr. The females vibrate their abdomen and the excited males either flutter their wing or dance before mating. The evening time was the best time for their mating. Although 2 to 3 males followed a single female but ultimately, they mate once with a single potential male and mating period lasted for 162±10.32 sec. Long et al.(2012) also reported that successful mating in *N. lugens* depended on the exchange of acoustic signals between male and female. As soon as mating was completed the female gave some vertical cut over the mid region of leaf sheath and inserted about 21 eggs in the first day and they laid total of 197±13.37 eggs in nearby 5 to 10 tillers within 8 to 10 days. The egg laying sites appeared as brownish streaks over the leaf sheath. The macropterous male survived up to 9.5±2.06 days whereas brachypterous male could not survive after 7.3±1.33 days; female survived up to 13.50±1.08 days and remained beside the slits till the first instar nymphs emerged out. The incubation period was 7.6±0.69 days, nymphal developmental period was 16.2±0.85 days. There were five instars requiring 2.55± 0.37, 2.45± 0.43, 2.72± 0.26, 3.55± 0.43 and 4.70± 0.67 days each. These results corroborate those of Kumar et al. (2015). The net fecundity rate, mean length of generation, finite rate of increase, mean generation time, weekly multiplication of the population and doubling time of the population were also measured. The net fecundity rate (*Ro*) and net reproductive rate (R_t) were 31.30 and 64.91, respectively (Table 1).

The present results corroborate with those of van Lenterenand Noldus (1990) who found that short developmental time and high reproduction rate on a host reflect suitability of the plant tested. Win et al. (2011) reported that the basic reproductive rate of BPH (*Ro*) was also 10.02; the population increased by a factor of 10. The study also showed that innate capacity (*rc*), intrinsic rate of increase (*rm*) and finite rate of increase (λm) were 0.117, 0.122 and 0.129, respectively during wet season in West Bengal. The intrinsic birth rate (2.79) and intrinsic death rate (2.73) were equal and it took only 5.68 days for doubling up their population. Therefore *S. furcifera* population could not maintain its *rm* value over an indefinite period under a given set of environmental condition. The data supports the observation of Win et al. (2009) who recorded the innate capacity (*rc*) and intrinsic rate of natural increase (*rm*) for BPH as 0.117 and 0.122 respectively. The *rm*, T_c and DT are useful in dices of population growth of *S. furcifera* on one of the most susceptible variety MTU 7029 during wet season in West Bengal. The *rm* value of a life table data of a particular species provide insight into characteristic life patterns of different species (Satpute et al. 2005). The intrinsic birth rate and death rate varied with some biotic and abiotic factors.

Andrewartha and Birch (1954) reported that both birth and death rates remain constant in a population having stable age distribution when grown in unlimited space. Quantifying the birth and death rate could predict the growth and decline of WBPH population in future in a particular variety of rice crop. The study showed that survival potential (*lx*) of the female and the age specific fecundity rate (*mx*) were maximum during the second day and then gradually declined with advancement of its pivotal ages. The survival potential (*lx*) of WBPH showed that high mortality was found in early stage followed by a gradual decline in the population densities throughout its life span over the study period. Studies on seasonal incidence of *S. furcifera* indicated that it appeared with very small average population of 0.10 macropterous adult per hill at 60 DAT during first week of September. Nymphs appeared at 75DAT during

Table 1. Biology and reproductive parameters of *S. furcifera* on rice (2019 & 2020)

Stage	Life period in days at 25 to 30ºC, 80 to 85% rh	
	Days (hour/second)	References
(A) Adult stage		
(i) Premating period	3.4± 0.459	-
(ii) Mating period	162± 10.32 second	148 second (Ahmed et al., 2016)
(iii) Preoviposition period	5.3 days± 1.76 days	3 to 8 days (Dale, 1994) 3.7± 0.3 days (Kumar et al., 2015)
(iv) Oviposition period	5.6 days± 1.26 days	10.2± 0.51 (Kumar et al., 2015)
(vi) Adult longevity		
Male (Macropterous)	9.5± 2.06 days	14.4 days (Kumar et al., 2015), 12. 5 days (Ammar et al.,1980)
Male (Brachypterous)	7.3± 1.33	
Female (Brachypterous)	13.50± 1.08	15.9 days (Kumar et al., 2015) 18.7 days (Ammar et al., 1980)
(vii) Sex ratio(Female: Male)	1.2: 0.98	1.0 : 0.78 (Kumar et al., 2015) 0.512:0.488. (Win et al. 2009)
(B) Egg stage		
Fecundity	197± 13.37	164 eggs (Vaidya and Kalode, 1981), 300 to 350 (Suenaga, 1963), 119 to 158 eggs (Kumar et al., 2015) (129 to 198 eggs Sandhu and Suri, 2018)
Incubation Period	7.6± 0.69	8.6± 0.24 (Kumar et al., 2015)
(C) Nymphal stage		
First instar	2.55± 0.37	2.05± 0.05 days (Kumar et al., 2015)
Second instar	2.45± 0.43	2.3± 0.12 days (Kumar et al., 2015)
Third instar	2.72± 0.26	2.6± 0.11 days (Kumar et al., 2015)
Fourth instar	3.55± 0.43	2.7± 0.10 days (Kumar et al., 2015)
Fifth instar	4.70± 0.67	2.95± 0.11 days (Kumar et al., 2015)
Total nymphal developmental period	16.2± 0.85	17. 7 days (Sandhu and Suri, 2018)
(D) Total life cycle(from egg to adult)	24.5± 0.99	23 days (Dale, 1994)
(E) Approximate generation time (T_c)= $\sum xlxmx/ lxmx$	29.29 days	-
(F) Net fecundity rate (R_0)= $\sum lxmx$	31.30	-
(G) Innate capacity(rc)= $\ln R_0/ T_c$	0.117	-
(H) Intrinsic rate of natural increase(rm)= $\sum e^{-rm.X} lXmX = 1$	0.122	-
(I) Finite rate of increase (λm) = antilog e^{rm}	1.129	-
(J) Doubling time of population (DT) in days=ln2/rm	5.68 days	-
(K) Intrinsic birth rate(B)=1/ $\sum e^{-rmx} lx$	2.79	-
(L) Intrinsic death rate(D)= B- rm	2.738	-
(M) Gross reproductive rate= $\sum mx$	108.6	-
(N) Net reproductive rate(R_t)= $\sum lxtx$	64.91	-

Lifetable for female *S. furcifera* (25ºC, 85 % RH)

Pivotal age in days(x)	*lx*	*mx*	*lxmx*	*xlxmx*	*tx*	*lxtx*	*xlxtx*
0-25.5	-	-	-	-	-	-	-
26.5	-	-	-	-	-	-	-
27.5	0.36	8.00	2.88	79.20	18.00	6.48	178.20
28.5	0.34	32.00	10.88	310.00	70.00	23.8	678.30
29.5	0.32	41.00	13.12	389.4	80.00	25.6	755.20
30.5	0.30	9.00	2.70	82.35	20.00	6.00	183.00

(contd.)

(contd. Table 1)

31.5	0.12	6.00	0.72	22.68	12.00	1.44	45.36
32.5	0.10	5.00	0.50	16.25	8.00	0.80	26.00
33.5	0.08	4.00	0.32	10.72	6.00	0.48	16.08
34.5	0.06	3.00	0.18	6.21	5.00	0.30	10.35
35.5	0.01	0.6	0.006	0.201	1.00	0.01	0.355
Total		108.6	31.306	917.01		64.91	1892.84

Correlation coefficients- *S. furcifera* incidence vs. weather factors

Stages	Temperature				Relative humidity			
	2019		2020		2019		2020	
	Max	Min	Max	Min	Max	Min	Max	Min
Nymph	0.172	-0.168	0.156	-0.147	0.212	-0.179	0.173	-0.112
Adult	0.492	-0.547**	0.162	-0.172	0.682**	-0.248	0.562**	-0.219

Max= Maximum; Min = Minimum; ** Significant at p≤0.05

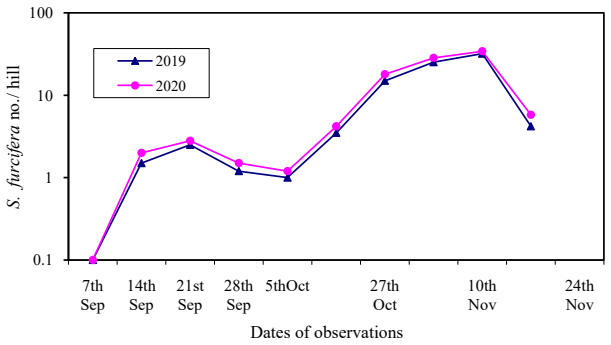


Fig. 1. Incidence of *S. furcifera* in West Bengal (2019 and 2020)

second week of September with an average population of 1.5 and 2.0/ hill during 2019 and 2020, respectively. The population increased when the second-generation nymphs emerged out averaging 3.5 and 4.2/ hill, respectively at 105 DAT during second week of October for subsequent 2 years of study (Table 1; Fig. 1).

Sogatella furcifera incidence peaked to 32.00 and 34.20/ hill at 120 DAT during 2nd week of November followed by sudden disappearance from the 3rd week of November with the onset of winter. The insect had the habit of clustering at the base of plant or on the under surface of leaf. Correlation coefficients of incidence with weather parameters showed a positive relationship with maximum (r= 0.172 to 0.492) temperature but had significantly negative association with minimum temperature (r= -0.168 to -0.547) during 2019. The trend was identical during 2020 with the correlation coefficient value of 0.156 to 0.162 and -0.147 to -0.172 for maximum and minimum temperature, respectively. A positive association was observed with maximum RH (r= 0.212 to 0.682) but was negatively correlated (r=-0.179 to -0.248) with minimum RH; similar positive (r= 0.173 to 0.562) and negative (-0.112 to -0.219) correlations were also found during 2020 (Table 1). The present results are similar to those of Sarkar et al. (2018) as regards temperature with high relative humidity. Khan and Misra (2003) also reported a positive correlation with temperature and RH. The upper temperature tolerances for adult female was estimated as 37-41°C (Ali et al. 2019).

The incidence of *S. furcifera* is gradually becoming to an alarming state in new Gangetic Alluvial Zones of West Bengal. Among the lifetable parameters the high net reproductive rate (R_0) and finite rate of increase (λm) with lower doubling time (DT) indicate that *S. furcifera* has a capacity for rapid buildup in a short period of time. The intrinsic birth rate and death rate may be regarded as the key factors for regulating its population. Therefore, based on lifetable study, a time-based management practices can be prepared including conservation of the natural enemies to reduce the negative impact on environment. The present study suggested that the key period for adoption of control measures is 2ndweek of October when the second-generation nymphs emerge out and the prevailing favourable climate is conducive to build up effective pest population in rice fields.

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EFFECT OF MAGNETIC FIELD ON *CORCYRA CEPHALONICA* EGGS FOR PARASITIZATION BY *TRICHOGRAMMA CHILONIS*

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ABSTRACT

This study evaluated the effect of permanent magnetic field on *Corcyra cephalonica* Stainton eggs and effect on parasitisation by the egg parasitoid *Trichogramma chilonis* Ishii. *Corcyra* eggs were exposed to magnetic field for different time periods. It was observed that three hours magnetized eggs were significantly more preferred for parasitisation and revealed maximum adult emergence (76.23%, 98.67%) than non-magnetized *Corcyra* eggs (63.50%, 85.96%). When UV irradiated *Corcyra e*ggs were kept under magnetic field for parasitisation, with increase in the time of exposure to magnetic field, parasitisation also increases along with adult emergence (89.85%, 97.16%) up to 3 hr. Then, it gradually decreases as compared with control (86.80%, 94.54%). Thus, 3 hr magnetized *Corcyra* eggs prevent the emergence of larva and was preferred by *Trichogramma* for maximum parasitisation and adult emergence.

Key words: *Trichogramma chilonis*, *Corcyra cephalonica*, magnetic field, magnetization, parasitisation, adult emergence, *Corcyra* eggs card, U V treated eggs, parasitoid

Trichogramma chilonis Ishii is an egg parasitoid measuring only 0.3 mm in length, belonging to the family Trichogrammatidae of the order Hymenoptera. *Trichogramma* spp., are widely used as egg parasitoids for biological control of insect pests through augmentation and field release (Funde et al., 2020). *Corcyra cephalonica* (Stainton) is being used as a factitious host for mass production of predators and parasitoids under laboratory condition. For production of tricho-card, sterilization of *Corcyra* eggs is essential (Singh and Kumar, 2017). Sometimes, larvae of the host insect emerge from unparasitized eggs and attack parasitized eggs, hampering the performance of the tricho-card in the field. Biocontrol agents constitute an important component of IPM, and demand for these is increasing, and there is potential to boost production and usage of these (Barratt et al., 2017). Insect possess ferromagnetic resonance, and magnetic material is present in the head, thorax and abdomen, and these magnetic nanoparticles in social insects act as geomagnetic sensors (Esquivel, 2007). The magnetic field is a safe, effective, economical and convenient alternative method. It has an effect on orientation (Jones and Macfadden, 1982), oviposition, development, fecundity, and behaviour for a wide variety of insects (Ramirez et al., 1983). Considering these, the present study evaluated the acceptability of magnetized *C. cephalonica* eggs for parasitisation by *T. chilonis* and assessed the effect of magnetic field on parasitisation.

MATERIALS AND METHODS

Trichogramma chilonis and *C. cephalonica* cultures were maintained in the Biocontrol Laboratory, Department of Entomology, Dr. PDKV Akola during 2018-19. Fresh *Corcyra* eggs produced were cleaned and then egg cards (6 x 4 cm) were prepared by sprinkling eggs on yellow coloured cards having acacia gum as sticking material with proper drying (Shinde et al., 2016). Experiments were conducted in two sets, in first set, *C. cephalonica* egg cards were exposed to permanent magnetic field for different time periods as per treatments and magnetized eggs were immediately allowed to be parasitized by freshly emerged female *T. chilonis* adults as a nucleus card (6:1) in a polythene bag containing 50% honey as food under incubation chamber. The preference of magnetized eggs for parasitisation by *T. chilonis* adults was assessed (Pandir et al., 2013). In a second set, *C. cephalonica* egg cards were UV irradiated and then immediately exposed to freshly emerged *T. chilonis* adults (6:1) for parasitisation in a polythene bag under magnetic field condition for different time periods as per treatments. To know the effect of magnetic field on parasitisation was assessed. A complete set of control was maintained and replicated thrice. Observations were made on parasitisation of

eggs at 4th day when eggs turned black (indicating development of parasitoid) and emergence of *T. chilonis* adults on 7th day. Obtained data were converted into % and analysed in completely randomized block design (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Study revealed that magnetic field treated *C. cephalonica* eggs had significant effect on parasitisation by *T. chilonis* (Table 1); maximum parasitisation and emergence was observed in 3 hr magnetized eggs (76.23%, 98.67%) which was at par with 2½ hr magnetized eggs (74.88%, 96.21%); and in control i.e., without magnetic field these values were 63.50 and 85.96%, which was at par with 2 hr, 1½ hr, 1 hr and 45 min magnetized eggs. Group of 30 and 15 min magnetized eggs revealed 57.58%, 80.04% and 56.09 %, 78.30% parasitisation and emergence, respectively and at par with each other. Both these treatments were significantly superior to 4, 5 and 6 hr magnetic field exposure. Similar observations were made by Pandir et al. (2013) with eggs of *E. kuehniella* upon 3 hr magnetization for its effect on *T. embryophagum* for parasitisation. Pan (1996) observed negative effects due to exposure to 7 T magnetic field with egg hatching and maturation of *Corcyra* eggs death causing death of embryo effectively used for parasitisation by *Trichogramma*. Eclosion of eggs in the 7-T field was delayed, and the hatching rate was reduced. Magnetic field for > 4 hrs had negative effect on *Corcyra* eggs

Table 1. Parasitisation/ adult emergence of *T. chilonis* from magnetized *C. cephalonica* eggs

Treatments (Magnetic field exposure)	Magnetic field treated eggs		UV irradiated and magnetic field treated eggs	
	Parasitisation %	Emergence %	Parasitisation %	Emergence %
T_1 – 0 hr	63.50	85.96	86.80	94.54
	(52.82)	(68.02)	(68.71)	(76.64)
T_2 – 15 min.	56.09	78.30	77.92	83.88
	(48.50)	(62.25)	(61.97)	(76.64)
T_3 – 30 min.	57.58	80.04	78.49	84.35
	(49.36)	(63.47)	(62.37)	(66.75)
T_4 – 45 min.	60.78	82.56	78.93	86.54
	(51.22)	(65.40)	(62.68)	(68.53)
T_5 – 1 hr	61.90	83.19	79.69	85.89
	(51.88)	(64.82)	(63.21)	(67.94)
T_6 – 1½ hr	62.72	84.05	82.34	87.40
	(52.36)	(66.49)	(65.15)	(69.21)
T_7 – 2 hr	63.47	85.28	87.18	95.56
	(52.81)	(67.44)	(69.03)	(78.05)
T_8 – 2½ hr	74.88	96.21	88.76	96.06
	(59.92)	(79.03)	(70.42)	(78.65)
T_9 – 3 hr	76.23	98.67	89.85	97.16
	(60.82)	(83.87)	(71.45)	(80.81)
T_{10} – 4 hr	44.30	66.33	67.77	72.84
	(41.72)	(54.23)	(55.41)	(58.59)
T_{11} – 5 hr	41.43	61.02	52.24	65.02
	(40.05)	(51.36)	(46.28)	(53.77)
T_{12} – 6 hr	39.29	63.36	49.95	57.61
	(38.80)	(52.75)	(44.96)	(49.37)
T_{13} – 12 hr	22.42	44.17	43.91	49.09
	(28.24)	(41.56)	(41.49)	(44.47)
T_{14} – 24 hr	20.02	40.80	42.57	48.15
	(26.54)	(39.68)	(40.71)	(43.94)
F test	Sig.	Sig.	Sig.	Sig.
SE (m)±	0.65	0.88	0.50	0.97
CD (p=0.05)	1.86	2.52	1.43	2.78

Figures in parentheses arc sin transformed values

hatching, larval growth and development (Madavi et al., 2020; Zein and Hussein, 2019; Chandrawanshi et al. (2018). Magnetic field significantly affects the orientation and behaviour of *Trichogramma* adults for parasitisation on UV irradiated *Corcyra* eggs (Table 1). Significantly maximum parasitisation and adult emergence was observed in 3 hr magnetic field treated eggs (89.85%, 97.16%, respectively) which was at par with 2½ hrs magnetic field treated ones (87.18%, 95.56%, respectively), which in turn was at par with untreated ones. Thus eggs exposed to magnetic field for 3 hr gave maximum parasitisation. Ram and Irulandi (1989) reported maximum egg parasitisation by *Trichogramma exiguum* with UV treated eggs of *Corcyra.* The nature of the response by insect is depended upon the power supply generating by the magnetic field (Starick et al. 2005)

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A NEW HOST RECORD FOR THE INVASIVE THRIPS *THRIPS PARVISPINUS* KARNY FROM INDIA

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ABSTRACT

The present study on the diversity of thrips on cotton in Tamil Nadu revealed the occurrence of the invasive species *Thrips parvispinus* Karny during kharif 2019-20. The thrips were found to occur on *Gossypium barbadens* variety Suvin. The diagnostics of this species are illustrated herein. The status of new record is also discussed.

Globalisation and climate change warrant the need to monitor the occurrence of invasive insects in different cropping systems. The present study evaluates the diversity of thrips in cotton and the flowering stage was observed to harbour more thrips, and hence the species diversity was explored in detail. The results including the occurrence of *Thrips parvispinus* Karny are discussed herein.

MATERIALS AND METHODS

The study was done at the farm of ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu (11^{o}N, 77^{o}E and 427.6 masl) during 2019-20 kharif season. Thrips were collected from *Gossypium barbadense* variety Suvin. During flowering stage, ten plants were randomly selected for sampling. From each plant, thrips were collected from five flowers. Sampling repeated for three times at weekly intervals during flowering stage. Collected thrips were stored in 70% alcohol. Among the collected specimens *Thrips parvispinus* Karny were mounted in Canada balsam for permanent preservation and subsequently sorted out and identified using appropriate keys (Mound, 2005).

RESULTS AND DISCUSSION

Among the three thrips species recorded on cotton flowers, *Thrips parvispinus* Karny was also observed along with other common flower thrips *Thrips florum* Schmutz and *Thrips hawaiiensis* (Morgan). This is the first record of *T. parvispinus* on cotton in India. The following features segregate *T. parvispinus* from other known species of the genus *Thrips*, especially which falls within *T. orientalis* group (Fig. 1)- Ocellar setae pair III at the anterior margin of ocellar triangle; postocular setae III shorter than postocular setae I and IV (Fig. 1F); Metanotum with median reticulations; median setae placed well behind the anterior margin; campaniform sensilla absent (Fig. 1C); First and second veins of fore wing with continuous setal row (Fig. 1I); Posterior margin of abdominal tergite VIII without comb (Fig. 1G); and abdominal sternites III–VI with discal setae, but absent on II and VII (Fig. 1D).

Thrips parvispinus, which is designated as one of the notorious pest species from South East Asia, is a serious pest on numerous agricultural and horticultural crops. *T. parvispinus*, a member of "*Thrips orientalis* group" (Mound, 2005), is a thrips of quarantine importance and has been reported from Thailand to Australia (Mound and Collins, 2000) as a serious pest on innumerable unrelated plant families. It is reported on papaya in Hawaii, greenhouse Gardenia plants in Greece, vegetable crops like chili, green beans, potato, and eggplant from other countries (Murai *et al*. 2009). Occurrence of this species in India has been first reported by Tyagi et al. (2015) on papaya from Bangalore. Later, Rachana et al. (2018) reported the same on flowers of *Dahlia rosea* in Puttur, Karnataka. This is the first record of *T. parvispinus* on cotton. The presence of this invasive pest on an agriculturally important host plant raises serious concern and is an apprehension for quarantine officials. Earlier reports of this species on different economically important host plants in Karnataka and the current report on an agriculturally important host in Tamil Nadu hint that the pest may be slowly moving across states and measures need to be strengthened so as to prevent the spread of this invasive thrips to other parts of India. Regular monitoring of *T. parvispinus* in other parts of India is also essential as it may attain pest status especially within the territory of our country. Considering the

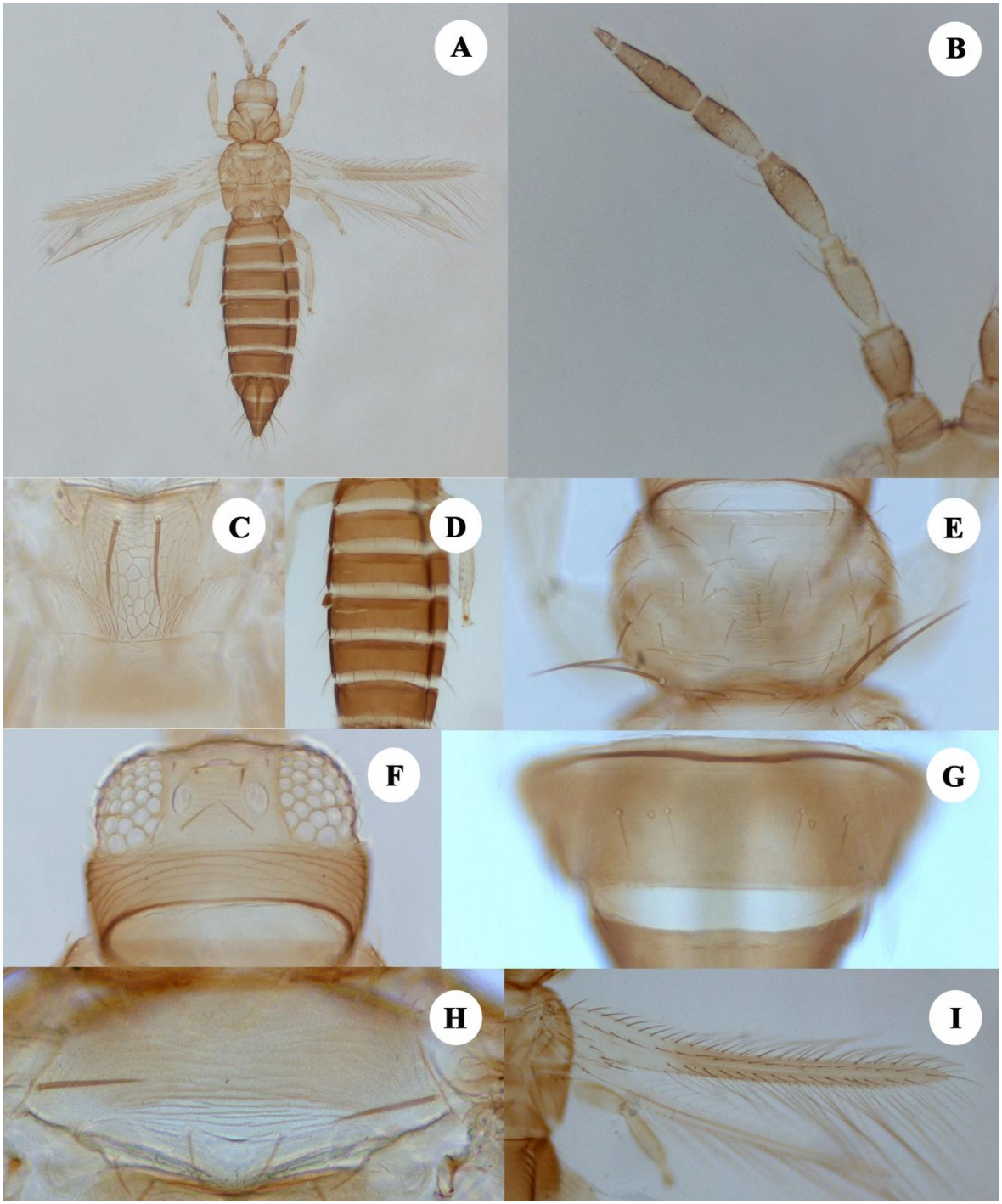


Fig. 1. *Thrips parvispinus*: A. Female; B. Antenna; C. Metanotum; D. Abdominal sternites II-VII; E. Pronotum; F. Head; G. Abdominal tergite VIII; H. Mesonotum; I. Fore wing

polyphagous nature of this thrips species, the chances of spread on economically important crops canot be ruled out. Since *T. parvispinus* may attain major pest status on cotton, the report demands regular monitoring and surveys for this thrips species on cotton.

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EFFICACY OF INSECTICIDES AGAINST POD BORERS OF INDIAN BEAN

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ABSTRACT

Among the various insecticides evaluated for their field efficacy against pod borers of Indian bean, the treatment of emamectin benzoate 5SG at 0.002%, indoxacarb 14.5SC at 0.007% and lambdacyhalothrin 5SC at 0.005% were found to be most effective against *Helicoverpa armigera* (Hubner) and *Maruca vitrata* Geyer. While, thiacloprid 21.7SC at 0.012% and novaluron 10EC at 0.01% were moderately effective. The least pod damage was observed with emamectin benzoate 5SG at 0.002% (13.16%) which was at par with indoxacarb 14.5SC at 0.007% (14.16%) and lambdacyhalothrin 5SC at 0.005% (16.33%). Maximum pod yield (21.75 q/ ha), increase in yield over control (95.76%) and % of avoidable loss (48.91%) was observed with emamectin benzoate 5SG at 0.002%.

Key words: Indian bean, *Helicoverpa armigera*, *Maruca vitrata,* emamectin benzoate, indoxacarb, lambda-cyhalothrin, thiacloprid, novaluron, pod damage, yield

Indian bean *Lablab purpureus* L. is a legume crop widely grown as vegetable or pulse crop. In Gujarat, this crop is mainly attacked by aphid *Aphis craccivora* Koch, leaf hopper *Empoasca kerri* Pruthi, whitefly *Bemisia tabaci* (Gennadius), thrips *Megaleurothrips distalis* Karny and pod borer *Helicoverpa armigera* (Hubner) (Chaudhari et al., 2016). Of these, pod borers are most important regularly causing crop loss to the tune of 80-100% (Reddy et al., 2017), and thus a key impediment for productivity; nearly 54% loss occurs due to these in field beans. The major pod feeders include *Maruca vitrata* Geyer besides *H. armigera*. Many insecticides are effective against the pod borers of Indian bean, but resistance to common insecticides is known and it occurs due to its injudicious use. Therefore, this study to evaluate efficacy of some newer molecules.

MATERIALS AND METHODS

The field experiments on the evaluation of field efficacy of insecticides were conducted at the College Farm, N M College of Agriculture, Navsari Agricultural University, Navsari, Gujarat during 2019-20. The variety GNIB-22 was used with sowing done in plots of size 11 m^2 at 60x 30 cm spacing. The crop was sown in the second fortnight of October. Nine treatments were evaluated along with untreated control, each replicated thrice. The insecticides i.e., thiamethoxam 25WG (1 g/ l), thiacloprid 21.7SC (0.6 ml/ l), buprofezin 25SC (2.0 ml/ l), acetamiprid 20SP (0.2 g/ l), indoxacarb 14.5SC (0.5 ml/ l), emamectin benzoate 5SG (0.4g/ l), lambdacyhalothrin 5SC (1ml/ l) and novaluron 10EC (1 ml/ l) were evaluated. These were applied as foliar spray using pre-calibrated knapsack sprayer when the pest incidence was sufficiently builtup. Second spray was repeated after 15 days of the first spray. The observations were recorded a day before spray as well as 1st, 3rd, 5th, 7th and 14th days after each spray, from 5 randomly selected plants/ plot. Number of *H. armigera* and *M vitrata* larvae were counted and mean was calculated. For recording observations on pod damage, total and damaged pods were counted at each picking. The yield of green pods was recorded plot-wise during each picking, and plot-wise yield obtained was converted into kg ha^{-1}. The data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Results of pooled data over two years revealed that the significantly minimum incidence of *H. armigera* and *M. vitrata* larvae was recorded in plots treated with emamectin benzoate 5SG (1.54, 2.17 larvae/ plant) and it was at par with indoxacarb 14.5SC (1.60, 2.29 larvae/ plant) and lambdacyhalothrin 5SC (1.69, 2.36 larvae/ plant, respectively). Thiacloprid 21.7SC (2.57, 3.68 larvae/ plant) was the next effective and it was at par with novaluron 10EC (2.65, 3.78 larvae/ plant, respectively). Significantly minimum pod damage was

Table 1. Efficacy of insecticides against *H. armigera* on Indian bean

Tr. No.	Treatments	Mean no. of *H. armigera* larvae/ plant											
		Before-spray	First spray					Second spray					Pooled
			1DAS	3DAS	5DAS	7DAS	14DAS	1DAS	3DAS	5DAS	7DAS	14DAS	
T_1	Thiamethoxam 25 WG at 0.025%	2.25 (5.08)	2.05 (4.20)	2.12 (4.49)	2.09 (4.35)	1.89 (3.59)	2.05 (4.20)	1.89 (3.58)	1.95 (3.82)	1.86 (3.47)	1.77 (3.13)	1.70 (2.89)	1.96 (3.85)
T_2	Thiacloprid 21.7 SC at 0.012%	2.32 (5.40)	1.70 (2.90)	1.81 (3.27)	1.75 (3.08)	1.52 (2.31)	1.72 (2.94)	1.50 (2.24)	1.58 (2.49)	1.47 (2.16)	1.41 (1.98)	1.29 (1.66)	1.60 (2.57)
T_3	Buprofezin 25 SC at0.05%	2.18 (4.77)	2.03 (4.13)	2.10 (4.42)	2.07 (4.28)	1.84 (3.38)	2.02 (4.08)	1.84 (3.39)	1.90 (3.62)	1.82 (3.30)	1.76 (3.10)	1.66 (2.76)	1.92 (3.72)
T_4	Acetamiprid 20 SP at 0.004%	2.24 (5.03)	2.06 (4.26)	2.12 (4.49)	2.10 (4.24)	1.91 (3.66)	2.07 (4.28)	1.91 (3.65)	1.97 (3.88)	1.92 (3.69)	1.85 (3.43)	1.72 (2.96)	1.98 (3.95)
T_5	Indoxacarb 14.5 SC at 0.007%	2.31 (5.32)	1.40 (1.97)	1.51 (2.28)	1.41 (1.99)	1.10 (1.21)	1.44 (2.07)	1.12 (1.25)	1.25 (1.55)	1.11 (1.23)	1.01 (1.03)	0.95 (0.90)	1.26 (1.61)
T_6	Emamectin Benzoate 5 SG at 0.002%	2.28 (5.18)	1.38 (1.90)	1.48 (2.20)	1.36 (1.86)	1.05 (1.10)	1.42 (2.01)	1.05 (1.10)	1.19 (1.42)	1.07 (1.15)	0.98 (0.97)	0.91 (0.83)	1.23 (1.54)
T_7	Lambda-cyhalothrin 5 SC at 0.005%	2.25 (5.06)	1.43 (2.04)	1.53 (2.35)	1.46 (2.15)	1.14 (1.31)	1.47 (2.17)	1.13 (1.29)	1.27 (1.61)	1.14 (1.29)	1.04 (1.08)	0.98 (0.97)	1.29 (1.69)
T_8	Novaluron 10 EC at 0.01%	2.29 (5.27)	1.72 (2.98)	1.83 (3.33)	1.77 (3.14)	1.47 (2.17)	1.73 (2.99)	1.51 (2.29)	1.61 (2.60)	1.53 (2.33)	1.46 (2.13)	1.35 (1.81)	1.62 (2.65)
T_9	Control (Treated with water)	2.32 (5.39)	2.36 (5.60)	2.49 (6.23)	2.45 (6.03)	2.36 (5.60)	2.44 (5.97)	2.33 (5.46)	2.39 (5.71)	2.33 (5.43)	2.29 (5.23)	2.26 (5.11)	2.34 (5.73)
	S.E.m ±	0.10	0.09	0.08	0.09	0.09	0.09	0.10	0.09	0.07	0.09	0.07	0.02
	S.E.m ± (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.03
	C.D (p= 0.05)	NS	0.26	0.24	0.26	0.29	0.27	0.31	0.26	0.21	0.27	0.22	0.06
	C.D (p= 0.05) (P×T)	-	-	-	-	-	-	-	-	-	-	-	NS

DAS = Days after spraying; Figure in parentheses original value whereas, those outside $\sqrt{x + 0.5}$ transformed values.

Table 2. Efficacy of insecticides against *M. vitrata* on Indian bean

Tr. No.	Treatments	Mean no. of *M. vitrata* larvae/ plant												Yield (q/ ha)	Increase in yield over control (%)
		Before spray	First spray					Second spray					Pooled		
			1DAS	3DAS	5DAS	7DAS	14DAS	1DAS	3DAS	5DAS	7DAS	14DAS			
T_1	Thiamethoxam 25 WG at 0.025%	2.63 (6.93)	2.27 (5.14)	2.33 (5.44)	2.30 (5.30)	2.27 (5.15)	2.43 (5.88)	2.22 (4.91)	2.26 (5.09)	2.15 (4.61)	2.05 (4.20)	1.92 (3.69)	2.24 (5.02)	14.58	31.23
T_2	Thiacloprid 21.7 SC at 0.012%	2.57 (6.62)	2.22 (4.91)	2.27 (5.15)	2.13 (4.54)	1.95 (3.81)	2.05 (4.21)	1.77 (3.12)	1.80 (3.25)	1.71 (2.92)	1.59 (2.52)	1.46 (2.13)	1.92 (3.68)	17.12	54.09
T_3	Buprofezin 25 SC at0.05%	2.67 (7.13)	2.26 (5.12)	2.31 (5.36)	2.29 (5.23)	2.25 (5.09)	2.42 (5.84)	2.19 (4.78)	2.22 (4.94)	2.13 (4.54)	2.03 (4.13)	1.90 (3.60)	2.22 (4.94)	15.04	35.37
T_4	Acetamiprid 20 SP at 0.004%	2.63 (6.92)	2.28 (5.20)	2.36 (5.58)	2.32 (5.38)	2.28 (5.22)	2.44 (5.97)	2.23 (4.99)	2.28 (5.21)	2.19 (4.81)	2.10 (4.41)	1.94 (3.75)	2.26 (5.13)	13.65	22.86
T_5	Indoxacarb 14.5 SC at 0.007%	2.57 (6.59)	1.81 (3.26)	1.84 (3.37)	1.67 (2.80)	1.58 (2.48)	1.70 (2.87)	1.36 (1.84)	1.39 (1.94)	1.27 (1.60)	1.16 (1.34)	1.07 (1.15)	1.51 (2.29)	20.60	85.41
T_6	Emamectin Benzoate 5 SG at 0.002%	2.51 (6.28)	1.80 (3.23)	1.82 (3.31)	1.64 (2.69)	1.52 (2.32)	1.64 (2.70)	1.28 (1.63)	1.36 (1.85)	1.24 (1.53)	1.13 (1.27)	1.00 (1.01)	1.47 (2.17)	21.75	95.76
T_7	Lambda-cyhalothrin 5 SC at 0.005%	2.62 (6.88)	1.82 (3.33)	1.85 (3.42)	1.69 (2.87)	1.60 (2.56)	1.72 (2.95)	1.38 (1.91)	1.42 (2.02)	1.29 (1.67)	1.18 (1.40)	1.10 (1.21)	1.53 (2.36)	19.81	78.30
T_8	Novaluron 10 EC at 0.01%	2.48 (6.16)	2.25 (5.06)	2.27 (5.17)	2.16 (4.66)	1.98 (3.93)	2.08 (4.35)	1.80 (3.24)	1.83 (3.34)	1.74 (3.02)	1.61 (2.60)	1.48 (2.19)	1.94 (3.78)	17.59	58.32
T_9	Control (Treated with water)	2.51 (6.32)	2.67 (7.12)	2.72 (7.42)	2.71 (7.34)	2.70 (7.29)	2.77 (7.69)	2.62 (6.88)	2.69 (7.22)	2.65 (7.02)	2.60 (6.76)	2.64 (6.97)	2.69 (7.26)	11.11	-
	S.E.m ±	0.13	0.12	0.12	0.12	0.09	0.10	0.12	0.11	0.11	0.12	0.10	0.08	0.98	-
	S.E.m ± (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
	C.D (p= 0.05)	NS	0.37	0.36	0.38	0.29	0.31	0.35	0.33	0.34	0.36	0.31	0.25	2.95	-
	C.D (p= 0.05) (P×T)	-	-	-	-	-	-	-	-	-	-	-	0.12		-

DAS = Days after spraying; Figure in parentheses original value whereas, those outside $\sqrt{x + 0.5}$ transformed values.

recorded in the plots treated with emamectin benzoate 5SG (13.16%) which was at par with indoxacarb 14.5SC (14.16%) and lambdacyhalothrin 5SC (16.33%). The next effective treatments were thiacloprid 21.7SC (24.50%) and novaluron 10EC (27.83%). Maximum pod yield was obtained with emamectin benzoate 5SG (21.75 q/ ha) followed by indoxacarb 14.5SC (20.60 q/ ha) (Table 1, 2). Mohapatra and Srivastava (2002) observed that lambdacyhalothrin 5EC @ 25 g a.i./ ha was the most effective against *M. vitrata* in pigeon pea. Rao et al. (2007) showed that the indoxacarb 14.5SC @ 1 ml/ l was the most effective against *M. vitrata* in pigeonpea. Srinivasan and Durairaj (2007) found that spinosad 45SC @ 73 g a.i./ ha was the most effective against *H. armigera* followed by indoxacarb 14.8SC in pigeon pea. Babariya et al. (2010) with indoxacarb 0.0075% observed maximum mortality of *H. armigera* in pigeon pea. Sonune et al. (2010) observed that the indoxacarb 0.008% and lambdacyhalothrin 0.005% were the most effective in against *M. vitrata* in black gram. Nebapure and Sagar (2019) revealed that chlorantraniliprole 18.5SC @ 30g a.i./ ha followed by indoxacarb 15.8EC @ 73g a.i./ ha at 15 days interval were effective against *M. vitrata* on pigeonpea. Ahmed et al. (2020) found emamectin benzoate @ 1.0 g/ l as the most effective against *M. vitrata.* Haripriya et al. (2021) revealed that spinosad 45SC @ 75 ml/ ha followed by emamectin benzoate 5SG @ 200 ml/ ha were effective against *M. vitrata* on lablab and green gram. Thus, emamectin benzoate 5SG at 0.002%, indoxacarb 14.5SC at 0.007% and lambda-cyhalothrin 5SC at 0.005% can be recommended against *H. armigera* and *M. vitrata*.

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BIORATIONAL MANAGEMENT OF *SPODOPTERA FRUGIPERDA* (J E SMITH) ON MAIZE

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ABSTRACT

The present study on the biorational management of *Spodoptera frugiperda* (J E Smith) on maize was conducted in the farm of Department of Agricultural Entomology, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola during kharif 2019-20. The experiment was laid out in randomized block design with eight treatments replicated thrice. The treatments included- *Nomuraea rileyi* 1x10^8 cfu/ g @ 30 g/ 10 l, *Metarhizium anisopliae* 1x10^8 cfu/ g @ 50 g/ 10 l, *Beauveria bassiana* 1x10^9 cfu/ g @ 40 g/ 10 l, NSKE 5%, Bt 85% @ 20 g/ 10 l, SLNPV 1x10^9 POB/ ml (500 LE), azadirachtin 1500 ppm @ 50 ml/ 10 l, and control (untreated). Four sprays were applied at 12 days interval. The results revealed that with fourth spray, Bt 85% @ 20 g/ 10 l was the most effective.

Key words: Maize, *Spodoptera frugiperda*, *Nomuraea rileyi*, SLNPV, Bt, *Metarhizium anisopliae*, *Beauveria bassiana*, azadirachtin.

Maize is a cereal crop grown in > 160 countries in tropical, subtropical and temperate regions, with India having a productivity of 109 kg/ ha, which is much less than the US yield of 863 kg/ ha. This low productivity might be due to several reasons viz., environmental factors, low mechanization, pest and diseases etc. Of these, insect pests are the major constraints as these attacking maize not only directly as borers, sap suckers, stem and root feeders etc. but also indirectly as vectors of diseases. In India, the fall army worm *Spodoptera frugiperda* (J E Smith) was reported in May 2018 on maize for the first time from Karnataka (Sharanabasappa et al., 2018a). Molecular diversity of this from different states of India indicated prevalence of R strain (Mahadevaswamy et al., 2018). The total life of male and female was observed to be 32-43 and 34-46 days, respectively (Sharanabasappa et al. 2018b). The extent of its damage varied from 20 to 80% on maize (Sharanabasappa et al., 2019b). Considering adverse effect of insecticides there is need for environmentally safe and cost-effective management strategy in the form of biopesticides in IPM. The present study evaluates some biorational approaches including biopesticides against *S. frugiperda* on maize.

MATERIALS AND METHODS

The experiment was conducted on the farm of Department of Agricultural Entomology Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during kharif 2019-20. The experiment was laid out in randomized block design (RBD) with eight treatments and three replications, with plot size 5.4x 3 m and spacing of 60x 20 cm. Uday (Mahabeej-1114) cultivar was used and sowing was done on 5th July 2019. All the agronomical practices were carried out as per the recommendations except, plant protection. The treatments include: T_1: *Nomuraea rileyi* 1x10^8 cfu/ g - 30 g, T_2: *Metarhizium anisopliae* 1x10^8 cfu/g - 50 g, T_3: *Beauveria bassiana* 1x10^9 cfu/ g - 40 g, T_4: Neem seed kernel extract (NSKE) 5% - 50 g , T_5: *Bacillus thuriengiensis* 85% - 20 g, T_6: *Sl*NPV 1x10^9 POB/ml - 500 LE, T_7: Azadirachtin 1500 ppm - 50 ml, T_8: Control. (The doses used are in 10 l of water); Source: *N. rileyi, M. anisopliae, B. bassiana*- Plant Pathology department, Dr PDKV, Akola; neem seed kernel extract- neem seeds from area of Dr PDKV, Akola; *Sl*NPV- Department of Agricultural Entomology, Dr PDKV, Akola; *Bacillus thuringiensis* var. *kurstaki*- Margo Company's Delfin WG; azadirachtin 1500 ppm- MBF Company Neem Fighter. The spray solution was freshly prepared with the required quantity of water for spraying each plot estimated by spraying plain water in untreated control plot. The required quantity of biopesticides and botanical was worked out and spray solution was prepared by mixing them in water thoroughly. The following formula was used- $V = \frac{C \times A}{\% \, a.i.}$ where, V= quantity of biopesticides and botanical, C= concentration of spray required, A= quantity of water, % a.i. = active ingredient in commercial product.

Table 1. Efficacy of biopesticides and botanicals against *S. frugiperda*

Tr. No.	Treatment Dose/ 10 l water	% infestation of *S. frugiperda*																
		1 DBS	3 DAS	7 DAS	10 DAS	1 DBS	3 DAS	7 DAS	10 DAS	1 DBS	3 DAS	7 DAS	10 DAS	1 DBS	3 DAS	7 DAS	10 DAS	Overall mean
T_1	*Nomuraea rileyi* 1x10^8 cfu/ g 30 g	63.33 (52.91)	33.00 (34.97)	26.67 (31.04)	38.00 (38.03)	39.30 (38.80)	31.33 (34.01)	26.10 (30.69)	33.33 (35.24)	33.91 (35.59)	32.05 (34.46)	31.50 (34.12)	32.90 (34.97)	32.92 (34.98)	32.43 (34.69)	31.83 (34.32)	31.48 (34.10)	31.72 (34.57)
T_2	*Metarhizium anisopliae* 1x10^8 cfu/ g 50 g	78.33 (62.48)	27.67 (31.72)	23.00 (28.64)	32.67 (34.84)	33.33 (35.23)	26.27 (30.77)	23.65 (29.05)	26.47 (30.91)	26.60 (30.99)	25.00 (29.94)	24.01 (29.26)	25.28 (30.10)	25.58 (30.30)	24.80 (29.78)	24.08 (29.29)	23.65 (29.00)	25.54 (30.35)
T_3	*Beauveria bassiana* 1x10^9 cfu/g 40 g	68.33 (56.03)	26.00 (30.64)	16.67 (24.07)	30.00 (33.20)	30.33 (33.40)	22.89 (28.51)	20.92 (27.12)	23.15 (28.69)	23.33 (28.81)	22.19 (28.02)	20.48 (26.80)	21.83 (27.76)	21.96 (27.85)	20.81 (27.04)	19.97 (26.43)	18.65 (25.46)	21.96 (27.93)
T_4	Neem seed extract (NSKE) 5% 50 g	65.00 (54.84)	51.00 (45.57)	44.67 (41.93)	57.67 (49.41)	57.89 (49.54)	48.90 (44.36)	44.24 (41.67)	49.24 (44.56)	49.75 (44.86)	48.68 (44.24)	47.67 (43.66)	48.87 (44.35)	49.20 (44.54)	48.52 (44.15)	48.24 (43.99)	47.81 (43.74)	48.79 (44.31)
T_5	*Bt* 85% 20 g	71.67 (58.26)	23.33 (28.67)	15.33 (22.79)	26.33 (30.77)	26.33 (30.77)	15.33 (22.97)	13.29 (21.33)	15.99 (23.47)	16.03 (23.49)	14.00 (21.87)	12.63 (20.72)	14.33 (22.13)	14.55 (22.31)	13.50 (21.41)	12.33 (20.42)	10.52 (18.79)	15.57 (23.22)
T_6	*Sl*NPV 1x10^9 POB/ml 500 LE	65.00 (54.84)	75.67 (61.12)	79.97 (64.93)	80.00 (63.84)	82.02 (67.27)	82.89 (68.17)	84.13 (69.07)	83.30 (68.46)	83.73 (66.59)	85.96 (70.41)	86.50 (69.77)	86.67 (70.36)	88.12 (71.37)	87.76 (70.40)	87.80 (71.22)	87.77 (71.15)	84.03 (65.70)
T_7	Azadirachtin 1500 ppm 50 ml	70.00 (57.00)	36.33 (37.06)	29.67 (32.96)	39.33 (38.83)	40.33 (39.42)	43.67 (41.36)	38.20 (38.17)	44.26 (41.70)	44.49 (41.83)	43.11 (41.04)	42.04 (40.41)	43.00 (40.97)	43.62 (41.33)	42.76 (40.83)	42.07 (40.43)	41.88 (40.32)	40.52 (39.53)
T_8	Control -	63.33 (53.07)	75.00 (60.78)	79.83 (63.79)	80.22 (65.90)	81.89 (65.04)	82.77 (65.78)	84.00 (66.77)	83.22 (68.71)	84.32 (69.21)	86.00 (68.34)	86.17 (68.56)	86.50 (68.76)	87.67 (69.76)	87.63 (69.69)	87.74 (69.80)	87.63 (69.69)	83.89 (65.53)
'F' test		NS	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±		-	3.27	3.19	3.77	3.56	3.75	3.54	3.70	3.62	3.38	3.18	3.06	2.85	2.29	2.95	2.92	3.25
CD (p= 0.05		-	9.92	9.68	11.44	10.80	11.38	10.75	11.22	10.97	10.25	9.64	9.29	8.63	8.90	8.95	8.86	10.02
CV (%)		-	13.71	14.26	14.73	13.73	15.47	15.17	15.12	14.69	13.84	13.18	12.52	11.52	12.00	12.17	12.18	13.7

*Figures in parentheses arc sin transformed values; DBS- Day before spraying, DAS-Day after spraying

Table 2. Efficacy of biopesticides and botanicals against *S. frugiperda*

Tr. No.	Treatment	% infestation of fall armyworm			
		3 DAS	7 DAS	10 DAS	Overall mean
T_1	*Nomuraea rileyi* 1x10^8 cfu /g 30 g	32.20 (34.57)	29.02 (32.60)	33.93 (35.62)	31.72 (34.57)
T_2	*Metarhizium anisopliae* 1x10^8 cfu /g 50 g	25.93 (30.61)	23.68 (29.11)	27.02 (31.32)	25.54 (30.35)
T_3	*Beauveria bassiana* 1x10^9 cfu /g 40 g	22.97 (28.64)	19.51 (26.21)	23.40 (28.93)	21.96 (27.93)
T_4	Neem seed extract (NSE) 5% 5 g	49.27 (44.58)	46.20 (42.82)	50.90 (45.52)	48.79 (44.31)
T_5	*Bt* 85% 20 g	16.54 (23.99)	13.39 (21.46)	16.79 (24.19)	15.57 (23.22)
T_6	*Sl*NPV 1x10^9 POB/ ml 500 LE	83.07 (65.70)	84.6 (66.89)	84.43 (66.76)	84.03 (65.70)
T_7	Azadirachtin1500 PPM 50 ml	41.46 (40.08)	37.99 (38.05)	42.12 (40.47)	40.52 (39.53)
T_8	Control	82.85 (65.54)	84.44 (66.77)	84.39 (66.72)	83.89 (65.53)
	'F' test	Sig	Sig	Sig	Sig
	SE(m)±	3.17	3.22	3.36	3.25
	CD at 5%	10.11	9.76	10.20	10.02
	CV (%)	13.76	13.70	13.64	13.7

*Figures in parentheses corresponding arc sin transformation values; DBS- Day Before Spraying; DAS-Day after spraying.

Neem seed extract was prepared following standard methodology. Pretreatment observations were made one day before spray, with further observations made on 3rd, 7th, and 10th days after spray (DAS) taking 10 plants/ plot selected randomly and % worked out. The data were subjected to statistical analysis as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The pretreatment observations of *S. frugiperda* were found statistically non-significant – ranging from from 63.33 to 78.33/ 10 plants. Three DAS, the treatments were found to significantly superior over control; minimum incidence of 23.33% was observed with T_5: *Bacillus thuriengiensis* 85%- 20 g; and T_6: *Sl*NPV 1x10^9 POB/ml - 500 LE was inferior to control (75.67%). Seven DAS, again T_5 was found superior (15.33%), statistically at par with T_3, T_2 and *N. rileyi* @ 30 g/ 10 l and T_6 was found inferior to control (79.97%). Ten DAS too T_5 was superior (26.33%), and T_6 was inferior. Thus, after first spray, six treatments were significantly effective. With second spray after 3 DAS, the least incidence was observed plots treated with T_5, statistically at par with T_3, T_2 and T_1 and T_6 was inferior to control. At 7 and 10 DAS, similar trend was observed. Thus, with mean % incidence after second spray six treatments were significantly effective, with T_5 resulting in minimum infestation (14.87%), found at par with T_3, T_2 and T_1. After third spray, at 3 DAS the least incidence was observed with T_5, statistically at par with T_3 and T_2; T_6 showed 85.96% infestation as against 86% in control. Seven DAS (after third spray) the incidence reduced to 12.63% with T_5, found statistically at par with T_3 and T_2. Ten days after third spray again similar trend was observed. Thus, after third spray T_5 resulted in minimum infestation of 13.65%, found statistically at par with T_3 and T_2. Similar results were obtained with fourth spray leading to 13.50% incidence with T_5 (Table 1).

The cumulative effect of treatments given in Table 2 reveal that at three days after spray, T_5 proved effective by recording minimum infestation of *S. frugiperda* (16.54%), However, this treatment was found statistically at par with T_3 and T_2 recorded 22.97% and 25.93% infestation respectively. Whereas the treatments T_1, T_7 and T_4 concentration with 32.20%,

41.46% and 49.27%, infestation respectively. The control plot recorded 82.85% infestation. T_6 treatment was inferior to control and recorded maximum (83.07) % infestation. The data on cumulative effect of different treatments at seven days after spray revealed similar trend. T_{5-} *Bacillus thuriengiensis* 85%- 20 g led to minimum incidence (13.39%), and found statistically at par with, T_3 and T_2, and T_6 treatment was inferior to control. Polanczyk et al. (1999) evaluated in vivo activities of *Bt* strains on *S. frugiperda*, and observed that suspensions of *Bt aizawai* HD 68 and *Bt thuringiensis* 4412, containing 3x 10^8 cells/ ml, induced effective mortality. Similar results were obtained by Hernandez (1988). Ramanujam et al. (2020) with field trials observed efficacy of three sprays of *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45. Ahirwar et al. (2013) in soybean reported that *B. thuringiensis* var. *kurstaki* was the most effective. Ramos et al. (2020) with biological control assays revealed that, both *B. bassiana* and *M. anisopliae* caused (100%) mortality. Gutierrez et al. (1996) evaluated entomopathogenic fungi and observed that isolates of *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, and *Paecilomyces javanicus* were highly pathogenic. Mallapur et al. (2018) in studies on *N. rileyi* revealed effective reduction of incidence of *S. frugiperda.*

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POPULATION DYNAMICS OF MAJOR INSECT PESTS OF INDIAN BEAN

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ABSTRACT

The incidence of aphid *Aphis craccivora* Koch in Indian bean *Lablab purpureus* (L.) reached its peak during 2nd week of January (2nd SMW) with 25.27 aphids/ twig of 10 cm. Maximum incidence of jassid *Empoasca kerri* Pruthi (8.20/ leaf) and whitefly *Bemisia tabaci* (Gennadius) (7.12/ leaf) was observed during 4th week of December (52nd SMW). The thrips *Megalurothrips distalis* Karny started appearing from 4th week of November (48th SMW) reaching its peak (10.35/ flower) during 1st SMW. Maximum larval counts of *Helicoverpa armigera* (Hubner) (5.79/ plant) was observed during 2nd week of January (2nd SMW), while in case of *Maruca vitrata* Geyer (7.21/ plant) it was during 2nd week of January (2nd SMW). Maximum and minimum temperature showed significant negative influence on the incidence of all these pests; and morning relative humidity showed significant negative effect on all except *M. distalis.* While morning vapour pressure showed significant negative influence on the incidence of all pests except *E. kerri* and *B. tabaci.*

Key words: *Lablab purpureus*, *Empoasca kerri, Bemisia tabaci*, *Megalurothrips distalis*, *Helicoverpa armigera, Maruca vitrata,* seasonal incidence, weather factors, correlation coefficients

Indian bean *Lablab purpureus* (L.) is an important pulse cum vegetable crop in India and it is also used as cattle feed. The protein content of field bean is quite high varying from 20.0 to 28.0% (Reddy et al., 2017). Insect pests are the major constraints for the productivity of Indian bean, as it is attacked by a number of insect pests viz. aphid *Aphis craccivora* Koch.; jassids *Empoasca fabae* (Harris); *E. krameri* Ross & Moore and *E. kerri* Pruthi; pod borers *Etiella zinckenella* (Treit.), *Maruca vitrata* Geyer and *Helicoverpa armigera* (Hubner); whitefly *Bemisia tabaci* (Genn.); stem fly *Ophiomyia phaseoli* (Tryon); Bihar hairy caterpillar *Spilosoma obliqua* (Wlk.) (Jakhar et al., 2017; Reddy et al., 2017). For development of successful IPM strategies, detailed information on the population buildup in particular the influence of weather factors is of great significance. This information will be helpful in preferring predictive models in forecasting the pest outbreaks. Hence, this study on the population dynamics of major pests of Indian bean.

MATERIALS AND METHODS

Study was carried out at the College Farm, N M College of Agriculture, Navsari Agricultural University, Navsari, Gujarat during 2019-20. The Indian bean variety GNIB-22 was sown on 22nd October 2019 in 400 m^2 area. All the recommended post-sowing agronomical practices were followed except the plant protection. Observations were recorded at an interval of seven days starting from one week after germination till harvesting from five randomly selected plants from each of five quadrates. Aphid population was recorded on three randomly selected twigs (about 10 cm long) from each tagged plant and mean number of aphids/ twig were counted. Jassid and whitefly were counted from three leaves (from top, middle and bottom), while that of thrips was assessed by shaking flowers with twigs over a white paper and counting thrips fallen on the paper piece. Number of pod borer *H. armigera* and *M vitrata* larvae were recorded and mean number of larvae/ plants was calculated. The data on weather parameters obtained from the Agricultural Meteorological observatory, Department of Meteorology, Navsari Agricultural University, Navsari were utilized for computing correlation coefficients.

RESULTS AND DISCUSSION

The results obtained revealed that the incidence of aphid *Aphis craccivora* commenced from 2nd week after sowing (WAS) i.e. 2nd week of November (46th SMW) and persisted till 1st week of February (6th SMW, 14th WAS); and ranged from 1.94 to 25.27 aphids/ twig of 10 cm, and their incidence increased from 8th WAS to reach its peak during 10th WAS (2nd SMW- 25.27 aphids/ twig) (Fig. 1). The relationship with weather factors revealed that maximum temperature (r=-0.782**),

minimum temperature (r=-0.726**), morning vapour pressure (r=-0.775**) and evening vapour pressure (r=-0.738**) exhibited highly significant negative correlation; while, wind speed (0.546*) had a significant positive correlation and morning relative humidity- RH (-0.539*) had a significant negative correlation (Table 1). These results agree with those of Chaudhary et al. (2016) on the seasonal incidence. Dalwadi et al. (2007) stated that minimum and mean temperature and vapour pressure showed significant negative association with aphids, while RH, sunshine hours and wind speed correlated positively.

The activity of jassid *E. kerri* started from of 2nd WAS i.e. 2nd week of November (46th SMW) and persisted till 1st week of February (6th SMW, 14th WAS) with peak (8.20 jassids/ leaf) during 4thweek of December (52nd SMW, 8th WAS). There was a significant negative correlation with maximum temperature (r= -0.632*), minimum temperature (r=-0.556*) and morning RH (r=-0.537*) (Fig. 1; Table 1). Dalwadi et al. (2007) reported that RH had significant negative correlation with jassids. Singh et al. (2019) revealed that jassid, *Empoasca motti* incidence had a negative correlation with maximum and minimum temperature, RH and rainfall. With whitefly *B. tabaci*, the incidence was found to commence from of 2nd WAS i.e., 2nd week of November (46th SMW) and persisted till 1st week of February (6th SMW, 14th WAS) with peak (7.12 whiteflies/ leaf) during 4th week of December (52nd SMW, 8th WAS). Correlation coefficients with weather parameters showed significant negative correlation with maximum temperature (r= -0.594*) and morning RH (r=-0.541*) (Fig. 1; Table 1). Marabi et al. (2017) observed that whitefly exhibited significant positive correlation with maximum and minimum temperature, morning and evening vapour pressure and evaporation in soybean; whereas, negative correlation was observed with morning RH % during rabi season. The activity of thrips *M. distalis* started from of 4th WAS i.e. 4th week of November (48th SMW) and persisted till 2nd week of February (6th SMW, 14th WAS) with peak (10.35 thrips/ flower) during 1st SMW (9th WAS). Its incidence

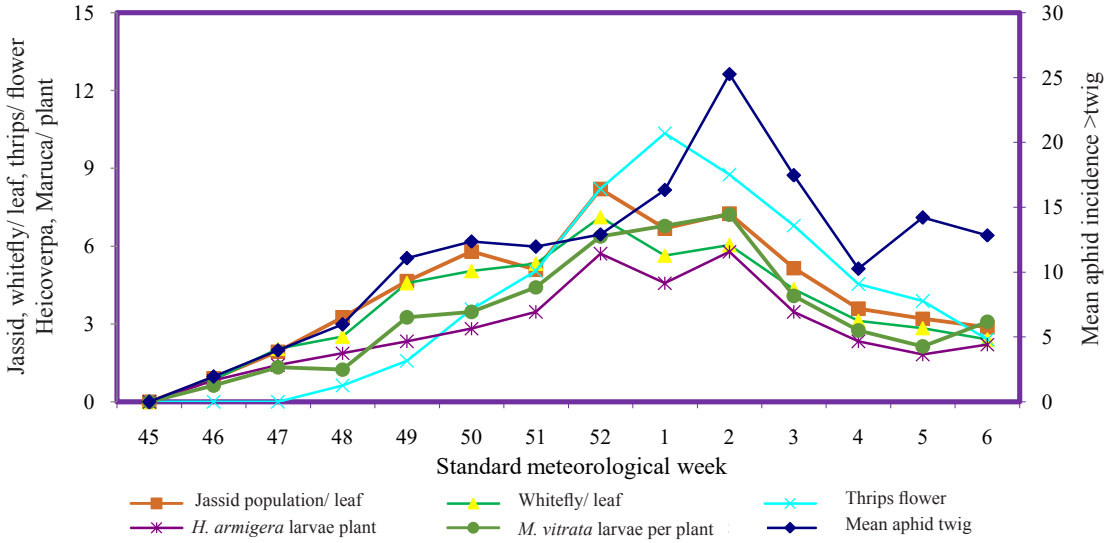


Fig. 1. Population dynamics of insect pests on Indian bean

Table 1. Correlation coefficient(r) between weather parameters and pests on Indian bean

Weather factors	Aphid	Jassid	Whitefly	Thrips	*H. armigera*	*M. vitrata*
Maximum temperature (^{0}C)	-0.782**	-0.632*	-0.594*	-0.830**	-0.644*	-0.701**
Minimum temperature (^{0}C)	-0.726**	-0.556*	-0.505	-0.727**	-0.553*	-0.570*
Morning relative humidity (%)	-0.539*	-0.537*	-0.541*	-0.296	-0.535*	-0.534*
Evening relative humidity (%)	-0.444	-0.051	0.001	-0.096	-0.097	-0.113
Bright Sunshine(h/ day)	0.084	-0.379	-0.430	-0.189	-0.328	-0.281
Wind speed (km/ hr)	0.546*	0.188	0.209	0.126	0.159	0.288
Morning vapour pressure (mm)	-0.775**	-0.474	-0.349	-0.669**	-0.543*	-0.593*
Evening vapour pressure (mm)	-0.738**	-0.406	-0.349	-0.565*	-0.427	-0.480

**Significant at p=0.01 (r=±0.66); *Significant at p=0.05 (r=± 0.53)

exhibited a highly significant negative correlation with the maximum temperature (r=-0.830**), minimum temperature (r=-0.727*) and morning vapour pressure (r=-0.669*), ehile, evening vapour pressure (r=-0.565*) showed significant negative correlation (Fig. 1, Table 1).

The larvae of *H. armigera* appeared starting 2nd week of November (46th SMW, 2nd WAS) with 0.83 larva/ plant and remained on the crop till 1st week of February (6th SMW, 14th WAS); maximum counts were during 2nd week of January (2nd SMW) i.e. 5.80 larvae/ plant. Maximum temperature (r= -0.644*), minimum temperature (r= -553*), morning RH (r=-0.535*) and morning vapour pressure (r=- 543*) were found to exhibit a significantly negative correlation with *H. armigera* (Fig. 1; Table 1). These results are in agreement with those of Rashmi et al. (2019), who reported that the highest infestation of this pest was found during the month of December and January and reported a significant negative association with maximum temperature. Chavan et al. (2020) and Dindor et al. (2020) revealed that *H. armigera* exhibited significant negative correlation with RH. *Maruca vitrata* incidence was observed from 2nd week of November (46th SMW, 2nd WAS) with 0.63 larva/ plant and remained till 1st week of February (6th SMW, 14th WAS); maximum counts were observed during 2nd week of January (2nd SMW- 7.21/ plant; the incidence was showing a highly significant negative correlation with maximum temperature (r= -0.701**), while, minimum temperature (r=-570*), morning RH (r=-0.534*) and morning vapour pressure (r=-593*) exhibited a significantly negative correlation (Fig. 1, Table 1). These results are in agreement with those of Rashmi et al. (2019), who observed that *M. vitrata* was found in large numbers during December and January; and maximum temperature, minimum temperature and evaporation showed significant negative correlation.

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ASSESSMENT OF *LECANICILLUM LECANII* (ZIMMERMEN) AGAINST SUCKING PESTS IN BT COTTON

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ABSTRACT

An on-farm trial on the assessment of *Lecanicillum lecanii* (Zimmermen) on management of sucking pests in cotton on farmer's fields was conducted by the Krishi Vigyan Kendra, Kampasagar during kharif 2015 to 2017. Three sprayings of *L. lecanii* @ 5 g/ l from 25 DAS (days after sowing) at an interval of 10 days were taken up. Incidence of leaf hopper *Amrasca biguttula biguttula* (Ishida), thrips *Thrips tabaci* (L.) and whitefly *Bemisia tabaci* (Genn) were observed as counts three leaves. Significantly less incidence i.e. 2.9, 4.3 and 4.5, respectively, was observed in improved practice (spraying of entomopathogenic fungi *L. lecanii* @ 5 g/ l three times with an interval of 10 days from 25 DAS) as against farmers' practice (i.e. 4.9, 9.0 and 7.9, respectively). Seed cotton yield was significantly more with improved practice (2335.7 kg/ ha) with 18.9% increase. Higher gross and net returns, and benefit cost ratio were obtained with improved practice.

Key words: Cotton, on farm trial, *Lecanicillum lecanii*, *Amrasca biguttula biguttula*, *Thrips tabaci*, *Bemisia tabaci,* yield, gross returns, net returns, benefit cost ratio

Cotton (*Gossypisum hirustum* L.) is one of the most important cultivated commercial crop in India which is subjected to the ravages of a number of insect pests. The insect pest spectrum of cotton is quite complex with 1326 pests, of which in India, 162 insect pests had been reported with 24 species attaining pest status (Dhawan, 2000; Puri, 1998). These cause yield losses up to 30-80% and it is a major limiting factor (Sundaramurthy, 1985; Kranthi et al., 2009). The major ones are the boll worms and sucking pest complex. Sucking pests cause significantly more damage, and these include leafhopper *Amrasca biguttula biguttula* (Ishida), thrips *Thrips tabaci* (L.) and whitefly *Bemisia tabaci* (Genn). Estimated yield losses are to an extent of 21.2% (Dhawan and Sindhu, 1986), 28.13% (Chavan et al., 2010) and 33.02% (Tukaram et al., 2017). In conventional methods of pest management, dependence on widespread use of chemical pesticides has resulted in environmental pollution and human health hazards (Perry et al., 1998). Continuous and indiscriminate use of pesticides results in ability of sucking pests to develop resistance, requiring ecofriendly measures (Koul, 2008). Microbial biopesticides reduce pesticide load and entomopathogenic fungi preferably decrease human toxicity, with low persistence in the environment (Isman et al*.,* 2001), more biodegradable and less toxic to humans and natural enemies (Isman, 1984). Entomopathogenic fungi play an important role in IPM especially against sucking pests i.e. aphids and whiteflies in vegetables (Saito and Sugiyama, 2005; Singh and Kaur, 2020), and thrips, mites, aphids, whiteflies and mealybugs (Khan et al., 2012). *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch) and *Lecanicillium lecanii* (Zimmerman) are the major entomopathogenic fungi (Daniel and Wyss, 2010; Shah and Shukla, 2014). *Lecanium lecanii* is the most effective entomopathogenic fungi against all stages of the sucking pests and is available in liquid and powder formulations. It is effective for the control of sap feeding pests like aphids, whiteflies, scale insects, thrips, mealy bugs (Kanagaratnam et al., 1982). The mode of action of *L. lecanii* is of contact with host integument, it gets adhere to the epicuticle, germinate and internal colonization which results in death of host insect (Rabindra and Ramanujam, 2007). *Verticillium lecanii* was found more effective against sucking pests (Wadhani et al., 2020; Ghelani et al., 2014); NSKE 5% and *V. lecanii* 0.5% (Gore et al., 2021) in cotton, aphid and jassid in okra (Janghel et al., 2015; Suraj et al., 2016). The use of such microbial formulations are ecofriendly and safe to human beings (Singh and Kaur, 2020). Hence, this study to assess the effectiveness of the *L. lecanii* on sucking pests in cotton at Nalgonda District.

Table 1. Efficacy of *Lecanicillum lecanii* against sucking pests of Bt cotton- improved *vs* farmer's practice (kharif 2015 to 2017)

Year	Improved practice			Farmers practice			Yield (kg/ha)		% increase of yield over the control	Gross returns (Rs/ha)		Cost of cultivation (Rs/ha)		Net returns (Rs/ha)		B:C ratio	
	Leaf hoppers/ 3 leaves	Whiteflies/ 3 leaves	Thrips/ 3 leaves	Leaf hoppers/ 3 leaves	Whiteflies/ 3 leaves	Thrips/ 3 leaves	Improved practice	Farmers practice		Improved practice	Farmers practice	Improved practice	Farmers practice	Improved practice	Farmers practice	Improved practice	Farmers practice
2015-16	1.4	2.6	2.6	4.8	9.0	7.2	2107.0	1694.0	18.4	79276.2	60136.4	36706.0	37702.0	42570.2	22434.4	2.2	1.6
2016-17	1.4	2.4	2.0	3.4	9.2	6.6	2175.0	1762.5	23..4	101375.0	80362.5	64750.0	41275.0	36625.0	39087.5	2.8	2.1
2017-18	6.0	7.8	8.9	6.5	8.7	9.8	2725.0	2437.5	10.4	115450.0	100362.5	41412.5	42950.0	74037.5	57412.5	2.8	2.3
Mean	2.9	4.3	4.5	4.9	9.0	7.9	2335.7	1964.7	18.9	98700.4	80287.1	47622.8	40642.3	51077.6	39644.8	2.6	2.0

MATERIALS AND METHODS

The experiments were carried out by the Krishi Vigyan Kendra, Kampasagar in farmer's fields of Nalgonda District, Telangana during kharif 2015 to 2017. During the study period, 5 farmers were selected in each season under on farm trials. The improved technology comprised of spraying of *L. lecanii* @ 5 g/ l at 25 days after sowing (DAS) as three sprays with an interval of 10 days. Whereas farmers' practice includes use of insecticides like monocrotophos @ 1.6 ml/ l and acephate @ 1.5 g/ l at various stages of crop growth period. Observations were recorded on population of sucking pests- *A. biguttula biguttula, T. tabaci,* and *B. tabaci* on three leaves (upper, middle and lower) each on five randomly selected plants in each field. Seed cotton yield was also recorded and economics were worked out.

RESULTS AND DISCUSSION

Efficacy of improved practice of spraying entomopathogenic fungi *L. lecanii* @ 5 g/ l three times at an interval of 10 days from 25 DAS vs farmers practice is presented in Table 1; these data reveal significantly less incidence of *A. biguttulla biguttula*, *B. tabaci* and *T. tabaci* (2.9, 4.3 and 4.5, respectively) in improved practice, during kharif 2015-2017. These results agree with those of Gore et al. (2021) on the application of NSKE 5%+ *V. lecanii* @ 0.5%. Sasikumar et al. (2018) reported that *L. lecanii* @ 5 g/ l was highly effective against thrips in cotton. Ghelani et al. (2006; 2014) found that *V. lecanii* @ 5 g/ l was effective against *Aphis gossypii*; and *V. lecanii* @ 2.5 kg/ ha combined with azadirachtin @ 0.0009% were found moderately effective against major sucking pests of Bt cotton. Raghunandan et al. (2018) observed that sucking pests in cotton get reduced significantly with spraying of *V. lecanii* @ 4 g/ l. Hole et al. (2015) observed effectiveness of *V. lecanii* (2 x 10^{12}cfu/g) @ 2000 g/ ha against sucking pests in Bt cotton. Wawdhane et al. (2020) also observed such effectiveness. Whitefly incidence reduced with spraying of *V. lecanii* @ 3 g/ l in cotton (Ritasharma and Sudha, 2017), and *V. lecanii* @ 7 g/ l gave higher mortality of jassids in okra (Baladaniya et al., 2010). The cotton yield in improved practice ranged from 2107.0 kg/ ha to 2725.0 kg/ ha over farmers practice of 1694.0 kg/ ha to 2437.5 kg/ ha. Similar results were obtained by Raghunandan et al. (2018) and Patil et al. (2012) with *V. lecanii*, as also observed by Harischandranaik and Shekharappa (2009); maximum gross returns of Rs. 98,700/ ha, net returns of Rs. 51,078/ ha and benefit cost ratio 2.6 were observed with such an improved practice. These results agree

with those of Raghunandan et al. (2018) and Patel et al. (2019). Meena et al. (2013) obtained maximum cost benefit ratio with application of *V. lecanii* in mustard.

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DISTRIBUTIONS PATTERN OF PROTECTIVE SILKEN DISCS WEAVED BY LARVAE OF SUGARCANE TOP SHOOT BORER *SCIRPOPHAGA EXCERPTALIS* (WALKER)

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ABSTRACT

The distribution pattern of protective silken discs weaved by larvae of five broods (generations) of top shoot borer *Scirpophaga excerptalis* (Walker) was studied in three early maturing varieties (Co 0238, CoPk 09151 and CoLk 94184) and two mid late varieties (CoLk 13204 and CoLk 8102). The mean number of silken discs guarding the female pupa was more in first, second, third, fourth and fifth broods than male pupa in all varieties. The ranges of the discs protecting the female pupae in all broods (first, second, third, fourth and fifth) varied 3-12, 2-21, 2-17, 3-13 and 2-9, respectively. The male pupae were surrounded by 5.23 (CoLk 8102), 6.93, 8.73, 6.67 and 3.92 (CoPk 05191) discs, respectively which were higher than the other varieties in respective broods. The results allow us to better understand the distribution pattern of silken discs weaved by larvae of different broods of *S. excerptalis* in early and mid-late varieties.

Key words: *Scirpophaga excerptalis*, Crambidae, Lepidoptera, pupal chamber, exit hole, parasitoids, temperature, humidity, protective discs, broods, varietal differences, dynamics

The top shoot borer *Scirpophaga excerptalis* (Walker) (Crambidae: Lepidoptera) is a major sugarcane borer in almost all the important cane growing areas of subtropical India. Its incidence is usually more serious in north Indian sugarcane belt where it causes considerable reduction in yield and quality (Gupta, 1959 and Kalra, 1972). It spends major portion of life cycle within the plant tissues, the short period passed outside is as adult, egg stage and neonate larva (Majumder, 2020). The young caterpillars enter the leaf mid-ribs within a few hours of their hatching from the eggs, and after that the pest remains hidden inside the plant tissues till its emergence as moths. The five broods (generations) of *S.excerptalis* in a calendar year have been observed at Lucknow conditions (Gupta, 1959 and Majumder, 2018).

The first, second, third, fourth and fifth broods appears in March, April, June, August, September and March, respectively. The first and second broods are very distinct, but from the third brood, overlapping of generations is common. On an average, top shoot borer usually completes the life cycle in 45-55 days (6-8 weeks). The top borer pupates within the cane and the exit hole (for subsequent emergence of moths) is formed by the grownup larva 3 to 5 days before pupation under normal conditions but during adverse conditions, the fully grown larva of the fourth and fifth broods (October- November) would form this hole before they enter hibernation. Before pupation, the larva spins out pupal chamber within which it pupates and reduces its size (Gupta, 1954 and David et al., 1986). Usually the exit hole is formed in the nodal region through the bud, but variations are noticed in different agroclimatic conditions (Singh, 1978). The protective silken discs were made in the pupal chamber before the larva actually enters pupation (Kalra, 1972).Since no active defence (development of any protections or spines in the abdomen) against parasitoids at pupal stage is possible, an adequate prophylactic protection (silken discs) is engineered by the larva. It is commonly known that several circular silken protective discs exist in the pupal chamber, their exact number and their distribution pattern (anterior/posterior end of pupa) with reference to gender or broods of the top borer is either inadequately reported. Therefore, the present study was undertaken to gather useful information on number and distribution pattern of discs guarding the male and female pupa of all five broods of *S. excerptalis*.

MATERIALS AND METHODS

The observations were made at the ICAR-Indian Institute of Sugarcane Research, Lucknow with three early maturing varieties (Co 0238, CoLk 94184 and CoPk 09151) and two mid late varieties (CoLk 13204 and CoLk 8102). The damaged shoots of each brood were collected from autumn planted cane at exit hole

stage (the stage before the emergence of moths) in the month of April, June, July, September and March, respectively during 2019-2020. The autumn planted crop faces all the five broods of *S. excerptalis*. The damaged shoots were split longitudinally and total number of silken discs observed along with anterior and posterior end of pupa. The gender of male and female pupa was determined on the basis of buff coloured or crimson abdomen in females due to the formation of the anal tuft (Majumder, 2018). Posterior end of the female pupa is initially white and later turning orange while male pupa remains white. The data on mean number of silken discs in the pupal chamber along with their distribution at the anterior and posterior end of pupa were analysed statistically.

RESULTS AND DISCUSSION

The mean number of silken discs guarding the female pupa in first brood of *S. excerptalis* was 9.60 (maximum) comprised of 8.00 at anterior and 1.60 discs at the posterior end in CoLk 13204 (Table 1). However, male pupa was protected by 5.23 discs with 4.00 at the anterior and 1.23 at the posterior end of pupa which was maximum in CoLk 8102. The maximum number of silken discs protecting female and male pupa (second brood) was 8.06 and 6.93 discs in CoPk 05191. However, the anterior and posterior ends of female and male pupa were guarded by 6.28 and 1.78 discs and 5.62 and 1.34 discs, respectively. The mean number of discs in female pupa was found to vary 4-21 between in CoPk 05191 whereas in male pupa it varied 2-12 in CoLk 8102. Baitha et al. (2021) reported that 6 to 17 silken discs are made towards the exit hole (anterior side) by female pupa where as 5 to 12 discs by male pupa in variety CoLk 11203. Four to twelve discs are made towards the exit hole and sometimes a few are formed at the posterior end of pupae (Kalra and Sidhu, 1965), without mentioned variety and gender of pupa.

In third brood, mean number of discs protecting female pupa was 7.38 (maximum) which was distributed as 6.33 and 1.05 discs at the anterior and posterior end of pupae, respectively in CoLk 13204. However male pupa was guarded by 8.73 discs (maximum) in CoPk 05191 with 6.82 at anterior and 1.91 discs at the posterior end (Table 1).The anterior end of pupa in all varieties towards exit hole was protected by more number of discs than posterior end. This exit hole (covered with several protective silken discs) renders the grown up caterpillar or the newly formed pupa of top borer vulnerable to parasitisation by *Isotima javensis* and newly hatched grubs gain access to the host pupa by biting its way through the nest of 7 to 12 silken discs (Gupta, 1954; Kalra and David, 1967 and Kalra et al., 1980). The female pupa of third brood always showed more number of discs than male pupa (Majumder, 2018). But in our studies male pupa was guarded more number of discs, it may due to reactions of early and mid late varieties.

The mean number of silken discs protecting female pupa in fourth brood was 8.83 (maximum) with 7.00 and 1.83 at the anterior and posterior end of CoPk 05191 whereas in Co 0238, 6.40 discs were distributed with 5.50 and 0.90, respectively. On the other hand, the male pupae were surrounded by 6.67 discs (maximum) with 5.33 and 1.33 at the anterior and posterior end of pupae in CoPk 05191. As many as 14 discs were observed in the fourth brood (averaging eight) which is higher in comparison to earlier broods (Majumder, 2018). The fifth brood or the winter brood or the over winter brood hibernates in winter, it didn't cut exit hole immediately. The larva prepared an exit hole only after the completion of hibernation and it was sealed by few silken protective discs and retreats in the pupal chamber. The mean number of discs varied from 3.17- 5.08 with 2.50-3.05 and 0.67- 2.17, respectively at the anterior and posterior end of female pupae in all varieties. However, male pupae were surrounded by 3.00 to 3.92 discs in observed varieties. A larva of the fifth brood weaved protecting disc (2 to 3) after the hibernation and if the leaf sheath covering is removed, the larva produces a longer exit tunnel extending beyond the cane epidermis (Majumder, 2018). In this stage pupation takes place very close to the exit hole.

The wide variations are observed in the number of silken discs weaved by larvae of different broods (first to fifth) and greatly influenced by the cane variety and growth stage of the cane. Temperature is the most dominant factor in the sugarcane agro ecosystem that modulates the development, survival, feeding, fecundity, disc formation and dispersal of *S. excerptalis* (Gupta, 1959). It is not clear what influences the larva to weave so many protective discs in early as well as mid late varieties and how the larva determines how many discs will be needed for its safety. The emerging moth from the pupating tunnel pushes the silken discs forward with its head and by partially dislodging the discs it makes it way forward (Majumder, 2018). The silken discs are not broken or punctured; rather these discs remain mostly intact at the centre. In the process of emergence of the moth a lot of scales are left behind in the tunnel on dislodged silken discs. Probably these

Table 1. Distribution of silken discs guarding female/ male pupa of *S.excerptalis* in sugarcane

Variety	Brood	♀/ ♂ Pupa	Mean± S.Em No. of silken discs	Anterior	Posterior
		♀	6.27± 0.69 (3-12)	4.82± 0.57 (3-9)	1.45± 0.28 (0-3)
Co 0238	I	♂	4.56± 0.41 (3-7)	4.22± 0.43 (3-7)	0.22± 0.15 (0-1)
CoLk 94184	I	♀	8.45± 0.43 (6-11)	6.27± 0.65 (4-10)	2.18± 0.44 (0-5)
		♂	4.22± 0.36 (3-6)	3.33± 0.24 (3-5)	0.89± 0.31(0-2)
CoPk 05191	I	♀	6.56± 0.56 (5-10)	5.44± 0.60 (3-9)	1.11± 0.31 (0-2)
		♂	4.40± 0.68(2-6)	4.00± 0.55 (2-5)	0.40± 0.24 (0-1)
CoLk 13204	I	♀	9.60± 0.40 (9-11)	8.00± 0.71(6-10)	1.60± 0.51 (0-3)
		♂	5.00± 0.32 (4-6)	3.80± 0.58 (2-5)	1.20± 0.49 (0-3)
CoLk 8102	I	♀	6.86± 1.06 (3-12)	5.00± 1.00(2-10)	1.86± 0.40 (1-4)
		♂	5.23± 0.46 (3-8)	4.00±0.36 (3-7)	1.23± 0.26 (0-3)
Co 0238	II	♀	4.0± 0.26 (0-5)	4.0± 0.26 (0-5)	0.00
		♂	3.83± 0.70 (2-7)	3.50± 0. 62 (2-6)	0.50± 0.22 (0-1)
CoLk 94184	II	♀	4.93± 0.41(2-8)	4.07± 0.38 (2-7)	0.93± 0.21 (0-2)
		♂	4.17± 0.40 (3-5)	3.67± 0.49 (2-5)	0.59± 0.34 (0-2)
CoPk 05191	II	♀	8.06± 0.60 (4-21)	6.28± 0.60 (2-19)	1.78± 0.22 (0-5)
		♂	6.93± 0.44 (3-11)	5.62± 0.44 (1-11)	1.34± 0.24 (0-4)
CoLk 13204	II	♀	6.13± 0.91 (3-9)	5.00± 0.78 (3-9)	1.13± 0.44 (0-3)
		♂	4.86± 0.55 (3-7)	3.86± 0.67 (2-7)	1.14± 0.40 (0-3)
CoLk 8102	II	♀	7.27± 0.42 (2-12)	6.35± 0.40 (2-12)	0.92± 0.17 (0-2)
		♂	6.67± 0.89 (2-15)	5.67± 0.73 (2-12)	1.00± 0.32 (0-4)
Co 0238	III	♀	5.87± 0.53 (3-10)	5.13± 0.52 (2-9)	0.73± 0.15 (0-2)
		♂	6.00± 1.20 (2-14)	5.60± 0.96 (2-11)	0.50± 0.31 (0-3)
CoLk 94184	III	♀	5.25± 0.80 (2-9)	4.88± 0.61 (2-7)	0.38± 0.26 (0-2)
		♂	5.36± 1.11 (3-15)	5.36± 1.11 (3-15)	0.00
CoPk 05191	III	♀	6.77± 0.90 (2-13)	4.92± 0.88 (1-11)	1.31± 0.26 (0-3)
		♂	8.73± 0.94 (4-14)	6.82± 0.76 (3-11)	1.91± 0.55 (0-6)
CoLk 13204		♀	7.38± 0.70 (3-17)	6.33± 0.49 (2-11)	1.05± 0.33 (0-6)
	III	♂	5.75± 0.48 (2-13)	5.14± 0.40 (2-12)	0.64± 0.16 (0-3)
CoLk 8102		♀	5.92± 0.71 (2-14)	5.38± 0.63 (2-14)	0.54± 0.19 (0-3)
	III	♂	5.38± 0.39 (3-9)	5.05± 0.39 (2-9)	0.43± 0.15 (0-2)
Co 0238	IV	♀	6.40± 0.37 (5-8)	5.50± 0.34 (4-7)	0.90± 0.35 (0-3)
		♂	5.60± 0.48 (3-8)	4.70± 0.40 (3-7)	0.90± 0.23 (0-2)
CoLk 94184	IV	♀	4.33± 0.67 (3-7)	4.00± 0.45 (3-5)	0.33± 0.33 (0-2)
		♂	4.45± 0.67 (2-9)	4.00± 0.45 (2-7)	0.45± 0.25 (0-2)
CoPk 05191	IV	♀	8.83± 1.01 (6-13)	7.00± 0.86 (5-10)	1.83± 0.60 (0-4)
		♂	6.67± 0.88 (4-9)	5.33± 0.80 (3-8)	1.33± 0.42 (0-3)
CoLk 13204	IV	♀	5.18± 0.32 (3-9)	4.97± 0.29 (3-8)	0.21± 0.11 (0-3)
		♂	4.74± 0.25 (1-10)	4.20± 0.18 (1-6)	0.54± 0.14 (0-4)
CoLk 8102	IV	♀	5.00± 1.05 (3-8)	4.40± 0.93(2-7)	0.60± 0.24 (0-1)
		♂	4.50± 0.96 (2-6)	3.50± 0.50(2-4)	1.00± 0.58 (0-2)
Co 0238	V	♀	3.91± 0.31 (3-6)	2.82± 0.26 (2-4)	1.09± 0.21 (0-2)
		♂	3.33± 0.21 (3-4)	2.33± 0.33 (2-4)	1.09± 0.21 (0-2)
CoLk 94184	V	♀	3.17± 0.48 (2-5)	2.50± 0.34 (2-4)	0.67± 0.33 (0-2)
		♂	3.00± 0.27 (2-4)	2.38± 0.26 (1-3)	0.75± 0.25 (0-2)
CoPk 05191	V	♀	4.00± 0.37 (3-5)	2.50± 0.22 (2-3)	1.50± 0.22 (1-2)
		♂	3.92± 0.26 (3-5)	2.83± 0.21 (2-4)	1.08± 0.15 (0-2)
CoLk 13204	V	♀	4.63± 0.32 (2-7)	3.05± 0.25 (1-5)	1.58± 0.22 (0-4)
		♂	3.48± 0.16 (3-5)	2.57± 0.19 (2-5)	0.90± 0.07 (0-1)
CoLk 8102	V	♀	5.08± 0.56 (2-9)	2.92± 0.26 (2-4)	2.17± 0.41 (0-5)
		♂	3.27± 0.34 (1-6)	2.20± 0.28 (1-5)	1.09± 0.23 (0-3)

Figures in parentheses range are of disc numbers; Co 0238, CoLk 94184 and CoPk 09151 (early maturing varieties) and CoLk 13204 and CoLk 8102 (mid late varieties)

scales act as the solid lubricant for the safe movement of the adult body in the tunnel. The larva could apparently sense the ambient situation well and accordingly weaves the silken discs to maintain the required temperature and humidity around the pupa in pupal chamber and to protect possible ingress of the parasitoids (Gupta, 1958 and Baitha et al., 2021). The results allow us to better understand the distribution pattern of silken discs weaved by larvae of different broods of *S. excerptalis* in early and mid-late varieties.

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AUTHOR CONTRIBUTION STATEMENT

The authors (AB, AKJ, AK) contributed to the study conception and field experimentation). Materials preparation, data collection and analysis were performed by AB, AKJ, AK The first draft of the manuscript was written by AB and all authors commented on previous version of the manuscript. All authors read and approved the final manuscript. No ethical issue related to animal is involved in the experimentation.

CONFLICT OF INTEREST

No conflict of interest.

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FIELD EVALUATION OF BIOPESTICIDES AGAINST RICE INSECT PESTS UNDER ORGANIC FARMING

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ABSTRACT

Neem based commercial formulations Ecotin (azadirachtin 5%; 50,000 ppm) @ 150,175 and 200 ml ha^{-1}, and standard checks- Achook (azadirachtin 0.15%; 1500 ppm) @ 2500 ml ha^{-1} were applied at pest appearance for evaluating them for the management of rice stem borers Scirpophaga spp. and leaf folder *Cnaphalocrocis medinalis* (Guenee) on basmati and non-basmati rice under organic cultivation conditions. In basmati, the reduction of deadhearts due to stem borers was observed to be maximum with Ecotin @ 200 ml ha^{-1} as 55.79 and 56.84% at 5 and 10 DAS, respectively; while white ears were at 55.99% in 2019; and reduction of deadhearts was 50.45 and 45.75% at 5 and 10 DAS, respectively, while white ears reduced up to 50.71% in 2020. Similarly, reduction of damaged leaves due to leaf folder was 55.80 and 59.62% at 5 and 10 DAS, respectively; and in 2020, it was 50.45 and 45.75% at 5 and 10 DAS, respectively. Ecotin 5% @ 200 ml ha^{-1} gave maximum crop yield (31.29 q ha^{-1}). In non-basmati rice too the reduction of deadhearts was maximum with Ecotin @ 200 ml ha^{-1}, while white ears reduced by 50.47% in 2019, with similar trend in 2020; also results were of similar trend with leaf folder. The occurrence of spiders revealed a non-significant difference among the treatments. The biopesticides caused no residual or phytotoxic effects and were safe to the environment and predatory fauna.

Key words: Field evaluation, rice, Scirpophaga sp., *Cnaphalocrocis medinalis*, Ecotin, Achook, basmati, non-basmati, stem borers, deadhearts, damaged leaves, rice yield, organic farming

Rice is the important staple crop of India, and occupied 3.14 million ha in Punjab with total production of 18.92 million mt during 2019-20 (Anonymous, 2021). Due to biotic factors approximately 52% losses occur in rice production worldwide and about 21% of these are due to the insect pests (Sogawa et al., 2003). Rice crop is attacked by more than hundred insect pests, of which 20 are very serious. Under Punjab conditions, major lepidopteran insects pests on rice include *Scirpophaga incertulas* (Walker), *S. innotata* (Walker), *Sesamia inferens* (Walker) and leaf folder *Cnaphalocrocis medinalis* (Guenee). After cotton, rice cultivation is much dependent on insecticides due to serious pest damage, which causes resistance in insect pests and environmental pollution. The increasing demand of insecticide residue free products has resulted in farmer's acceptance of biopesticides in organic basmati and non-basmati rice. Keeping these in mind, this study on the field evaluation of some biopesticides against rice stem borers and leaf folder under organic conditions.

MATERIALS AND METHODS

The basmati and non-basmati rice crops were raised under organic conditions during kharif 2019 and 2020 at the Research Farm of School of Organic Farming following package of practices of Punjab Agricultural University, Ludhiana (PAU). The organic inputs viz. land, seed and organic manures were used as per PAU recommendations for organic farming. In the treatments, neem based commercial formulations, Ecotin (azadirachtin 5%; 50,000 ppm) @ 150, 175 and 200 ml ha^{-1}, standard checks- Achook 0.15% (azadirachtin 0.15%) @ 2500 ml ha^{-1} were evaluated along with untreated control. The trial was replicated thrice in randomized block design, and the pesticides were sprayed at pest appearance. The observations on stem borers' incidence as % deadhearts and leaf folder damage as % damaged leaves at vegetative stage were recorded one day before, and then 5 and 10 days after spray (DAS); and white ear incidence was observed about a week before harvest. Data on spider counts were also made. Crop yield data was observed at harvest, and the data analysed after arc sine transformation.

RESULTS AND DISCUSSION

In basmati rice, deadhearts due to stem borers ranged from 1.73-1.81 and 1.93-2.07% in 2019 and

2020, respectively, in the treatments as pretreatment data with differences being non-significant (Table 1); all biopesticide treatments were significantly better than the untreated control after 5 and 10 DAS; Ecotin @ 200 ml ha^{-1} registered i.e. 1.07 and 1.23, against 1.67 and 1.98% deadhearts at 5 and 10 DAS in 2019 and 2020, respectively, which were significantly lower than its lower dosages (1.36-1.39 and 1.46-1.53%), standard check, Achook @ 1000 ml ha^{-1} (1.58 and 1.73%) and untreated control (2.42 and 2.85%). Mean white ears were lower i.e., 1.91 and 2.07% in Ecotin @ 200 ml ha^{-1} during 2019 and 2020, respectively, but were significantly higher than its lower dosages (2.74-2.79 and 2.80-3.00%), Achook (2.98 and 2.97%) and untreated control (4.34 and 2.97%). In 2019, Ecotin @ 200 ml ha^{-1} at vegetative stage registered more reduction of deadhearts (55.79 and 56.84 at 5 and 10 DAS, respectively), than its lower dosages (42.56-43.80 and 46.32-48.77%) and Achook (34.71 and 39.30%). About white ears, it was 55.99% reduction at harvest stage. In 2020, also similar trend was noticed with regard to deadhearts and white ears. Similarly, the leaf folder damaged leaves ranged from 3.07- 3.21 and 2.80- 4.30% in pretreatment, with differences being non-significant; Ecotin @ 200 ml ha^{-1} registered 2.21 and 2.54%, and 2.18 and 2.72% reduction in damaged leaves at 5 and 10 DAS during 2019, respectively, which were significantly less than its lower dosages (3.00-3.15 and 3.22-3.48%). Thus, Ecotin @ 200 ha^{-1} led to 55.80 and 59.62% reduction in damage at 5 and 10 DAS, respectively; in 2020 too similar trend was observed. The data on the occurrence of spiders revealed non-significant differences in all the treatments at pretreatment level, at pre-spray, 5 DAS and 10 DAS. Crop yield was significantly more with Ecotin 5% @ 200 ml ha^{-1} (31.29 q ha^{-1}) than its lower dosages (29.56-30.17 q ha^{-1}), Achook (29.27 q ha^{-1}) and the untreated control (27.63 q ha^{-1}) during 2019. Similar trend was observed in kharif 2020.

In non-basmati rice, incidence of stem borers varied from 3.83-4.29 and 4.19-4.80% during 2019 and 2020, respectively at the pretreatment level, with differences being non-significant (Table 2). All biopesticide treatments were significantly superior after 5 and 10 DAS; Ecotin @ 200 ml ha^{-1} recorded lower i.e. 3.13 and 3.28, and 2.52 and 2.90% deadhearts at 5 and 10 DAS in 2019 and 2020, respectively, but were significantly lower than its lower dosages (3.44-3.49 to 3.64-3.70 and 2.83-3.27 to 3.10-3.57 %), Achook (3.63 to 3.78 and 3.13 to 3.55%) and untreated control (5.90 to 6.71 and 4.43 to 4.75%. Similarly, Ecotin @ 200 ml ha^{-1} recorded 3.69 and 3.43 % white ears in 2019 and 2020, respectively, which were significantly more compared to its lower dosages (4.09-4.13 and 4.03-4.30 %), Achook (4.95 and 4.57 %) and untreated control (7.45 and 5.63 %). In 2019, Ecotin @ 200 ml ha^{-1} at vegetative crop stage gave maximum reduction in deadhearts and white ears. Similar trend was observed in 2020. With leaf folder, in terms of % damaged leaves Ecotin @ 200 ml ha^{-1} was superior (3.06 and 3.21, and 2.33 and 2.95 at 5 and 10 DAS in 2019 and 2020, respectively), registering significant reductions. The counts of spiders revealed on-significant differences prior to treatment and at 5 and 10 DAS. Crop yield was significantly more with Ecotin 5% @ 200 ml ha^{-1} (68.53 q ha^{-1}) than its lower dosages (64.80-66.40 q ha^{-1}), Achook (64.53 q ha^{-1}) and the untreated control (62.27 q ha^{-1}) in 2019. A similar yield trend was observed during kharif 2020.

The reduction in the pest incidence might be due to repellency, oviposition deterrence and antifeedant effects of azadirachtin. Ho et al. (1983) observed that neem oil can control borer menace at vegetative stage. Dhaliwal et al. (1998) also reported that neem based insecticides are effective against insect pests of cabbage. Nanda et al. (1996) and Murugabharathi and Balasubramanian (1999) suggested the application of 3% neem oil to suppress rice borers. Bora et al. (2004) found neem products as effective to control yellow rice stem borer. Ponnusamy (2003) reported a quantum jump in yield with neem formulations. Kaul et al. (1999) also observed positive impact of neem products on rice. Longkumer et al. (2017) observed less leaf damage due to leaf-folder with Achook (azadirachtin 1500 ppm). Mohapatra and Nayak (2015) reported that the foliar application of neemazal @1.0 ml l^{-1} at 60 and 70 DAS and foliar spraying of buprofezin 25SC @ 1.5 ml l^{-1} at 85DAS at ETL afford excellent control of major insect pests of rice like leaf folder and green leafhopper. Saikal and Parameswaran (2003) reported the EC formulations of neem and pungam oil combination proved to be highly effective against *Cnaphalocrocis medinalis* under laboratory conditions. Nigam et al. (2010) also studied the efficacy of neem oil @ 5% against leaf folder in basmati rice, and showed reduction in leaf damage and increased grain yield. The present results also corroborate with those of Sharma and Aggarwal (2014) on stem borer with neem azal 1% (1250 ml ha^{-1}).

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Table 1. Efficacy of neem based biopesticides against insect pests of *basmati* rice under organic conditions

Treatment	Dosage (ml ha^{-1})	% deadhearts due to stemborers*					% white ears		% damaged leaves due to leaf-folder					Spider population (nos./ hill)			Yield (q ha^{-1})
		Pre-spray	5 DAS		10 DAS		Mean	PROC	Pre-spray	5 DAS		10 DAS		Pre-spray	5DAS	10DAS	
			Mean	PROC	Mean	PROC				Mean	PROC	Mean	PROC				
2019																	
Ecotin (azadirachtin 5%)	150	1.73	1.39 (6.77)	42.56	1.53 (7.11)	46.32	2.79 (9.61)	35.71	3.21	3.15 (10.22)	37.00	3.48 (10.74)	44.67	0.23	0.20	0.23	29.56
Ecotin (azadirachtin 5%)	175	1.74	1.36 (6.70)	43.80	1.46 (6.94)	48.77	2.74 (9.52)	36.87	3.07	3.00 (9.96)	40.00	3.22 (10.31)	48.81	0.27	0.20	0.23	30.17
Ecotin (azadirachtin 5%)	200	1.78	1.07 (5.94)	55.79	1.23 (6.36)	56.84	1.91 (7.92)	55.99	3.09	2.21 (8.55)	55.80	2.54 (9.17)	59.62	0.23	0.17	0.20	31.29
Achook (azadirachtin 0.15%)	2500	1.81	1.58 (7.21)	34.71	1.73 (7.56)	39.30	2.98 (9.93)	31.34	3.13	3.58 (10.90)	28.40	3.93 (11.43)	37.52	0.23	0.17	0.20	29.27
Untreated control	-	1.80	2.42 (8.94)	-	2.85 (9.70)	-	4.34 (12.01)	-	3.18	5.00 (12.89)	-	6.29 (14.48)	-	0.23	0.27	0.27	27.63
CSD (p=5%)		NS	(0.14)	-	(0.41)	-	(1.34)	-	NS	(0.98)	-	(1.20)	-	NS	NS	NS	0.44
2020																	
Ecotin (azadirachtin 5%)	150	2.00	2.01 (8.16)	40.36	2.45 (8.91)	32.88	3.00 (9.95)	28.57	3.20	2.85 (9.71)	29.10	3.47 (10.73)	23.74	0.10	0.17	0.13	30.07
Ecotin (azadirachtin 5%)	175	2.00	1.88 (7.88)	44.21	2.35 (8.81)	35.62	2.80 (9.43)	33.33	3.00	2.62 (9.29)	34.83	3.25 (10.37)	28.57	0.20	0.20	0.20	30.30
Ecotin (azadirachtin 5%)	200	1.93	1.67 (7.41)	50.45	1.98 (8.08)	45.75	2.07 (8.17)	50.71	4.30	2.18 (8.49)	45.77	2.72 (9.48)	40.22	0.13	0.17	0.20	30.70
Achook (azadirachtin 0.15%)	2500	2.00	2.02 (8.16)	40.06	2.43 (8.95)	33.42	2.97 (9.73)	29.29	2.80	2.73 (9.50)	32.09	3.32 (10.49)	27.03	0.13	0.10	0.17	29.10
Untreated control	-	2.07	3.37 (10.55)	-	3.65 (10.97)	-	4.20 (11.68)	-	3.20	4.02 (11.55)	-	4.55 (12.31)		0.13	0.17	0.17	28.00
CSD (p=5%)		NS	(0.78)	-	(1.17)	-	(0.48)	-	NS	(0.24)	-	(0.37)		NS	NS	NS	0.22

* Figures in parentheses % arc sine transformed values; PROC: % reduction over control

Table 2. Efficacy of neem based biopesticides against insect pests of non-basmati rice under organic conditions

Treatment	Dosage (ml ha^{-1})	% deadhearts due to stemborers*					% white ears		% damaged leaves due to leaf-folder					Spider population (nos./ hill)			Yield (q ha^{-1})
		Pre-spray	5 DAS		10 DAS		Mean	PROC	Pre-spray	5 DAS		10 DAS		Pre-spray	5DAS	10DAS	
			Mean	PROC	Mean	PROC				Mean	PROC	Mean	PROC				
2019																	
Ecotin (azadirachtin 5%)	150	4.05	3.49 (10.76)	40.85	3.70 (11.08)	44.86	4.13 (11.72)	44.56	4.07	3.80 (11.23)	43.87	4.02 (11.55)	44.48	0.30	0.27	0.33	64.80
Ecotin (azadirachtin 5%)	175	4.06	3.44 (10.68)	41.69	3.64 (11.00)	45.75	4.09 (11.66)	45.10	4.74	3.66 (11.01)	45.94	3.88 (11.35)	46.41	0.26	0.23	0.30	66.40
Ecotin (azadirachtin 5%)	200	3.83	3.13 (10.19)	46.95	3.28 (10.43)	51.12	3.69 (11.07)	50.47	4.93	3.06 (10.06)	54.80	3.21 (10.31)	55.66	0.33	0.30	0.36	68.53
Achook (azadirachtin 0.15%)	2500	3.89	3.63 (10.98)	38.47	3.78 (11.21)	43.67	4.95 (12.85)	33.56	4.10	4.19 (11.81)	38.11	4.88 (12.75)	32.60	0.27	0.23	0.30	64.53
Untreated control	-	4.29	5.90 (14.06)	-	6.71 (15.00)	-	7.45 (15.82)	-	4.86	6.77 (15.08)	-	7.24 (15.60)	-	0.30	0.33	0.40	62.27
CSD (p=0.05)		NS	(0.15)	-	(0.38)	-	(0.52)	-	NS	(0.91)	-	(0.98)	-	NS	NS	NS	1.59
2020																	
Ecotin (azadirachtin 5%)	150	4.33	3.27 (10.39)	26.19	3.57 (10.86)	24.84	4.30 (11.96)	23.62	4.10	3.10 (10.14)	30.02	3.70 (11.08)	31.48	0.20	0.10	0.13	63.37
Ecotin (azadirachtin 5%)	175	4.45	2.83 (9.68)	36.12	3.10 (10.11)	34.74	4.03 (11.57)	28.42	4.37	2.70 (9.45)	39.05	3.38 (10.59)	37.41	0.23	0.27	0.20	63.90
Ecotin (azadirachtin 5%)	200	4.19	2.52 (9.11)	43.12	2.90 (9.78)	38.95	3.43 (10.67)	39.08	6.03	2.33 (8.78)	47.40	2.95 (9.87)	45.37	0.17	0.20	0.20	64.63
Achook (azadirachtin 0.15%)	2500	4.46	3.13 (10.17)	29.35	3.55 (10.83)	25.26	4.57 (11.95)	18.83	4.07	2.85 (9.71)	35.67	3.43 (10.67)	36.48	0.10	0.17	0.17	62.97
Untreated control	-	4.80	4.43 (12.13)	-	4.75 (12.55)	-	5.63 (13.72)	-	4.03	4.43 (12.15)	-	5.40 (13.42)	-	0.17	0.17	0.17	60.46
CSD (p=0.05)		NS	(0.37)	-	(0.39)	-	(0.70)	-	NS	(0.55)	-	(0.48)	-	NS	NS	NS	0.72

*Figures in parentheses % arc sine transformed values; PROC: % reduction over control

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EVALUATION OF IPM MODULES AGAINST *DIAPHANIA CAESALIS* (WALKER) IN JACK FRUIT

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ABSTRACT

Field trials were conducted to evaluate four IPM modules against jack shoot and fruit borer *Diaphania caesalis* (Walker). Among different canopy types, the spreading type canopy was the most susceptible. The fruit damage on covered and hooked fruits were significantly lesser compared to control. Among the five modules evaluated- Module I (*Nomuraea rileyi* (WP)@ 10g/ l, pongamia soap 5 gm/ l, *Trichogramma chilonis*@ 45,000 adults and *Apanteles stantoni*@ 450 adults/ acre) were more effective. The laboratory mass multiplied parasitoids and pathogens provided an effective control. Moreover, ecofriendly botanical insecticides i.e. neem and pongamia are equally effective.

Key words: Jack fruit, *Diaphania caesalis*, ecology, biocontrol, life history, chemical control, neem, pongamia, *Nomuraea rileyi*, *Trichogramma chilonis*, *Apanteles stantoni*, hooking, blagging

Jack fruit is the largest tree-borne tropical fruit species found in high rainfall, coastal and humid areas of the world (Sturrock, 1959). In India, about 39 species of insects are known to attack jack fruit (Butani, 1979). Among them, the shoot and fruit borer *Diaphania caesalis* (Walker) is a major (Soumya et al., 2015; Soumya et al., 2019). Though ample studies have been conducted on its biology (Manjunatha et al., 2014; Soumya et al., 2019), the literature available on its management through IPM is scanty. Although pesticides generally are used to kill a particular target pest, several other non-target pests and beneficial organisms also will be killed in the process (Patil and Bheemanna, 2015). In tree crops, especially with a large canopy, like jack fruit tree application of insecticides is more difficult, and aerial sprays are found effective (Hill, 2008). However, the application of pesticides by aerial spray method can cause adverse effects on different crops, livestock, waterways and environment. Hence, IPM is a more ecofriendly approach, especially in jack fruit, where insecticide sprays on huge canopy will be a difficult task. Although IPM represents a key strategy for the control of jack fruit pests, it has not been attempted. Therefore, this study to evaluate IPM modules against *D. caesalis.*

MATERIALS AND METHODS

Experiments were conducted in the laboratory and in the jack fruit orchards of the Indian Council of Agricultural Research- Indian Institute of Horticultural Research (ICAR- IIHR), Bengaluru (12 8'N; 77 35'E) during May 2014- December 2016. Trees selected for the study were of medium height (2.00 - 2.50 m), aged between 12 and 20 years and being pruned once in a year. Recommended agronomic practices (i.e. weeding, pruning, fertilization with minerals and organic nutrients) were followed, except pesticides. Laboratory assay was conducted with the treatments viz, neem soap (5 gm/ l), pongamia soap (5 gm/ l), *N. riley* WP (1.0x 10 ^{9}conidia/ ml @ 10 gm/ l), *B. bassiana* WP (1.0x 10^{9} conidia/ ml @ 10 gm/ l), and *M. anisopliae* oil formulation (1.0 ×10 ^{9}conidia/ml @ 0.5 ml/ l). Fresh shoots with 2 - 3 leaves were kept individually in small transparent plastic trays (12x 12x 2 cm) and were sprayed with fungal pathogens and botanicals using a hand sprayer. Different larval instars of *D. caesalis* were allowed to feed on treated leaves. Five replications were maintained for each treatment. Each replication consisted of 10 larvae of first, second, third, fourth and fifth larval instars. The efficacy of treatments against larval instars was evaluated in the laboratory. The mortality of larval instars was observed daily up to seven days after treatments.

Field trials were conducted in a randomized complete block design (RCBD) with different treatments in ten

replications, each consisting of one tree. All treatments were imposed simultaneously once a week. Foliar sprays (5 l/ tree) of botanicals i.e. neem soap and *Pongamia* soap, microbial agents i.e. *N. rileyi*, *B. bassiana* and *M. anisopliae* and organic insecticides i.e. Dipel (*Bacillus thuringiensis*) and One Up (spinosad) were applied to the crop in the morning (9.00- 10.00 hr) using knapsack sprayer (Lotus knapsack sprayer, 16l). The effect of canopy shape on *D. caesalis* infestation was evaluated. The jack fruit trees were grouped into four categories as open type (T1), oval type (T2), round type (T3) and spreading type (T4) based on their canopy shape. Fruit bagging and hooking experiments were conducted to evaluate their effect on *D. caesalis* infestation. Fruits were bagged using polyethene bags (55x 85cm) after fruit set. Tiny holes were made at the bottom of the polyethene bag for aeration and escape of transpiration water/ rainwater. Hooking was done using a small sharp forceps (10x 12x 2 cm). Wax was applied on the hooked portion to prevent water from entering into the damaged fruit, which causes fruit rotting. Data on fruit infestation and fruit weight was recorded both from bagging and hooking experiments.

Light traps (white light) were installed within the field on a pole (9 m) and were secured firmly on the ground. In order to rear *T. chilonis* and *A. stantoni* in the laboratory, rice moth, *C. cephalonica* was cultured following the methods of Kumar and Murthy (2000) and Nathan et al. (2006). Rearing of *A. stantoni* was carried out from the field-collected parasitoid cocoons as well as from parasitised *D. caesalis* larvae. The mated females of *A. stantoni* were released in the field during evening hours @ 450 adults/acre/week as followed by Mitchell et al.(1997) for *Cotesia* (=*Apanteles*) *plutellae.* Different IPM modules evaluated- Module I (*Nomuraea rileyi* (WP)@10 gm/ l, pongamia soap 5 gm/ l, *T. chilonis*@ 45,000 adults/ ha and *Apanteles stantoni*@ 450 adults/ ha); Module II (*Beauveria bassiana* @10 gm/ l, neem soap 5 gm/ l and *Apanteles stantoni* @450 adults/ ha); Module III (*Beauveria bassiana* @10 gm/ l, *T. chilonis* @ 45,000 adults/ ha and *Apanteles stantoni* @450 adults/ ha); Module IV (*Nomuraea rileyi* WP @10 gm/ l, *Apanteles stantoni* @ 450 adults/ ha) and Module V (*T. chilonis* @ 45,000 adults/ ha and *Apanteles stantoni* @ 450 adults/ ha). Observations were recorded for assessing the suppression level of *D. caesalis* and population of natural enemies. From each selected plant, 30 buds were selected randomly from the upper, middle and lower canopies to record the pest population. Infestation level was recorded before and after treatments. Incidences of pests and natural enemies were assessed one day before treatment as a pretreatment observation and on the 7th day after treatment as post-treatment observation. The data on *D. caesalis* damage in different canopy-types jack fruit trees, mean mortality of *D. caesalis* in the laboratory and in the field was subjected to one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) multiple range test (p=0.05) to find the significant difference. Student 't'- test was used to compare the % fruit damaged by *D. caesalis* in hooking and bagging experiments.

RESULTS AND DISCUSSION

The extent of *D. caessalis* infestation among different canopy types of jackfruit trees was significantly different ($F = 304.49.8$, $df = 3, 36$; $P < 0.05$). In open and oval type of canopies, the number of damaged buds significantly less and ranged between 0.00-0.45 and 0.45 - 1.10 damaged buds/tree respectively. The pest incidence was relatively higher (6.42 - 11.00 damaged buds/tree) in trees with round canopy when compared to open and oval types. The spreading type canopy was most susceptible to the attack of the pest, which showed 10.87 - 14.40 damaged buds and significantly higher when compared to the other three canopy types. The results regarding the influence of canopy on *D. caesalis* infestation was similar to that of Singh and Verma (2013) who reported the greater outbreak of mango leaf webber *Orthaga euadrusalis* Hampson (Pyralidae: Lepidoptera) in mango orchards with a dense canopy. Greater infestation of *D. caesalis* in spreading type canopy could be due to the availability of more fresh shoots in these trees. The percentage of *D. caesalis* infestation in treated and control fruits was significantly different in both bagging ($t (38) = 24.63$; $p < 0.05$) and hooking ($t (38) = 16.74$; $p < 0.05$) experiments. The fruit damage in covered fruits was 0.5% while it was 31.65% in control. However, average fruit weight was 6.90 kg in bagging and 7.00 kg in control, which was not significantly different. The fruit infestation in hooked fruits was 5.50% while it was 39.5% fruits in control. Colour of the covered fruits was bright greenish-yellow and appearance was better than that of control fruits. The infestation of *D. caesalis* was lesser in bagged jack fruit as reported by Abbasi et al. (2014) in bagged guava fruits which were less infested with *Bactrocera dorsalis* Hendel (Tephritidae: Diptera).

The effect of biopesticides and botanicals screened against *D. caesalis* in the laboratory is given in Table 1. The effectiveness of *N. rileyi*, *B. bassiana,* neem soap and *Pongamia* soap on larvae was more in all larval

Table 1. Efficacy biopesticides against larval instars of *D. caesalis*

Treatments	Mortality (%)					
	1st instar	2nd instar	3rd instar	4th instar	5th instar	Average
N. rileyi	99.00	89.29	86.85	65.71	59.29	80.03
	(9.95)[a]	(9.45)[a]	(9.32)[a]	(8.11)[a]	(7.70)[b]	(8.95)[a]
B. bassiana	95.71	85.57	87.14	65.14	64.29	79.57
	(9.78)[a]	(9.25)[a]	(9.34)[a]	(8.07)[a]	(8.02)[a]	(8.92)[a]
M. anisopliae	40.86	32.00	24.29	20.00	18.57	27.14
	(6.39)[b]	(5.66)[b]	(4.93)[b]	(4.47)[b]	(4.31)[c]	(5.21)[b]
Neem Soap	74.29	70.00	71.43	70.00	68.33	70.81
	(8.62)[a]	(8.37)[a]	(8.45)[a]	(8.37)[a]	(8.27)[a]	(8.41)[a]
Pongamia Soap	91.43	85.71	85.71	86.67	87.14	87.33
	(9.56)[a]	(9.26)[a]	(9.26)[a]	(9.31)[a]	(9.34)[a]	(9.35)[a]
Control	2.50	1.00	0.00	0.00	0.00	0.70
	(1.58)[c]	(1.00)[c]	(0.00)[c]	(0.00)[c]	(0.00)[d]	(0.82)[c]

Values in parentheses square root-transformed. Means in columns followed by different alphabets significantly different (Tukey's HSD test, $p < 0.05$, ANOVA).

Table 2. Effect of IPM modules on bud/ fruit damage by *D. caesalis* and on natural enemies in jackfruit orchard

Module	Daamge on beds	Damage on fruits	Reduviids (No/ plant)		Spiders (No/ plant)		Coccinellids (No/ plant)		*A. stantoni* (% parasitism)	
			BT	AT	BT	AT	BT	AT	BT	AT
Module-I	3.43[a]	5.50[a]	0.17[a]	0.18[a]	0.88[a]	0.90[a]	1.54[a]	1.45[a]	36.00[b]	93.50[a]
Module-II	11.50[b]	10.00[b]	0.16[a]	0.14[a]	0.80[a]	0.78[a]	1.60[a]	1.80[a]	31.46[b]	87.90[a]
Module-III	10.10[b]	10.59[b]	0.18[a]	0.18[a]	0.90[a]	0.88[a]	1.49[a]	1.50[a]	33.25[b]	88.50[a]
Module-IV	16.75[b]	11.50[b]	0.45[a]	0.4[a]	1.15[a]	1.10[a]	2.20[a]	2.10[a]	37.10[b]	87.00[a]
Module-V	17.50[b]	12.10[b]	0.17[a]	0.13[a]	1.60[a]	1.50[a]	1.40[a]	1.90[a]	32.66[b]	85.90[a]
Control	97.25[c]	35.40[c]	0.41[a]	0.42[a]	1.10[a]	1.10[a]	1.50[a]	1.70[a]	38.15[a]	37.50[a]

Means in columns followed by different alphabets significantly different ($p < 0.05$,'t' test).
BT- Before treatment, AT- After treatment.

instars of *D. caesalis* except in fifth. The mortality in fifth instar larvae was highest with pongamia soap, neem soap and *B. bassiana* treatments when compared to neem soap *N. rileyi* and *M. anisopliae;* mortality of *D. caesalis* was highest in pongamia soap (87.33%) followed by *N. rileyi (*80.03%), *B. bassiana* (79.57%), neem soap (70.81%) and *M. anisopliae* (27.14%) (Table 1). The present result with *N. rileyi* is similar to Burges (1998) and Ramanujan et al. (2003) who reported greater efficacy of *N. rileyi* formulation, against lepidopteran pests. Yi and Qui (1999) reported that Bt insecticide was effective in controlling *D. indica*. The effectiveness of *T. chilonis* in controlling *D. caesalis* was similar to that reported by Jalali and Singh (1992), Singh et al. (2004). Krishnamoorthy (2012) and Sardana et al. (2005), who reported that the inundative release of *T. chilonis* reduced the incidence of several lepidopteran pests. The mortality of *D. caesalis* was more in all the five modules of IPM. The bud damage by *D. caesalis* on the seventh day after treatment was significantly lesser in M- I (3.43%) followed by M- II (10.10 %), M- III (11.50 %) and M- IV (16.75 %) and M- V (17.50 %). The fruit damage in different IPM modules remained significantly lowest in M-I (5.50%) %) followed by M-II (10.50%), M- III (10.59%), M- IV (11.50%) and M- V (12.50%) as compared to control (35.40 %) (Table 2).

The natural enemy (reduviids, spiders, coccinellids and *A. stantoni)* population did not significantly differ with IPM modules evaluated. However the parasitization by the larval parasitoid *A. stantoni* expressed as percentage differed significantly among the modules (Table 2). Dipel and *B. bassiana* has been widely used for the control of different economically important lepidopteran pests, i.e. *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) and *S. litura* (Chandrakar et al., 1999; Mohammed et al., 2005). The results regarding the effect of neem in controlling *D. caesalis* was similar to that of Gowda (2000) who reported that neem seed kernel extract (NSKE) and

neem oil and pungam (*Pongamia pinnata)* seed extract were effective in controlling *D. pulvernlentalis* in mulberry crop.

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OCCURRENCE OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J E SMITH) IN ASSAM

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ABSTRACT

The fall army worm (FAW) has spread to locations at 1222 masl in the northeast region of India. In Assam, its incidence was first noticed on the two banks of the Brahmaputra. In Biswanath district, infestation on maize was observed to be of 15.2-64.3% at farmers' field and 2.15% in Biswanath College of Agriculture campus of the Assam Agricultural University. This variation might be attributed to the rich avian diversity in the college campus. No larval parasite was observed in the field collected larvae. A large section of farmers observed high pest incidence in maize as compared to previous years.

Key words: *Spodoptera frugiperda*, maize, Assam, Brahmaputra, papaya mealybug, rugose spiralling whitefly, field incidence, parasites, avian diversity

The fall army worm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is a pest native to tropical and subtropical regions of the America. It is much invasive that within three years of its first report from West Africa, it spread to more than 40 countries in Africa (Sisay et al., 2019). In India, FAW was first detected in Indian state of Karnataka at the College of Agriculture, Shivamogga in May 2018 (Sharanabasappa et. al., 2018). Since then it has been reported from different parts of India (Deole et al., 2018; Mallapur et al., 2018; Shylesha et al., 2018; Srikanth et al., 2018; Babu et al., 2019; Chormule et al., 2019; Dhar et al. 2019; Firake et al., 2019; Kumar et al., 2020; Sarma, 2020). In northeast India, its outbreak was first detected in Ngasih area in south Mizoram's Lunglei district in the 1st week of March, 2019. Subsequently, it has been observed causing massive outbreaks during April in Mizoram and Nagaland. As per the government report, > 8,900 families in 332 villages have been affected in Mizoram; it was found causing damage to maize crop during early May in Meghalaya, Manipur, Sikkim and Arunachal Pradesh (Firake et al., 2019). The pest infested around 2961.95 ha in seven villages of Mokokchung district during early part of May 2020 (The Assam Tribune, May 21, 2020). In Assam, it was observed during different months from the two banks of the Brahmaputra; in south bank its first was reported from Karbi Anglong district in May, 2019, while its initial attack was noticed in north bank in Kokrajhar district during kharif, 2018. It is noteworthy that the state of Assam is under two global biodiversity hotspots i.e. the Indo-Burma and the Himalayan (Myers et al., 2000). Hence invasion of any exotic pest may bring threat to the local flora and fauna. Because of this, it warrants survey to know its spread and extent of invasion. In this context a study was conducted in the Biswanath district, and the details presented herein.

MATERIALS AND METHODS

The study was conducted in both the summer and winter maize during 2020-2021 in the Biswanath district (92°16'- 93°43'E, 26°30'- 27°01'N). Observations were made in crops grown in the campus of Biswanath College of Agriculture, Assam Agricultural University as well as in the farmers' fields of 47 villages located in 7 ADO circles in the district (viz., Pabhoi, Behali, Biswanath, Jinjia, Kalabari, Gahpur and Sootea). Infestation was recorded at early-mid whorl stage before applying any insecticide. Infestation was based on the fresh leaf damage or frass in whorl symptoms (FAO, 2018). Larvae were identified morphologically (FAO and CABI, 2019), with taxonomic confirmation made by the ICAR- National Bureau of Agricultural Insect Resources, Bengaluru, India. A lot of 100 larvae of different instars collected from the farmers' fields were reared in the laboratory till adult emergence. Pheromone traps (Pheromone Chemicals, Plot No. 23, TSIIC Techpark, IDA Nacharam, Hyderabad) were also

installed in the maize fields for adult trapping. Trapped adults were also identified morphologically and further confirmed by comparing with the adults emerged from the lot maintained in the laboratory. Infested plants (%) was estimated at early-mid whorl stage before applying any insecticides. While assigning the intensity, the action thresholds of crop stages was considered as laid out by Suby et al. (2019); damage intensity below and above the threshold has been considered as low and high. Altogether 64 farmers were interviewed to know their view on the pest in relation its population increase over the last two years, insecticide use etc

RESULTS AND DISCUSSION

The results revealed that the FAW is an exotic species that has invaded Assam recently after the invasion of papaya mealy bug (Sarma, 2013) and rugose spiraling whitefly (Mohan et al., 2018; Sarma et al., 2021). In northeast India, the outbreak of FAW was first reported on 3rd March, 2019 from the Ngasih area in south Mizoram's Lunglei district. Next was West Tripura district in the same month. The aerial distance of Lunglei district (Mizoram) and West Tripura district from Shivamogga (the first reported place in India) is about 2070 and 2000 km, respectively (https://www.distancefromto.net), indicating that the pest had travelled >2000 km in 10 months (May, 2018- March, 2019) and attack maize crop. It is noteworthy that said period includes both the months of high rainfall and severe cold. Moreover, Lunglei district at 1221 masl (https://en.wikipedia.org/wiki/Lunglei), showing pest's high dispersive nature. As per report, the pest spread to > 40 countries in Africa within three years (Sisay et al., 2019).

In Assam, the first invasion was reported from the two banks of Brahmaputra, in north bank, initial attack was noticed at Kokrajhar district (village: Matktaigaon, Bajugaon etc.) during kharif, 2018; whereas, in south bank its first was attack was reported from Lumbajong Block of Karbi Anglong district (villages: Dhanshiri Nepali Basti, Nagon Basti & Matipung) on 7th May, 2019. The first attack on maize was received from the farmers of Biswanath district during April - May, 2020; however, the occurrence was scientifically confirmed during October-November, 2020 in the campus of Biswanath College of Agriculture, Assam Agricultural University. The pest has also been detected in the maize fields of farmers in different localities of the district in subsequent periods (Table 1). Periodic surveys indicate that FAW is spreading progressively from west to east in the north bank plain region of the Brahmaputra River. The pest has established in this zone and has been detected in the farmers' fields this year too. The State Department of Agriculture is distributing maize seeds to farmers for field-demonstrations and also as compensatory input to the flood-affected farmers. The farmers have experienced the assured return from this crop and thus, the acreage under maize crop is increasing recently. It is noteworthy that the state had experienced the worst outbreak of swarming caterpillar in 2016 (Sarma and Salam, 2018). Biswanath district receives a high rainfall with monsoon period (June 4- September 30) receiving 1070 mm, and FAW can thus can tolerate high rainfall, and can establish in temperature of 23.1- 33.8^{0}C).

The FAW infestation in Assam reached the level which brings hue and cry situation among the farmers in later dates as compared to that in other northeastern states. Presumably, insectivorous bird species have played a crucial role in reducing the infestation in some parts of Assam; of the 818 species of birds of northeast India, 689 are known in Assam (Saikia et al., 2000) and 166 species in the agricultural landscape (Saikia, 2019). In the present study, it was observed that the infestation was less in maize fields which support insectivorous birds; at Biswanath College of Agriculture campus it was 2.15% as against 15.2-64.3% in the farmers' fields. The bird diversity in the BNCA campus is high because of the congenial environment. The nocturnal birds, especially the owls, were very active and found hovering the maize crop; they were also seen preying on the insects coming to light sources, including the street-lights. Studies in Central America have demonstrated significant impacts of birds on infestation levels of the FAW (FAO, 2018). The presence of diurnal bird species was less. The compactness of the crop canopy, coarse texture of the maize leaves, internal feeding of army worms and nocturnal behaviour of FAW-adults may be the prime reasons of inefficacy of diurnal birds. In a preliminary study, the randomly collected mature larvae (50 nos.) were reared in the laboratory till emergence of adults to see the larval parasites, if any. All the reared larvae pupated and emerged successfully showing an indication of absence of larval parasites.

The ability of peasant farmers in the third world to monitor the environmental occurrence around them has been ignored (Atteh, 1984) which is also true for the farmers of northeastern region of India. However, they can provide preliminary genuine information on agricultural issues like pest outbreak and new invasion

Table 1. Incidence of FAW in villages of Biswanath district, Assam (2020-21)

S. No.	Village	GPS	Infestation*
1	Japowbari	26°46’32.9”N 93°09’23.1”E	++
2	Sakomatha	26°47’24.5”N 93°07’01.0”E	++
3	Kherbari	26°49’22.9”N 93°05’35.0”E	+++
4	Bamunipathar	26°48’20.6”N 93°08’21.4”E	+++
5	Petulibari	26°45’32.0”N 93°08’03.6”E	+++
6	Borkura	26°47’33.3”N 93°09’33.4”E	++
7	Selaikhati	26°52’04.5”N 93°03’22.1”E	++
8	Joypur	26°51’54.1”N 93°09’32.8”E	++
9	Majulibasti	26°50’18.4”N 93°03’51.2”E	++
10	Bhimajuli	26°51’28.4”N 93°05’06.4”E	++
11	Samukjuli	26°52’14.3”N 93°11’00.2”E	++
12	Simaluguri	26°47’26.7”N 93°10’41.3”E	++
13	Thanbihali	26°48’14.3”N 93°20’54.8”E	++
14	Roumari	26°46’56.2”N 93°24’57.8”E	+++
15	Nizbihali	26°47’45.4”N 93°21’15.7”E	+++
16	Kamanpukhuri	26°48’44.8”N 93°21’28.6”E	+++
17	Niralabasti	26°43’59.0”N 93°12’11.5”E	+++
18	Chuwaguri	26°40’20.6”N 93°08’50.0”E	+++
19	Silamari	26°43’08.9”N 93°12’44.0”E	+++
20	Gaibandha	26°40’11.7”N 93°08’58.2”E	+++
21	Kumalia	26°42’07.8”N 93°06’26.1”E	+++
22	Garehagi	26°43’00.2”N 93°08’22.4”E	+++
23	Disiri pathar	26°47’04.3”N 93°12’51.9”E	+++
24	Niz Solmari	26°47’17.0”N 93°11’20.6”E	+++
25	Ratowa	26°47’39.3”N 93°14’48.0”E	+++
26	Pub-Jinjia	26°49’38.0”N 93°14’51.5”E	+++
27	Pachim Jinjia	26°49’32.2”N 93°14’11.5”E	+++
28	Monabaribasti	26°46’29.1”N 93°14’44.2”E	+++
29	Monabaripathar	26°47’07.4”N 93°13’29.0”E	+++
30	Tengabari	26°51’17.3”N 93°15’53.2”E	+++
31	Niz Baghmari	26°45’50.2”N 93°12’46.1”E	+++
32	Dath Kola	26°48’47.0”N 93°32’49.6”E	++
33	Bakori Doloni	26°49’11.7”N 93°33’44.3”E	++
34	Khatarbari	26°49’37.7”N 93°31’42.1”E	++
35	Toltoli	26°46’29.2”N 93°34’26.0”E	++
36	Akhoiphuta	26°49’44.0”N 93°33’55.6”E	++
37	Bortamuli	26°47’56.7”N 93°37’17.3”E	++
38	Dhandipathar	26°50’08.5”N 93°41’46.8”E	++
39	Rajabari	26°49’57.2”N 93°41’14.7”E	++
40	Tinisukia	26°49’42.9”N 93°43’00.4”E	++
41	Malargaon	26°45’05.8”N 93°02’14.1”E	++
42	Magurmara	26°46’38.3”N 93°03’06.4”E	++
43	Rawnamukh	26°48’06.0”N 93°40’36.7”E	++
44	Louguti	26°47’53.5”N 93°35’54.1”E	++
45	Chirakhowa Chapori	26°47’14.3”N 93°40’06.8”E	++
46	Paken	26°48’06.0”N 93°37’55.0”E	++
47	Chawguri Chapori	26°44’06.9”N 93°45’26.2”E	++
48	BNCA campus	26°43’28.8”N 93°08’08.1”E	+

Infestation level: + = Low (<5%); ++ = High (5-30%); +++ = Very high (>30%)
*Infestation at early-mid whorl stage before applying insecticides.

of pest. Even though it is time consuming, it is felt necessary to survey the farmers individually to know the spread of FAW. Out of the 64 farmers interviewed, 89.1% (57/64) perceived that the infestation is increasing in maize; 84.4% farmers reported infestation in 2021 is manifold now; most of the farmer reported FAW wrongly as *Spodoptera mauritia* from their previous experience on massive outbreak experienced in 2016; except for few locations all farmers applied insecticides viz., Celcron, Profex, Rogor etc. against FAW.

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ASSESSMENT OF YIELD LOSSES DUE TO SUCKING PESTS IN BT COTTON

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ABSTRACT

Field experiment was conducted during kharif 2019 to determine the avoidable yield losses due to sucking insect pests on four cotton cultivars BG II (US 81& RCH 776), non Bt F-2228 and Desi LD 949 at two sowing dates (timely-25th April; and late 27th May) at the Punjab Agricultural University, Ludhiana. Significantly higher incidence of whitefly *Bemisia tabaci* (Genn.) (20.61 adults), jassid *Amrasca biguttula biguttula* (Ishida) (12.97 nymphs) and thrips *Thrips tabaci* (Lindeman) (7.32 adults/ 3 leaves) was observed under late sown conditions, compared to the timely sown one with less incidence- *B. tabaci* (14.51 adults), *A. biguttula biguttula* (11.37 nymphs) and *T. tabaci* (6.58 adults)/ 3 leaves. Significantly less *B. tabaci* (16.69 adults), *A. biguttula biguttula* (13.74 nymphs) and *T. tabaci* (5.86 adults) were observed with desi cotton (LD 949) as compared BG II cultivars (RCH 776, US 81) and non Bt F 2228. Significantly less avoidable yield loss (16.41%) was observed with timely sown crop as compared to 18.45% losses under late sown conditions. Significantly less yield loss was observed in desi cotton (16.01%) as compared to US 81 (16.55%), RCH 776 (17.20) and F 2228 (19.98%).

Key words: Bt, desi, non Bt cotton, avoidable yield losses, *Bemisia tabaci*, *Amrasca biguttula biguttula*, *Thrips tabaci*, seed cotton yield

India leads in cotton cultivation and it is the only country where all the four species of *Gossypium arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense* along with intra and interspecific hybrids are cultivated (Rajendran and Jain, 2004; AICCIP 2019). In Punjab cotton occupied an area of 2.63 lakh ha with production of 12.06 lakh bales during 2019-20 (Anonymous, 2020). Cotton has an inventory of >1300 insect pest species in the world and there are about 130 arthropod species recorded from Punjab, out of which 54 belongs to the category of insect pests (Bal and Dhawan, 2008). The sap sucking insects like whitefly, jassid, mealy bug, aphid and mirids are emerging as serious threat and they are inflicting heavy losses to Bt cotton. The insect pest complex of cotton crop is broadly categorized into three categories, viz. sucking pests [leafhopper {*Amrasca biguttula biguttula* (Ishida)}, whitefly {*Bemisia tabaci* (Gennadius)}, thrips {*Thrips tabaci* (Lindemann)}, aphid {*Aphis gossypii* (Glover)} and mealybug {*Phenacoccus solenopsis* (Tinsley)}]; foliage feeder [tobacco caterpillar {*Spodoptera litura* (Fabricius)}] and bollworms [American bollworm {*Helicoverpa armigera* (Hübner)}, spotted bollworm {*Earias vittella* (Fabricius)}, spiny bollworm {*E. insulana* (Boisduval)} and pink bollworm {*Pectinophora gossypiella* (Saunders)}]. These cause damage to various plant parts at different growth stages throughout the cropping season. Since 2005, the adoption of transgenic Bt cotton has not only changed the cultivation profile, but also the pest scenario in Punjab. Owing to the introduction of Bt cotton having gene from Bacillus thuringiensis Berliner expressing delta endotoxin, the pest status of bollworm complex has declined (Dhawan et al., 2011). Though genetically engineered Bt cotton provides effective management of bollworm complex, sucking pests still pose a great threat in cultivation of Bt cotton, and hence the present study.

MATERIALS AND METHODS

The field experiment was conducted using four cotton cultivars under protected and unprotected conditions during kharif 2019 at the Entomology Research Farm, Department of Entomology, Punjab Agricultural University, Ludhiana. The crop was raised in split plot design (SPD) with three replications following the recommendations by PAU, Ludhiana except the date of sowing and crop protection. Each cultivar was sown in individual plots measuring 60.75 m^2 with recommended spacing. Unprotected plots were kept free from insecticides and were subjected to natural infestation, whereas protected plots were kept free from insects by applying the foliar spray of insecticide flonicamid 50WG @ 200 g/ ha and profenophos 50EC @ 1250 ml/ ha. The crop sown at two dates namely 25th April (timely sown) and 27th May (late sown).

Table 1. Effect of dates of sowing on the incidence of sucking pests and yield losses in cotton cultivars

Treatment	Number of whitefly adults*/ 3 leaves			Number of jassid nymphs*/ 3 leaves			Number of thrips adults*/ 3 leaves			Seed cotton yield* (q/ acre)					Avoidable yield losses %		
	Timely sowing (25th April)	Late sowing (27th May)	Mean	Timely sowing (25th April)	Late sowing (27th May)	Mean	Timely sowing (25th April)	Late sowing (27th May)	Mean	Timely sown (25th April)		Late sown (27th May)		Mean	Timely sown (25th April)	Late sown (27th May)	Mean
										Sprayed	Unsprayed	Sprayed	Unsprayed				
BG II cotton hybrid (US 81)	14.51 (3.79)[a]	21.50 (4.61)[e]	18.01 (4.20)	11.30	13.15	12.23 (3.63)[b]	6.52	7.41	6.97 (2.63)[b]	11.32	9.48	10.80	8.98	10.14	16.25	16.85	16.55
BG II cotton hybrid (RCH 776)	14.86 (3.83)[b]	21.10 (4.56)[d]	17.98 (4.20)	11.72	13.31	12.51 (3.67)[c]	7.19	7.80	7.50 (2.73)[c]	10.35	8.62	9.94	8.18	9.27	16.71	17.70	17.20
Non Bt cotton cultivar (F 2228)	14.43 (3.78)[a]	20.71 (4.52)[d]	17.57 (4.15)	12.90	14.57	13.74 (3.83)[d]	7.05	7.89	7.47 (2.72)[c]	6.27	5.15	5.75	4.48	5.41	17.86	22.08	19.98
Desi cotton cultivar (LD 949)	14.24 (3.74)[a]	19.14 (4.35)[c]	16.69 (4.05)	9.58	10.83	10.20 (3.34)[a]	5.55	6.16	5.86 (2.41)[a]	8.36	7.12	7.97	6.60	7.51	14.83	17.18	16.01
Mean	14.51 (3.79)[a]	20.61 (4.51)[b]		11.37 (3.51)	12.97 (3.73)		6.58	7.32		8.33		7.84			16.41	18.45	
LSD (p=0.05)	Date of sowing = 0.72; cultivars = 0.06; date of sowing × cultivars = 0.08			Date of sowing = 0.03; cultivars = 0.07; date of sowing × cultivars = NS			Date of sowing = NS; cultivars = 0.10; date of sowing × cultivars = NS			Date of sowing = 0.37; sprayed & unsprayed = 0.32; cultivars = 0.41					Date of sowing = 0.37; cultivars = 0.39; date of sowing × cultivars = NS		

*Mean of three replications; Figures in parentheses square root transformed values

Table 2. Incidence of sucking insect pests on cotton cultivars under protected and unprotected conditions

Treatment	Number of whitefly adults*/ 3 leaves			Number of jassid nymphs*/ 3 leaves			Number of Thrips adults*/ 3 leaves		
	Sprayed	Unsprayed	Mean	Sprayed	Unsprayed	Mean	Sprayed	Unsprayed	Mean
BG II cotton hybrid (US 81)	15.01	21.00	18.01 (4.20)^{c}	11.81	12.64	12.23 (3.63)^{b}	7.5 (2.73)^{f}	6.44 (2.53)^{c}	6.97 (2.63)^{b}
BG II cotton hybrid (RCH 776)	14.57	21.40	17.98 (4.20)^{c}	12.27	12.76	12.51 (3.67)^{c}	7.22 (2.68)^{e}	7.78 (2.78)^{g}	7.50 (2.73)^{c}
Non Bt cotton cultivar (F 2228)	14.29	20.85	17.57 (4.15)^{b}	13.40	14.07	13.74 (3.83)^{d}	6.61 (2.56)^{d}	8.33 (2.88)^{h}	7.47 (2.72)^{c}
Desi cotton cultivar (LD 949)	13.71	19.66	16.69 (4.05)^{a}	10.34	10.07	10.20 (3.34)^{a}	5.52 (2.34)^{a}	6.19 (2.47)^{b}	5.86 (2.41)^{a}
Mean	14.40 (3.77)	20.73 (4.52)		11.95 (3.59)^{a}	12.39 (3.65)^{b}		6.71 (2.58)	7.18 (2.67)	
LSD (p=0.05)	Sprayed and unsprayed = 0.11; Cultivars = 0.06 Sprayed and unsprayed × cultivars = NS			Sprayed and unsprayed = 0.03; cultivars = 0.07 Sprayed and unsprayed × Cultivars = NS			Sprayed and unsprayed = NS; Cultivars = 0.10 Sprayed and unsprayed × Cultivars = 0.15		

*Mean of three replications; Figures in parentheses square root transformed values

The observations on the incidence of sucking pests namely whitefly, jassid and thrips were recorded from 3 fully formed leaves of 10 randomly selected plants at weekly intervals throughout the cropping system. The seed cotton yield of the protected and unprotected plots was recorded on whole plot basis, and avoidable yield losses was computed as per the formula given by Pradhan (1964). Data was analyzed using ANOVA to test for significance of difference among the treatments.

RESULTS AND DISCUSSION

Effect of date of sowing on the incidence of *B. tabaci* revealed significantly low incidence (14.51 whitefly adults/ 3 leaves) under timely sown conditions vs late sown (20.61 whitefly adults/ 3 leaves); among the cultivars tested, significantly less incidence (16.69 whitefly adults/ 3 leaves) was on Desi cotton (LD 949); population density revealed significantly higher incidence (20.73 whitefly adults/ 3 leaves) under unsprayed as compared to sprayed conditions (14.40 whitefly adults/ 3 leaves). With *A. biguttula biguttula* significantly less incidence was in timely sown crop (11.37 jassid nymph/ 3 leaves) as compared to late sown (12.97 jassid nymphs/ 3 leaves); and significantly less incidence (10.20 jassid nymph/ 3 leaves) was on Desi cotton (LD 949); and significantly high incidence was under unsprayed conditions. The incidence of *T. tabaci* was significantly less in timely sown crop (6.58 thrips adults/ 3 leaves), compared to late sown one (7.32 thrips adults/ 3 leaves); of the cultivars significantly less infested was Desi cotton (LD949) (5.86 thrips adults/ 3 leaves); and significantly high incidence (7.18 thrips adults/ 3 leaves) was under sprayed conditions (Table 1, 2).

The results obtained from the present study on the effect of date of late sowing (with high incidence) are corroborated with those of Devi and Ram (2018). Late sown condition supports higher population of *A. biguttula biguttula* in cotton (Devi et al.,2019). Kumar et al. (2020) also showed the effect of late sown cotton on the incidence of *B. tabaci* in Punjab. The late sown cotton crop is attacked more by sucking pests had been shown with results of Abhilasha and Shekharappa (2017), Magundmder (2013), Karavina et al. (2012) and Butter et al. (1992). Significantly high *B. tabaci* incidence was observed on BG II cultivars (US 81 and RCH 776) and non Bt variety (F 2228) whereas, lowest incidence was on Desi cotton hybrid (LD 949). Pathania et al. (2020) observed that *B. tabaci* incidence was more in BG II cultivars. Several workers have observed that growth, development, survival and reproduction of insects were affected by characteristics of host plant (Liu et al., 2004; Akkopru et al., 2015).

Seed cotton yield illustrating interaction of date of sowing cultivars under sprayed and unsprayed conditions showed that significantly low seed cotton yield was recorded on F 2228 (5.41 q/ acre); maximum yield was in US 81 (10.14 q/ acre). The data on seed cotton yield of different Bt hybrid under sprayed and unsprayed conditions revealed significantly more yield in US 81 (10.14 q/ acre). Avoidable yield losses due to date of sowing was significantly less (16.41%) in

timely sown crop, and with LD 949 (16.01%) (Table 1). These results on avoidable yield losses correspond with those of Shera et al. (2012), Surulivelu et al. (2009) and Dhawan et al. (2008); also those of Satpute et al. (1988).

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TOXICITY OF IMIDACLOPRID 17.8%SL AND TRIFLUMEZOPYRIM 10%SC TO RICE BROWN PLANTHOPPER *NILAPARVATA LUGENS*

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ABSTRACT

The rice brown planthopper *Nilaparvata lugens* (Stål.) is a threat to rice growers and it has developed resistance to many insecticides. Hence, newer molecules like triflumezopyrim 10%SC are available. Its toxicity has been investigated in this study, with four populations from Tamil Nadu along with imidacloprid 17.8%SL using rice seedling dip method. Lower LC_{50} values of 0.538 ppm and 0.280 ppm were observed with the susceptible populations against imidacloprid 17.8%SL and triflumezopyrim 10%SC, respectively. LC_{50} values were in range of 0.280-0.848 ppm for triflumezopyrim 10%SC indicating its superior toxicity. Nagapattinam population exhibited moderate resistance (15.36 folds) to imidacloprid 17.8%SL and low resistance (3.02 folds) to triflumezopyrim 10%SC.

Key words: *Nilaparvata lugens*, rice, triflumezopyrim 10%SC, imidacloprid 17.8%SL insecticide resistance, susceptibility, bioassay, seedling dip method, resistance ratio

Rice production amounts to 42% of food production and 45% of cereals production in India (Mohapatra, 2014; Kumar and Sharma, 2021). Rice is affected by a number of pests, and among these, brown planthopper (BPH) *Nilaparvata lugens* (Stal) causes typical hopper burn symptoms with a yield loss of about 40-70% (Anand et al., 2020), and in the 1960's it has become a major threat with the introduction of high yielding varieties and use of pesticides (Peshin, 2021). BPH has developed resistance to most of the earlier used insecticide molecules due to indiscriminate use leading to use of neonicotinoids and phenyl pyrazoles, as these effectively control pests like aphids, thrips, planthoppers and whiteflies (Bose et al., 2020). More than 25% in total global sales of insecticides are accounted for by the neonicotinoids, in particular against *N. lugens* (Ihara and Matsuda, 2018). The first of these, imidacloprid was developed against *N. lugens* in the early 1990s but after a decade, across Asia resistance to imidacloprid had been known (Bass et al., 2015; Matsumura et al., 2014). Continuous monitoring/ detection of insecticide resistance revealed resistance to sulfoxaflor 21.8%SC (Priyadharshini et al., 2020). Recently, new molecules that are analogous to neonicotinoids are introduced like triflumezopyrim 10%SC of mesoionic class that exhibits high potential against BPH with a unique mode of action which acts as a weak agonist at the orthosteric site of nAChRs and produces lethargic nature instead of excitatory symptoms produced by other neonicotinoids (Holyoke et al., 2016). The present study carried out during 2019-2020 aims to determine baseline toxicity and the existence of cross resistance to imidacloprid 17.8%SL and triflumezopyrim 10%SC against four populations of BPH from Tamil Nadu.

MATERIALS AND METHODS

The *N. lugens* collected from Coimbatore, Bhavani and Nagapattinam sites during 2019 were used, with >50 healthy female adults and 500 nymphs collected from the collar region using an aspirator or sweep nets. These were initially kept in mylar films filled with cut rice stems covered with muslin cloth on both sides. One to two days old female adults of these populations were used for the bioassay. A susceptible strain from the Paddy Breeding Station, TNAU, Coimbatore reared in the glasshouse was also used. The field collected populations were used as a starting culture reared on 10 days old TN-1 seedlings transplanted in plastic pots maintained at the Toxicology unit in glasshouse, TNAU, Coimbatore ($11^0$0'11"N, $76^0$55'26"E); it was observed that 25-35 days old plants ideal for feeding and oviposition. These were mass cultured in the glasshouse (25± 1°C, 70-80% RH). Adults confined to the 35 to 45 days old potted plants were placed in plastic trays and kept in aluminium cages (Heinrichs, 1981). The stands were placed in trays with water to prevent ants. Adults were allowed to oviposit on 55 to 60 days old plants and freshly hatched nymphs were offered young seedlings. Continuous pure culture of the *N. lugens* was

thus maintained. The bioassay were carried out with these using seedling dip method- IRAC method No. 5 (IRAC, 2009) and 10 days old rice seedling (TN-1) were used. Six concentrations of each insecticide replicated thrice were laid out in a completely randomized block design. Preliminary range-finding tests were done to fix the test dose causing 20-80% mortality approximately for constructing log-concentration-probit mortality (LCPM) lines. The test mortality was corrected against untreated mortality as per Abbott's formula (Abbott, 1925) and subjected to Finney's probit analysis to estimate the median lethal concentrations for the dose-mortality response (Finney, 1971). The resistance ratio (RR) was calculated by dividing the LC_{50} value of the field population by that of LC_{50} of the susceptible one (Baehaki et al., 2017; Liao et al., 2017).

RESULTS AND DISCUSSION

Baseline toxicity data of imidacloprid 17.8%SL and triflumezopyrim 10%SC against *N. lugens* adult females were expressed in terms of median lethal concentration (LC_{50}); the order of resistance was observed to be- Coimbatore > Bhavani > Nagapattinam (Table 1). The LC_{50} value was 0.538 ppm for the TNAU susceptible population which is in accordance with Gorman et al. (2008). The susceptibility remains unaltered with TNAU susceptible population. Maximum LC_{50} value (8.265 ppm) was observed in the Nagapattinam population; LD_{50} for imidacloprid for the susceptible strain was 0.6 mg/ l which was maintained for 130 generations without exposure to insecticides (Garrood et al., 2017). The insect resistance declines and susceptibility increases over succeeding generations under continuous mass culturing in laboratory conditions. The field population developed moderate to high resistance for imidacloprid 17.8%SL during the last decade (Liu and Han, 2006). Basanth et al. (2013) reported Kathalagere *N. lugens* population developed resistance (8.90 folds) to imidacloprid 17.8%SL, while a resistance ratio of 13.42 folds was reported by Khan et al. (2020). Imidacloprid resistance is due to the enhanced activity of P450 monooxygenases detoxification (Wen et al., 2009). In the present study, Nagapattinam population displayed low to moderate level of resistance. A similar trend of higher resistance to imidacloprid 17.8%SL was seen in WBPH populations collected from the Nagapattinam region (Surya Raj et al., 2020).

The Nagapattinam population exhibited higher resistance to older molecules compared to newer neonicotinoid, triflumezopyrim 10%SC- the values were: Bhavani (0.688 ppm) and Coimbatore (0.570 ppm). LC_{50} value with third instar nymphs fixed by rice stem dipping bioassay method was 0.042 mg/l to triflumezopyrim 10%SC (Zhang et al., 2020); field populations exhibited a low level of resistance (1.3-7.3 fold) to triflumezopyrim 10%SC (Liao et al., 2020). In the present study, *N. lugens* exhibited less resistance (RR 2.03- 3.02 folds). The unique action of triflumezopyrim 10%SC which has a distinct chemotype might increase the susceptibility to *N. lugens* and very effective in managing *N. lugens* population resistant to other neonicotinoids (Baehaki et al., 2018). The reliable baseline data assessed for newer insecticide molecules would be more useful for field resistance monitoring studies and as well for further applications (Tang et al., 2013).

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Table 1. Baseline toxicity of neonicotinoids to *N. lugens*

Population	Insecticide	χ^2	LC_{50} (ppm)	Fiducial limits LL	Fiducial limits UL	LC_{95} (ppm)	Fiducial limits LL	Fiducial limits UL	RR
Susceptible	Imidacloprid	0.145	0.538	0.485	0.596	1.704	1.217	2.387	-
	Triflumezopyrim	0.073	0.280	0.253	0.310	0.879	0.623	1.240	-
Coimbatore	Imidacloprid	0.115	2.784	2.629	2.948	5.303	4.400	6.392	5.17
	Triflumezopyrim	0.367	0.570	0.520	0.625	1.614	1.203	2.166	2.03
Bhavani	Imidacloprid	0.043	4.893	4.741	5.049	6.966	6.303	7.698	9.09
	Triflumezopyrim	0.071	0.688	0.633	0.749	1.791	1.334	2.404	2.45
Nagapattinam	Imidacloprid	0.094	8.265	8.034	8.503	11.414	10.310	12.637	15.36
	Triflumezopyrim	0.098	0.848	0.787	0.914	1.980	1.496	2.621	3.02

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TOXICITY OF THIAMETHOXAM 25WG AND SULFOXAFLOR 21.8SC TO RICE WHITE BACKED PLANTHOPPER *SOGATELLA FURCIFERA* (HORVATH)

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ABSTRACT

Baseline toxicity of neonicotinoid insecticides viz., thiamethoxam 25WG and sulfoxaflor 21.8SC was assessed in the field collected populations of the rice white backed planthopper *Sogatella furcifera* (Horvath). The major rice growing regions of Tamil Nadu when studied revealed that the field populations vary in their susceptibility. Coimbatore and Bhavani populations were more susceptible compared to the Nagapattinam one. Baseline toxicity of thiamethoxam 25WG was relatively high with narrow variation (LC_{50}- 0.3130 to 1.7882 ppm; LC_{95}- 1.0993 to 6.3840 ppm); LC_{50} of sulfoxaflor 21.8SC ranged from 1.3121 to 3.8392 ppm, while the LC_{95} values were 3.1507 to 5.2754 ppm. The resistance ratios varied among the populations viz., thiamethoxam 25WG (2.5 to 5.6 folds) and sulfoxaflor 21.8SC (1.8 to 2.9 folds). Thus, the population from Nagapattinam exhibited maximum resistance.

Key words: Rice, *Sogatella furcifera*, field populations, baseline toxicity, lethal concentration, neonicotinoids, susceptibility, resistance, thiamethoxam, sulfoxaflor, resistance ratios

Rice is the staple food in India (Prasad et al., 2017) and it is attacked by >800 insect pests, of which planthoppers are serious causing significant economic loss. Planthoppers constitute a large group of phytophagous insects (Catindig et al., 2009). Brown planthopper (BPH) *Nilaparvata lugens* (Stal) and white backed planthopper (WBPH) *Sogatella furcifera* (Horvath) are the major pests considered as green revolution induced pests (Gunathilagaraj and Ganesh Kumar, 1997). Besides, the direct damage caused by the feeding, planthoppers serve as a vector for virus disease such as southern rice black streak dwarf virus (SRBSDV) (Zhang et al., 2008; Zhou et al., 2008). Chemical control remains the most efficient means to manage planthoppers. However, due to the large scale and intensive use of these, resistance to insecticides has been reported in rice planthoppers. Neonicotinoid and phenyl pyrazole insecticides were used in mid-1990's in many East Asian countries. At present neonicotinoid insecticides including imidacloprid, thiamethoxam, and nitenpyram are most frequently used in China (Su et al., 2013b; Zhang et al., 2014). Currently, *S. furcifera* has developed resistance to 12 compounds as reported in the Arthropod Pesticide Resistance Database (APRD), and this involve various detoxification enzymes (Denholm and Rowland, 1992; Whalon et al., 2008). Systemic neonicotinoids like imidacloprid, thiamethoxam and their analogues sulfoxaflor, triflumezopyrim and flupyradifurone have similar mode of action (Casida, 2018; Taillebois et al., 2018). Imidacloprid and thiamethoxam exhibit more resistance in planthoppers. Resistance monitoring is necessary to understand the current status of susceptibility of field populations of *S. furcifera*. Early detection of changes can prompt adoption of alternative measures. The use of insecticides alternatively with same mode of action helps the farmer overcome resistance (Liao et al., 2019). For newer molecules, baseline susceptibility helps in determining the resistance level and enables location specific management practices. Hence, the present study to assess the current status of resistance levels of field populations of *S. furcifera* to thiamethoxam 25WG and sulfoxaflor 21.8SC in different locations of Tamil Nadu.

MATERIALS AND METHODS

The laboratory reared TNAU susceptible *S. furcifera* population and populations collected from major rice growing locations of Tamil Nadu viz., Coimbatore ($11^0$0'11"N; $76^0$55'26"E), Bhavani ($11^0$36'15"N, $77^0$43'8"E) and Nagapattinam ($10^0$47'43"N, $79^0$44'5"E) during December 2019 were mass cultured separately and maintained as stock culture in glasshouse (25± 1ºC, 70-80% RH). Susceptible variety TN1 was used for rearing *S. furcifera.* in aluminium cages (45x 45x 60 cm) on 10 days old seedlings transplanted in plastic pots (10 cm dia). The potted plants maintained in the glass house

were covered with the mylar film cages. Different aged rice seedlings were maintained separately for nymphs and adult rearing. Initially five pairs of *S. furcifera* were released on 35 days old seedlings and allowed for oviposition (Heinrichs, 1985). The seedlings with eggs were placed in separate cages for the nymphal emergence and further emerged nymphs were released on 7-10 days old seedlings. These were maintained without exposure of insecticides up to F_2 generation and used for the bioassay studies. For baseline susceptibility studies, standard insects (1 to 2 days old female adult) were collected from the rearing cages daily. Dried and wilted plants together with *S. furcifera* were removed from the cages and plants were tapped gently to dislodge the hoppers and the insects were transferred to fresh rice seedlings (Heong et al., 2011).

Technical grade thiamethoxam 25WG and sulfoxaflor 21.8SC were purchased from UPL India Private Ltd., Mumbai for the bioassay; and the TNAU susceptible *S. furcifera* population (maintained for more than 20 generation without the exposure of insecticide) was taken as a baseline and preliminary range test was conducted to fix dose that gives 50% mortality (LC_{50}). Initially the dose was fixed from wider to narrow range. The range which gave 20 to 80% mortality was taken finally for the study. Mortality data was taken at 24 hr interval, with each concentration replicated thrice by releasing 15 adult female insects/ mylar film cage. F_1 generation of the susceptible population was taken for the preliminary range test and the x dose from the preliminary range test was selected. Dose for the other field populations (resistant population) were fixed at different levels and baseline susceptibility test was undertaken. For the resistant population, F_2 generation insects were taken for the baseline susceptibility study. Dose response bioassay for the selected insecticides was performed using seedling dip method (IRAC, 2012; Tsujimoto et al., 2016; Murtiati et al., 2021).

Initially, series of insecticide concentrations were prepared and the seedlings were dipped in these for about 10-30 sec and allowed to dry for 15 min. Suitable hoppers were collected from the rearing cage using a suction device. It was ensured that, only one target life stage was used, with care to ensure that life stages of short/ long winged forms do not get mixed in one test (Zhuang et al., 2000). Forty-five adult female insects were used against one insecticide concentration with three replications. Number of live and dead insects at 24 hr interval was counted and recorded. Moribund insect was counted as dead. Mortality in the control treatment was also recorded. Results were expressed as % mortality and corrected using Abbott's formula (Abbott, 1925). Concentration mortality results were subjected to probit analysis (Finney, 1971). Regression equation for dosage-mortality responses were computed to determine the LC_{50} value. Log-concentration-probit mortality lines were computed by Finney's probit analysis (Regupathy and Dhamu, 1990). The resistance ratio (RR) was calculated by dividing the LC_{50} value of field population by susceptible one. Resistance levels were classified on the basis of the standard (Su et al., 2013a; Zhang et al., 2014) as susceptible (RR < 3-folds), minor resistance (RR = 3-5 folds), low resistance (RR = 5-10 fold), medium resistance (RR = 10-40 folds) and high resistance (RR = 40-160 folds).

RESULTS AND DISCUSSION

Baseline toxicity to thiamethoxam 25WG of *S. furcifera* from different regions was relatively high with narrow variation in LC_{50} (0.3523 to 1.9861 ppm) and LC_{95} values (1.0993 to 6.3840 ppm). Among the resistant ones, Nagapattinam population showed 5.6 folds resistance followed by the one from Bhavani (3.7 folds) and Coimbatore (2.5 folds) (Figs. 1, 2; Table 1); the Coimbatore population was susceptible to thiamethoxam while Nagapattinam one was found comparatively more resistant. Application of insecticides at frequent intervals to manage rice planthoppers lead to development of resistance in rice growing areas of Nagapattinam. The results on lower resistance level to thiamethoxam 25WG (2.5- 5.6 folds) are in conformity with those of Su et al. (2013b) where 1.5 to 8.0 folds resistance was seen. The present findings agree with Zhang et al. (2014) that *S. furcifera* is showing less resistance of 0.6 to 3.1 folds with LC_{50} ranging from 0.06 to 0.28 ppm. Zhang et al. (2017) reported that the field population of *S. furcifera* from China is exhibiting lower to moderate resistance (0.8 to 14.9 folds) to thiamethoxam with LC_{50} value of 0.1 to 1.4 ppm. These results are in agreement with present ones. Nagapattinam population showed more resistance (6.2 folds) to imidacloprid 17.8SL followed by Bhavani (4.1 folds) and Coimbatore populations (2.8 folds) with LC_{50} values ranging from 0.4 to 2.5 ppm (Surya Raj et al., 2020).

The LC_{50} of sulfoxaflor 21.8SC to field populations of *S. furcifera* ranged from 1.3121 to 3.8392 ppm Nagapattinam population recorded higher LC_{50} (3.8392 ppm) followed by Bhavani (3.2116 ppm) and Coimbatore populations (2.3782 ppm). LC_{95} values

Table 1. Toxicity of thiamethoxam 25WG and sulfoxaflor 21.8SC to *S. furcifera*

Insecticide	Locations	Regression equation	X^2	LC_{50} (ppm)	Fiducial limits Lower	Fiducial limits Upper	LC_{95} (ppm)	Fiducial limits Lower	Fiducial limits Upper	RR
Thiamethoxam 25 % WG	TNAU susceptible	y = 3.351x + 3.166	0.1557	0.3523	0.3130	0.3965	1.0993	0.8182	1.4770	-
	Coimbatore	y = 5.351x - 0.095	0.1192	0.8947	0.8401	0.9530	1.8201	1.4667	2.2586	2.5
	Bhavani	y = 4.318x + 0.163	0.0520	1.3180	1.2188	1.4252	3.1648	2.4804	4.0382	3.7
	Nagapattinam	y = 3.229x + 0.810	0.0708	1.9861	1.7882	2.2059	6.3840	4.6022	8.8557	5.6
Sulfoxaflor 21.8% SC	TNAU susceptible	y = 4.357x + 0.120	0.4450	1.3121	1.2158	1.4225	3.1507	2.4690	4.0205	-
	Coimbatore	y = 7.308x - 5.060	0.2573	2.3782	2.2701	2.4913	4.0059	3.4227	4.6885	1.8
	Bhavani	y = 9.649x - 9.545	0.3111	3.2116	3.1018	3.3254	4.7538	4.2516	5.3154	2.4
	Nagapattinam	y = 11.98x - 13.98	0.4255	3.8392	3.7316	3.9499	5.2754	4.7908	5.8090	2.9

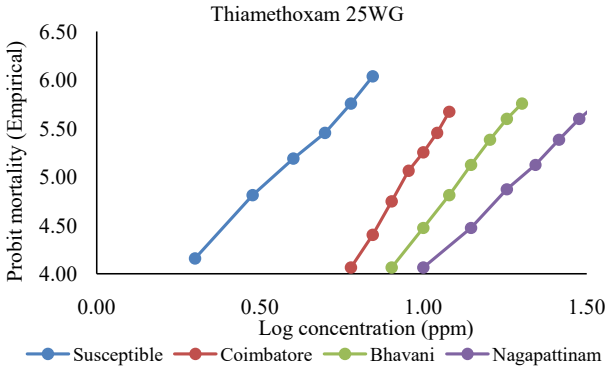


Fig. 1. LCPM regression lines for populations of *S. furcifera* to thiamethoxam 25WG

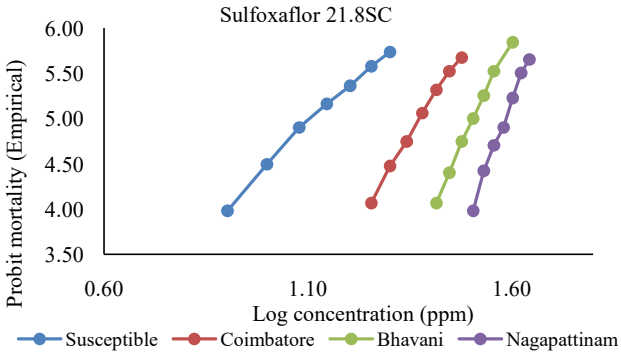


Fig. 2. LCPM regression lines for populations of *S. furcifera* to sulfoxaflor 21.8SC

followed similar trend and varied from 3.1507 to 5.2754 ppm. The resistant ratio was 2.9, 2.4 and 1.8 folds as compared to the susceptible one (1.3121 ppm) (Table 1). These findings derive support from Kapasi et al. (2017) from northeastern Karnataka. Priyadharshini et al. (2020) observed that *N. lugens* sulfoxaflor 21.8SC shows resistance of 1.9 to 3.35 folds. The present results contradict with those of Ghosh et al. (2013) showing comparatively lower LC_{50} values for sulfoxaflor (0.382 to 2.986 ppm) to selected *N. lugens* populations of West Bengal.

Thus, *S. furcifera* populations from different regions of Tamil Nadu differed significantly in their response to sulfoxaflor. The regional variation in susceptibility of different *S. furcifera* populations had been already reported in India (Basanth et al., 2013). Elevated activity of glutathione-S-transferase, peroxidase and mixed function oxidase (MFO) might be responsible for this (Vontas et al., 2002). The planthoppers showing resistance to imidacloprid was due to the increased P450 monooxygenase activity (Nakao, 2017).

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EFFECTS OF DATE OF TRANSPLANTING ON THE INCIDENCE OF RICE LEAF FOLDER *CNAPHALOCROCIS MEDINALIS* (GUENÉE) AND ITS POPULATION DYNAMICS

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ABSTRACT

Field studies were conducted to evaluate the impact of transplanting dates and weather parameters on the incidence of rice leaf folder *Cnaphalocrocis medinalis* (Guenee). During 2018 kharif season, the first incidence was noticed during 27th standard meteorological week (SMW) irrespective of the transplanting dates, with peak being in the 28th SMW (1.67% damaged leaves/ hill). Least damage was observed in 4th date of transplanting (20th July), while maximum incidence was with 20th June transplanting. The correlation of incidence with temperature showed negative correlation, while rainfall and relative humidity showed positive relationship.

Key words: *Cnaphalocrocis medinalis*, population dynamics, rainfall, relative humidity, temperature, correlation coefficients, transplanting dates, seasonal incidecne

Rice (*Oryza sativa*) is an important cereal crop, and it is attacked by 800 species of insect out of which 20 (e.g. rice leaf folder, stem borer, plant hopper, grass hopper and gall midge etc.) cause economic damage and are considered as major pests (Jena et al., 2018). Among these, the rice leaf folder *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) is a predominant foliage feeder and is one of the most devastating pests (Luo, 2010). It occurs in all growth stages of the crop. Larvae damage the crop by feeding on chlorophyll content of leaves after folding (Bisen et al., 2019). Its damage at reproductive stage can reduce yield up to 6.2%, and overall losses range from 63 to 80% (Satish et al., 2007; Teng et al., 1993). Growers rely heavily on pesticides to protect their rice crop. The weather parameters and the transplanting dates have an influence on the appearance of the pests. This experiment evaluates the seasonal incidence in relation to date of transplanting and the population dynamic is in relation to weather factors.

MATERIALS AND METHODS

A field experiment was carried out at the agriculture research farm, Lovely Professional University, Jalandhar (31 24' N, 75 69' E, 4342 masl) during 2018 kharif season. Thirty days' old seedlings of Pusa 1509 variety were transplanted, and randomized block design was followed in 5x 5 m plots replicated thrice. Recommended agronomic practices by the PAU, Ludhiana were followed. Four transplanting dates evaluated were: 20th June/ 25th standard meteorological week (SMW), 30th June/ 26th SMW, 10th July/ 28th SMW and 20th July/ 29th SMW, 2018. Incidence of *C. medinalis* was recorded at weekly intervals from randomly selected six hills from untreated plots. Weather data were obtained from the meteorological observatory, agriculture research farm, LPU, Phagwara (Punjab). The incidence data was subjected to angular transformation prior to statistical analysis. Pearson correlation was obtained to find out the relationship with weather factors. The statistical analysis was done using SPSS (version 22).

RESULTS AND DISCUSSION

Incidence of *C. medinalis* was observed during 27th SMW (0.32/ hill) which reached its peak during 28th SMW (1.67/ hill) (Fig. 1); and with 29th SMW it decreased till 36th SMW (0.08/ hill) and then increased during 38th SMW (0.82/ hill), while at maturity stage of crop (39th and 40th SMW) the incidence declined abruptly. The weather factors viz., temperature, humidity and rainfall showed a non-significant effect on the incidence; maximum (r = -0.214) and minimum (r = -0.154) temperature showed a negative correlation

while relative humidity- RH (r = 0.420 and r = 0.375) and rainfall (r = 0.211) showed a positive one (Fig. 2). Similar results were obtained by Singh et al. (2017) and Zainab et al. (2017) showing a negative correlation with temperature and positive correlation with rainfall. Tiwari et al. (2021) observed a positive correlation with the maximum temperature but negative with the rainfall and evening RH. The present results agree with those of Singh et al. (2017) in terms of positive correlation of rainfall and RH. Yimjenjang and Mishra (2020) also observed that maximum (r= 0.527*, 0.590) and minimum temperature (r=0.849**, 0.853**), morning (r=0.244, 0.458) and evening RH (r=0.518*, 0.269) and wind velocity (r=0.356, 0.361) had significant positive relationship. Haider et al. (2020) obtained similar results with weather factors as having an insignificant relationship. Kakde and Patel (2015) observed non-significant positive relationship of maximum temperature, RH, rainfall and sunshine hours with the leaf damage by leaf folder; and non-significant negative correlation of minimum temperature was observed under SRI planting method.

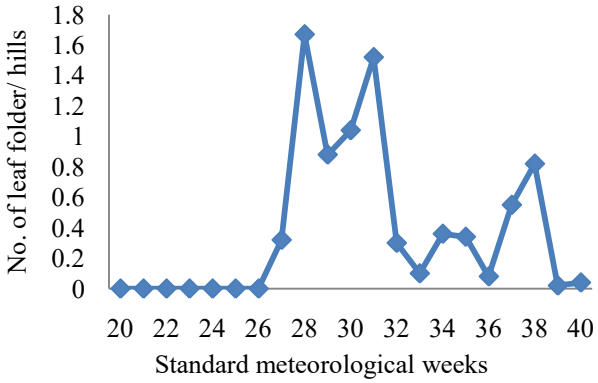


Fig. 1. Seasonal incidence of *C. medinalis*

About the dates of transplanting, least damage (0.03 -1.34% leaves/ hill) was observed in fourth transplanting date (20th July/ 29th SMW), followed by third date of transplanting (10th July/ 28th SMW); maximum damage was observed with the first date of transplanting (20th June/ 25th SMW, 0.35-9.58%) Rautaray et al. (2019) observed that the rice transplanted late (30th August) had higher infestation. In the present study June transplanting was observed to be the best with least infestation. Rautaray et al. (2019) concluded that transplanting rice on first July would be a better option. As far as the crop stage is concerned, maximum damage (0.35-9.58%/ hill) was observed during vegetative phase of the crop, 30 days after transplanting (DAT) followed by 45 DAT (Table 1). Thus, the rice leaf folder incidence was more when crop was sown early. Similar observations had been reported by Ma and Lee (1996) and Chaudhari et al. (2018).

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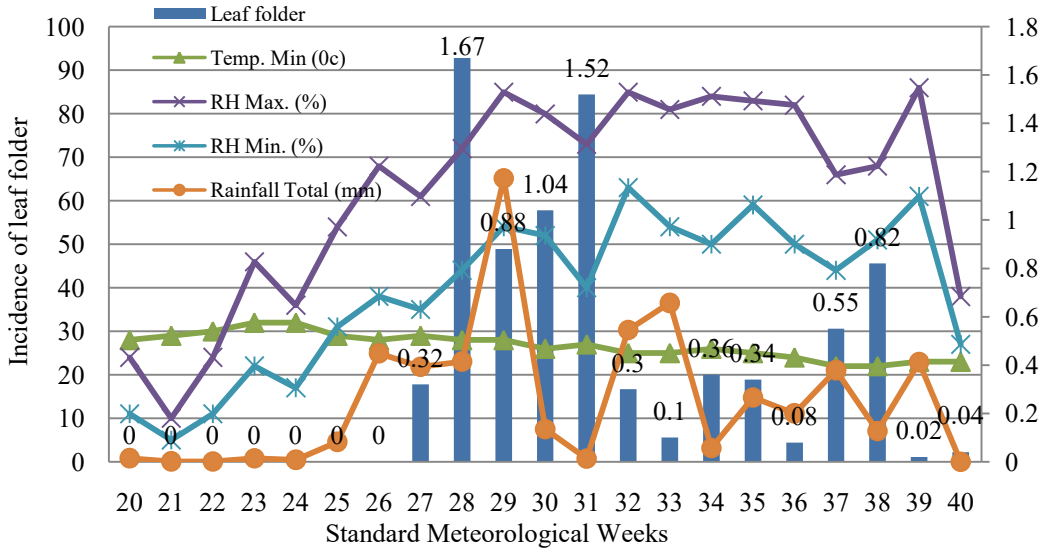


Fig. 2. Population dynamics of *C. medinalis*

Table 1. Effect of transplanting dates on *C. medinalis* incidence

Factors	Vegetative stage				Reproductive stage			
	30 DAT		45 DAT		60 DAT		90 DAT	
Transplanting	Mean % damage/hill*							
20th June	9.58 (17.78) a		3.13 (9.28) a		1.34 (4.04) c		0.35 (2.11) b	
30th June	4.91 (12.62) b		1.89 (7.22) b		2.00 (7.81) a		0.36 (3.34) a	
10th July	0.76 (4.55) c		1.24 (6.33) d		0.69 (4.69) b		0.08 (0.88)c	
20th July	0.56 (4.16) d		1.34 (6.61) c		0.03 (0.31) d		0.00 (0.14)d	
DAT	S.Em (±)	CD@ 0.05%	S.Em (±)	CD@ 0.05%	S.Em (±)	CD@ 0.05%	S.Em (±)	CD@ 0.05%
	0.12	0.36	0.08	0.24	0.12	0.36	0.002	0.006

*Figures in parentheses angular transformed values (p≥0.05)

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SEASONAL INCIDENCE OF STRIPED FLEA BEETLE *PHYLLOTRETA STRIOLATA* F. ON CRUCIFEROUS CROPS IN NORTH KASHMIR

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ABSTRACT

This study is on the seasonal incidence of *Phyllotreta striolata* F. during 2015 and 2016, on turnip (*Brassica rapa var. rapa*), radish (*Raphanus sativus*) and cabbage (*B. oleracea* var. *capitata*). The incidence observed at weekly intervals was correlated with weather factors. The pest was first observed during 35th standard meteorological week (SMW), and in 2015, peak was observed on turnip (22.8 beetles/ plant) and radish (22.2 beetles/ plant) during the 40th SMW; during 2016, peak was (19.4 beetles/ plant on turnip; 22.2 beetles/ plant on radish) in the 39th SMW; and on cabbage, the peak incidence (4.8 beetles/ plant- 2015; 9.2 beetles/ plant- 2016) was during 38th SMW. The incidence showed a positive correlation with temperature and sunshine hours, and a negative correlation with relative humidity and rainfall.

Key words: *Phyllotreta striolata,* cruciferous crops, turnip, radish, cabbage, seasonal incidence, correlation, regression, temperature, relative humidity, rainfall, sunshine hours

Cruciferous crops are important group of rabi season crops in India (Kumaranag et al*.,* 2014). The productivity of these crops in India is low, as these are attacked by a complex of specialist and generalist insect pests. These include cabbage butterfly (*Pieris brassicae* L.); aphid *viz*., cabbage aphid (*Brevicoryne brassicae* L.) and mustard aphid (*Lipaphis erysimi* K.); diamondback moth (*Plutella xyllostella* L.); cabbage semilooper (*Thysanaplucia orichalcea*); flea beetles (*Phyllotreta* sp.); mustard sawfly (*Athalia colibri* F.); cut worm (*Agrotis ipsilon* H.) and leaf miner (*Chromotomyia horticola* G.) (Hooks and Johnson, 2003; Bana et al*.,* 2012). Flea beetles (Coleoptera: Chrysomelidae), belonging to the genus *Phyllotreta* are important economic pests of cruciferous crops. *Phyllotreta striolata* F., is prevalent as pest of cruciferous crops in Kashmir (Rasool et al., 2019). *Phyllotreta* spp., are found in all geographical regions (≥ 60%) including Palearctic, Afrotropical, and Nearctic regions (≥ 80%) (Gikonyo et al*.,* 2019). Factors like temperature, rainfall, relative humidity and other weather factors affect the seasonal incidence of insects. Hence, studying the effect of these factors on the seasonal incidence is important (Day, 2006; Shang et al*.,* 2010; Shang et al*.,* 2012). In IPM programme, need based application of insecticides is recommended for which monitoring and surveillance is necessary. During last few years, the vegetable growers of Kashmir valley are affected due to the damage caused by flea beetles, earlier considered as minor pests on these crops. These pests have increased their host range, as these are now attacking other fruit crops like apple, grapes, apricot etc. (Ahad et al*.,* 2011). Due to changing climatic conditions, the pests of minor importance are showing severity (Lone et al*.,* 2009). The present study evaluates the seasonal incidence of *P. striolata* on turnip, radish and cabbage crops, so that we can precisely predict its outbreaks.

MATERIALS AND METHODS

The field study was carried out at the Regional Research Station, Faculty of Agriculture, Wadura, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir (34°20′51″, 74°23′54″ E, 1586.8 masl). The crops were sown during rabi season, on 7th of August, 2015 and 2016, and raised as per the recommend agronomical practices of the Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir. Recommended varieties of turnip (Purple Top White Bottom), radish (White Round) and cabbage (Golden Acre) were sown in plots of 4x 3 m with row to row spacing of 45 cm and plant to plant spacing of 30 cm. Only adult flea beetles were observed, and counts of *P. striolata* were recorded at weekly

intervals on five tagged plants from each replication (3 replications). From these mean numbers were worked out. *P. striolata* samples were sent to National Bureau of Agriculturally Important Resources, Bangalore for identification. Data on temperature (°C), relative humidity (%), sunshine (hrs.) and rainfall (mm) were obtained from the Meteorological Centre, Srinagar, India. The data was subjected to correlation and regression analysis, using the SPSS Software. Data were arc-sine transformed before performing ANOVA. Means were separated at $p<0.05$ and adjusted with least significant difference (LSD).

RESULTS AND DISCUSSION

Flea beetle species studied was identified as *Phyllotreta striolata* (Accession No. KU752539.1), with the characters of shiny black with yellowish elytral stripes incurved at the base not extending to the posterior end of the elytra. Seasonal incidence of *P. striolata* on turnip, radish and cabbage depicted by Fig. 1 indicate that its incidence commenced during 35th SMW- in 2015 it was @ 1.8, 2.6 and 1.8 beetles/ plant on turnip, radish and cabbage, respectively; and during 2016, it was 0.8, 0.6 and 0.8 beetles/plant, respectively. Initial occurrence on turnip, radish and cabbage was observed just after cotyledonary leaf emergence (35th SMW). Similar findings were reported by Patel and Purohit (2016) with flea beetles (*Chaetochnema indica*) on sorghum. Ulmer and Dosdall (2006) reported that progeny of the overwintering generation of *P. striolata*, already present in the surrounding fields attack newly emerged crops as soon as cotyledons emerge. During 2015, *P. striolata* on turnip and radish reached a peak incidence of 22.8 and 22.2 beetles/ plant, respectively during 40th SMW (1st week of October) at temperature, relative humidity, sunshine and rainfall of 19.06°C, 62.14 %, 8.15 hrs. and 0.00 mm, respectively. These results are in conformity with those of Samoon (2004) who observed maximum catches of *Psylliodes tenebrosus* in October during emergence of the crop.

However, on cabbage, *P. striolata* reached a peak level of 4.8/ plant during 38th SMW (3rd week of September) when temperature, relative humidity, sunshine and rainfall were 17.74 °C, 69.71%, 6.12 hr. and 8.28 mm, respectively; and then there was decline in all the three crops to counts of 0.8, 0.8 and 0.2 beetles/ plant on turnip, radish and cabbage, respectively, during 45th SMW; at this time temperature, relative humidity, sunshine and rainfall were 7.51°C, 80.28%, 0.68 hrs. and 2.8 mm, respectively. During 2016, the incidence on turnip and radish was maximum viz., 19.4 and 22.2 beetles/ plant, respectively during 39th SMW, when temperature, relative humidity, sunshine and rainfall were 19.61 °C, 63.35%, 8.44 hrs. and 0.00 mm, respectively. In case of cabbage, it was maximum (9.2 beetles/ plant) during 38th SMW at a temperature, relative humidity, sunshine and rainfall of 19.53°C, 63.99%, 7.54 hrs. and 0.00 mm, respectively. During 45th SMW, incidence declined to 3.4, 1.4 and 0.1 beetles/ plant on turnip, radish and cabbage, respectively at temperature, relative humidity, sunshine and rainfall of 10.31°C, 64.42%, 3.7 hrs and 0.00 mm, respectively.

The correlation coefficients (r) and regression equation for weekly *P. striolata* counts with weather parameters presented in Table 1. Reveal the following- during 2015, incidence on turnip, radish and cabbage showed a significant positive correlation with temperature (r = +0.607, +0.607, +0.819) and sunshine hrs. (r = +0.625, +0.660, +0.738) and a significant negative correlation with relative humidity-RH (r = -0.605, -0.659, -0.677); but with rainfall it was non-significant. During 2016, incidence on radish and cabbage with temperature was significantly positive (r

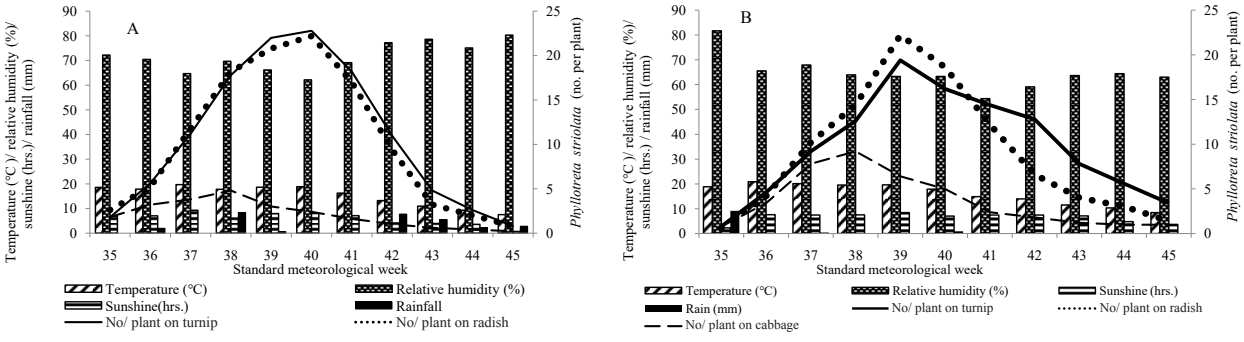


Fig. 1. Population dynamics of *P. striolata* on turnip, radish and cabbage- 2015 (A) and 2016 (B)

Table 1. Seasonal incidence of *P. striolata* on cruciferous crops (2015, 2016)

Year	Factors	Turnip		Radish		Cabbage	
		Correlation coefficient (r)	Regression equation	Correlation coefficient (r)	Regression equation	Correlation coefficient (r)	Regression equation
2015	Temperature (°C)	0.607**	y = 0.3158x + 11.903 R^2 = 0.3642	0.654**	y = 0.3473x + 11.734 R^2 = 0.4227	0.819*	y = 2.3663x + 10.403 R^2 = 0.6743
	Relative humidity (%)	-0.605**	y = -0.5332x + 77.180 R^2 = 0.5616	-0.659**	y = -0.5739x + 77.336 R^2 = 0.6243	-0.677*	y = -2.8362x + 77.309 R^2 = 0.524
	Sunshine (hrs.)	0.625**	y = 0.191x + 3.817 R^2 = 0.3906	0.660**	y = 0.2058x + 3.758 R^2 = 0.4352	0.738*	y = 1.2423x + 3.301 R^2 = 0.5449
	Rainfall (mm)	-0.088^{NS}	y = -0.0332x + 2.988 R^2 = 0.0077	-0.114^{NS}	y = -0.0442x + 3.085 R^2 = 0.013	0.001^{NS}	y = 0.0017x + 2.626 R^2 = 6E-07
2016	Temperature (°C)	0.490^{NS}	y = 0.2126x + 13.941 R^2 = 0.0802	0.655**	y = 0.2994x + 13.354 R^2 = 0.2441	0.787**	y = 1.0021x + 12.361 R^2 = 0.472
	Relative humidity (%)	-0.599^{NS}	y = -0.6841x + 71.237 R^2 = 0.3585	-0.369^{NS}	y = -0.3404x + 67.609 R^2 = 0.1363	-0.070^{NS}	y = -0.1554x + 65.161 R^2 = 0.0049
	Sunshine (hrs.)	0.772*	y = 0.2558x + 3.965 R^2 = 0.5961	0.687**	y = 0.1837x + 4.822 R^2 = 0.472	0.597^{NS}	y = 0.384x + 5.053 R^2 = 0.3559
	Rainfall (mm)	-0.474^{NS}	y = -0.2215x + 3.104 R^2 = 0.225	-0.333^{NS}	y = -0.1257x + 2.066 R^2 = 0.1112	-0.278^{NS}	y = -0.2522x + 1.871 R^2 = 0.0773

*Significant at p=0.01; **Significant at p= 0.05; NS (Non-significant).

= +0.655, +0.787); and a significant positive correlation was also observed between sunshine (hrs.) on turnip and radish (r = +0.772, +0.687); and non-significant values were observed on turnip, radish and cabbage with weather factors. Ghosh et al. (2006) also observed a significantly positive correlation between flea beetle incidence and temperature; however, Shukla and Kumar (2003) reported a negative correlation between *Phyllotreta cruciferae* incidence with temperature. Temperature plays an important role in determining growth rate of insects (Narayanasamy et al*.,* 2014). Patel and Purohit (2016) reported that flea beetle incidence was negatively influenced by morning and evening, and mean RH. The present results are in conformity with results of Yadav et al. (2010) who observed a significant positive correlation in radish with temperature (r = +0.890) and significant negative correlation (r = -0.830) with RH. In mustard it was a positive correlation (r = +0.293) with sunshine.

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EVALUATION OF SOME ECOFRIENDLY IPM APPROACHES IN SORGHUM

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ABSTRACT

Some ecofriendly IPM modules were evaluated against insect pests of sorghum. These included: timely sowing (41st standard week-SW); seed treatment with imidacloprid 70WS @ 3 g/ kg seed; installing fishmeal trap @ 12/ ha up to 30 days after emergence (DAE); bund cropping with sorghum + bhendi; releasing egg parasitoid, *Trichogramma chilonis* at 35 DAE; spraying NSKE 5% at 60 DAE. These recorded minimum pest load with highest cost benefit ratio. This IPM package was compared to local common farmers' practice and the results proved that these approaches are superior.

Key words: Sorghum, *Atherigona soccata*, *Chilo partellus*, *Stenodiplosis sorghicola*, IPM modules, timely sowing, imidacloprid, NSKE, fishmeal trap, bund cropping, bhendi, *Trichogramma chilonis*

Sorghum (*Sorghum bicolor* L.) is an important cereal crop (FAO, 2018), and in Tamil Nadu, it is grown in drought-prone, marginal areas. In Tamil Nadu it is cultivated in an area of 3.85 lakh ha with a productivity of 1117 kg/ ha (AICSIP, 2021). The yield of sorghum is largely affected by biotic constraints and >150 insect species infest sorghum. Damage inflicted by these cause serious losses (Daware et al., 2012). In India, shoot fly and stem borer significantly reduce the yield, at times as high as 100% in grain and crop stand (Padmaja et al., 2010). In Tamil Nadu, shoot fly *Atherigona soccata* Rondani, stem borer *Chilo partellus* Swinhoe and grain midge *Stenodiplosis sorghicola* Coquillett are the regular pests in southern districts causing 2 to 48% damage (Anandhi and Sankarapandian, 2013; Anandhi et al., 2015). An array of sprayable and granular insecticides are recommended against these (Patil et al., 1992), but these have several limitations (Anandhi et al., 2017). Resource poor farmers find problem in chemical spraying due to increased cost of cultivation and possible phytotoxic effects (Sharma, 1993). Current recommendation of soil application with carbofuran 3G @ 30kg/ ha is very expensive (Shekharappa and Bhuti, 2007). Hence, improved IPM approaches are required. Habitat manipulation to enhance the activities of natural enemies aim at minimal or zero insecticide use (Gurr et al., 2004). Prior researches on ecological engineering model in cotton (Muthukrishnan et al., 2015), bhendi (Deepika, 2016) and rice (Chandrasekar, 2016) have demonstrated the use of these. The present study aims to assess the advantages of sorghum- based bund crop, along with other approaches as IPM modules in sorghum along with their validation under rainfed condition in southern region of Tamil Nadu.

MATERIALS AND METHODS

The experiment was conducted during 2017 and 2018 at the Agricultural Research Station, Kovilpatti, Tamil Nadu, with eight treatments replicated thrice in a randomized block design (RBD). The K-8 sorghum variety was used in plots of 24.3m^2 (5.4 x 4.5m) with spacing of 45 x 15 cm. The treatments included neem oil (NO) 3%, neem seed kernel extract (NSKE) 5%, *Vitex negundo* 10% leaf extract, *Ocimum sanctum* 10% leaf extract, *Aloe vera* 10% leaf extract, *Calotropis gigantea* 10% leaf extract, *Datura stramonium* 10% leaf extract and an untreated control. Botanicals such as leaves of *V. negundo*, *O. sanctum, A. vera, C. gigantea* and *D. stramonium* were collected and cut into small pieces. The cut leaves were mixed with water at 10% and boiled for 30-50 min and afterward allowed to cool for about 2 hr and was then filtered through muslin cloth (Shakti Khajuria et al., 2015). The extracts were sprayed on the crop on 25, 40 and 55 DAE (days after emergence). A total of 3 sprays at 15 days interval were given using knapsack sprayer @ 500 l spray fluid/ ha. Another experiment was conducted with seven treatments including an untreated check with three replications. Six bund crops viz., coriander, sesamum, cowpea, cluster bean, black gram and bhendi were selected. No chemical pesticides were applied throughout the crop season. The IPM modules consisted of five treatments including farmers practice with four replications were validated.

The details of the treatments are as follows- T_1- Insecticide module with only insecticide application (i. seed treatment with imidacloprid 70 WS @ 3 g/ kg seed; ii. spraying of carbaryl 50WP @ 1 kg/ ha at 28 DAE for shoot fly; iii. Dusting of carbaryl 10D @25 kg/ ha at 60 DAE for stem borer; iv. dusting of carbaryl 10D @25 kg/ ha at 90 DAE for midge), T_2- Ecofriendly module (i. bund cropping sorghum + bhendi; ii. installing fish meal trap up to 30 DAE; iii. releasing of egg parasitoid *Trichogramma chilonis* at 35 DAE for stem borer; iv. spraying of spinosad 45 % SC @ 75 g ai/ ha at 50 DAE for stem borer; v. spraying neem oil 3% at 60 DAE for midge), T_3- need based integrated module (i. spraying of spinosad 45% SC @ 75 g ai/ ha at 50 DAE for stem borer (stem borer-10% deadheart ETL crossed), ii. releasing of egg parasitoid *T. chilonis* at 35 DAE for stem borer; iii. spraying neem oil 3% at 60 DAE for midge- (ETL (5/ earhead) crossed), T_4- neem based module with application of only neem products (i. incorporation of neem cake 150 kg/ ha; ii. neem seed kernel extract (5%) at 28 DAE; iii. neem seed kernel extract (5%) at 45 DAE; iv. spraying of neem oil 3% at 60 DAE); and T_5- untreated check. Based on the above modules, the best treatments were assorted and compared with the farmers practice. To validate, large scale field experiments were conducted in farmer's field with two modules including a farmers practice as a control plot. The IPM modules imposed are as follows: T1- IPM Package **(**i. timely sowing (41st standard week); ii. seed treatment with imidacloprid 70 WS @ 3 g/ kg seed; iii. installing fishmeal trap @ 12/ ha up to 30 DAE; iv. bund cropping (sorghum + bhendi); v. releasing egg parasitoid *T. chilonis* at 35 DAE; vi. Spraying NSKE 5% at 60 DAE); and T2- farmers' practice compared as check. In case of farmers practice, broadcasting the seeds 15 kg/ ha without any plant protection.

The shoot fly deadheart at 28 days after emergence (%), stem borer deadhearts at 45 days after emergence (%), midge spikelet damage (%) panicle maturation stage were observed in each treatment following protocols of AICRIP, Sorghum, DSR, Hyderabad. The occurrence of predatory coccinellids (grubs and adults) and parasitoids (number/ plant) were recorded, and emergences rate of adult parasitoids were represented as % parasitism. In bund crop and IPM validation, cost benefit ratio was calculated considering additional cost (cost of insecticide and operational charge) and benefit (compared to untreated control) in the respective treatments. Data values in % were arc sine transformed prior to ANOVA and treatment means were compared by Least Significant Difference (LSD) (Gomez and Gomez, 1984). IPM Experiments were subjected to student's t- test.

RESULTS AND DISCUSSION

Effect of plant products against the major pests of sorghum study was consistent over two years and hence the pooled results are discussed. The deadhearts due to shoot fly and stem borer (%) at 28 and 45 DAE revealed that significantly less values in neem oil 3% (11.01 and 11.44%) and NSKE 5% treated plots (13.58 and 12.40%) as compared to control (50.96 and 41.50%). Maximum incidence of midge spikelet damage rating was of 9-grade in control, followed by *Aloe vera* 10% leaf extract (6-grade), and the least values were in neem oil 3% and NSKE 5% (3 and 3.33-grade). Significantly more grain yield was obtained with neem oil 3% (1907.67 kg/ ha) and NSKE 5% (1869.55 kg/ ha). Maximum cost benefit (C:B) ratio (1:1.95) was NSKE 5% followed by neem oil 3% treated plots (1:1.70) (Table 1). Prabakaran et al. (2019) observed maximum azadirachtin content in the neem tree populations growing in the southern part of India. Shrinivas and Mudigoudra Shekharappa (2009) and Sable (2009) reported that NSKE (5%) and neem oil (2%) spray in sorghum at 21DAT reduced deadhearts and gave maximum yield; similar results were observed with 3% neem oil (Gautam et al., 2014). The antifeedant properties of neem oil 3%, NSKE with shoot fly had been earlier reported (Zongo et al., 1993; Adane Tesfaye and Asmare Dejan, 2006). About the suitable bund crop, least deadhearts (%) due to shoot fly was observed in sorghum + bhendi (11.52%) and sorghum+ sesamum (13.92%); deadhearts due to stem borer was in sorghum + cowpea (6.11%) and sorghum + bhendi (10.20%); with incidence of midge, spikelet damage rating was the least with sorghum+ cowpea, sorghum+ sesamum and sorghum+ bhendi (2-3 grade).

Larval parasitism on sorghum shoot fly, stem borer, midge and general predators such as coccinellids (*Menochilus sexmaculata*) and spiders were more with sorghum+ sesamum (28.0% and 14/ plot) and sorghum + bhendi (25.00 % and 15/ plot). Maximum grain yield was obtained from sorghum+ sesamum (2046.00 kg/ ha) followed by sorghum+ bhendi (1815.77 kg/ ha). Maximum C: B ratio (1: 2.25) was also obtained in sorghum+ bhendi followed by sorghum+ sesamum (1: 2.19) (Table 1). Karibasavaraja et al. (2005) reported that sorghum+ coriander, sorghum+ sesamum intercropping resulted in lowest number of deadhearts due to shoot fly. Sorghum+ bhendi best intercropping combination was reported by Degri and Richard (2014) and sorghum

+ groundnut, sorghum + pegionpea intercropping was suggested by Rana et al. (1998).

Validation of the IPM modules against major pests of sorghum revealed that the deadhearts due to shoot fly and stem borer at 28 and 45 DAE were significantly minimum in insecticide applied module (12.76%) followed by need based integrated module (14.97%) as compared to control (62.08%); similar was the trend in insecticide applied module (7.22) and ecofriendly module (7.30%) as compared to control (42.19%). The least incidence of midge spikelet/ damage rating was observed in need based integrated module and insecticide applied module (3-grade). Grain yield was maximum with insecticide module with only insecticide application (2396.25 kg/ ha) followed by need based integrated module consisting of insecticides, botanicals and natural enemies (2115.75 kg/ ha); however,

Table 1. Effect of plant products and bund cropping on *A. soccata, C. partellus* and *S. sorghicola* in sorghum (Pooled, 2017, 2018)

Treatments		Deadheart (%)		*Stenodiplosis sorghicola*	Grain yield (kg/ ha)	C:B ratio
		Atherigona soccata at 28 DAE*	*Chilo partellus* at 45 DAE*	Panicle damage scale (1-9)		
T_1	Neem oil 3%	11.01 (19.05)**	11.44 (19.90)	3.00	1907.67	1: 1.70
T_2	Neem Seed Kernal Extract 5%	13.58 (21.31)	12.40 (21.37)	3.33	1869.55	1: 1.95
T_3	Notchi (*Vitex negundo*) 10% leaf extract	18.33 (23.97)	24.79 (27.57)	5.88	1511.55	1: 1.66
T_4	Tulsi (*Ocimum sanctum*) 10% leaf extract	19.47 (25.96)	19.48 (26.02)	5.44	1435.66	1: 1.54
T_5	Sothukathalai (*Aloe vera*)10% leaf extract	20.89 (27.15)	20.16 (27.25)	6.00	1241.22	1: 1.15
T_6	*Calotropis gigantea* 10% leaf extract	25.75 (30.44)	21.85 (29.20)	5.22	1482.44	1: 1.74
T_7	*Datura stramonium* 10% leaf extract	28.04 (31.91)	19.43 (29.34)	5.77	1363.77	1: 1.59
T8	Untreated control	50.96 (45.56)	41.50 (43.17)	9.00	790.55	1: 0.84
	SE	2.14	1.07	0.76	83.94	
	CD (p=0.05)	4.59	3.54	1.63	169.88	

Efficacy of bund cropping

Treatments		Deadheart (%)		*Stenodiplosis sorghicola*	Natural enemies		Grain yield (kg/ha)	C:B ratio
		Atherigona soccata at 28 DAE*	*Chilo partellus* at 45 DAE*	Panicle Damage scale	% Parasitism (Midge)	Predators *** (No./ plot)		
T_1	Sorghum alone	56.32 (44.44)**	47.78 (42.43)	9.00	2.50 (1.56)	2.00 (1.56) **	952.00	1: 0.86
T_2	Sorghum+ coriander	17.14 (27.54)	17.88 (21.03)	4.00	8.00 (2.86)	8.00 (2.86)	1185.11	1: 2.01
T_3	Sorghum+ sesamum	13.92 (25.70)	11.83 (21.75)	2.33	28.00 (3.73)	14.00 (3.73)	2046.00	1: 2.19
T_4	Sorghum+ cowpea	24.0 (30.78)	6.11 (16.36)	3.00	21.00 (3.02)	9.00 (3.02)	1747.33	1: 1.72
T_5	Sorghum+ clusterbean	29.90 (33.06)	14.07 (67.07)	3.67	15.00 (3.17)	10.00 (3.17)	1575.77	1: 1.63
T_6	Sorghum+ blackgram	30.29 (33.68)	16.98 (24.29)	5.33	12.00 (2.30)	5.00 (2.30)	1176.33	1: 1.27
T_7	Sorghum+ bhendi	11.52 (24.12)	10.20 (22.13)	2.33	25.00 (3.85)	15.00 (3.85)	1815.77	1: 2.25
	SE	2.15	4.42	0.97	0.55	0.18	141.95	
	CD (0.05)	4.69	9.62	2.11	1.19	0.40	283.91	

(contd.)

(Table 1 contd.)

Efficacy of IPM modules (rabi 2018)						
Treatments		Deadheart (%)		*Stenodiplosis sorghicola*	Grain yield (kg/ ha)	C:B ratio
		Atherigona soccata at 28 DAE	*Chilo partellus* (%) at 45 DAE	Panicle Damage scale (1-9)		
T_1	Insecticide module with only insecticide application.	12.76 (20.90)[a]	7.22 (15.64)**[a]	3	2396.25	1: 1.11
T_2	Eco-friendly module	18.03 (25.10)[b]	7.30 (16.72)[a]	5	2173.75	1: 1.63
T_3	Need based integrated module consists of insecticides, botanicals and natural enemies	14.97 (22.74)[ab]	11.38 (19.55)[b]	3	2115.75	1: 1.08
T_4	Neem based module	23.13 (28.73)[c]	10.86 (18.63)[ab]	5	1932.5	1: 1.31
T_5	Untreated control	62.08 (52.01)[d]	42.19 (41.10)[c]	9	754.75	1: 0.78
	SE	1.24	1.79	1.41	58.93	
	CD (p = 0.05)	2.70	3.91	2.30	128.0	

*DAE=Days after emergence; **Figures in parentheses arc sine transformed value; ¤¤¤ Azadirachtin % in neem oil and NSKE 0.33 and 0.03 % by the test method IS 14300 (1995) - Neem Based EC containing azadirachtin (FAD 1: Pesticides and Pesticides Residue Analysis); ***Square root transformation value; *Sorghum grain equivalent yield (SGEY) kg/ha; Cost of inputs based on prevailing market prices; Means followed by different letters significantly different (p=0.05)

Table 2. Validation of IPM modules against *A.soccata, C. partellus* and *S. sorghicola* in sorghum (rabi 2018)

Treatments		Deadheart (%)		*Stenodiplosis sorghicola*	Grain yield (kg/ ha)	C:B Ratio	% Parasitism (Midge)	Predators No./ plot
		Atherigona soccata at 28 DAE*	*Chilo partellus* at 45 DAE*	Panicle Damage scale (1-9)				
T_1	IPM	13.02	7.61	2.58	2700.0	1:2.27	42.00	15.80
T_2	Farmers practices compared as check	45.97	36.67	8.08	810.00	1:0.84	6.8	2.0
	SED	0.54	0.57	0.57	59.70	-	10.16	5.43
		11.73	3.59					
	CD	4.14	2.41	1.31	127.80	-	21.0	11.00
		11.23	11.55					
	t value	8.81	7.66	12.70	3.46	-	3.46	2.48
	P value	-	-	-	-	-	-	0.02

*DAE=Days after emergence; IPM includes**:** Timely sowing (41st standard week); Seed treatment with imidacloprid 70 WS @ 3 g/kg seed; Installing fishmeal trap @ 12/ha upto 30 DAE; Bund cropping sorghum + bhendi; Releasing egg parasitoid, *T. chilonis* at 35 DAE; Spraying NSKE 5% at 60 DAE

the maximum C: B ratio (1:1.63) was obtained in ecofriendly module followed by neem based module (1:1.31) (Table 2). The best performing ecofriendly IPM module was reframed as modified IPM module and was conducted in large scale. The results revealed that deadhearts due to shoot fly and stem borer at 28 and 45 DAE get significantly reduced in IPM module (13.02 and 7.61%) as compared to control (45.97 and 36.67%); also lease incidence of midge/ spikelet damage rating was in the integrated module (2.58 grade). Yield was significantly more from IPM module (2700 kg/ ha) with maximum C: B ratio (1:2.27). A fine tuning of advocated sorghum-IPM has proven to maximize the yield generation (Table 2).

Daware and Ambilwade (2014) and Karabhantanal et al, 2018 identified IPM module with different components for management of sorghum pests in India. In the present study, the IPM module validated in large scale including timely sowing (41st standard week), seed treatment with imidacloprid 70 WS @ 3 g/ kg seed, installing fishmeal trap @ 12/ ha upto 30 DAE; bund cropping (sorghum + bhendi), releasing egg parasitoid, *T. chilonis* at 35 DAE; followed by spraying

NSKE 5% at 60 DAE was found economically prudent to suppress major pests incidence and increased the production. Hence, this module can be recommended as part of an integrated pest control system, since the module recorded minimum pests infestation and increased population of free living natural enemies with highest cost benefit ratio of (1: 2.27) in large scale demonstrations against major pests of sorghum.

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ECOLOGY OF MANGO NUT WEEVIL *STERNOCHAETUS MANGIFERAE* (F.) AND EFFICACY OF INSECTICIDES

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ABSTRACT

An experiment was conducted for six years at the Horticulture Research Station, Anantharajupet to study the behavior and management of mango nut weevil *Sternochaetus mangiferae* (F.). Maximum number of adults (99.50%) emerged from middle portion of the fruit, while only negligible numbers (0.50%) emerged from sinus (lower) portion (3rd year), in 4th to 6th years it was 98.50, 98.00 and 98.50%, respectively. Most of these emerged adults hide in bark, cracks and crevices in the trunk followed by collected nuts, fallen leaves and debris as the offseason shelter. The evaluation of efficacy of insecticides carried out for three years revealed the least damage of (8.66%) with spinosad treated mango trees followed by profenophos (11.33%), emamectin benzoate (15.33%), indoxacarb (16.33%), thiodicarb (19.33%), triazophos (23.33%), while in control it was 32.00%.

Key words: *Sternochaetus mangiferae*, mango, behaviour, fruit parts, emergence, oviposition, off season shelter, spinosad, profenophos, emamectin benzoate, indoxacarb, thiodicarb

Mango is an important fruit crop and it is attacked by about 492 species of insects, 17 species of mites and 26 species of nematodes of which 188 species are from India (Tandon and Verghese, 1985; Srivastava, 1998). The mango nut weevil *Sternochetus mangiferae* is a specific pest on mango and it is known from all principal mango growing areas (Dey and Pande 1987), and mainly from south India including Andhra Pradesh (Ramakrishna Ayyar 1940). The grubs and adults feed on the endocarp of the fruit and hasten its maturity resulting in early fruit drop (Subramanyam, 1925; Peter Follett, 2002). It causes losses up to 90% of marketable fruits in the export market (Bagle and Prasad, 1985; Verghese, 2000). The weevil also reduces seeds germination and seed as a source of rootstocks is a concern. The import of Indian mangoes into USA has been withheld for the reason that it might be introduced there (Sundarababu, 1966). The quarantine restrictions prevent the export of fresh mangoes into uninfected areas (Hansen, 1983). Prevention of nut weevil incidence is essential to get better price for produce. Considering the economic importance of the pest, experiments on the behavior and management of *S. mangiferae* were carried out under field conditions in the present study.

MATERIALS AND METHODS

Six-year studies were carried out at the Horticultural Research Station Farm, Ananthrajupeta (13.9976°N, 79.3304°E). The data was collected for off season shelter by examining the adult weevils/ 100 trees, number of eggs in 200 fruits for the preferential sites of oviposition within the fruit of mango, and for adult emerging sites 100 fruits were inspected and expressed as %. For management aspect, experiment was conducted for three years period at the HRS Farm, on Neelum cultivar with seven treatments viz., spinosad 45SC @0.3ml/ l, triazophos 40EC @1ml/ l, profenophos 50EC @2ml/ l, thiodicarb 70WP@1g/ l, indoxacarb 14.5SC @1ml/ l, and emamectin benzoate 5SG @0.75g/ l with three replications and in randomized block design (RBD). The spraying of insecticides was carried at marble stage of the fruit and for data recording on nut weevil damage, fruits were collected at the time of harvest and destructive sampling followed. The sample size was 50 fruits/ replication and expressed as % damage. The data was subjected to statistical analysis using ANOVA.

RESULTS AND DISCUSSION

The results on the preferred sites for oviposition of *S. mangiferae* revealed that in the first year of observation, female *S. mangiferae* prefer to lay eggs in sinus (lower) portion of the fruit (18 eggs) followed by middle (7) and pedicel portion (4); while the third year data s showed a different trend- maximum number of eggs were laid in middle portion of fruit (25 eggs)

followed by sinus (9 eggs) and pedicel (upper) with 7 eggs; and same trend was noticed later (IV, V and VI years) indicating that the *S. mangiferae* female prefers the fruit middle portion for oviposition (Table 1). In contrast, females were observed to oviposit on infested fruit (Hansen et al., 1989), and lay eggs mostly on the sinus of the fruit or sometimes on the stems (Shukla et al., 1985). The observations with regard to emerging sites of *S. mangiferae* indicated that maximum weevils emerged from middle portion of the fruit and negligible (0.50%) emerged from sinus (lower) portion (III year data); similarly in fourth, fifth and sixth years, 98.50, 98.00 and 98.50% emerging from middle portion, respectively; the mean data over a four-years period revealed 98.62% emergence was from middle portion followed by 1.37% from sinus and negligible of 0.25% from pedicel part. Maximum number of *S.* weevils prefer to hide in trunk bark, cracks and crevices (11.67) followed by leftover and collected nuts (3.83) and fallen leaves and debris (2.17). Pinese and Holmes (2005) indicated that adult weevils can live for two years, so even with a crop failure in one season some weevils can survive into the following year through hide in trunk bark. Robert et al. (2006) noted that weevils overwinter under loose bark around the base of mango trees or in the forks of branches.

Table 1. Behavioural biology of *S. mangiferae*

Portion of fruit part	I year	II year	III year	IV year	V year	VI year	Mean
Oviposition sites							
Pedicel (Upper)	4*	8	7	9	9	9	7.67
Middle	7	21	25	25	25	23	21.00
Sinus (lower)	18	5	9	12	12	12	11.33
Emergence of adults (%)							
Pedicel (Upper)	0.00	0.00	1.00	0.00	0.25	0.00	0.00
Middle	99.50	98.50	98.00	98.50	98.62	99.50	98.50
Sinus (lower)	0.50	1.50	2.00	1.50	1.37	0.50	1.50
Off season shelters							
Trunk bark, crack and crevices	12#	8	13	14	12	11	11.67
Fallen leaves and debris	2	2	2	2	2	3	2.17
Leftover and collected nuts	3	3	4	4	5	4	3.83

*Number of eggs/200 fruits; #Number of weevils/ 100 trees

Table 2. Efficacy of insecticides against *S. mangiferae*

Treatments	damage (%)			
	I year	II year	III year	Pooled Mean
Spinosad 45SC @0.3 ml/ l	8.00	10.00	8.00	8.66
	(16.42)	(18.43)	(16.42)	(17.11)
Triazophos 40EC @1 ml/ l	24.00	25.00	21.00	23.33
	(29.32)	(29.99)	(27.26)	(28.87)
Profenophos 50EC@2 ml/ l	10.00	13.00	11.00	11.33
	(18.43)	(21.13)	(19.36)	(19.66)
Thiodicarb 70WP@1g/ l	17.00	20.00	21.00	19.33
	(24.34)	(26.55)	(27.26)	(26.07)
Indoxacarb14.5SC@1 ml/ l	15.00	16.00	18.00	16.33
	(22.78)	(23.57)	(25.09)	(23.83)
Emamectin benzoate 5SG @0.75g/l	14.00	15.00	17.00	15.33
	(21.96)	(22.78)	(24.34)	(23.04)
Control	30.00	32.00	34.00	32.00
	(33.20)	(34.44)	(35.65)	(34.44)
CD (p=0.05)	4.04	3.83	3.77	2.92
SEM	1.85	1.24	1.22	0.94

Figures in parentheses arc sign √ % transformed values

The efficacy of insecticides evaluated for three years revealed mean infestation levels between 8.66 to 23.33% in treated plots and 32.00% in untreated control. The pooled data showed that spinosad was the best treatment (8.66% damage) and the next best was profenophos (11.33%). These observations corroborate those of Karuppaiah (2015) on spinosad 2.5SC, and indoxacarb 14.5EC also found to be superior in ber). Muriuki et al. (2011) noted that chlorpyriphos band applied once per month + sanitation reduced the infestation. Ramakrishna Rao (2015) observed that carbaryl was effective followed by endosulfan and malathion. Emamectin benzoate (15.33%), indoxacarb (16.33%), thiodicarb (19.330%), triazophos (23.33%) reduced the damage (Table 2). Verghese et al. (2004) in their studies observed infestation levels between 3.3 and 14.8% from plots treated with synthetic insecticides against a mean of 33.0% in the untreated control plot.

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EFFICACY OF NEEM BASED FORMULATIONS AGAINST WHEAT APHIDS UNDER SEMI-ARID IRRIGATED CONDITIONS IN SOUTH-WEST PUNJAB

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ABSTRACT

Neem formulations viz. aqueous extract of neem and NSKE along with standard check thiamethoxam were evaluated for their efficacy against wheat aphids *Rhopalosiphum padi* (L.) and *Rhopalosiphum maidis* (Fitch). It was observed that aphids' incidence on shoots started in the first week of January with a peak (40.90 aphids/ shoot) during third week of March. Thiamethoxam 25 WG @ 50 g/ ha was found significantly superior giving 93.53% reduction after 7 DAS (days of spray). Different formulations of neem also yielded comparable results in controlling wheat aphids. Aqueous extract of neem @ 5 l/ ha resulted maximum (74.21%) reduction followed by NSKE @ 10% (69.04%) after 7 DAS. Occurrence of coccinellids was more in treatments with neem formulations than in thiamethoxam. The treatments resulted in 2.48- 9.25% increase in yield and it can be concluded that aqueous extract of neem and NSKE can be recommended over the most commonly used thiamethoxam for controlling wheat aphids.

Key words: Wheat, aphids, *Rhopalosiphum padi*, *R. maidis*, coccinellids, neem formulations, thiamethoxam, incidence, population dynamics, efficacy, NSKE, yield

In Punjab wheat (*Triticum aestivum* L.) is cultivated as a major rabi crop on about 35.20 lakh haa with the production of 182.62 lakh mt (Anonymous 2019). Several abiotic and biotic factors like insect pests and diseases are responsible for the low productivity of wheat. Among insect pests, commonly aphids and termites affect the crop. In India, aphids cause yield losses and became regular pest of wheat in almost all the wheat cultivating zones. Out of the nine aphid species infesting wheat crop in India, *Sitobion avenae* (F.), and *Rhopalosiphum maidis* (Fitch) are the major ones (Joshi and Sharma 2009). However, bird cherry oat aphid *Rhopalosiphum padi* L. and corn leaf aphid *R. maidis* are the major ones in Punjab (Singh, 1983; Singh et al., 2018). The severe incidence of wheat aphid complex causes 3.53-21.05% yield losses in wheat varieties in Punjab (Singh and Deol, 2003). Aphids can be successfully managed with foliar sprays of neonicotinoids; however, because of their frequent usage certain issues like their persistence in soil and water, side effects to pollinators and natural enemies and residue in grains are the foremost environmental concerns (Goulson, 2013). To avoid or to minimize these ill effects, ecofriendly and cost effective control methods are required. Among the available non-chemical options, neem (*Azadirachta indica* L.) has shown the potential to be used as a substitute of synthetic insecticides (Schmutterer, 1995). Seeds, leaves, kernels and other parts of the neem tree are rich source of azadirachtin and various other compounds (Shafeek et al., 2004; Adhikari et al., 2020). These were found promising against many insects in different crops (Gahukar, 2000; Liang et al., 2003; Nathan et al., 2005). The present study evaluates different neem based formulations for the management of wheat aphids and also to verify their effects on arthropod natural enemies.

MATERIALS AND METHODS

The experiments were done during the winters of 2019 and 2020, at the experimental farm of Punjab Agricultural University Regional Research Station, Bathinda, India (30°17'N, 74°58'E, 211 masl). This station is in the extreme south west of Punjab. The fields selected had long history of wheat and cotton in rotation. Four set of experiments were laid out in randomized block design (RBD) with each treatment replicated thrice, in plots of size of 5 x 5 m. Seeds of variety Unnat PBW343 @ 100 kg/ ha was sown at 20 cm row to row spacing, and the crop raised as per the recommended practices of Punjab Agricultural University (Anonymous, 2019) except for crop protection. Treatments include- T1: NSKE @ 5%, T2: NSKE @ 7.5%; T3: NSKE @ 10%; T4: aqueous extract of neem @ 3 l/ ha; T5: aqueous extract of neem

@ 3.75 l/ ha; T6: aqueous extract of neem @ 5l/ ha; T7: Thiamethoxam 25 WG @ 50 g/ ha; and T7: untreated control. The NSKE was soaked in water overnight and the extract collected during next morning was used for the spray after diluting it to required dosages. To make aqueous extract of neem 4 kg of terminal parts of shoots of neem trees including leaves, green branches and fruits were boiled in 10 l of water for 30 min and then filtered through muslin cloth, and the filtrate was used. Thiamethoxam (Actara 25 WG, Syngenta India Pvt. Ltd.) procured from local market was taken as a standard. The treatments were applied at heading stage when the aphid infestation exceeds economic threshold level of 5 aphids/ earhead. Aphid counts were taken from ten tagged plants/ replication before and 1, 2, 7 days of spray application (DAS). The counts of coccinellids grubs and adults/ m^2 were also recorded from five spots in each treatment. At the harvest, grain yield was recorded and % increase in yield over untreated control was worked out. The % data were subjected to arc sine transformation and numerical data to square root transformation for statistical analyses. The data generated from four trials was pooled and subjected to ANOVA to evaluate the treatment effects with the means separated using Least Significant Difference (LSD, p=0.05).

RESULTS AND DISCUSSION

The pooled data of 2019-20 on aphid's incidence in wheat given in Fig. 1 reveals that it ranged from

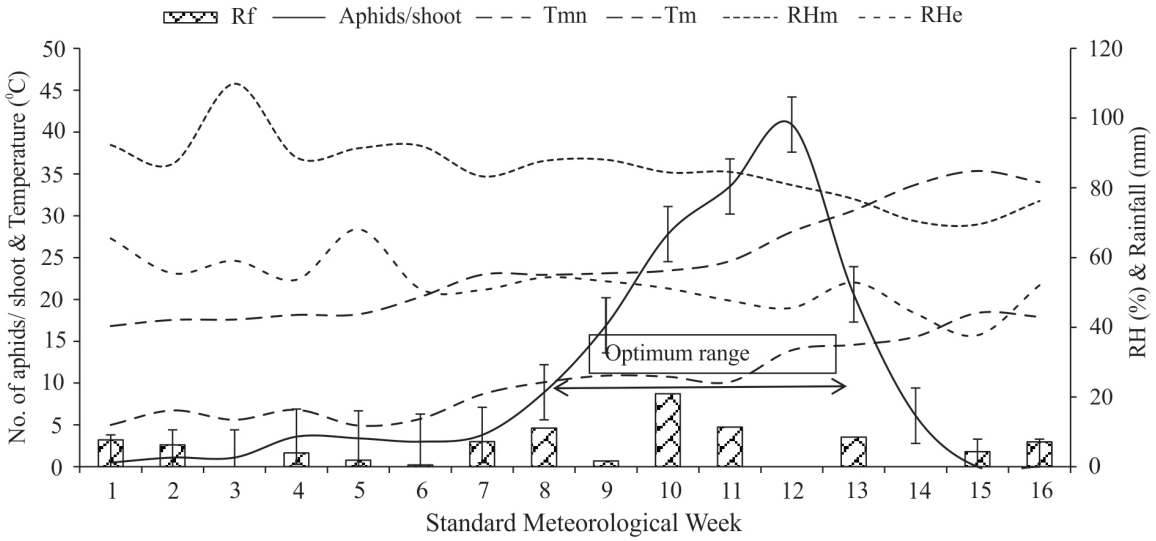


Fig. 1. Population dynamics of *R. maidis* and *R. padi* in wheat cv. *Unnat* PBW 343 (2019-2020)

Table 1. Efficacy of neem formulations against *R. maidis* and *R. padi* in wheat (pooled data, 2019-20)

Treatment	No. of aphids/ plant (10 plants/ replication)				% reduction after 7 DAS	No. of coccinellids/ sq. m			Yield (q/ ha)	% Increase in yield over control
	BS	1 DAS	2 DAS	7 DAS		BS	1 DAS	7 DAS		
T1	20.53^{a}	14.10^{c}	7.80^{c}	8.38^{c}	59.20	3.65^{a}	2.45^{b}	3.55^{a}	58.30	4.05
T2	20.60^{a}	13.48^{c}	6.65b^{c}	7.03^{c}	65.90	4.45^{a}	3.30^{ab}	3.60^{a}	57.35	2.48
T3	18.25^{a}	10.28^{b}	5.58^{b}	5.65^{bc}	69.04	3.95^{a}	3.60^{ab}	3.30^{a}	61.62	9.25
T4	20.13^{a}	13.18^{c}	6.95b^{c}	7.85^{c}	60.99	4.35^{a}	2.75^{b}	3.35^{a}	57.57	2.84
T5	20.10^{a}	13.13^{c}	6.03b^{c}	6.60^{b}	67.16	4.20^{a}	3.90^{a}	3.80^{a}	59.22	5.55
T6	20.28^{a}	13.08^{c}	5.20^{b}	5.23^{b}	74.23	3.70^{a}	3.15^{ab}	3.25^{a}	60.00	6.79
T7	21.25^{a}	7.75^{a}	3.20^{a}	1.38^{a}	93.53	3.55^{a}	0.80^{c}	0.55^{b}	59.95	6.71
T8	20.55^{a}	22.33^{d}	24.53^{d}	30.63^{d}	-	4.30^{a}	3.00^{b}	3.70^{a}	55.92	-

T1: NSKE @ 5%, T2: NSKE @ 7.5%; T3: NSKE @ 10%; T4: aqueous extract of neem @ 3 l/ ha; T5: aqueous extract of neem @ 3.75 l/ ha; T6: aqueous extract of neem @ 5l/ ha; T7: Thiamethoxam 25 WG @ 50 g/ ha; and T7: untreated control.

0.00- 40.90± 1.43 (mean ± SE; N=30) from 1st SMW (Standard Meteorological Week) 1 to 16th SMW; peak was during 12th SMW, then it declines and almost disappeared in second week of April. These data revealed that the corresponding weather are- optimum temperature (T_{max} 22.96- 28.08 °C; T_{min} 8.66-13.93 °C) and humidity (RHm 80.79- 88.06%; RHe 45.57%-51.14%). Thus, the peak was in the third week of March during the heading stage of the wheat, and declined with maturity during the first fortnight of April. Karimullah and Ahmad (1989) observed that aphids' infestation started in the beginning of February with its peak in the latter half of March. Such an increase in population towards heading stage was also observed by Xiong (1990) and Nawaz (2000). Table 1 depicts the reduction in incidence due to treatments with neem, which reveal that all the treatments were found effective; significant differences were observed after 1 ($F_{7,21}$ = 42.66, p < 0.01), 2 ($F_{7,21}$ = 158.77, p < 0.01) and 7 ($F_{7,21}$ = 395.17, p < 0.01) DAS. Foliar application of thiamethoxam 25WG @ 50 g/ ha was found significantly superior, followed by aqueous extract of neem @ 5 l/ ha and NSKE @ 10%. A little increase in aphid's population in neem treated plots was recorded after 7 days of spray so it is advised to repeat the spray of botanicals after seven days. Overall, the spray of aqueous extract of neem @ 5 l/ ha and NSKE @ 10% brings about 74.23 and 69.04% reduction after 7 DAS. Babu and Sharma (2003) advocated that thiamethoxam was most effective. Shah et al. (2017) evaluated the neem seed extract (NSE), moringa leaf extract (MLE) and used imidacloprid as check for aphid management in late sown wheat and found that imidacloprid was the best followed by neem seed extract. Homemade formulations of neem viz. aqueous extracts of neem, NSKE and neem seed cake etc. have been successfully used against insect pests (Dougoud et al., 2019). These are comparatively safe to natural enemies, economical, easy to prepare at farmer levels and would reduce the harmful effects of pesticides to humans and environment (Agbo et al., 2019).

The results reveal that the counts of coccinellids (grub and adult) varied from 3.55 to 4.45 in different treatments before spray (Table 1); and significant differences between insecticide and botanicals were observed after one ($F_{7,21}$ = 6.81, p < 0.01) and seven DAS ($F_{7,21}$ = 15.12, p < 0.01). All the botanical treatments were at par with each other in bringing 2.74-22.99% reduction in coccinellids grubs/ m^2 after 7 DAS; thiamethoxam 25 WG @ 50 g/ ha caused maximum reduction (84.51%). These results are in accordance with those of Jiang et al. (2018) on the sublethal and transgenerational effects of thiamethoxam on *Coccinella septumpunctata*. Khan et al. (2011) reported that neem oil formulations were safer for beneficial insects. The results ont the yield were statistically non-significant but maximum yield was in plots treated with NSKE @ 10 % (61.62 q/ ha) followed by aqueous extract of neem @ 5 l/ ha (60 q/ ha), thiamethoxam 25 WG @ 100 g/ha (Table 1).

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DIVERSITY OF ODONATA OF DISTRICT PULWAMA OF KASHMIR

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ABSTRACT

This baseline field study carried out from April 2019 to September 2020, in the Pulwama district of Kashmir Himalayan Region reported a total of 11 species of Odonata- under two suborders namely Anisoptera (Dragonflies: 7 genera and 9 species) and Zygoptera (Damselflies: 2 genera and species each). Family Libellulidae, suborder Anisoptera (Dragonflies) with seven species distributed in five genera was the most dominant family. *Pantala flavescens* was the most common species.

Key words: Odonata, dragonflies, damselflies, Pulwama, Kashmir Himalayan Region, biodiversity, distribution, status, *Pantala flavescens*

The Insect order Odonata predatory insects viz., dragonflies and damselflies (Kalkman et al., 2008). Their biodiversity, ecology and evolutionary biology is well documented, are relatively easy to identify and are key components of wetland ecosystem. They are amphibiotic insects, since adults are terrestrial and the larvae aquatic (Subramanian and Babu, 2018). These are often used as indicators for environmental health and conservation management (Kalkman et al., 2008). Dragonflies and Damselflies are predators throughout their life, and act as an important natural biocontrol agent of various harmful insects. The district Pulwama of Kashmir is with a temperate climate (33.8716°N, 74.8946°E, 1300-2000 masl). The Odonate fauna occurring in the localities of Kashmir Himalayan Region (KHR) including district Pulwama are not fully known. Very few workers like Fraser (1933, 1934, 1936), Singh and Baijal (1954), Kumar and Prasad (1981), Lahiri and Das (1991), Dar et al., (2002), Mitra (2003), and Subramanian and Babu (2018), have contributed to the Odonata biodiversity of this Himalayan Region. Dar et al. (2002) gave a list of 31 species of Odonata from the Kashmir valley; and Mitra (2003) listed 24 species under 16 genera from Kashmir. Odonates of this Himalayan Region are also facing serious threats. Hence, Odonata biodiversity of the KHR needs to be brought out and the present preliminary work provides data pertaining to the biodiversity of Odonata of district Pulwama.

MATERIALS AND METHODS

Field surveys were conducted from April 2019 to September 2020 in habitats like marshy lands, aquatic areas, gardens, parks, mountains, forests, orchids, vegetated and open fields, agricultural areas etc of district Pulwama. Specimens were mostly identified in the field and collected by either using aerial net or by handpicking. Borror et al. (1976) was followed for field surveys and preservation. Species were identified following field guides of Mitra (2002; 2006), Subramanian (2005) and Andrew et al., (2008). The collected specimens have been deposited in the Biodiversity Museum, Biodiversity, Biosystematics & Climate Change (B2C2) Research Lab, Mantaqi Centre for Science & Society, Islamic University of Science & Technology, Awantipora, Kashmir.

RESULTS AND DISCUSSION

Surveys from April 2019 to September 2020 in various habitats/ areas of district Pulwama revealed a total of 11 species of Odonata under two suborders Anisoptera (dragonflies) and Zygoptera (damselflies) under 3 superfamilies and families each falling in nine genera (Table 1). Anisoptera is the most dominant with nine species under seven genera; Zygoptera is represented by only two. The surveyed areas include Aripal (34.0175° N, 75.0713° E), Awantipora (33.9232° N, 75.0128° E), Barsoo (33.9515° N, 74.9910°

Table 1. Distribution of Odonata in Pulwama

Species	Common name	Status	Remarks
Zygoptera			
Lestoidea			
Lestidae			
1. *Lestes barbarus* (Fabricius, 1798)	Southern Emerald Damselfly, Shy Emerald Damselfly, Migrant Spreadwing	Least concern (Clausnitzer, 2020)	It is a migrant and highly erratic species (Assandri, 2019). Samraoui and Corbet (2000), gave its occurrence in Algeria from April to October. Now, it was found active from June to November. Earlier, Fraser (1933), Dar et al., (2002) and Mitra (2003) gave its distribution in Kashmir Himalayan Region.
2. *Sympecma paedisca* (Brauer, 1877)	Siberian Winter Damselfly	Least concern (Kalkman, 2014)	It was seen active from June to October. Fraser (1933), Dar et al., (2002) and Mitra (2003) gave its distribution from Kashmir. It is regarded as critically endangered as per Italian Red List (Assandri, 2019)
Anisoptera			
Superfamily: Aeshnoidea			
Aeshnidae			
3. *Aeshna juncea* (Linnaeus, 1758)	Common Hawker, Moorland Hawker, Sedge Darner	Least concern (Boudot, 2017)	Found flying from June to September. Distribution in Kashmir known (Fraser, 1936; Dar et al., 2002; Mitra, 2003). Found up to 1200 m
4. *Anax parthenope* (Selys, 1839)	Lessor Emperor	Least concern (Ferreira, 2010; Ferreira et al., 2010; Kalkman, 2010)	Found active from June to September. Samraoui and Corbet (2000) reported it from April to October in Algeria. Fraser (1936), Kumar and Prasad (1981), Dar et al., (2002), and Mitra (2003) gave its distribution in Kashmir.
Libelluloidea			
Libellulidae			
5. *Crocothemis erythraea* Brulle, 1832	Broad Scarlet, Common Scarlet-Darter, Scarlet Darter, Scarlet Dragonfly	Least concern (Clausnitzer, 2016)	Found active from June to October. Samraoui and Corbet (2000) found it active from April to October in Algeria
6. *Crocothemis servilia* (Drury, 1770)	Scarlet Skimmer, Ruddy Marsh Skimmer	Least concern (Dow, 2017)	Found active from June to October. They have been observed flying in the altitudinal range up to 1000 m. During the present investigations, Observed frequently during mid-day flying up to 10 m, some flying during morning hours. Nair (2011) gave its flight period from May to September in India. Widely distributed in Oriental and Australian region (Subramanian, 2005). Andrew et al., (2008) called it as a commonest red dragonfly.
7. *Orthetrum luzonicum* (Brauer, 1868)	Marsh Skimmer, Tricolored Marsh Hawk, Slender Blue Skimmer, Luzon Skimmer	Least concern (Dow, 2010a)	Breeds in habitats like marshes, wet areas, rice fields and open and disturbed areas; widespread at upland streams, ponds, marshland, edge of forests, etc. No conservation measures are needed (Dow, 2010a). Seen up to 1500 m (Dow, 2010a). Found flying from June to September. Lahiri and Das (1991) reported it from Kashmir.

(contd.)

8. *Orthetrum triangulare* (Selys, 1878)	Blue Tailed Forest Hawk	Least concern (Dow, 2010b)	Found mostly during mid-day time and comes to the same perch/ place in the next days, and sit under sunny spots. Fraser (1936), Dar et al., (2002) and Mitra (2003) gave its distribution from Kashmir. Fraser (1936) gave its altitudinal range between 1200-2400 m. Found flying from June to November.
9. *Pantala flavescens* (Fabricius, 1798)	Wandering Glider, Globe Skimmer, Globe Wanderer	Least concern (Boudot, et al., 2016)	Highly migratory species (Andrew et al., 2008; Nair, 2011). It is widely distributed in the Indian subcontinent/ Oriental region and the most common (Mitra, 2003; Andrew, et al., 2008). Found active from June to November, commonly in paddy fields, playgrounds, wetlands, orchids, forests, open areas, etc. Mitra (2003), gave its distribution from Kashmir.
10. *Sympetrum commixtum* (Selys, 1844)	Mountain Meadow Hawk	Least concern (Dow, 2010c)	Widely distributed in Himalayan Region and India, Nepal and Bhutan (Mitra, 2006). Found near ponds, orchids, paddy fields, flying from June to September. Fraser (1936), (Dar et al. (2002), and Mitra (2003) gave its distribution in Kashmir.
11. *Trithemis aurora* (Burmeister, 1839)	Crimson Marsh Skimmer	Least concern (Subramanian and Dow, 2010)	It is distributed throughout India (Sharma, 2014) and is common (Subramanian and Dow, 2010). Found in both terrestrial and freshwater, such as ponds, lakes, marshes, wet paddy fields, streams, open areas, rivers, and irrigation canals. Found flying from June to September. Subramanian and Dow (2010) mentioned its distribution from Kashmir.

E), Beighpora (33.8784° N, 75.0093° E), Chersoo (33.8852° N, 75.0254° E), Dadsara (33.9043° N, 75.0855° E), Kakapora (33.9468° N, 74.9296° E), Kandizal (33.9925° N, 74.9136° E), Khrew (34.0209° N, 74.9998° E), Koil (33.8782° N, 74.9472° E), Ladhu (33.9925° N, 74.9935° E), Lajoora (33.8989° N, 74.9588° E), Lassipora (33.8134° N 74.9408° E), Lethpora (33.9675° N, 74.9647° E), Lurgam (33.9710° N, 75.0918° E), Malangpora (33.8918° N, 74.9811° E), Midoora (33.9301° N, 75.0609° E), Newa (33.9159° N, 74.8818° E), Naibugh (33.9138° N, 75.0918° E), Newa (33.9159° N, 74.8818° E), Padgampora (33.9092° N, 74.9972° E), Pampore (34.0060° N, 74.9238° E), Pastuna (33.9931° N, 75.0758° E), Pulwama town (33.8716° N, 74.8946° E), Rajpora (33.8243° N, 74.8531° E), Ratnipora (33.9209° N, 74.9438° E), Shadimarg (33.8040° N, 74.8229° E), and Tral (33.9342° N, 75.1137° E). The family Libellulidae was the most dominant with 7 species under 5 genera. *Pantala flavescens* was the most common and dominant species. More sighting was during summer season. As per the IUCN Red List of Threatened Species, all the species reported here are categorized under least concern species category.

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RIND SCARRING CAUSED BY BIOTIC AND ABIOTIC FACTORS INFLUENCES KINNOW FRUIT QUALITY

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ABSTRACT

The relationship between rind scarring [caused by *Brevipalpus phoenicis* (Geijskes), *Scirtothrips citri* (Moulton) and wind] and physiochemical parameters was studied in three main kinnow producing zones of Punjab (Abohar, Ludhiana and Hoshiarpur), India. The results showed a nonsignificant increase in TSS value with increase in rind damage (caused by mites + thrips + wind) upto 50% but later on it was significantly decreased with increase in damage from 50 to 100%. Other parameters like vitamin C, phenols (pulp and peel), pectin, total carotenoids and fruit firmness were decreased with the increment in damage and lowest values were observed in 100% damaged fruits but the titrable acidity and sugars (non reducing, reducing & total sugar) parameters were not affected by rind scarring. The wind damaged fruits showed noncomparable data for all studied physiochemical parameters which indicated that wind doesn't affect the fruit quality. It may also be concluded that only thrips and mites are responsible for deterioration of kinnow fruits quality.

Key words: Acidity, *Brevipalpus phoenicis*, carotenoids, fruit firmness, kinnow, non reducing sugars, pectin, phenols, reducing sugars, rind scarring, *Scirtothrips citri*, total soluble solids, total sugars, vitamin C

India is the third largest producer of citrus fruits in the world (after China and Brazil) and it contributes about 8.2% of total world's citrus production (FAO, 2020). Citrus orchards occupies an area of 1028 thousand ha with total production of 13404 thousand mt (NHB, 2020). Among Indian states, Punjab covers 59.9 thousand ha area with total 134.9 thousand mt of production and 39.8 mt/ha productivity (Anonymous, 2020). Punjab contributes about 12.26% citrus mainly Kinnow to the nation's citrus pool (Kumar et al., 2010). Rind scarring (blemishes) is becoming very important issue affecting the choice of the produce by the consumers in the domestic and international market. Morphologically the blemish is collapsed hypodermis tissue related to shrunken or destroyed oil glands which might be due to some biotic (thrips and mites) and abiotic reasons (wind). Red and black flat mite, *Brevipalpus phoenicis* (Geijskes) has been reported to feeds both on leaves and fruits but damage is more severe on younger fruits. *B. phoenicis* was found to be associated with rind-disorder of different citrus fruits from November to February in Punjab (Dhooria et al., 1997). Thrips, *Scirtothrips citri* (Moulton) cause damage by feeding near calyx-end of the fruit and by developing a "halo" or ring having brownish tissues, which turn into grey colored scar and can also cause injury to the flowers (Sharma 2007, Kaur 2020). Freeman (1976) reported that wind scarring is a major blemish of kinnow fruit. Rind scarring problem makes kinnow production unprofitable because of high rejection and price difference in various quality grades. There is also a possibility of loss in the nutritional quality of kinnow fruits due to rind scarring. At present, there is no information available in the literature on this aspect in Punjab as well as India. So the present study was planned to determine the impact of rind disorder on quality parameters of kinnow.

MATERIALS AND METHODS

The experiments of various quality and biochemical parameters were conducted in the laboratories of Punjab Horticultural Postharvest Technology Center (PHPTC), Punjab Agricultural University, Ludhiana. To study the impact of rind disorder caused by mites thrips and wind on quality of kinnow fruits, fifty kinnow fruits of different injury levels (0, 25, 50, 75, and 100%) were collected from the orchards (Fruit Research Farm/ College Orchard, PAU, Ludhiana; Regional Research Station, Abohar and Department of Horticulture (Punjab), Hoshiarpur (Bhunga) during 2016 and

2017 at harvest time to analyze the different quality parameters. Fruits having 0, 10 and 20% rind damage were selected to study the effect of rind disorder caused by wind on quality parameters of kinnow. These locations were selected in such a manner that it represented the three different horticultural zones of Punjab (Abohar from arid zone, Ludhiana from central zone and Hoshiarpur from submontane zone) where kinnow is grown.

Fruit firmness of five randomly selected kinnow fruits was measured with the help of a Penetrometer (Fruit Pressure Tester, Model FT-327). The total soluble solids (TSSs) of the fruits were determined at room temperature with Pocket Refractometer PAL-1. The values of the TSSs were expressed in term of degree brix (%). Titrable acidity and sugars (reducing sugars, total sugars and non-reducing sugars) were estimated by method given by AOAC (2005) and expressed in per cent. Ascorbic acid and total carotenoid were estimated following the method described by Ranganna (1991). Total phenols were determined by Folin-Ciocalteu (FC) reagent as described by Bray and Thorpe (1954). The concentration of total phenolics was calculated by comparing the data with the standard graph of gallic acid (0 to 300 ppm) and expressed as mg gallic acid equivalents/ 100 g pulp. The pectin content was estimated by following the method given by Thimmaiah (1999) and expressed as % calcium pectate/ 100 g of sample. Statistical analysis of quality parameters was done through ANOVA and LSD All-Pairwise Comparisons Test by using computer programme Statistix-10.

RESULTS AND DISCUSSION

The studies for finding the impact of rind scarring caused by mites, thrips and wind (mixed damage) on quality parameters of Kinnow fruits revealed that there was insignificant increase in total soluble solids (TSSs) (Brix) and acidity (%) with increase in damaging level upto 50% whereas healthy fruits had less value as compared to 50% damaged fruits. Later on there was decrease in values as damaging level increased from 50 to 100% and least was observed at 100% damage. Kinnow fruits of Abohar and Hoshiarpur locations had more TSS value as compared to Ludhiana because of suitable climatic conditions for kinnow production at these locations. A significant variation in vitamin C (mg/ 100 g) was observed at different damaging levels at Abohar, Hoshiarpur and Ludhiana. Maximum vitamin C content was obtained from healthy fruits (0% damage) and was significantly higher than damaged fruits. Minimum vitamin C content was obtained from 100% damaged fruits. The effect of rind scarring on reducing sugars, nonreducing sugars and total sugars (%) cannot be correlated with damaging levels because there was not any particular trend of increase or decrease in sugar levels. The maximum value of phenol (pulp and peel) (mg/ 100 g), pectin (% calcium pectate/ 100 g) and total carotenoids (mg/ 100 g) were measured from healthy fruits and it was significantly higher than from damaged fruits. Minimum values were recorded from fully damaged fruits at all three locations. It was also found that damage (%) significantly affected the firmness (lbf) of fruits. The maximum fruit firmness was obtained from healthy fruits and minimum fruit firmness was recorded from 100% damaged fruits of all three tested locations (Table 1).

Wind damaged fruits had nonsignificant results or negligible differences between values of all physiochemical parameters (TSS, acidity, vitamin C, total sugars, reducing sugars, non reducing sugars, total phenol, pectin, total carotenoids and fruit firmness) of healthy fruits and wind scarred fruits.

From the results as obtained from the investigation carried out on fruits from Abohar, Hoshiarpur and Ludhiana, it was concluded that there is a relationship of infestation by mites thrips and wind (mixed damage) to the kinnow fruits with the contents of different physiochemical parameters. From the overall results as obtained from investigation, it was concluded that the TSS value was nonsignificantly increased when the mixed infestation increased upto 50% and later on significantly decreased when the infestation increased from 50 to 100%. Healthy fruits had more vitamin C, pulp phenol, peel phenol, pectin, total carotenoids and fruit firmness as compared to 100% damaged fruits (mixed damage). It was also analysed that rind scarring did not have any effect on titrable acidity, reducing sugar, non reducing sugar and total sugar content. On the other hand, wind damaged fruits had nonsignificant results or negligible differences between values of all physiochemical parameters of healthy fruits and wind scarred fruits. So it was concluded that these losses might be due to deterioration caused by only thrips and mites and not wind. There was no information available in the literature regarding impact of rind disorder caused by *S. citri* and *B. phoenicis* on biochemical characteristics of kinnow fruits but various other workers like, Kalaisekar et al (2000) studied the effect of rust mite on Sathgudi sweet orange and

Table 1. Impact of fruit rind damage by mites, thrips and wind on quality of kinnow fruits pooled data, (2016 and 2017) at Abohar, Hoshiarpur and Ludhiana

Damage (%)	TSS (°Brix)	Acidity (%)	Vitamin C (mg/ 100 g)	Reducing sugar (%)	Total sugar (%)	Non reducing sugar (%)	Total phenol (mg/ 100 g)		Pectin (% calcium pectate/ 100 g)	Total carotenoids (mg/ 100 g)	Fruit firmness (lbf)
							Peel	Pulp			
Mixed damaged fruits (mites, thrips and wind)											
Abohar											
0	12.80^{a}	0.87^{bc}	27.47^{a}	2.69^{b}	6.01^{c}	3.16^{c}	270.63^{a}	179.30^{a}	5.93^{a}	0.62^{a}	11.50^{a}
25	12.90^{a}	0.90^{ab}	25.35^{b}	2.30^{d}	6.50^{b}	3.99^{b}	268.05^{a}	169.75^{b}	5.10^{b}	0.48^{b}	11.10^{a}
50	12.90^{a}	0.93^{a}	24.33^{c}	3.24^{a}	6.73^{b}	3.32^{c}	262.27^{b}	158.40^{c}	4.62^{c}	0.43^{c}	11.23^{a}
75	11.70^{b}	0.85^{c}	20.90^{d}	2.33^{d}	7.55^{a}	4.95^{a}	245.47^{c}	144.80^{d}	3.99^{d}	0.36^{d}	9.53^{b}
100	10.50^{c}	0.77^{d}	18.00^{e}	2.48^{c}	6.55^{b}	3.87^{b}	224.60^{d}	130.53^{c}	2.11^{e}	0.19^{e}	8.50^{c}
LSD ($p\leq0.05$)	0.18	0.03	0.26	0.11	0.27	0.28	3.75	1.68	0.30	0.02	0.35
F value	324.90	31.35	2130.12	118.05	41.28	61.84	259.78	1319.47	224.20	437.17	135.25
Hoshiarpur											
0	12.53^{b}	0.95^{a}	28.20^{a}	2.22^{c}	6.96^{b}	4.51^{b}	270.80^{a}	179.97^{a}	5.67^{a}	0.55^{a}	11.37^{a}
25	12.60^{b}	0.87^{b}	27.33^{b}	2.41^{b}	6.80^{b}	4.17^{c}	265.60^{b}	161.87^{b}	4.53^{b}	0.50^{b}	11.03^{b}
50	12.77^{a}	0.93^{a}	25.50^{c}	2.45^{b}	5.66^{c}	3.05^{d}	253.00^{c}	153.73^{c}	4.23^{c}	0.43^{c}	10.23^{c}
75	11.60^{c}	0.88^{b}	20.80^{d}	3.40^{a}	8.52^{a}	4.86^{a}	244.93^{d}	141.63^{d}	3.90^{d}	0.36^{d}	9.53^{d}
100	10.80^{d}	0.85^{b}	19.90^{e}	2.29^{c}	5.45^{c}	3.00^{d}	228.13^{e}	120.77^{e}	3.53^{e}	0.26^{e}	7.43^{e}
LSD ($p\leq0.05$)	0.15	0.03	0.55	0.10	0.22	0.23	1.84	2.27	0.20	0.01	0.28
F value	287.82	22.20	475.23	228.20	286.85	137.42	840.29	942.53	156.76	1953.95	313.83
Ludhiana											
0	11.73^{a}	0.96^{a}	24.43^{a}	2.62^{b}	7.08^{b}	4.24^{c}	265.10^{a}	174.57^{a}	4.73^{a}	0.52^{a}	10.30^{a}
25	11.70^{a}	0.97^{a}	22.47^{b}	3.04^{a}	5.55^{c}	2.38^{e}	251.30^{b}	156.40^{b}	3.63^{b}	0.50^{b}	10.13^{b}
50	11.80^{a}	0.76^{d}	22.07^{b}	3.02^{a}	7.68^{a}	4.43^{b}	239.77^{c}	140.20^{c}	3.33^{c}	0.43^{c}	9.53^{c}
75	11.30^{b}	0.86^{b}	19.70^{c}	2.59^{b}	5.59^{c}	2.85^{d}	227.87^{d}	128.40^{d}	2.83^{d}	0.36^{d}	9.17^{d}
100	9.53^{c}	0.83^{c}	17.43^{d}	2.44^{c}	7.63^{a}	4.93^{a}	217.60^{e}	117.33^{e}	2.03^{e}	0.26^{e}	7.97^{e}
LSD ($p\leq0.05$)	0.17	0.02	0.43	0.06	0.10	0.11	3.96	3.85	0.25	0.01	0.15
F value	295.82	276.62	387.08	223.69	1009.3	986.16	222.16	343.33	154.10	1248.14	390.65
Wind damaged fruits											
Abohar											
0	12.80	0.87^{b}	27.47^{a}	2.69^{b}	6.01^{c}	3.16^{c}	270.63^{a}	179.30^{a}	5.93^{a}	0.62^{a}	11.50
10	12.70	0.91^{a}	26.03^{b}	3.06^{a}	6.72^{b}	3.48^{b}	269.40^{a}	172.33^{a}	5.27^{b}	0.60^{ab}	11.60
20	12.67	0.83^{c}	27.63^{a}	2.79^{b}	6.99^{a}	3.99^{a}	259.27^{b}	164.17^{b}	4.65^{c}	0.59^{b}	11.40
LSD ($p\leq0.05$)	NS	0.03	0.95	0.12	0.10	0.17	3.16	7.59	0.29	0.02	NS
F value	-	20.57	10.24	29.88	295.65	74.21	46.52	11.93	57.78	3.93	-
Hoshiarpur											
0	12.53	0.95^{a}	28.20^{a}	2.22^{b}	6.96^{b}	4.51^{a}	270.80^{a}	179.97^{a}	5.67^{a}	0.55^{a}	11.37^{a}
10	12.60	0.91^{b}	27.43^{b}	2.42^{a}	7.24^{a}	4.57^{a}	268.93^{a}	175.43^{b}	5.23^{b}	0.52^{b}	11.13^{b}
20	12.50	0.77^{c}	27.67^{ab}	2.41^{a}	6.85^{b}	4.22^{b}	261.00^{b}	161.77^{c}	4.33^{c}	0.51^{c}	11.47^{a}
LSD ($p\leq0.05$)	NS	0.02	0.54	0.12	0.15	0.14	3.05	4.37	0.43	0.01	0.23
F value	-	380.67	6.42	11.21	19.59	21.68	34.75	56.14	29.74	44.09	6.58
Ludhiana											
0	11.73	0.96^{a}	24.43^{a}	2.62^{b}	7.08^{a}	4.24^{a}	265.10^{a}	174.57^{a}	4.73^{a}	0.52	10.30
10	11.73	0.87^{b}	24.07^{b}	2.58^{b}	6.54^{b}	3.76^{b}	264.03^{a}	170.43^{b}	4.53^{a}	0.52	10.17
20	11.73	0.78^{c}	22.40^{c}	2.67^{a}	6.75^{b}	3.88^{b}	258.87^{b}	165.27^{c}	4.03^{b}	0.51	10.20
LSD ($p\leq0.05$)	NS	0.02	0.36	0.04	0.24	0.24	2.19	3.68	0.45	NS	NS
F value	-	198.91	109.34	16.13	14.87	13.53	27.66	19.21	7.80	-	-

Rangpur lime fruits. They reported that infected fruits had thicker skin as well as had rusty spots on skin due to mite attack which made them unacceptable in the consumer market. Rust mite damaged fruits (Sathgudi sweet orange and Rangpur lime) had less fruit weight, juice per cent, fruit size and titrable acidity. Other quality parameters values like TSS, sugars and vitamin C were higher in damaged fruits as compared to healthy fruits (Kalaisekar et al., 2003).

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EFFICACY OF MINERAL OILS AGAINST SAN JOSE SCALE AND EUROPEAN RED MITE ON APPLE

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ABSTRACT

Field efficacy of horticulture mineral oil Rilso 999 and MAK All Season at different concentrations were evaluated at two locations i.e., Lar (District Ganderbal) and Hajin (District Bandipora) of J&K during 2017 and 2018, all the treatments were significantly superior in terms of mortality of San Jose scale *Quadraspidiotus perniciosus* (Comstock) (SJS) and European red mite (ERM) *Panonychus ulmi* (Koch) as compared to untreated control. The cumulative mean mortality of SJS (58.08% and 62.20%) and ERM (58.69% and 53.65%) at recommended dose of 7.5 ml/ l of water both for standard check (MAK All Season) and Rilso999, respectively. Maximum (27.14 and 19.63 %) cumulative mortality of *Amblyseius* spp. and Coccinellids were recorded with Rilso999@ 10 ml/ l of water, respectively. No phytotoxicity symptoms viz., leaf injury, yellowing, wilting, necrosis, hyponasty and epinasty were observed with Rilso999 at 15 ml/ l of water. Maximum yield (246.16 kg/tree) was attained with MAK (standard check) @7.5 ml/ l of water.

Key words: Rilso999, MAK All Season, HMO, apple, San Jose scale, European red mite, natural enemies, phytotoxicity, coccinellid, *Amblyseius,* yield

India is blessed with diverse agroclimatic conditions and has the potential to produce almost all kinds of fruits. Apples are grown along the foot hills of Himalaya, ranging from Shillong obtained with (Assam) to Darjeeling (Bengal), in Kumaon Hills of Uttar Pradesh, hills of Punjab, Kullu Valley and Simla in Himachal Pradesh and whole of Jammu and Kashmir. Jammu and Kashmir is the largest producer of apple, with a production of 1882774 mt (Directorate of Horticulture Kashmir, 2018). Many insect pests cause significant economic damage on apple. These pests cause losses both directly and indirectly. Among all these pests, San Jose scale and European red mite are the serious pests of apple trees in Jammu and Kashmir (Anonumous, 2014; Bhalla and Gupta, 1993: Mahendiran and Ganie, 2018). The San Jose scale *Quadraspidiotus perniciosus* (Comstock) nymphs and female adults suck the sap from branches, twigs and fruits, weakens the plant and leaves, render the fruit unacceptable and unmarketable. European red mite*, Panonychus ulmi* (Koch) is another serious pest feeding on foliage. Keeping in view the severity of these an experiment was undertaken to evaluate horticulture mineral oil i.e. Rilso 999 along with standard check -MAK against European red mite and San Jose scale in the apple ecosystem of Kashmir Valley.

MATERIALS AND METHODS

Rilso999 was evaluated during 2017 and 2018 under field conditions. The experiments were laid at two locations i.e. Lar (District Ganderbal) and Hajin (District Bandipora). There were five treatments- Rilso999@ 5.0, 7.5 and 10.0 ml/ l of water, compared with MAK (standard check) @ 7.5 ml/ l of water, with only water spray as control. Spraying was done by high volume sprayer @ 15 l/ tree. 12 branches/ tree were randomly selected for counting *Q. perniciosus*/ cm^2 area and for *P. ulmi* counting was from randomly selected 12 leaves/ tree. Coccinellids were counted from 4 branches/ tree and *Amblyseius* sp., was from 20 leaves/ tree. Pretreatment observations were recorded before spray and post treatment ones after 0, 1, 3, 5, 7, 14 and 21 days after spray; similarly it was followed for the coccinellids and *Amblyseius* spp. Phytotoxicity on leaf injury on tips/ surface, yellowing, wilting, necrosis, hyponasty and epinasty were observed with Rilso999@ 15ml/ l of water after 1, 3, 5, 7 and 15 days after spray. Yield was computed as kg/tree at the time of harvest and compared.

RESULTS AND DISCUSSION

The results revealed that maximum mortality of

Table 1. Evaluation of mineral oils against *Q. perniciosus* and *P. ulmi* and their natural enemies in apple

Treatment	Dose (ml/l)	Mortality (%) of *Q. perniciosus*				Cumulative (%) mortality of SJS	Mortality (%) of *P. ulmi*				Cumulative Mean (%) mortality
		2017		2018			2017		2018		
		Location 1 (Ganderbal)	Location 2 (Bandipora)	Location 1 (Ganderbal)	Location 2 (Bandipora)		Location 1 (Ganderbal)	Location 2 (Bandipora)	Location 1 (Ganderbal)	Location 2 (Bandipora)	
	5.00	60.90	61.25	53.13	50.46	56.44	50.14	51.55	44.83	45.09	47.90
Rilso999	7.5	66.18	66.90	58.86	56.85	62.20	56.56	56.66	49.99	51.40	53.65
	10.0	69.90	70.18	60.10	58.46	64.66	57.90	58.90	51.86	53.87	55.63
MAK(Sd. Check)	7.5	73.14	73.65	63.83	61.69	68.08	61.90	61.98	54.47	56.39	58.69
Water (Control)	-	5.57	6.00	4.83	8.07	6.12	7.56	8.32	7.19	6.31	7.35
C.D ($p \leq 0.05$)		7.75	7.75	6.91	6.04	-	6.20	6.16	5.52	5.84	-
SE (m)		2.34	2.34	2.09	1.82		1.87	1.86	1.67	1.76	
		Mortality (%) of *Amblyseius* spp					Mortality (%) of coccinellids				
	5.00	17.74	17.29	16.36	17.95	17.33	8.25	6.96	7.07	7.33	7.40
Rilso999	7.5	21.41	22.03	23.18	20.18	21.70	15.65	14.32	13.43	16.44	14.96
	10.0	28.18	25.80	27.85	26.75	27.14	20.09	18.74	19.83	19.86	19.63
MAK(Sd. Check)	7.5	20.07	20.95	21.95	19.95	20.72	13.85	14.36	12.87	15.10	14.04
Water (Control)	-	8.83	8.99	7.33	9.15	8.57	3.65	3.85	4.26	4.40	4.04
CD ($p \leq 0.05$)		1.97	1.79	2.22	1.79	-	1.81	1.71	1.71	1.83	-
SE(m)		0.59	0.54	0.67	0.54		0.55	0.52	0.52	0.55	

		Apple yield (kg) /tree			
		2017		2018	
		Location 1 (Ganderbal)	Location 2 (Bandipora)	Location 1 (Ganderbal)	Location 2 (Bandipora)
	5.00	76.66	73.00	75.66	76.33
Rilso999	7.5	242.33	241.66	247.66	249.66
	10.0	216.66	217.00	220.33	223.66
MAK(Sd. Check)	7.5	243.33	241.66	249.33	250.33
Water (Control)	-	53.00	54.33	54.00	52.00

Q. perniciosus (68.08%) was observed with the MAK (standard check) @ 7.5 ml/ l of water, followed by Rilso999 @ 10 and 7.5 ml/ l (64.66 and 62.20%, respectively. Maximum (58.69%) mortality of *P.ulmi* was observed again with MAK (standard check) followed by Rilso999@ 10 Ml/ l (Table 1). Mir et al. (2015); Sherwani et al. (2017; 2018) and Bano et al. (2021) also observed that horticulture mineral oil (HMO) causes considerable reduction of both the pests. Maximum (27.14 and 19.63%) cumulative mortality of *Amblyseius* spp. and coccinellids were observed with Rilso999@ 10 ml/ l water, respectively; this was followed by Rilso999 and MAK @ 7.5 ml/ l of water. No phytotoxic symptoms were observed and maximum yield (246.16 kg/ tree) being obtained with MAK (standard check) followed by Rilso999.

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INFLUENCE OF TRICHOMES ON THE INCIDENCE OF TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* ON BRINJAL

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ABSTRACT

The present study evaluates the effect of host plant resistance by antixenosis (trichomes) in 40 brinjal entries to the two spotted spider mite *Tetranychus urticae* Koch, an important pest of brinjal. The maximum trichome density was observed (719.15/ 0.5cm^2 leaf area) with the variety Namakkal local and minimum (276.82/ 0.5cm^2 leaf area) was with Tiruppur local. Stellate type of trichomes were observed on all the entries, with maximum length on the upper surface of the leaves being 200.18 µm on Namakkal local, and on lower surface of leaves (269.36 µm) on Chinthapalli local-2. Manapparai local measured 247.40 µm long on midrib and 206.84 µm on veins. The correlation coefficients between the trichome density, length and *T. urticae* incidence revealed a significant negative correlation. Arka Nidhi was found to be resistant (2.07/ cm^2 leaf area) with low trichome density (427.50 µm).

Key words: *Tetranychus urticae*, brinjal, varietal resistance, leaf trichomes, upper and under surface, density, length, antixenosis, correlation coefficients

The brinjal *Solanum melongena* L. is cultivated in an area of 728.0 lakh ha with the annual production of about 126.60 lakh tonnes in India (Anonymous, 2018). This crop is attacked by more than eleven species of spider mites (Gupta, 1985). Among these, the two spotted spider mite *Tetranychus urticae* Koch is an extremely polyphagous and serious pest of at least 150 economically important crops including brinjal (Jeppson et al., 1975). It causes an estimated yield loss of 26.94 to 64.93% during severe infestation (Shukla et al., 2017). The host plant resistance through antixenosis results from the physical barrier of the plant that reduces the interaction between plant and herbivore (Gowthish et al., 2018). Ayyanar (2017) reported that the trichome density and length in brinjal had a negative impact on the incidence of sucking pests especially mites and observed that trichomes will hinder their movement and feeding. Jayabal et al. (2017) and Sheeba (2010) also confirmed these exhibiting a negative impact on the mites in okra varieties. The host plant resistance can be an important component to manage *T. urticae*. Hence, the present study evaluated the impact of trichome density and length in brinjal entries.

MATERIALS AND METHODS

Seed materials including released popular varieties, hybrids and local land races were collected from the Department of Olericulture, Horticultural College and Research Institute, TNAU, Coimbatore; Annamalai University, Chidambaram; Vegetable Research Station, TNAU, Palur; Department of Vegetable Science, College of Horticulture, KAU, Vellanikkara and Indian Institute of Horticultural Research, Bengaluru; National Seeds Corporation (NSC). Local popular land races were obtained from the farmers directly and the popular hybrids were procured from seed companies. The seeds were sown in mud pots of convenient size for screening under in-vitro conditions. The screening was carried out in a polyhouse located in orchard of the Department of Horticulture, AC&RI, Madurai adopting CRD design with two replications. The trichome density and length were assessed after sufficient population buildup and damage symptoms. In the experiment, the plants were infested with 30 mites on 25th day after transplanting @ one seedling/ pot. The mite incidence was assessed at 10 days interval after inoculation, and continued till the incidence is nil. Mite counts were made from the top, middle and bottom leaves of each plant, in 1 cm^2 area and the means were subjected to statistical analysis.

The trichome density from the leaf lamina, midrib and veins of all the entries were assessed adopting the methodology suggested by Maite et al. (1980). The

Table 1. Screening of brinjal entries for resistance to *T. urticae*

S. No.	Entries	Mite incidence/ cm^2 leaf area	Trichome length (µm)				No. of trichomes/ 0.5 cm^2 area of leaf
			Upper surface	Lower surface	Midrib	Veins	
1	Ariyalur local-1	7.42 (2.81)^{ef}	152.52 (12.37)	202.48 (14.25)	172.41 (13.15)	181.85 (13.50)	560.98 (23.70)
2	Ariyalur local-2	16.22 (4.09)^{ikl}	120.76 (11.01)	160.20 (12.68)	135.96 (11.68)	158.39 (12.61)	372.75 (19.32)
3	Arka Anand	3.27 (1.94)^{b}	164.33 (12.84)	232.75 (15.27)	216.68 (14.74)	193.35 (13.92)	680.07 (26.09)
4	Arka Harshitha	7.28 (2.79)^{def}	156.75 (12.54)	205.53 (14.35)	192.51 (13.89)	183.63 (13.57)	553.32 (23.53)
5	Arka Keshav	6.82 (2.71)^{cde}	155.20 (12.48)	209.50 (14.49)	180.15 (13.44)	184.50 (13.60)	540.00 (23.25)
6	Arka Nidhi	2.07 (1.60)^{a}	186.65 13.68)	232.57 (15.27)	210.22 (14.52)	201.94 (14.23)	427.50 (20.69)
7	Annamalai-1	17.9 (4.29)^{m}	123.50 (11.14)	162.25 (12.76)	149.75 (12.26)	151.79 (12.34)	365.32 (19.13)
8	Bhavani local	6.55 (2.66)^{cd}	159.78 12.66)	227.60 (15.10)	205.66 (14.36)	192.05 (13.88)	588.00 (24.26)
9	CO-2	16.32 (4.10)^{jkl}	122.78 (11.1)	162.00 (12.75)	147.54 (12.17)	157.80 (12.58)	388.75 (19.73)
10	Chinthapalli local-1	7.40 (2.81)^{ef}	157.43 (12.57)	217.58 (14.77)	199.95 (14.16)	181.57 (13.49)	574.98 (23.99)
11	Chinthapalli local-2	2.25 (1.66)^{a}	166.21 (12.91)	269.36 (16.43)	247.03 (15.73)	203.50 (14.28)	703.77 (26.54)
12	Gottivada local	14.27 (3.84)^{i}	130.00 (11.42)	157.25 (12.56)	151.30 (12.32)	161.30 (12.72)	377.33 (19.44)
13	Guntur local-1	15.47 (4.00)^{j}	106.52 (10.34)	159.30 (12.64)	144.56 (12.04)	153.70 (12.42)	379.17 (19.49)
14	Guntur local-2	17.37 (4.23)^{lm}	111.48 (10.58)	136.65 (11.71)	124.55 (11.18)	126.79 (11.28)	349.87 (18.72)
15	Haritha	7.48 (2.82)^{ef}	148.65 (12.21)	228.13 (15.12)	169.57 (13.04)	186.99 (13.69)	550.25 (23.47)
16	Irapaduguda local	16.30 (4.10)^{jkl}	125.95 (11.24)	158.85 (12.62)	149.67 (12.25)	158.84 (12.62)	328.33 (18.13)
17	Kovilpatti local	7.05 (2.75)^{cdef}	146.88 (12.14)	222.88 (14.95)	171.25 (13.11)	174.80 (13.24)	534.50 (23.13)
18	Manapparai local	3.47 (1.99)^{b}	165.72 (12.89)	236.31 (15.39)	247.40 (15.74)	206.84 (14.40)	684.00 (26.16)
19	Madurai local	7.38 (2.81)^{ef}	147.38 (12.16)	221.48 (14.90)	180.52 (13.45)	185.28 (13.63)	584.78 (24.19)
20	Mahy-91	15.52 (4.00)^{j}	106.98 (10.37)	140.05 (11.86)	149.36 (12.24)	159.90 (12.67)	346.00 (18.61)
21	MDU-1	15.82 (4.04)^{jk}	114.63 (10.73)	143.69 (12.01)	139.75 (11.84)	145.30 (12.07)	392.82 (19.83)
22	Musiri local-1	7.08 (2.75)^{cdef}	150.38 (12.28)	222.60 (14.94)	198.71 (14.11)	172.62 (13.16)	543.17 (23.32)
23	Musiri local-2	7.15 (2.77)^{cdef}	147.88 (12.18)	219.08 (14.82)	198.02 (14.09)	187.87 (13.72)	566.92 (23.82)
24	Musiri local-3	7.33 (2.8)^{def}	152.07 (12.35)	217.95 (14.78)	178.71 (13.39)	189.90 (13.80)	556.42 (23.60)
25	Namakkal local	3.20 (1.92)^{b}	200.18 (14.17)	271.59 (16.50)	223.15 (14.95)	198.26 (14.10)	719.15 (26.83)
26	Palur-2	11.65 (3.49)^{g}	134.90 (11.64)	195.15 (13.99)	154.31 (12.44)	173.66 (13.20)	449.92 (21.22)
27	Pusa purple long	11.48 (3.46)^{g}	138.25 (11.78)	169.55 (13.04)	153.75 (12.42)	166.95 (12.94)	450.92 (21.25)
28	Swetha	7.72 (2.87)^{f}	150.49 (12.29)	218.86 (14.81)	186.30 (13.67)	188.40 (13.74)	540.83 (23.27)
29	Sungro (No. 132)	18.10 (4.31)^{m}	129.17 (11.39)	214.43 (14.66)	193.79 (13.94)	175.96 (13.28)	317.25 (17.83)
30	Simran	15.12 (3.95)^{ij}	131.20 (11.480	151.04 (12.31)	151.61 (12.33)	122.40 (11.09)	395.83 (19.91)
31	Surya	7.47 (2.82)^{ef}	159.71 (12.66)	203.58 (14.29)	184.02 (13.58)	172.33 (13.15)	546.15 (23.38)
32	Telikecherla local	16.00 (4.06)^{jk}	112.93 (10.65)	154.68 (12.46)	150.08 (12.27)	160.21 (12.68)	361.23 (19.02)
33	Tiruppur local	20.52 (4.58)^{n}	119.60 (10.96)	120.38 (10.99)	112.79 (10.64)	138.19 (11.78)	276.82 (16.65)
34	Tuni local	6.45 (2.64)^{c}	157.38 (12.56)	219.90 (14.85)	185.96 (13.65)	188.97 (13.76)	591.58 (24.33)
35	UGIE- 4411	12.78 (3.64)^{h}	145.21 (12.07)	200.56 (14.18)	156.35 (12.52)	167.38 (12.96)	471.83 (21.73)
36	Ujala	7.37 (2.81)^{def}	149.53 (12.25)	222.65 (14.94)	193.73 (13.94)	181.70 (13.50)	553.60 (23.54)
37	Vijay (ARBH-905)	16.97 (4.18)^{klm}	128.25 (11.35)	157.28 (12.56)	151.16 (12.32)	163.93 (12.82)	376.83 (19.43)
38	VNR Kanchi	11.37 (3.45)^{g}	135.97 (11.68)	186.50 (13.67)	152.90 (12.39)	160.56 (12.69)	474.83 (21.80)
39	VNR Kirti (ARBH-555)	7.03 (2.74)^{cdef}	157.63 (12.57)	211.73 (14.57)	207.31 (14.42)	179.32 (13.41)	571.82 (23.92)
40	VNR Utkal	6.92 (2.72)^{cdef}	152.81 (12.38)	216.50 (14.73)	204.85 (14.33)	172.03 (13.14)	551.50 (23.49)
SEd		0.0777	0.5919	0.5933	0.5123	0.5398	0.3505
CD (p=0.05)		0.1570	1.1964	1.1991	1.0355	1.0910	0.7084

***** Each value mean of two replications; Figures in parentheses square root transformed values

number of trichomes/ half cm^2 was counted under a stereozoom microscope (Carl Zeiss Stemi 508) at 10x magnification. The trichome length was also measured by using the same leaf bit used for trichome density assessment (Laichattiwar et al., 2018). The data from pot culture and laboratory experiments were subjected to ANOVA using AGRESS software, and significance evaluated by F-tests, while the significance of difference between the treatment mean values was compared by LSD (p=0.05). Correlation and regression coefficients were computed to find out the relationship between the mite incidence and trichome density and length, using SPSS ver.17.0 software.

RESULTS AND DISCUSSION

The trichome density on leaves revealed highly significant differences among the brinjal entries screened, maximum being in the leaves of Namakkal local (719.15/ 0.5 cm^2) while the minimum was with the Tiruppur local (276.82/ 0.5 cm^2) (Table 1). Ayyanar (2017) also observed maximum trichome density on resistant entry IC 249358 (652/ cm^2) and the least (236/ cm^2) with IC 261801 which is a highly susceptible one. Jayanthi et al. (2018) also observed similar variations in density of trichomes in brinjal varieties on both adaxial (76.66± 1.90) and abaxial surfaces (121.58± 2.02), exhibiting a positive correlation between trichomes on adaxial and abaxial surfaces. Nain et al. (2017) with seven genotypes of okra observed similar effects of trichomes in okra. Exceptionally, Arka Nidhi which was identified as a resistant entry (2.07/ cm^2 leaf area) recorded low trichome density of (427.50/ 0.5 cm^2 leaf area). Sarma (2010) observed Arka Nidhi with the least mite incidence (8.15 mites/ 6.25 cm^2) among 12 brinjal entries screened against *T. macfarlanei.* The correlation coefficients (r) between total trichome density and *T. urticae* incidence revealed that the number of trichomes/ 0.5 cm^2 leaf area exhibits a significant negative correlation (r = -0.9195). These observations agree with those of Sarma (2010), Mutthuraju (2013), Mahendrakumar (2016), Ayyanar (2017) who observed that trichome density exhibited a significant negative correlation with mite incidence in brinjal.

The data on trichome length revealed highly significant differences among the brinjal entries, with maximum being observed on the upper surface of Namakkal local (200.18 µm), lower surface of Chinthapalli local-2 (269.36 µm), midrib of Manapparai local (247.40 µm), and veins of Manapparai local (206.84 µm). The least trichome length was observed with Guntur local-1 (106.52 µm) on upper surface, Tiruppur local (120.38 µm) on lower surface, Tiruppur local (112.79 µm) on midrib, and Simran (122.40 µm) on vein. Ayyanar (2017) observed that the incidence decreased with increase in length of trichomes, and trichomes in the lower surface of leaves were longer in brinjal. Star shaped (multi-radiate, stellate and non-glandular) trichome having one vertical hair and 7-8 horizontal hairs were observed in all the entries. Bayoumy et al. (2017) also reported that brinjal leaves had smooth and soft surfaces with stellate trichomes on both adaxial and abaxial surfaces of leaves. Jayabal et al. (2017) also observed similar trichomes in okra, with longer ones being observed in highly resistant and resistant entries. The correlation coefficients (r) between trichome length and *T. urticae* incidence revealed that the length on the upper and lower surfaces, those on midrib and veins exhibit a significant negative relationship (r = -0.905, -0.901, -0.862, -0.861, respectively). These observations are in conformity with those of Jayabal et al. (2017) and Ayyanar (2017). The multiple linear regression between *T. urticae* incidence and trichome length revealed a significantly high value ($R^2 = 0.87$), implying that these trichome length contributed directly towards the population buildup of *T. urticae* on brinjal entries to an extent of 87.65%. Thus, it is concluded that host plant resistance (antixenosis) observed with brinjal's morphological characters like trichome density and length exhibit a significant negative impact on the incidence of *T. urticae*.

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POTENTIAL INVASIVE TERMITES IN INDIA AND IMPORTANCE OF INTEGRATIVE TAXONOMY

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ABSTRACT

Termites are ubiquitously abundant in the tropics and subtropics. Globally 28 species are considered invasive among the 2937 species in nine families known. Among the invasive species, most are considered nuisance organisms to humans in urban areas, and a few have invaded natural forests. Generally, the invasive termites share three qualities: (i) they eat wood, (ii) make nests in wood, and (iii) they quickly generate secondary reproductives. These qualities are the most common in the Kalotermitidae and Rhinotermitidae (Blattodea: Isoptera), which enhance their opportunities for producing viable and reproductively capable individuals. Species of the Termitidae cannot produce secondary reproductives, and this is attributed as a reason for their lack of invasiveness. The 28 species recognized as strongly invasive have the potential to widen their geographical range from their present distribution locations. According to available data, 10 out of the 17 recognized invasive species have expanded their area of occurrence since 1969. Among the 28, three are known in India, although their exact distribution data in the Indian subcontinent, the damage they inflict, and consequent economic loss are yet to be worked out in detail. Climate change, intensifying urbanization, and globalization, acting either individually or in combination, are likely to exacerbate ecological and economic effects. Strict quarantine measures and appropriate treatment of every wood material imported are imperative. Efforts have been made to list the potential invasive termites in India based on imported-wood material. Approximately 70 species reported in the GenBank database for about 300 species known from India, emphasizing a dire need for accurate morphological and molecular determinations. Gene sequences of some of the Indian termite species submitted are incorrectly identified, as their presence and distribution in India is doubtful. Therefore, in this article, I discuss the importance of the combined and efficient use of morphological and molecular taxonomy in determining termites in India.

Key words: Identification, Kalotermitidae, Rhinotermitidae, traded commodity, woodeating, nest constructing, places of origin, invasives, number, Indian perspectives, NCBI accessions, threats to biodiversity

Exotic species invasion is one among the many of the world's serious environmental threats (Buczkowski and Bertelsmeier, 2017). Although growing human population and their injudicious actions are the critical reasons for climate change and urbanization, invasions by exotic species can be traced back to mobility of humans and movement of products to meet human needs. Huge numbers of exotic, pestiferous insects are moving from continent to continent as a result of globalization and trade, resulting in substantial economic loss (Sharma et al., 2018). In the last decade, various invasive insects are known from India and have been found to be establishing in 'new' habitats (Kalleshwaraswamy et al., 2015; Sharanabasappa et al., 2018; Sundararaj et al., 2020) and termites are no exception (Evans et al., 2013). Many species of termites are serious nuisance organisms, inflicting damage to the wood used in both human-made structures and agricultural and forest environments (Kalleshwaraswamy et al., 2018). They occur more abundantly in the tropical and subtropical regions of the world. We now know that the Isoptera have evolved from the subsocial Cryptocercidae (Blattodea: Blattoidea) (Inward et al., 2007; Evangelista, 2019). Hence, the termites are now classified under Isoptera, Epifamily Termitoidae within Blattodea. However, the family status of all extant termites is retained to avoid confusion arising out of this modified classification. About 3000 species of Isoptera were described from nine families in 2013 (Krishna et al., 2013) and from then onwards, a few new species have been added from different parts of the world. Among them, 1160 species are from the Oriental region. Two hundred and ninety-five species placed under 52 genera in six families are known from India. Among the 295, 188 are endemic to India (Rajamohana et al., 2019). Out

of *c*. 3000 species known around the globe, 366 affect human dwellings in different parts of the world. Yet, we need to recognize that these taxa are one among the many critical invertebrate decomposers in arid and semi-arid environments, and they contribute to improving the physical and chemical properties of soil by their activities and especially by the construction of structures such as mounds, galleries, sheetings with varying physical and chemical qualities (Jouquet et al., 2018). On an average, the termites increase water infiltration above the natural rate by a factor of 1 to 4, depending on their activity, soil type, and rainfall intensity (Kaiser et al., 2017).

Diverged from cockroaches (Cryptocercidae : Blattodea) *c*. 150 mya (late Jurassic), the termites developed advanced social nature and caste system, which their ancestors lacked. They live in colonies with a large number of workers and soldiers that are usually sterile. Colonies have fertile males, the 'kings', and one or more fertile females, the 'queens'. Pheromones maintain the caste system, preventing all but a few termites from becoming reproductives and the rest will remain sterile, assuring a division of labour (Simpson et al., 2011). Primary reproductives and secondary reproductives are the two types of reproductives found in the Isoptera. Nymphs give raise to adults, which mate and reproduce the primary reproductive, hemimetabolically. In some species, such as *Coptotermes,* when primary reproductives are either lost or immatures are separated from their parent colony, nymphs or workers (= pseudergates) can give rise to either a king or a queen, referred to as secondary reproductives (Myles, 1999).

With the increased trade of wood, more termites are becoming invasive in different countries; India is no exception. Therefore, anticipating this potential megaproblem strict policy measures are vitally necessary. In this article, I have made an effort to enumerate the potentially invasive termite species in India, based on wood import from different countries, especially in the recent past. Monitoring and treatment of wood material imported are critical to minimize entry of exotic Isoptera into India. Examples of success stories restricting the entry of nuisance species of Isoptera have also been highlighted in this article. The importance of both morphological and molecular taxonomy of Indian Isoptera for correct determination of taxa enabling better management is also indicated.

A. Characteristics of invasive termites

Although various termite species are considered nuisance organisms of different crops (Rana et al., 2021), forest (Junqueira and Florencio, 2018) and urban ecosystems (Olaniyan et al., 2015), all of these are not invasive. Most of the invasive Isoptera have three qualities in common: (i) they eat wood, (ii) construct nests in the wood, and (iii) quickly generate secondary reproductives (Evans et al., 2013; Donovan et al., 2001). Many a time, in combination of the above, the termites have an increased likelihood of producing reproductively viable offspring. These qualities are especially common in the Kalotermitidae and Rhinotermitidae, which collectively constitute 23 species in the invasive termites list, in genera such as *Cryptotermes*, *Heterotermes*, and *Coptotermes* (19 species) (Table 1). The Termitidae comprising *c*. 70% of the Isoptera, includes only one invasive species, *Nasutitermes corniger.* The innate characters that make this species invasive need to be studied.

(i) Wood eating

In general, four types of food habits occur among the Isoptera (Donovon et al., 2001; Yamada et al., 2007; Jones and Eggleton, 2000; Kaur et al., 2013; Kaur et al., 2017). Type I and II eat undecomposed plant matter such as wood, grass, and leaf litter. Those of type III and IV eat decomposed plant matter; those of type III eat on the decomposing wood either covered with or embedded within soil (plant matter–soil interface), whereas those of type IV are exclusive soil feeders. Those of types III and IV make up *c*. 50% of all Isoptera. Wood eating is a common characteristic among the known invasives, that means they belong to either Type I or Type II.

(ii) Nest constructing

Nest construction is classified into four categories (Abe, 1987):

Single-piece nesters: Isoptera of this category nest and forage in either one piece of wood or wood pieces bound tightly together and feed within: *e.g.*, species of *Cryptotermes* (Kalotermitidae) and Archotermopsidae

Intermediate-piece nesters: Colonize a single piece of wood, but they move out searching food material and eat pieces of wood, where they construct nests and start new nests once the originally occupied piece of wood is completely eaten: *e.g.*, species of *Coptotermes* (Rhinotermitidae).

Separate-piece nesters: build a nest in soil or nest separate from their food and forage away from their nest to find food: *e.g.*, mound-building Macrotermitinae (Termitidae).

Continuously mobile (no permanent nest): Do not settle in a nest permanently and are mobile continuously. Found among the Type III and IV soil-feeders that eat their way through the soil.

A majority of the invasive species are either single-piece nesters (12— Archotermopsidae and Kalotermitidae) or intermediate-piece nesters (Rhinotermitidae) (Table 1). The possible reason is either presence of all the castes within the nest or the ability to produce viable secondary reproductives (explained in the following paragraph).

(iii) Can generate secondary reproductive

If the nymphs or workers develop as reproductives, they are collectively referred as the nymphoids and ergatoids, respectively. Here sexual maturity is achieved without attaining a fully winged adult stage, and hence they are 'secondary reproductives'. There may be a situation when an infested wood log is transported, a small number of nymphs may be carried from their original habitat, there is a chance that secondary reproductives produced from those nymphs in a new habitat, making that species invasive. These few nymphs carried from the infested material could act as propagules. Because of this trait, many termite species are potential invaders in new habitats even though primary reproductives are not transported.

B. Number of invasive termite species

Termites were not thought to be invasive previously, despite accounts of their spread away from their native ranges. A first worldwide enumeration by Gay published in 1969 recorded 17 species of termites as invasive with the evidence of their establishment in new habitats. All the 17 determined species appear to be associated with either buildings or cultivated crops. Presently 28 species of termites are considered invasive worldwide, (Evans et al., 2013). This implies that the numbers of invasive species have increased from 17 in 1969 to 28 today and are expanding their geographic locations. In the past 50 years, 14 species have been added to the list; 10 of them have extensive distributions and four have no reported changes in distribution, and three species are no more considered invasive (Evans et al., 2013). It appears among the invasive termite species, most are pestiferous in urban areas, although six species have been found to invade natural forests (Evans et al., 2013; Chouvenc et al., 2016).

C. Transportation of live stages of termites

Most wood feeding termites (Kalotemitidae, Rhinotermitidae and Archotermopsidae) display the above-said three qualities and they can be transported from their original habitats to new habitats through the movement of wood. Many countries export wood and wooden material to different parts of the world. If the transported wood includes any developmental stage of the Isoptera, then they serve as propogules and become secondary reproductives (Evans, 2011; Myles, 1999). A recent report with dead stages of *C. gestroi* in the seaport of Goa (15.50° N, 73.83° E) (Venkateshan et al, 2021) from an imported wood consignment sends an alarm signal and seeks a close monitoring of the invasive Isoptera. Species-rich Termitidae cannot produce secondary reproductive, hence they lack the invasive capability.

D. Places of origin and continents invaded

Initial reports of termite invasion were mainly restricted to movement of Isoptera from continents to nearby islands (Gay, 1969). This is attributed to free trade of wood products. However, the reverse could be true, since species such as *Cryptotermes brevis* and *C. formosanus*, have reached the coast of Queensland, Australia, and Hawaii (USA) in the North Pacific Ocean and have invaded inland as well (Constantino, 2002; Austin et al., 2006; Jenkins et al., 2001). Among the 28 invasive species known to date a perusal of subcontinents or land mass from which the invasive species originated, Indo-Malayan region includes a maximum of seven species, indicating many endemic species have moved from this region to different parts of the world (Table 1). This is followed by South America (six), Australia (five), Africa (three), North America (two), the Caribbean Islands (one), East Asia (one), and Europe (one). *Heterotermes perfidus* and *Coptotermes truncatus* are of unknown origin.

Similarly, a perusal of invaded subcontinents indicates that the islands of the Pacific Ocean are the most invaded that include 13 species, followed by the Caribbean islands (nine), North America (eight), Indian Ocean (six), South America (five), Australia (four), Atlantic Ocean islands (four), East Asia (three), Africa (three), South and Southeast Asia (two), and Europe (one) (Evans et al., 2013). Coastal regions are more prone to invasion by the Isoptera and inflict damage to built-structures consisting of wood and crop ecosystems (Ferreira et al., 2013; Szalanski., 2004). The probable reason for this pattern is that the wood is mostly imported through sea-routes and the invasive Termitidae first establish themselves in coastal areas, and then propagate inwards.

Table 1. Invasive Isoptera, their country or bioregion of origin, and the country or continent invaded

Name of the species	Family	Native country or bioregion	Country or continent invaded and (probable year of invasion)	References
Mastotermes darwiniensis	Mastotermitidae	Northern Australia	Papua New Guinea (before 1959)	Gray, 1968; Thistleton et al., 2007
Zootermopsis angusticollis	Archotermopsidae	Western North America	Hawaii (1999)	Haverty et al., 2000; Grace et al., 2002
Zootermopsis nevadensis	Archotermopsidae	Western North America	Kawanishi, Japan (2000)	Kiritani and Morimoto, 2004
Porotermes adamsoni	Stolotermitidae	Southeast Australia	New Zealand 1941	Bain and Jenkins, 1983; Phillip et al., 2008
Incisitermes immigrans	Kalotermitidae	Pacific coast of Panama to Peru	Pacific Islands, Polynesia, Hawaii, Japan (1995)	Gay, 1969; Constantino, 1998; Grace et al., 2002; Kiritani and Morimoto, 2004
Incisitermes minor	Kalotermitidae	Southwestern United States and northern Mexico	Japan (1975), Eastern United States (1995); Toronto Canada (1989), Ninghai, Zhejiang Province, China (1937), Hawaii (1999)	Grace et al., 1991; Scheffrahn et al., 2001; Xie et al., 2001; Grace et al., 2002
Kalotermes banksiae	Kalotermitidae	Southeast coast of Australia	New Zealand (1942)	Bain and Jenkins, 1983
Glyptotermes breviconis	Kalotermitidae	Southeast coast of Australia	New Zealand (Pre 1983), Fiji (pre 1942)	Gay, 1969; Bain and Jenkins, 1983; Evenhuis, 2007
Cryptotermes brevis	Kalotermitidae	Coastal deserts of Peru and Chile	Egypt, Queensland, Australia (≃ 1941), Azores (2002), Canary Island, Lisbon, Portugal (87)	Peters, 1990; Borges et al, 2007; Scheffrahn et al., 2009; Nunes et al., 2010; Ferreira et al., 2013
Cryptotermes cynocephalus	Kalotermitidae	Philippines	Throughout Southeast Asia, Australia (before 1942), Hawaii (2000), Sri Lanka	Gay and Watson, 1982; Scheffrahn et al., 2000; Hemachandra et al., 2012
Cryptotermes domesticus	Kalotermitidae	Southeast Asia	China, Australia (before 1942), Panama in Central America	Gay and Watson, 1982; Evans, 2010
Cryptotermes dudleyi	Kalotermitidae	Southeast Asia	India (Orissa in Lower Bengal) and Bangladesh (before 1950), East Africa, northern Australia (before 1942), Caribbean islands (Jamaica and Trinidad), South America (Panama, Costa Rica, Colombia and Brazil)	Williams, 1976; Gay and Watson, 1982; Constantino, 1998; Scheffrahn and Krecek, 1999; Constantino, 2002; Schabel 2006
Cryptotermes havilandi	Kalotermitidae	Tropical West Africa	Caribbean Islands, Brazil, East Africa, Tanzania, Indian Ocean (Madagascar), India and Bangladesh	Maiti, 1983; Bose, 1984; Constantino, 1998; Scheffrahn and Krecek, 1999; Schabel 2006; Scheffrahn et al., 2009
Heterotermes convexinotatus	Rhinotermitidae	South America, from Mexico to Argentina	Greater (Hispaniola and Puerto Rico) and Lesser Antilles (Antigua, Barbados etc) and in Gal´apagos	Peck, 2001; Szalanski, 2004
*Heterotermes perfidus**	Rhinotermitidae	Unknown origin	south Atlantic Ocean	-
Heterotermes philippinensis	Rhinotermitidae	Philippines	Madagascar and Mauritius	Cachan, 1949
Heterotermes tenuis	Rhinotermitidae	Central America	Lesser Antilles; Trinidad and Tobago	Szalanski, 2004

(contd.)

Table 1 contd...

Heterotermes sp.	Rhinotermitidae	Probably Caribbean Island	Miami, Florida	Szalanski, 2004
Reticulitermes flavipes	Rhinotermitidae	Eastern United States	Canada, Bahamas (1998), Europe (Vienna- 1837), Germany (before 1937),and Southwestern France (before 1840), South America (Uruguay -1960), Chile (1986)	Grace et al., 1991; Scheffrahn et al., 1999; Dronnet et al., 2005; Austin et al., 2005; Su et al., 2006
Reticulitermes grassei	Rhinotermitidae	Southwestern Europe (France and Spain)	Britain (Saunton, 1994), Faial Island of the Azores (2000)	Jenkins et al., 2001; DeHeer et al., 2005
Coptotermes acinaciformis	Rhinotermitidae	Australia	Auckland and New Plymouth, New Zealand (1930), Suva, Fiji (1939)	Phillip et al., 2008;
Coptotermes curvignathus	Rhinotermitidae	Southeast Asia	Southern China	Xie et al., 2001
*Coptotermes formosanus***	Rhinotermitidae	Southern China and Taiwan	Continental United States (~ 1950s), Japan (1700), Hawaii (1907), South Africa (1974)	King and Spink, 1969; Vargo et al., 2003; Sun et al., 2007
Coptotermes frenchi	Rhinotermitidae	Southern Australia	New Zealand	Miller, 1941
Coptotermes gestroi	Rhinotermitidae	Southeast Asia	Mauritius (1936), R´eunion Island (1957), Taiwan (2001), Hawaii (1963), Polynesia 1999) and Micronesia and Fiji (before 2009). Mexico (2000),Florida (1996), Greater Antilles and lesser Antilles (1937), India	Roonwal and Chhotani, 1965; Scheffrahn et al.,1994; Kirton and Brown, 2003; Tsai and Chen., 2003 ; Vargo et al., 2003; Scheffrahn and Su., 2005; Li et al., 2009
Coptotermes sjostedti	Rhinotermitidae	Tropical West Africa	Lesser Antilles (Guadeloupe Island, 1999)	Scheffrahn et al., 2005
*Coptotermes truncates**	Rhinotermitidae	Unknown origin	Madagascar, Republic of Seychelles (1897)	-
Nasutitermes corniger	Termitidae	Central to South America and the Caribbean Islands	Abaco Island of the Bahamas, Florida (before 2001), New Guinea	Scheffrahn et al., 2005; Evans, 2010

*Not a valid species; **The establishment and spread of this species in the United States is the best-documented termite invasion

Buczkowski and Bertelsmeier (2017) employed two alternative representative concentration pathways (RCPs) to predict climate scenarios: RCP 4.5 and RCP 8.5, and two projection years (2050 and 2070 AD) to offer the first worldwide risk assessment for 13 of the world's most invasive termites. Representative concentration pathways (RCPs) are widely used to describe different climate future depending on the volume of greenhouse gases (GHG) emitted in the years to come and thereby predict population dynamics. Buczkowski and Bertelsmeier's findings reveal that, regardless of the climate or year, most of the species will have a substantial rise in their global spread by 2050 AD. The most appropriate places for invasion are the tropics and subtropics. All the continents have large land spaces and natural conditions suitable for more than four species to coexist. Climate change, growing urbanization, and accelerating economic globalization, working singly or in concert, are anticipated to exacerbate the enormous economic and ecological damage caused by invasive termites.

E. Invasive termites: an Indian perspective

India, being one of the largest importers of wood and wood-related material, is vulnerable to Isoptera invasives. Its tropical and subtropical climates also support rapid multiplication of individuals after they arrive. Earlier *Cryptotermes dudleyi* (Kalotermitidae), *C. havilandi* (Kalotermitidae), and *C. gestroi* (Rhinotermitidae) were the invasives known in India. Among these, the taxonomic authenticity of *C. dudleyi* is confirmed presently. The incidence of

C. havilandi is questionable, in spite of its reported spread from India to Bangladesh (Maiti, 1983, Bose 1984). As per Krishna et al. (2013), a world authority on the Isoptera, it is distributed in West Africa (from Sierra Leone to Nigeria), Brazil, Trinidad, the Guianas, Barbados and other west-Indian Islands. Similarly, *C. gestroi*'s original locality is Myanmar (Wasmann, 1896) and its report in Assam (Roonwal and Chhotani, 1965) and in north-eastern Puducherry (Harit et al., 2014) needs validation by further collection and molecular determination. Not many serious efforts have been made to determine the taxonomic identity, distribution, and damage of these species in the Indian landscape. There is no NCBI sequence for this species submitted for specimens collected from India, which may offer a valid confirmation of its presence in India. Originally described from Myanmar, *C. gestroi* is considered to occur in north-eastern India and Thailand. This species inflicts serious economic losses by feeding on wood in built structures in other Asian countries, such as Malaysia, Taiwan, Indonesia and in Brazil, the Caribbean islands, and in the peninsular Florida, USA. *Coptotermes gestroi* is the predominant termite species attacking buildings in urban area of Taiwan (Sornnuwat, 1996). Up to 27% of the trees in the city of São Paulo, Brazil, found infesting contributed by four subterranean species of termites but *C. gestroi* (Wasmann) being the dominant species (Zorzenon and Campos, 2014). However, such a serious damage has not been reported from India and hence intensive surveys and data collection on damage by the Isoptera may shed light on its presence and its impact on the economy.

Venkateshan et al. (2021) offer substantial evidences of both morphological and molecular characters of *C. gestroi* collected from the wooden packing material of a consignment received in Goa coming from Harrisonburg, Virginia, USA. In another report made in December 2018, a few Isoptera were received for identification from Visakapatnam Plant Quarantine Station collected from the timber of *Maclora tinctoria* (Moraceae) imported from Guyana (South America). In July and September 2019, I received different specimens of Isoptera for determination intercepted from the wood of a species of *Gmelina* (Verbenaceae) in Tuticorin port (Tamil Nadu) imported from Columbia, *M. tinctoria* and a species of *Erythrophleum* (Fabaceae) wood logs from Suriname. Using morphological and molecular characters, the intercepted specimens were identified as *C. testaceus* and C. *sjöstedti*. *Coptotermes testaceus*'s 16S rRNA gene sequences were deposited with NCBI GenBank with accession numbers MK559590, MK559591, MK559592, and MK559593. Whereas *C. sjöestedti* 16S rRNA gene got NCBI GenBank accession number MN540914 (Nagaraju et al., 2020). Such clarity is needed in taxonomy for identifying and reporting of any termites intercepted in India.

F. Potential invasive termites in India

The spatial spread of Termitidae is increasing due to trade, urbanization, and climate change (Buczkowski and Bertelsmeier, 2017). Extrapolating this scenario, India could be invaded by another 8-10 species by 2050 AD as predicted in the RCP 4.5 scenario, indicating a greater risk and potential damage to urban and agricultural ecosystems. India imports timber from South America, Africa, southeast Asia and from the Caribbean Island nations as evident in the data of non-compliance report of pest interceptions (Directorate of Plant Protection, Quarantine & Storage website, updated monthly http://plantquarantineindia.nic.in/PQISPub/html/ncrep.htm (accessed on 10 December 2021). There is a need for a thorough scrutiny of every wooden log imported into India. The countries mentioned have a high diversity of invasive Isoptera, which have the potential to enter and cause serious ecological and economic damage in India (Table 2). A list of potential invasive species from where wooden logs were imported into India in 2019 is presented in Table 2. This list is not comprehensive but will serve as a base for an early detection of species and to look for the possible entry of potentially damaging species from those countries.

G. Scope of integrative taxonomy

Because of their wide distribution and intraspecific character variations, taxonomic validity of many species of the Isoptera remains unclear. For example, *Coptotermes*, currently includes 69 named species, and among them, only 21 are considered valid (Chouvenc et al., 2016). According to Chouvenc et al. (2016), most of the described Chinese species of *Coptotermes* are invalid and need revision. Species identification in the Termitoidae is principally based on the measurement of different body parts of soldiers and hence intraspecific variation is highly possible. Hence adapting molecular taxonomy using more than one mitochondrial gene, such as mtCo1, 12S rRNA, and 16S rRNA and nuclear genes such as 28S rRNA, 18S rRNA may provide better clarity on valid species. There is a dire need to barcode all the morphologically identified Isoptera. Currently, barcode sequences are available only for approximately 70 Indian taxa, out of more than 300 species known. In this article, efforts have been to list the available NCBI

Table 2. Potential invasive Isoptera into India*

Country	Native or invasive species distributed at present
Brazil	*Nasutitermes corniger, Incisitermes immigrans, Cryptotermes havilandi, Cryptotermes brevis*
Cameroon	*Coptotermes sjostedti, Cryptotermes havilandi , Cryptotermes brevis*
Central African Republic	*Coptotermes sjostedti*
Colombia	*Heterotermes convexinotatus, Heterotermes tenuis*
Congo	*Cryptotermes havilandi*
Costa Rica	*Incisitermes immigrans, Nasutitermes corniger*
Ecuador	*Incisitermes immigrans, Nasutitermes corniger*
Equitorial Guinea	*Coptotermes sjostedti, Cryptotermes havilandi*
Ghana	*Coptotermes sjostedti, Cryptotermes havilandi*
Malaysia	*Coptotermes curvignathus, Coptotermes gestroi*
France	*Reticulitermes flavipes*
Guyana	*Nasutitermes corniger, Termes hispaniolae, Cryptotermes brevis*
Mexico	*Incisitermes minor, Heterotermes convexinotatus*
Papua New Guinea	*Mastotermes darwiniensis, Cryptotermes cynocephalus*
Suriname	*Nasutitermes corniger, Coptotermes testaceus*
Trinidad and Tobago	*Cryptotermes brevis, Cryptotermes domesticus, Cryptotermes dudleyi, Cryptotermes havilandi, Coptotermes testaceus, Heterotermes tenuis, Heterotermes convexinotatus, Nasutitermes corniger, Termes hispaniolae*
USA	*Cryptotermes brevis, Coptotermes formosanus* and *C. gestroi*
Benin	*Coptotermes sjostedti, Cryptotermes havilandi*
*Ecuador	*Heterotermes* sp.
Gabon	*Coptotermes sjostedti, Cryptotermes havilandi, Cryptotermes brevis*
Honduras	*Incisitermes immigrans, Nasutitermes corniger*
Indonesia	*Coptotermes curvignathus, Coptotermes gestroi*
Kenya	*Cryptotermes dudleyi*
New Zealand	*Cryptotermes brevis, Kalotermes banksiae Coptotermes acinaciformis, Porotermes adamsoni, Glyptotermes brevicornis*
Nigeria	*Coptotermes sjostedti, Cryptotermes havilandi*
Panama	*Incisitermes immigrans, Nasutitermes corniger*
Philippines	*Heterotermes philippinensis, Cryptotermes cynocephalus, Cryptotermes havilandi*
Sri Lanka***	*Cryptotermes cynocephalus, Cryptotermes domesticus, Cryptotermes perforans*, *Coptotermes formosanus*
Sudan**	*Coptotermes sjostedti*
Panama	*Cryptotermes dudleyi, Cryptotermes domesticus*
South Africa	*Coptotermes formosanus, Cryptotermes brevis*
Suriname	*Nasutitermes corniger,*
Togo	*Coptotermes sjostedti, Cryptotermes havilandi, Cryptotermes brevis*
Uruguay	*Reticulitermes flavipes*

*Based on Evans et al. (2013); *Dahlsjö et al., 2020; **Pears et al., 1995; *** Hemachandra et al., 2015

submissions so far made from India. Apparently, the genes targeted were 12s rRNA and 16s rRNA, that are not robust for species delimitation. A few submissions are with Mt COI and Mt COII used universally for molecular identification of insects. Moreover, there is a need of high-throughput mtCOI or mt COII DNA extraction procedure so as to determine valid species of the Isoptera. Vidyashree et al. (2018) successfully sequenced 12 Isoptera species from the Western Ghats and developed a DNA extraction procedure. However, they reported for 12S rRNA and hence there is a need of standardization of preservation technique of collected termite specimens and storage for successful DNA extraction.

The following example may clarify the imperative need for molecular data in resolving taxonomic confusions in the Indian species of *Coptotermes*. A total of seven species are known from India (Rajamohana et al., 2019): *C. beckeri*, *C. ceylonicus*, *C. gauri*, *C. gestroi*, *C. heimi*, *C. kishori*, and *C. premarasmii*. Among these, the presence and distribution of *C. gestroi* and

Table 3. NCBI GenBank database available for Indian Isoptera

Species	Gene	NCBI GenBank accession number	Authors	Remarks
Anacanthotermes viarum	28S ribosomal RNA	JQ957910	Deivendran et al., 2012	
Glyptotermes ceylonicus	12S ribosomal RNA	MZ191059	Joseph and Mathew, 2021	
	mtCOII	MZ191060	Joseph and Mathew, 2021	
Neotermes koshunensis	12S ribosomal RNA	KM657485	Ramya et al., 2014	
Neotermes nilumburensis	16S ribosomal RNA	MZ234128	Kalleshwaraswamy et al., 2021	
Neotermes viraktamathi	16S ribosomal RNA	MW699667	Kalleshwaraswamy et al., 2021	
Coptotermes gestroi	16S ribosomal RNA	MZ540797	Ranjith et al., 2021	
	mtCOI	MW575256	Ashika and Venkatesan, 2021	
Coptotermes heimi	12S ribosomal RNA	KT828711, KT828711 KT828710, KT828709 KU665478	Mahapatro and Singh., 2016	
		KR078331	Mahapatro and Singh, 2015	
		KT820660	Mahapatro and Singh, 2015	
		EU553818	Sobti et al., 2008	
		EU553816	Sobti et al., 2008	
	16S ribosomal RNA	AY558908	Scheffrahn et al., 2004	
		MW680951	Kalleshwaraswamy et al., 2021	
		GQ422885	Salunke et al., 2009	
		GQ422882	Salunke et al., 2009	
		EU553817	Sobti et al., 2008	
	28S ribosomal RNA	JQ957911	Deivendran et al., 2012	
Coptotermes sjoestedti	16S ribosomal RNA	MN540914	Mahadeva swamy et al., 2019	
Coptotermes testaceus	16S ribosomal RNA	MK559593	Mahadeva Swamy et al., 2019	
		MK559592	Mahadeva Swamy et al., 2019	
		MK559591	Mahadeva Swamy et al., 2019	
		MK559590	Mahadeva Swamy et al., 2019	
Coptotermes sp.	mtCOII	MN913607	Tiwari et al., 2020	
Heterotermes balwanthi	16S ribosomal RNA	KU574658	Vidyashree et al., 2016	
Heterotermes indicola	12S ribosomal RNA	KT820657	Mahapatro and Singh., 2016	
		KF170428	Mahapatro and Kumar, 2013	
		KF769546	Poonia and Sharma, 2013	
		KM077441	Poonia and Sharma, 2014	
	16S ribosomal RNA	HF968496	Mahapatro, G.K. and Kumar, 2013	
		MZ183973	Kalleshwaraswamy et al., 2021	
		KF170427	Mahapatro and Kumar, 2013	
		KF769554	Poonia and Sharma, 2013	
Heterotermes malabaricus	16S ribosomal RNA	KU574645	Vidyashree et al., 2016	
		MZ540855	Ranjith et al., 2021	
		MZ558168	Santhrupthi et al., 2021	
		OK285073	Joseph and Mathew, 2021	
	mtCOII	OK284904	Joseph and Mathew, 2021	

(contd...)

Table 3 contd...

Hypotermes makhamensis	*Mt CoI*	KT898536 KT898532 KT898526 KT724954	Patel and Jadhav., 2015	This species is not reported from India (native to Thailand, Vietnam and Cambodia). Hence needs collection details and morphological authentication
	Mt CoI	KY614388	Murthy and Lubna., 2017	
Hypotermes xenotermitis	12S ribosomal RNA	KY825251	Murthy and Lubna, 2017	
	mtCOI	KU687341	Murthy et al., 2016	As per the authors, specimens were collected from south India. This species is not reported from south India (reported only from North India). Needs morphological authenticity and morphometry data.
		KY293420	Murthy and Lubna, 2016	
		KT224387	Murthy et al., 2016	
		KX646190	Murthy et al., 2016	
		KU687340	Murthy et al., 2016	
		KT898553 to KT898566	Patel and Jadhav, 2015	
		KT898535 to KT898551		
		KT898527 to KT898531		
		KT898507 to KT898525		
		KT887698 to KT887717		
		16.KT879833 to KT879849		
		KT879830, KT724955, KT898533		
Macrotermes convulsionarius	16S ribosomal RNA gene	MZ540857	Ranjith et al., 2021	
	28S ribosomal RNA	JQ957912	Deivendran et al., 2012	
Microtermes incertoides	16S ribosomal RNA	MZ571483	Ranjith et al., 2021	
Microtermes mycophagus	12S ribosomal RNA	KU665477	Mahapatro and Singh, 2016	
		KP765715	Mahapatro and Singh, 2015	
		KP748241	Mahapatro and Singh, 2015	
		KF769547	Poonia and Sharma, 2013	
		KT820658	Mahapatro and Singh, 2015	
		KM657479	Ramya et al., 2014	
		JX263668	Singla et al., 2012	
		JX045651	Singla et al., 2012	
		EU553821	Sobti et al., 2008	
		EU553819	Sobti et al., 2008	
	16S ribosomal RNA	KF769555	Poonia and Sharma, 2013	
		EU553822	Sobti et al., 2008	
		EU553820	Sobti et al., 2008	
Microtermes obesi	12S ribosomal RNA	EU551158	Sobti et al., 2008	
		KM657488	Ramya et al., 2015	
		KT820661	Mahapatro and Singh, 2015	
	16S ribosomal RNA	MZ558085	Santhrupthi et al., 2021	
		EU306616	Sobti et al., 2007	
		KU574654	Vidyashree et al., 2016	
	mtCOI	EU242522	Sobti et al., 2007	
	mtND1	EU306614 EU306613 EU306615	Sobti et al., 2007	

(contd...)

Table 3 contd...

Microtermes unicolor	12S ribosomal RNA	JX263667	Singla et al., 2012	
Microtermes sp.	ITS-2	KX495579	Murthy et al., 2016	
	16S ribosomal RNA	KM275840	Poonia and Sharma, 2014	
	12S ribosomal RNA	KF703855	Poonia and Sharma, 2013	
Odontotermes anamallensis	16S ribosomal RNA	MZ562513	Santhrupthi et al., 2021	
Odontotermes assmuthi	16S ribosomal RNA	KF769556	Poonia and Sharma, 2013	
		KU574651	Vidyashree et al., 2016	
		MZ558070	Santhrupthi et al., 2021	
	28S ribosomal RNA	JF792835	Deivendran and Suresh, 2011	
		FJ966379	Suresh et al., 2009	
Odontotermes bellahuniensis	16S ribosomal RNA	KU574650	Vidyashree et al., 2016	
Odontotermes bhagwatii	12S ribosomal RNA	KF769552	Poonia and Sharma, 2013	
		KM523663	Ramya et al., 2014	
		KM523662	Ramya et al., 2014	
		EU551161	Sobti et al., 2008	
	16S ribosomal RNA	KF769559	Poonia and Sharma, 2013	
		EU258632	Kumari et al., 2007	
		EU258631	Kumari et al., 2007	
	mtCOII	EU242525	Sobti et al., 2008	
	mtND1	EU262586	Sobti et al., 2007	
		EU262585	Sobti et al., 2007	
		EU262587	Sobti et al., 2008	
Odontotermes boveni	16S ribosomal RNA	MZ344981	Kalleshwaraswamy et al., 2021	
Odontotermes brunneus	12S ribosomal RNA	KT820659	Mahapatro and Singh., 2016	
	16S ribosomal RNA	KF792982	Poonia and Sharma., 2017	
		MZ540811	Ranjith et al., 2021	
	28S ribosomal RNA	JF792836	Deivendran and Suresh, 2011	
	12S ribosomal RNA	KF769549	Poonia and Sharma, 2013	
		JX263664	Singla et al., 20012	
Odontotermes ceylonicus	12S ribosomal RNA	KY908410	Murthy et al., 2017	
Odontotermes escherichi	12S ribosomal RNA	KY495155	Murthy and Lubna., 2017	Not reported from India (recorded only from Sri Lanka). Needs morphological authenticity
	mtCOI	KT224389	Murthy et al., 2015	
		KY495154	Murthy and Lubna., 2017	
Odontotermes feae		KU947966	Mahapatro and Singh, 2016	
	12S ribosomal RNA	KY908402	Murthy et al., 2017	
		KY676779	Murthy and Lubna, 2017	
		KY908403	Murthy et al., 2018	
		KR296660	Mahapatro et al., 2015	
	16S ribosomal RNA	KU574649	Vidyashree et al., 2016	
Odontotermes gurdaspurensis	12S ribosomal RNA	KM523667	Ramya et al., 2015	
		KM523664		

(contd...)

Table 3 contd...

Odontotermes horni	12S ribosomal RNA	EU551159	Sobti, et al., 2008	
		GQ422892	Salunke et al., 2009	
		GQ422890	Salunke et al., 2009	
		GQ422889	Salunke et al., 2009	
		GQ422888	Salunke et al., 2009	
	16S ribosomal RNA	GQ422887	Salunke et al., 2009	
		GQ422886	Salunke et al., 2009	
		GQ422879	Salunke et al., 2009	
		EU258629	Kumari et al., 2007	
		KU574646	Vidyashree et al., 2016	
		EU258630	Sobti et al., 2007	
	28S ribosomal RNA	JF792837	Deivendran and Suresh, 2011	
	mtCOII	EU242523	Sobti, et al., 2007	
Odontotermes obesus	12S ribosomal RNA	KY908407	Murthy et al., 2017	
		KP410731	Mahapatro et al., 2015	
		EU551160	Sobti et al., 2008	
	16S ribosomal RNA	KU574648	Vidyashree et al., 2016	
		MZ423304	Kalleshwaraswamy et al., 2021	
		MN511317	Amina etal., 2019	
	mtCOI	KY474376	Murthy and Lubna, 2007	
		MZ823814	Ranjith et al., 2021	
	mtCOII	EU242524	Sobti, et al., 2007	
	mtND1	EU262594	Sobti, et al., 2007	
Odontotermes longignathus	*Mt CoI*	KY930907; KY930908; KY775488	Murthy and Lubna., 2019	This species is not reported from India (native to south east Asia). Needs morphological authenticity
	12S ribosomal RNA gene	KY495156; KY563712	Murthy and Lubna., 2017	
	Mt CoI	MN205551	Alina et al., 2019	
Odontotermes parvidens	12S ribosomal RNA	KF769551	Poonia and Sharma, 2013	
	16S ribosomal RNA	KF769558	Poonia and Sharma, 2013	
Odontotermes redemanni	12S ribosomal RNA	KF769553	Poonia and Sharma, 2013	
	16S ribosomal RNA	KU574647	Vidyashree et al., 2016	
		KF792983	Poonia and Sharma., 2017	
Odontotermes wallonensis	28S ribosomal RNA	JF792838	Deivendran et al., 2012	
Odontotermes yadevi	16S ribosomal RNA	KU574656, KU574655 MZ189521	Vidyashree et al., 2016 Kalleshwaraswamy et al., 2021	
Euhamitermes hamatus	12S ribosomal RNA	KM657484	Ramya et al., 2015	Not reported from India (recorded only from Thailand, Malaysia, Singpur, Bangladesh). Needs morphological authenticity
Eurytermes buddha	16S ribosomal RNA	MW678776	Kalleshwaraswamy et al., 2021	
	mtCOI	MW664866	Kalleshwaraswamy et al., 2021	

(contd...)

Table 3 contd...

Ampoulitermes wynaadensis	12S ribosomal RNA	MZ044682	Kalleshwaraswamy et al., 2021	
		MZ044681	Kalleshwaraswamy et al., 2021	
Grallatotermes niger	16S ribosomal RNA	MZ262750	Kalleshwaraswamy et al., 2021	
Nasutitermes anamalaiensis	16S ribosomal RNA	KU574659	Vidyashree et al., 2016	
		MW692352	Kalleshwaraswamy et al., 2021	
		MW694353	Kalleshwaraswamy et al., 2021	
Nasutitermes brunneus	16S ribosomal RNA	MZ262702	Kalleshwaraswamy et al., 2021	
		MZ540856	Ranjith et al., 2021	
Nasutitermes indicola	16S ribosomal RNA	KU574660	Vidyashree et al., 2016	
Nasutitermes kali	16S ribosomal RNA	MZ262715	Kalleshwaraswamy et al., 2021	
Nasutitermes octopilis	12S ribosomal RNA gene	KM657478	Ramya et al., 2015	Not reported from India (recorded only from Africa-Guyana). Needs morphological authenticity
Trinervitermes_ biformis	16S ribosomal RNA	KU574657	Vidyashree et al., 2016	
		MZ558074	Santhrupthi et al., 2021	
Trinervitermes togoensis	12S ribosomal RNA gene And MtCo1	KY569523		This species is not reported from India (native to Africa). Needs morphological authenticity.
		KX711183	Murthy and Lubna., 2017	
			Murthy etal., 2016	
Amitermes belli	12S ribosomal RNA	KR078330	Mahapatro and Singh., 2015	
	16S ribosomal RNA	MZ269706	Priya and Gupta, 2021	
Angulitermes sp.	12S ribosomal RNA	KP780274	Mahapatro and Singh, 2015	
Dicuspiditermes achankovili	mtCOI	MT272760	Amina et al., 2020	
		MT272755	Amina et al., 2020	
		MT272750	Amina et al., 2020	
Dicuspiditermes fontanellus	16S ribosomal RNA	MZ270643	Kalleshwaraswamy et al., 2021	
Dicuspiditermes gravelyi	12S ribosomal RNA	MZ825163	Ranjith et al., 2021	
		MZ825164	Ranjith et al., 2021	
	16S ribosomal RNA	MZ270644	Kalleshwaraswamy et al., 2021	
		MZ823812	Ranjith et al., 2021	
Dicuspiditermes obtusus	16S ribosomal RNA	MZ270642	Kalleshwaraswamy et al., 2021	
Homallotermes pilosus	mtCO1	MT272758	Amina et al., 2020	
		MT272753	Amina et al., 2020	
		MT272747	Amina et al., 2020	
Indocapritermes aruni	mtCOI	MT272742	Amina et al., 2020	
Krishnacapritermes thakuri	mtCOI	MT272759	Amina et al., 2020	
		MN507713 to MN507729	Amina et al., 2019	
Krishnacapritermes dineshan	mtCOI	MN507708 to MN507712	Amina et al., 2019	

(contd...)

Table 3 contd...

Labiocapritermes distortus	16S ribosomal RNA	MZ558073	Santhrupthi et al., 2021	
	mtCOI	MT272756	Amina et al., 2020	
		MT272752	Amina et al., 2020	
		MT272744	Amina et al., 2020	
		MT272743	Amina et al., 2020	
Microcerotermes beesoni	12S ribosomal RNA	JX263665	Singla et al., 2012	
Microcerotermes fletcheri	16S ribosomal RNA	KU574652	Vidyashree et al., 2016	
Microcerotermes pakistanicus	16S ribosomal RNA	KU574653	Vidyashree et al., 2016	
		MZ414220	Kalleshwaraswamy et al., 2021	
		MZ427480	Kalleshwaraswamy et al., 2021	
Pericapritermes topslipensis	mtCOI	MT272762	Amina et al., 2020	
		MT272761	Amina et al., 2020	
		MT272749	Amina et al., 2020	
		MT272745	Amina et al., 2020	
		MT272741	Amina et al., 2020	
Procapritermes keralai	mtCOI	MT272748	Amina et al., 2020	
		MT272757	Amina et al., 2020	
Pseudocapritermes fletcheri	16S ribosomal RNA	MW686909	Kalleshwaraswamy et al., 2021	
		MW687081	Kalleshwaraswamy et al., 2021	
	mtCOI	MT272754	Amina et al., 2020	
		MT272751	Amina et al., 2020	
		MT272746	Amina et al., 2020	
		MW672524	Kalleshwaraswamy et al., 2021	
Synhamitermes quadriceps	16S ribosomal RNA	MW680954	Kalleshwaraswamy et al., 2021	
	mtCOI	MW680303	Kalleshwaraswamy et al., 2021	
Neotermes koshunensis	12S ribosomal RNA gene	KM657485	Ramya et al., 2015	Not reported from India (recorded only from Japan). Needs morphological authenticity
Rinacapritermes abundans	mtCOI	MT274296	Amina et al. 2022	
Rinacapritermes silvius	mtCOI	MT274294	Amina et al. 2022	

C. travians are doubtful. Among the remaining five, taxonomic validity of *C. beckeri*, *C. ceylonicus* and *C. kishori* is uncertain, because they are possibly junior synonoms of *C. amanii*, *C. brunneus* and *C. kalshoveni* (Chouvenc et al., 2016). Among these, *C. amanii* is of African origin, *C. brunneus* is of Australian origin and *C. kalshoveni* is a serious nuisance organism in Indonesia and Malaysia. This example may throw light on the need for an integrated taxonomy of the Indian Isoptera. There is a greater need for extensive collection, morphological and molecular characterization of Indian Isoptera.

H. Threats to biodiversity

The ecological and economic importance of the Isoptera is extensive. The most positive environmental relevance is their role as soil engineers. The most important negative relevance is their activity as nuisance organisms since they damage built structures and agricultural crops, a majority of them belonging to the Termitidae, Rhinotermitidae, and Kalotermitidae (Jouquet et al., 2018). In recent decades the tendency of expansion of the range of harmful Isoptera has led to an increase in economic damage.

Invasive termites spread with infested timbers and invade human environments, before spreading into natural ecosystems. A recent study utilized occurrence data and climate modeling to predict the potential habitats of *C. formosanus* and *C. gestroi* in Florida and demonstrated that future distribution projections for both species are influenced by urban development

and climate change (Buczkowski and Bertelsmeier, 2017). Another negative outcome of increased Isoptera invasions is a potential increase in pesticide use in urban and natural landscapes. Although chemicals are hazardous to the environment, farmers and structural engineers all over the world use them extensively for the management of agricultural pests and in structures. Termites are eusocial, live in nests constructed with well protected soil or many inches below the soil surface and move in galleries which themselves protected from outside threats hence management of termites with termiticides is a difficult task. However, some termiticides such as imidacloprid, chlorpyrifos, fipronil, spinosad, chlorfenapyre, bifenthrin, cypermethrin, permethrin, disodium octaborate tetrahydrate, calcium arsenate, lindane, endosulfan, and chlorantraniliprole have been used worldwide for the management of termites (Ahmad et al., 2019). Leaching of termiticide is toxic to non-target organisms, due to indirect accumulation, which simultaneously affects human population through food chains (Arias-Estévez et al., 2008).

The known 28 invasive species have the potential to expand in the range of distribution, just as 10 of the 17 known invasive species have expanded between 1969 and today (Table 1). The spatial spread of the invasive Isoptera is a consequence of a combination of intrinsic and extrinsic factors that shape the population dynamics of the involved species. Intrinsic factors include dispersal, growth, survival, and reproductive constraints dictated by the species' physiological capabilities. Extrinsic factors include factors such as the spatial and temporal availability of suitable habitat for survival, growth, and reproduction. Human-induced environmental changes, most notably climate change and urbanization, are likely to affect both intrinsic and extrinsic factors. For example, invasive termites have been shown to adapt their reproductive phenology in response to climate change. In parts of Florida, the dispersal flight season of *C. formosanus* and *C. gestroi* has begun to overlap due to changes in local climate. Mating pairs of heterospecific individuals were observed in the field with *C. gestroi* males preferentially engaging in mating with *C. formosanus* females rather than females of *C. gestroi*. This leads to hybridization between the two species and the potential evolution of highly destructive "super-termites" due to hybrid vigour (Chouvenc et al., 2016).

I. Management of invasive termites

In most cases of invasive Isoptera, attempts were made to eradicate them applying synthetic pyrethroids and organochlorine insecticides, and by baiting. Baiting has been a successful tactic using area-wide management strategy. However, many attempts to eradicate the invasive Isoptera have proved futile (Bravey and Verkrek, 2010). Thus, only instances of successful management refer to those of *C. formosanus* in South Africa and *C. frenchi* in New Zealand (Chouvenc et al., 2016). Therefore, monitoring their entry and successful management are the viable options presently.

At the time of shipment, log fumigation must reduce the risk of introductions. It is possible to use a less environmentally harmful fumigant like sulfuryl fluoride. A strict policy for the importing country and strict monitoring of imported material could minimize the intrusion into a new geographical region, reducing both economic and ecological risks. Imported wood should be fumigated with methyl bromide @ 48g/ m^3 for 24 h to destroy infestation to avoid their entry (https://plantquarantineindia.nic.in/ accessed on 10 December 2021). The treatment is as per the Plant Quarantine (Regulation of Import into India) Order, 2003 which regulates import and prohibition of import of plant and plant products into India and amended recently (S.O. 3686 (E), dated 9 September 2021). The treatment should be endorsed on Phytosanitary Certificate issued at the country of origin or re-export (https://plantquarantineindia.nic.in/ accessed on 10 December 2021).

CONCLUSIONS

Tropical, subtropical conditions of India are highly congenial for the rapid multiplication of termites. If any species is introduced accidentally, there is every chance of successful establishment. Hence monitoring, observation, reporting their identity and intervention to avoid the establishment is the need of the hour. Training and recruiting taxonomists with a centralized diagnostic centre is one possible solution to strengthen the activity of the quarantine stations.

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EMERGING PHYTOPLASMA DISEASES IN FORESTRY AND THE ROLE OF DETERMINING THE INSECT VECTORS FOR THE MANAGEMENT OF THE DISEASES#

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ABSTRACT

Pathosystem associated with phytoplasmas in forest plants is an emerging threat to forest productivity and biodiversity. Diseases caused by or associated with phytoplasmas occur in hundreds of commercial and native plants, causing minor to extensive damage. Phytoplasmas are wall-less, phloem-limited, insect-transmitted, plant pathogenic bacteria that are transmitted by phloem-sap feeding insects of the order Hemiptera under the families such as Cicadellidae, Delphacidae, Cixiidae, Derbidae, Psyllidae and rarely by Pentatomidae and Tingidae. Disease transmission occurs very quickly, often plants become infected before adopting any management strategies. The single most effective management practice of managing the vector in agricultural/horticultural crop is to cover plants with insect exclusion netting but this is not feasible and practical in the case of forest trees. Because of these limitations, researchers are shifting to manipulation genetics in host plants to affect vector populations and transmission of pathogen but big lacunae exists in our knowledge on the vectors of phytoplasma diseases in Indian forestry. We review the literature addressing the phytoplasma diseases in forest species, the lacunae existing in the insect vectors and the need for determining the vectors for possible management of phytoplasma diseases in forest trees.

Key words: Hemipteran vectors, forest species, symptoms, management

Yellow diseases of trees and shrubs are among the most important diseases in forests as they cause very extensive and serious damage in perennial crops, particularly in woody plants. Such plants if infected once, the pathogen is carried throughout the life of the host plants. More than 60 forest species, trees, shrubs and herbs have been reported infected by the pathogens causing yellows and witches' broom (Nayar, 1988b). In early years, the causative organisms are termed as Mycoplasma-Like Organisms (MLOs) but presently they are classified as Phytoplasmas and are a large group of obligate, endophytic, cell wall-less bacterial parasites classified within the class Mollicutes (Wei et al., 2007). Pathosystem associated with phytoplasmas in forest trees is an emerging threat to forest productivity and biodiversity. They are fastidious prokaryotes that can survive and multiply only in hypotonic habitats such as plant phloem or insect haemolymph and they are strictly host dependent. They are known to be pathogenic to more than 300 plant species (Bertaccini et al., 2014). In India presently more than 172 plant species are reported to be the host of ten different groups of phytoplasmas associated with plants including vegetables, fruits, trees, ornamentals, sugarcane, palms, oil crops and weeds are increasing at an alarming rate (Rao, 2021). Phytoplasmas are mainly transmitted by phloem- sucking leafhopper species, primarily belonging to the three families, Cicadellidae, Cixiidae, and Psyllidae (Weintraub and Beanland, 2006). The distribution of phytoplasma diseases in different geographical areas and their impact depends on the host range of the phytoplasma as well as the feeding behaviour of the insect vectors. Phytoplasmas are complex syndrome with symptoms such as phyllody, forming sterile deformed flowers, proliferating auxiliary shoots, virescence and stunting in several hundreds of plant species (Lee et al., 2000). The major phytoplasma diseases causing severe economic losses in the country are sesame phyllody, brinjal little leaf, sugarcane grassy shoot, sandal spike, coconut root wilt and areca nut yellow (Rao et al., 2017). So far ten 16Sr groups of phytoplasmas have been identified in different plant species. The biology, ecology, vector interaction, and epidemiology of crop diseases caused by phytoplasmas, are reviewed extensively in recent years by Christensen et al. (2005), Weintraub and Beanland (2006),

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Bertaccini (2007), Rao et al. (2017), Marcone (2021) and Rao (2021). For many phytoplasma diseases in India that are known more than a century such as sandal spike disease, coconut root (wilt) and areca nut yellow leaf diseases, information on their confirmed insect vectors are not known, only putative vectors are known so far. Though insects remain the most significant vectors of these organisms, unfortunately, information on them as vectors of phytopathogens in universal and phytoplasmas in particular is meager. The significance of phytoplasma diseases in important forest species and necessitate to investigate their insect vectors are discussed in detail in this manuscript.

Phytoplasma diseases in forest plant species

Nayar (1988a; 1988b) reported phytoplasmas in more than 60 forestry plant species, including 19 economically important species (Table 1). Among the listed ones, four species belongs to the family Fabaceae, two each in the family Arecaceae, Poaceae, Rhamnaceae and Santalaceae. Remaining families viz., Acanthaceae, Lamiaceae, Meliaceae, Myrtaceae, Sapindaceae and Rubiaceae are represented by each one species.

Sandal spike disease (SSD) of Indian sandalwood

Indian sandalwood (*Santalum album* Linn.) is a hemiparasitic, perennial tree belonging to the family Santalaceae. It possesses a highly valuable wood, known for its scented oil and carving and which is acknowledged as "*Royal Tree*" in Indian continent is one of the most economically important tree species (Sundararaj, 2008). In India, sandal is found mainly in the Deccan Plateau and its extension and in small numbers in almost all parts except the Himalayas. Large natural stand of sandal occurs in Karnataka (5,245 km^2) followed by Tamil Nadu (3,040 km^2) accounting for nearly 90% of sandal in India (Venkatesan, 1981). The price of Indian sandalwood and its oil have risen significantly in the past decade mainly due to depletion in production annually at the rate of 20% since 1995 (Ananthapadmanabha, 2000). India's production during 1930s to 1950s was around 4000 tons of heartwood per year which had decreased to an average of 300 tons per year. Correspondingly the wood price from 1996 to 2012 has increased in arithmetic progression reached Rs. 60,00,000 per ton in 2013. It has impacted the oil export as the maximum quantity of 27,930 Kg sandalwood oil exported in the year 1997-98 has reduced to 2,330 kg in 2019-20 (Export-Import Data Bank V.7.1 Trade Stat, 2021).

The decline in sandalwood production is mainly due to the depletion of sandalwood trees in its natural habitats of forest. The major reasons for this reduction is illicit felling and smuggling and the other reasons being the impact of pests and disease (Rao et al., 1999). They cause serious disease of sandalwood, Sandal Spike Disease (SSD) known from 1850 in Karnataka state, caused havoc in the production of sandalwood (Nayar, 1988a) and are one of the major causes for the

Table 1. Phytoplasma diseases associated with important forestry plant species of India (From Nayar, 1988b)

Sl. No.	Tree/Shrub species	Family
1	*Barleria mysorensis* Roth.	Acanthaceae
2	*Areca catechu* L.	Arecaceae
3	*Phoenix sylvestris* (L.) Roxb.	Arecaceae
4	*Acacia catechu* Wild	Fabaceae
5	*Acacia intsia* Willd	Fabaceae
6	*Dichrostachys cinerea* W.et.A.	Fabaceae
7	*Pongamia pinnata* (L.) Pierre	Fabaceae
8	*Gmelina* sp.	Lamiaceae
9	*Cedrela toona* M. Roem	Meliaceae
10	*Eucalyptus* spp.	Myrtaceae
11	*Ochlandra travancorensis* (Bedd.) Benth. ex Gamble	Poaceae
12	*Dendrocalamus strictus* (Roxb.)	Poaceae
13	*Scutia indica* Bronga	Rhamnaceae
14	*Ziziphus oenoplia* (L.)	Rhamnaceae
15	*Randia candolleana* W.et A.	Rubiaceae
16	*Dodonea viscose* (L.) Jacq.	Sapindaceae
17	*Ozyris* sp.	Santalaceae
18	*Santalum album* L.	Santalaceae

decline in sandalwood production as the quantum of trees extracted due to SSD is enormous. Over a million of sandalwood trees had been removed in Coorg and Mysore (Karnataka) during 1903-1916 (Mc Carthy, 1899; Hodgson, 1918; Coleman, 1923; Rangaswami and Sreenivasya, 1934) and 98,734 trees during 1917-1925 in Salem (Tamil Nadu) alone due to SSD (John, 1957). In Karnataka, the growing stock has been condensed to 25% of its initial level in the last two decades of 20th century (Swaminathan et al., 1998). The disease is not known in Timor and does not affect other species of *Santalum* (Fox et al., 1994). All these mass extractions due to SSD in natural habitats pushed the sandalwood to be first classified as vulnerable by the International Union for Conservation of Nature in the year 1998. Even in the global scenario, sandalwood has developed into a commercial timber crop over the past 10 to 15 years, with substantial sandalwood plantations established in India, China and Australia, and more modest plantings being established in Fiji, Vanuatu, Hawaii and Indonesia. In this context, it is key note that the SSD is so far reported only from India and the research on SSD in India is more than 50 years old, Realizing the sharp decline in the sandalwood population in the states and having realized that they cannot be the custodian of sandalwood any more, Karnataka and Tamil Nadu forest departments have amended the Sandalwood act in 2001 and 2002, respectively and made growers themselves as the owners of the sandalwood as per the Amended Acts. The purpose of the amendment is to encourage farmers and corporate bodies to grow sandalwood plantations to meet the growing demand (Gowda et al., 2008). This is encouraging the farming community and private entrepreneurs to cultivate sandalwood in agro-forestry, farm forestry and varied agri-silvi-horticultural and mixed plantation systems as per their choice. Its hemiparasitic nature makes it suitable for tree species in any agro-forestry conditions (Sundararaj et al., 2019).

Causative agents of sandal spike disease

SSD is the most serious disease of *S. album* which is characterized by extreme reduction in the size of leaves and internodes accompanied by stiffening of the leaves. In advanced stage, owing to the progressive reduction in leaf size and internodes, the whole shoot looks like a 'spike inflorescence'. Though it was known from 1850 in Karnataka, it was first reported in Coorg, South India (Karnataka) in the year 1899 (Mc Carthy, 1899). It was initially considered as viral disease and then as the disease caused by Mycoplasma Like Organisms (MLO) (Parthasarathi et al., 1966; Hull et al., 1969), and presently as Phytoplasma disease. At present, natural populations of Sandalwood are mainly available in Marayur in Kerala and some patches of reserve forests of Karnataka. Recently it is confirmed that in these natural habitats the trees are heavily infected with SSD associated with *Candidatus* Phytoplasma asteris (Kirdat et al., 2019) and 1 to 5% trees are dying annually (Figs. 1-4). The evidence suggests that only 18 months is enough to cause the death of a sandalwood tree by SSD and it kills even very young naturally regenerating trees before reaching the age of heartwood formation. Disease incidences reaching up to 33 and 55% were recorded in southern Karnataka (Murali et al., 2019).

Rising threat of sandal spike disease

The situation in Marayur sandalwood reserve which had a density of about 1000 sandal trees per hectare (Nayar, 1988b) was considered relatively free from SSD till 1980 (Prasad, 2011). It recorded 21.4% density of sandalwood in 2005-06 (Sundararaj and Sharma, 2010) which is now reduced to 1.92% mainly due to the death of trees by SSD and consequent extraction on annual basis. Similar situation of dying of naturally regenerating trees happens in the natural habitats of sandalwood in Karnataka and the sandalwood plantations raised by the Karnataka Forest Department. The potential phase of spreading of the infection is primarily due to the fact of restriction on green felling in forests, which allow the infected tree to serve as inoculums till it dies and spread the phytoplasma pathogen to the maximum extent to the healthy trees by the potential vectors.

Phytoplasma diseases in bamboos

Bamboos are interesting plants that profusely grow in the tropical and subtropical zones and they form the most important non-woody species (Naithani, 1993). They represent the world's best natural and renewable resources with exceptionally diverse group of species (Seethalakshmi et al., 2008). There are more than 1250 species of bamboo under 75 genera worldwide, which are unevenly distributed in various parts of the humid tropical, sub-tropical and temperate regions of the earth (Subramaniam, 1998). A total of approximately 148 species in 29 genera of bamboos currently occur in India (Sharma and Nirmala, 2015). Bamboos form an important group of tropical woody monocotyledons that have been traditionally used by people in Asia for a wide variety of purposes and they have been regarded as the major resource that meets the multiple requirements of common people and also a poverty alleviator (IFAD, 2014). It is estimated that about 8.6 million people depend on bamboo for their livelihood in India (FSI, 2019). There is a growing trend of using bamboo in



Figs. 1-2. Sandalwood trees in various stages SSD in Marayur sandalwood reserves



Fig. 3- 4. Drying and dyeing of sandalwood trees due to SSD at Marayur sandalwood reserves

rural and urban areas of the country as a widespread, renewable, productive, versatile, low cost or no-cost, easily accessed, environment-enhancing resource. The importance of bamboo as a resource for livelihood development and alleviation of both environment and social problems is increasing (Singh et al., 2017). Many diseases such as leaf spot disease, leaf blight, and seedling wilt in nurseries, bamboo mosaic disease, witches' broom, little leaf disease, and emerging culm rot affect the bamboos in plantations (Harsh et al., 2005). Among these diseases, the little leaf disease is caused by phytoplasma and it is known to infect *Dendrocalamus strictus* (Yadav et al., 2015), *Bambusa bambos* (L.) Voss, *D. stocksii* (Munro.) and *D. asper* (Schult.) Baker (Mondal et al., 2019). The infected bamboos show typical disease symptoms of witches' broom with severe proliferative branching at nodal regions and reduction in the size of the leaf. The leaves dry prematurely and look burnt in appearance. The affected culms bend due to higher weight and then dry (Figs.5-7). It attracts the infestations of termites besides such dried culms may catch fire. *Ca* P. aurantifolia was known to cause the symptom of witches' broom disease in bamboos; however, so far no information is available on its insect vectors.

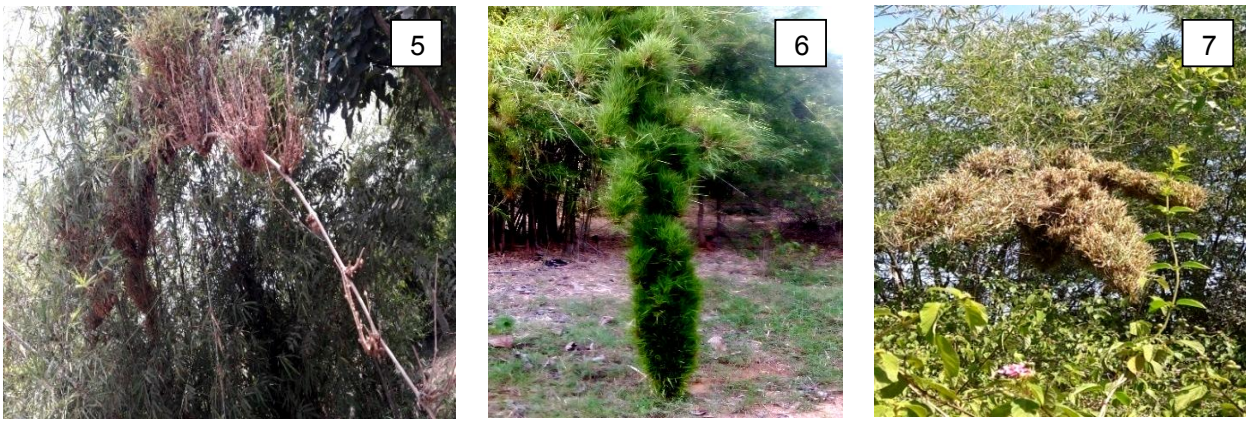
Phytoplasma diseases in other forestry tree species

Phytoplasma disease is also recorded in several Eucalyptus species. This is also called as Eucalyptus Little-Leaf (ELL). It was first observed in India in the year 1971 and was thought to be caused by a virus (Sastry et al., 1971). The disease was found to be induced by *Frankliniella occidentalis* (Pergande). Based on electron microscope observations of diseased eucalyptus trees, it was later proved as phytoplasma infection (Marcone, 2015). The symptoms described from various geographical regions and different *Eucalyptus* spp. are similar. They include small leaves, pale narrow lamina (little leaf), yellowing, and proliferation from axillary buds that confers a broom-like appearance and wilting of foliage. Diseased trees or their symptomatic parts are stunted with die-back symptoms, do not set fruits, and severely affected trees die. A 16SrV phytoplasma was identified in ELL-affected trees in Italy (Marcone et al., 1996).

Recent studies of SSD in Marayur sandalwood reserves in Kerala State exposed the presence of phytoplasma diseases in many trees like ficus (*Ficus* sp.) (Fig. 8), wild ber (*Zizyphus oenoplia*) (Fig. 9) and wild amla (*Emblica officinalis*) (Fig. 10). Similarly cutch tree (*Acacia catechu*) (Fig. 11) was found associated with phytoplasma symptoms in wesern parts of India. Phytoplasmas are harboured by weeds which may act as alternative natural hosts facilitating their spread to economically important plants and thereby increasing economic losses. About 43 weed species are also reported to be the hosts of phytoplasmas (Rao, 2021).

Management of phytoplasma diseases

Among the forestry plant species reported, utmost importance to be given to developing protocols for effective management of SSD as sandalwood is on the vulnerable list of the International Union for Conservation of Nature. No effective control measure of spike disease has so far been evolved. However, attempts have been made in sandalwood plantations on the following lines, where at the early stage of incidence spread of the disease may be lessened to some extent by eliminating the source of infection. Kristensen (1960) in his report, submitted to the Government of India for the control of SSD has made certain proposals under 3 headings viz.,



Figs. 5-7. Bamboos showing different symptoms of Witches' broom disease

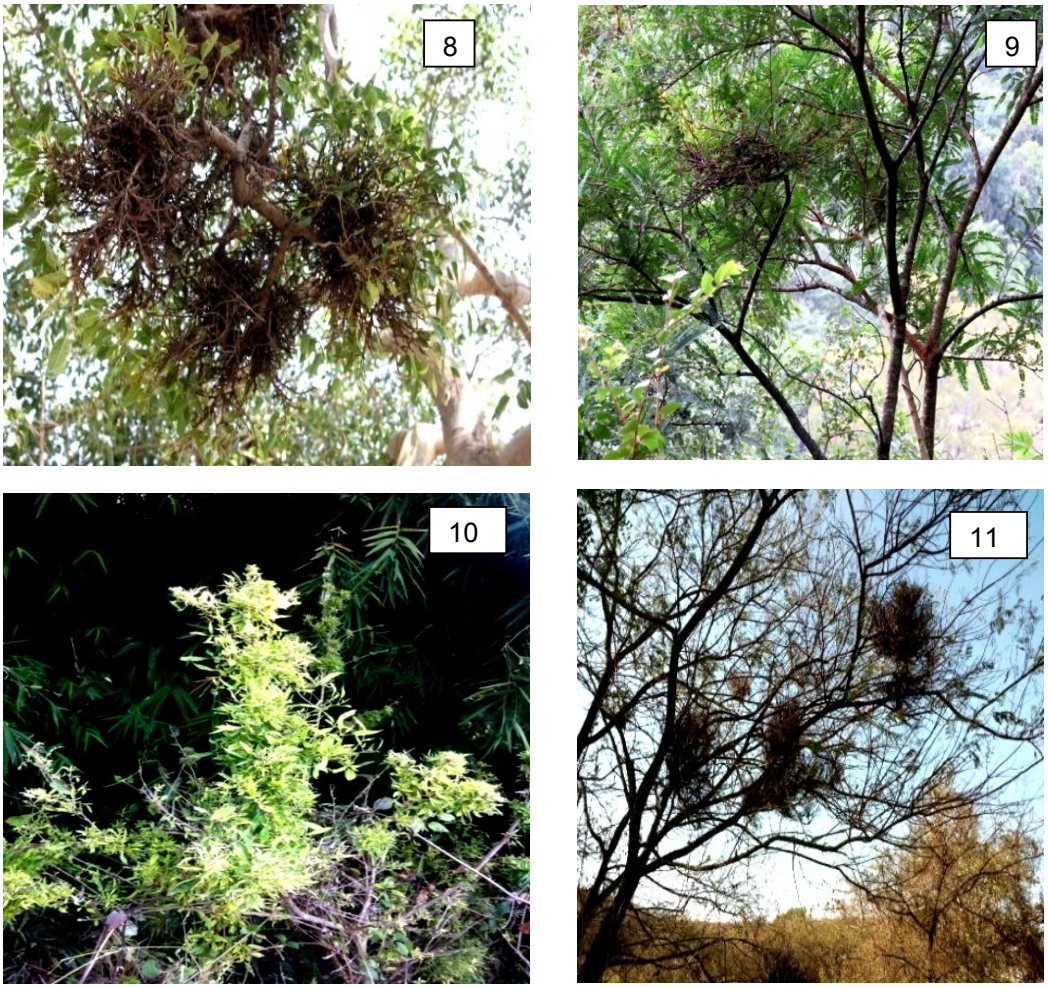


Fig. 8-11. Symptoms of phytoplasma diseases in forest plants. 8. *Ficus* sp.; 9. *Zizyphus oenoplia;* 10. *Emblica officinalis;* 11. *Acacia catechu.*

(i) Diagnostic work: Indexing of various test plants by various transmission methods, and serological work, (The main idea is to detect virus – infected plants and remove the source of infection as early as possible). Accurate diagnosis is necessary for any successful disease control. However, diagnosis of diseases caused by plant molecule has often been one of the difficult aspects. This is primarily due to the lack of methodologies for detecting the pathogens in the field or in quarantine plants (Davis and Lee, 1988). Detection and identification of phytoplasma using molecular techniques like Polymerase Chain Reaction (PCR) has become popular because of the high sensitivity of the test. PCR is preferred in situations where the concentration of phytoplasma is very low.

(ii) Transmission studies: Insect transmission studies to help in the identification of the insect vectors of spike disease. Identification of phytoplasma vectors requires the proof of successful transmission of the pathogens since non-vector species are able to acquire detectable quantities of phytoplasma from infected plants. However, transmission experiments on plants are time consuming and laborious. An alternative technique was developed recently to determine the inoculative ability of potential vectors. (Tanne et al., 2001).

(iii) Control measures: This includes removal of sources of infection, protection against possible vectors, selection and testing of apparently resistant mother-trees of sandalwood. Among these recommendations it is demoralizing that overshadowing all other recommendations only identification of infected plants and their extraction was done because of the financial returns. Considering the impact of SSD in the past, it is imperative to protect the sandalwood populations in their natural habitats from SSD and contain its spread in the farmer's field before it causes colossal damage.

Unlike other crops, the loss of a single tree is crucial for the sandalwood growers as every plant needs to be survived for better monetary returns. This will also guarantee the regaining of the lost glory of the Royal tree of India.

The other forestry important plant species affected by phytoplasmas are Bamboos which are of vital importance from ecological, commercial, and socio-economic points of view. They have more than 1500 documented uses and are widely used for paper and rayon manufacture, construction, architecture, engineering, handicraft, food and medicine. Bamboo forest constitutes about 13% of the total forest area of our country. The bamboos being C-4 plants absorb more carbon-dioxide from the atmosphere and give out oxygen at a faster rate and are better candidates for mitigating climate change. Roots are highly capable of VAM infection, and consequently improve soil conditions, and leaves absorb metals like Fe and Zn. In different tropical forests of the country, bamboos are found to occur widely which apart from being a source of livelihood for forest-dependent population and industry it plays an ecological role in sustainable forest management. Hence, protection of bamboo from phytoplasma diseases requires greater attention.

Importance of vector management for the control of phytoplasma diseases

Phytoplasma-induced diseases in agricultural/horticultural crops are controlled by proper management of insect vectors, removal of infected plants from the field, and by application of plant growth-promoting microbes that not only enhance nutrient levels but also provide protection against microbial and insect vectors (Trivedi et al., 2016). However, so far no potential vector of SSD has been confirmed by applying molecular tests and phytoplasma transmission assays though many insect vectors are suspected to be the transmitters of SSD (Table 2). Unconfirmed transmission of the spike disease by many insects was reported but the validity of the transmission appears doubtful. The disease is known to be transmitted from diseased sandal trees to healthy ones by bud, bark and twig grafting between stems of healthy plants. The idea that vectors transmitted the disease causing agent in nature occurred to early workers when they temporarily prevented the diseases spread by clearing a belt of sandal and *Zizyphus oenoplea* (Coleman, 1917) or when they observed infection in trap plants (Sreenivasaya and Rangaswami, 1934). Prompted by this success, researchers conducted elaborate studies of insect fauna that inhabited sandalwood between 1933 and 1937, and more than 200 species of probable vectors were tested for their ability to transmit spike disease. Hence, studies on vectors to provide conclusive explanation on phytoplasma-vector relationships, under different environmental and farming conditions were inconclusive. Similarly effective management strategies for phytoplasma epidemics are also lacking. Considerable progress has been made in the detection of vectors of phytoplasma diseases of trees outside India (Table 3), such progress is yet to be achieved in the context of Indian forests. Until recently, management of plant diseases caused by phytoplasma was focused on controlling the vector by insecticides, as it is feasible in agricultural/horticultural crops though it is costly, and also not ecologically-sound or economically-sustainable. However, it is practically impossible in forest environment. Reducing the alternative host plants

Table 2. Putative insect vectors of sandal spike disease in *S. album*

Scientific Name	Order: Family	Reference
Coelidia indica (Walker)	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Olidiana kirkaldyi (Nielson)	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Moonia albimaculata Dist.	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Nephotettix virescens (Dist.)	Hemiptera: Cicadellidae	Sivaramakrishan and Sen-Sarma (1977)
Redarator bimaculatus Distant	Hemiptera: Issidae	Balasundaran and Muralidharan (2004)
Jassus indicus (Walker)	Hemiptera: Cicadellidae	Rangaswami and Griffith (1941)
Indomias cretaceous (Faust)	Coleoptera: Cucurlionidae	Hart and Rengaswamy (1926)
Eurybrachys tomentosa (Fab.)	Hemiptera: Eurybrachydae	Chatterjee (1933a)
Coccosterphus tuberculatus Motsch.	Hemiptera: Membracidae	Chatterjee (1939)
Nezara viridula L.	Hemiptera: Pentatomidae	Chatterjee (1939)
Petalocephala nigrilinea Walk.	Hemiptera: Cicadellidae	Chatterjee (1934)
Sarimanigro clypeata Melichar	Hemiptera: Fulgoridae	Chatterjee (1933b)
Coccids	Hemiptera	Chatterjee and Ayyar (1936)

Table 3. Putative insect vectors of phytoplasma diseases in forest trees of outside India

Host plant	Scientific Name	Order: Family	Reference
Paulownia spp.	*Halyomorpha halys* Stal	Hemiptera: Cicadellidae	Hiruki (1999)
Hovenia tomentella L.	*Hishimonus sellatus* (Uhler)	Hemiptera: Cicadellidae	Kusunoki et al. (2002)
Ulmus minor and *Ulmus pumila*	*Macropsis mendex* Lewis	Hemiptera: Cicadellidae	Carraro et al. (2004)
Ulmus sp.	*Scaphoideus luteolus* Van Duzee	Hemiptera: Cicadellidae	Hart (1978)
	Philaenus spumarius L.	Hemiptera: Cercopidae	
	Allygus atomarius Fab.	Hemiptera: Cicadellidae	
	Macrosteles quadrilineatus (Fascifrons)	Hemiptera: Cicadellidae	
Alnus glutinosa (L.)	*Oncopsis alni* (Schrank)	Hemiptera: Cicadellidae	Maixner and Reinert (1999)
Eucalyptus spp.	*Frankliniella occidentalis*	Thysanoptera: Thripidae	Marcone, 2015).
Fraxinus sp.	*Oncopsis alni* (Schrank)	Hemiptera: Cercopidae	(Maixner and Reinert, 1999).
	Orientus ishidae (Mastumura)	Hemiptera: Cicadellidae	
	Allygus mixtus Fab.		
	Allygus modestus Scott	Hemiptera: Cicadellidae	
	Psylla alni (Linn.)	Hemiptera: Cicadellidae	
		Hemiptera: Psyllidae	

of the vectors and/or reservoirs of phytoplasma-infected crop plants and weeds or management of phytoplasma spread within the tree by habitat management and the use of genetically disease resistant trees are the ideal ways in the management of phytoplasma diseases in the forest environment. Such habitat management can reduce the incidence and breeding of vectors. Maintaining conducive vegetation that can increase the incidence and abundance of natural enemies of vectors needs to be explored. Hence, more efforts should be made to determine those elements of the forest/tree environment that enhance the survival of natural enemies without supporting the population of vectors. There is a lot of scope to expand research on the management of phytoplasma diseases by identification of vector resistant phenotype/genotype of trees and applying the technology of tissue culture and genetic modification to produce plants with reduced phytoplasma titers or with insect resistance. Thus identification of real vectors is of paramount importance to understand the entire pathosystem of phytoplasma, for designing effective and economically and environmentally sustainable means of disease management.

CONCLUSION

Management of phytoplasma diseases in forest species is a great challenge as there is a huge lacuna existing in determination of real vectors and unavailability of resistant varieties/populations of trees. Therefore, critical knowledge not only on the actual vectors but also on their distribution, host range and mode of disease transmission are crucial for integrated management of phytoplasma diseases in forestry.

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DIAGNOSIS OF PHYTOPLASMA ASSOCIATED WITH THE SANDALWOOD SPIKE DISEASE#

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ABSTRACT

Sandalwood spike disease (SSD) is a serious disease of Indian sandal tree (*Santalum album*). The disease is caused by a *Candidatus* Phytoplasma asteris strain of subgroup 16SrI-B. The disease is naturally spread through leafhopper and root-grafting. The infected plants take long time to express disease symptoms and eventually die. The diagnostic disease symptom of SSD is crowded small chlorotic leaves on stiff twigs that has spike like appearance. There are various biological, histopathological, electron microscopic and molecular methods available for the detection and identification of phytoplasma. Biological and histopathological tests are time consuming and ambiguous. Serological test like ELISA and molecular test like PCR are more versatile and accurate for the detection of phytoplasma. Application of various molecular techniques like nested PCR, RFLP and sequence analysis of 16S ribosomal RNA led to identify the SSD phytoplasma isolates as a member of 16SrI-B and 16SrXI-B phytoplasma subgroups. In this chapter, various diagnostic methods utilised for the detection and identification of SSD phytoplasma are summarised.

Key words: Detection, phytoplasma, sandal spike disease, *Santalum album*

Sandalwood tree (*Santalum album* L., family Santalaceae), a valuable commercial tree is well-known for its heartwood fragrance. Sandalwood is a semi-root parasitic tropical tree indigenous to Southern India and Southeast Asia. The oil extracted from its heartwood is widely used in cosmetics, aromatherapy and pharmaceuticals (Srinivasan et al., 1992; Coppen, 1995). The sandalwood trees are threatened species and are mostly grown under the government regulation in India, Pakistan and Nepal. The demand for sandalwood and oil is not as much due to slow growing nature of heartwood, poor *in vitro* regeneration, illegal cutting down and diseases.

The plant pathogens such as *Phytophthora* sp. and *Fusarium oxysporum* cause seedling diseases. *Ascochytasantali, Macrophomina phaseoli, Asterina congesta* and *Sphaceloma santali* causes leaf spot diseases. *Ganoderma applanatum* and *G. lucidum* cause basal stem rot disease. Among the various other diseases, sandal spike disease (SSD) caused by phytoplasma is a major disease of *Santalum* sp. (Mukerji and Bhasin, 1986; Ghosh et al., 1992; Srinivasan et al., 1992).The SSD occurs at any developmental stage of the tree. As the disease progresses, the new leaves become smaller, narrower or more pointed and fewer in number with each successive year until the new shoots give an appearance of fine spike. At the advanced stage, inter nodal distance on twigs becomes small, haustorial connection between the host and sandal breaks. Eventually, the affected tree dies within 2-3 years after the onset of spike disease. SSD is also known to occur in many alternate hosts such as *Vinca rosea* (Dijkstra and Lee, 1972; Hiruki and Dijkstra, 1973), *Santalum yassi, Dodonaea viscosa* and *Zizyphus onoplea* (Hull et al., 1970). In Karnataka, the growing stock has been reduced by 25% due to SSD compared to last two decades (Swaminathan et al., 1998).

SSD was reported to be caused by *Candidatus* Phytoplasma asteris strain belonging to subgroup 16SrI-B (Kirdat et al., 2019). The genus, phytoplasma are intracellular plant pathogens derived from gram positive a bacterium belongs to the class Mollicutes. Phytoplasmas are pathogenic parasites of plant and insects. Phytoplasmas are non-culturable, phloem

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restricted prokaryote that lack typical cell wall and are resistant to many antibiotics. Transmission of phytoplasma mainly occurs through phloem feeding insect vectors and infected plant materials. In general, phytoplasmas cause different types of symptoms such as stunting, phyllody, witches' broom, yellowing resulting in serious loss of yield in many economically important agricultural and horticultural crops worldwide (Arunkumar and Joshi, 2012).

Sandalwood spike disease was first reported in Indian sandal (*S. album*) in 1899 from Coorg state of Southern India (McCarthy, 1900; Barber, 1903). Over a million SSD infected trees have been removed in Mysore and Coorg states between 1903-1916 (Da Silva et al., 2016). Researchers had different opinions on the cause of SSD such as imbalanced circulation of sap (Hole, 1917; Venkatarama, 1918) and virus or virus like microorganism (Coleman, 1923; Dijkstra, 1968). In 1969, the presence of mycoplasma like bodies was confirmed in phloem cells of SSD infected trees through electron microscopy (Dijkstra and Le., 1969; Hull et al., 1969, Varma et al., 1969). SSD induces characteristic phytoplasma symptoms such as chlorosis, shortened internodes, reduced leaf size with a bushy appearance and stem show a spike like appearance (Barber, 1903; Hole, 1917). Infected young trees generally die within a year or two after the appearance of spike symptoms (Swaminathan et al., 1998; Balasundaran and Muralidharan, 2004). The production of *S. album* is significantly affected by SSD in Kerala and Karnataka (Swaminathan et al., 1998; Kumar, 2014; Arunkumar et al., 2016). The incidence of SSD in Karnataka and other regions in Southern India has increased since 1990 (Kumar, 2014). Survey among 24 Sandal plantations from five districts in southern Karnataka for assessment of SSD incidence revealed the occurrence of spike disease ranging from 0-18 per cent. The maximum incidence (18%) was recorded in Sirataluk of Tumkur district, followed by Shimoga (1.65%). The least incidence (0.33%) was recorded in plantations of Tygarthi village (Murali et al., 2019).

Even though the disease was reported a century ago from India, very few researches have been undertaken to understand the molecular nature of infection and management of pathogen. Diagnosis of SSD is very crucial for authentic identification. Phytoplasmas are intracellular parasite found in sterol rich phloem tissues of plant or in haemolymph of insect vectors. The detection of phytoplasma is challenging due to low titre, seasonal fluctuation and uneven distribution in planta, particularly in woody crops (Garcia-Chapa et al., 2003). The latency period of phytoplasma vary from several months to years before symptom appearance makes it more difficult for early detection and diagnosis. The distribution of phytoplasma in entire plant system is again a time taking process as a result there is a less chance of detection in the asymptomatic branches. The inhibitory enzymes, polysaccharide and polyphenolic compounds present in woody plants interfere with phytoplasma DNA isolation and further nucleic acid-based detection. Various approaches from biological to molecular techniques are employed for the detection of phytoplasma. In this chapter, we summarise the work conducted in the area of diagnosis of SSD.

Unique molecular features of phytoplasma

Phytoplasma genome consists of single chromosome with an average size of 600 - 880 kb and extra chromosomal plasmids. The genome contains the basic cellular functional genes responsible for replication, transcription, translation and protein translocation (Jung et al., 2003). Phytoplasma doesn't contain gene involved in several biosynthetic pathways, instead they completely depend on the host cell machinery. Mycoplasmas lack genes involved in tricarboxylic acid cycle and biosynthesis of sterol, fatty acid, de novo nucleotide and most amino acids (Razin et al., 1998). Phytoplasma have also lost many metabolic genes in comparison to mycoplasma (Oshima et al., 2004). The unique feature of phytoplasma that differ from mycoplasma and other bacteria is the absence of the gene encoding F1F0-type ATP synthase, i.e., involved in synthesis and hydrolysis of ATP. The two secretion systems found in phytoplasma are Sec system and YidC system. The Sec system essential for cell viability is involved in integration and secretion of proteins into host cell cytoplasm. The YidC system is involved in integration of membrane proteins (Oshima et al., 2013). The unique phytoplasma virulence factor TENGU causes sterility in plants by down regulating the jasmonic acid and auxin pathways. Secreted proteins and virulence factors of phytoplasma play a major role in symptom expression and survivability of the primitive bacteria inside the host cell. The fine diagnosis of phytoplasma is based on the specific membrane protein and genome sequence. The ribosomal RNA (16S and 23S) genes were used as a basis of molecular detection and classification of phytoplasma.

Biological diagnosis

Diagnostic symptoms

Biological method was the basis of identification of phytoplasma disease in earlier days, which included symptomology, host range, collateral hosts and transmission. Symptomatology was the major criterion employed in diagnosis of the disease caused by phytoplasma (Lee and Davis, 1992). SSD shows prominent yellowing of leaves with witches' broom appearance in infected trees. Sandal trees of all stages are affected by the phytoplasma and the trees die prematurely with the onset of spike disease. SSD shows two types of spike symptoms such as rosette spike and pendulous spike. The rosette spike is more commonly found in infected trees. The symptoms appear in one part of tree and gradually it spreads throughout the tree.

Young leaves become smaller, chlorotic, sometimes with purplish tinges and branches tend to stand out stiff with upright growth exhibiting a spike like appearance. Subsequently, the affected branch becomes bushy with distinct yellowing of leaves, which turn reddish shortly before the death of the tree (Fig. 1). The infected part of plant doesn't flower or bear any fruits, while the unaffected branch of the tree continues to bear flower and fruits. The haustoria and root tips of the infected sandal tree are degenerated. Whereas, in case of pendulous spiked symptom, the haustoria and root tips do not die. The infected shoots grow apically and have a drooping effect without rosette symptoms in younger leaves.

Graft and dodder transmission

In addition to the diagnostic symptoms, the



Fig. 1. Sandal spike disease (SSD) symptoms. A. A healthy twig showing dark green leaves and flowers; B. A SSD affected twig showing chlorotic and small leaves; C. A healthy sandal tree; D. An affected tree showing chlorotic bushy foliage; E. An affected tree with dead branches

successful transmission of the causal agent from the disease plant to a healthy indicator host plant is an important biological criterion of identification of a pathogen. Phytoplasma being phloem borne is not sap transmissible, and therefore, graft, dodder, and insect transmission methods were attempted to transmit the SSD. Coleman (1917) described the graft transmission of SSD through infected buds, barks and twigs. The natural root grafting through haustorial connections between healthy and infected plants could contribute up to 6 to 7% of SSD transmission. Later, in 1930, Sreenivasaya reported that, the disease could also be transmitted through infected leaves by inserting it between the wood and bark tissues of healthy plant. Dodder mediated transmission of SSD to *Vinca rosea* was reported by Nayar and Ananthapadmanabha (1970). The infected *V. rosea* plants showed vein clearing and interveinal chlorosis symptoms. In 1972, Dijkstra and Lee demonstrated that the SSD can be transmitted between sandal tree and *V. rosea* plants and *vice versa*. The SSD infected *V. rosea* plants developed witches broom symptom, while the dodder strand was connected between the *V. rosea* and infected sandalwood tree. The dodder also could transmit the disease from SSD infected *V. rosea* back to sandalwood trees. The infection was confirmed by the presence of mycoplasma-like bodies in the test plant tissues.

Indicator hosts

Collateral hosts play a major role in pathogen survivability in absence of the main host. *Lantana camera* and *Pongamia glabra* are reported as symptomless carriers of SSD (Nayarand Srimathi, 1968; Kristensen, 1960). Phytoplasma disease like symptoms was observed in many plants such as *Zizyphus oenoplea*, *Vinca rosea*, *Dodonea viscosa, Eucalyptus grandis, Stachytarpheta jamaicensis, Dendrocalamus strictus* etc., grown near the sandalwood tree. The report also indicated that from the collateral hosts viz., *Zizyphus* sp. and sesamum with spike symptoms; the disease might get transmitted to sandal trees within a period of 3-5 years. The causal agent could also be transmitted between the collateral host and sandal trees and *vice versa* (Nayar, 1981). *Vinca rosea* has been shown as an excellent experimental indicator plants for SSD or phytoplasma in general.

Vector transmission

As insect vector transmits virus or virus like pathogens with an extraordinary biological specificity, the vector transmission by a specific insect vector was considered as the convincing identification evidence of these types of pathogens in the absence of biochemical or molecular diagnostic assays in the early phase of the development of the subject. The first evidence of insect vector transmission of phytoplasma was demonstrated with a leafhopper, *Macrosteles fascifrons* that transmitted infectious agent from aster yellows (Kunkel, 1926). The insect vector transmission of phytoplasma was demonstrated way before the discovery of phytoplasma (Doi et al., 1967). Several leafhopper, planthopper and psyllid have been identified as the vectors of phytoplasma. Dover (1932) suspected that SSD can be transmitted by insects. Later in 1940, Rangaswami and Griffith reported that insect vectors are primary source of transmission of SSD in nature. Insects such as *Moonia albimaculata* Dist., (Dover and Appanna, 1933), *Coelidia indica* Walker (Rangaswami and Griffith, 1940) and *Nephotettix virescens* (Shivaramakrishnan and Sen-Sarma, 1978) were found to be vectors of SSD. Some of the vectors like *M. albimaculata* and *C. indica* need further confirmation (Sen-Sarma, 1982). The successful transmission of SSD through the known vector species is an important confirmatory bioassay of SSD. The authenticity of some of the vectors like *M. maculata* and *C. indica* needs further confirmation (Sen-Sarma, 1982). The successful transmission of SSD through the known vector species is an important confirmatory bioassay of SSD. It appears that there could be multiple vectors of SSD phytoplasma. However, after 1980s, there is a lack of vector transmission studies of SSD and as a result of that the data pertaining to the transmission parameters, efficiency of transmission by the different vectors listed in the literature are lacking.

Microscopic diagnosis

Light microscopy

Light microscopy is used as a preliminary and indirect method to detect phytoplasma. Light microscopy is limited when the pathogen titre is low in the disease tissues. Detection of abnormal levels of wounded callose produced in response to injured phloem is an indirect method for diagnosis of phytoplasma. The routinely used dyes for detection of phytoplasma are Diene's stain, which produce blue colour (Fig. 2) and DAPI (4'-6-diamidino-2-phenylindole), a DNA binding fluorochrome (Dienes et al., 1948; Seemuller, 1976). Ananthapadmanabha et al. (1973) used Glemsa and Dienes' stain for the detection of SSD phytoplasma. Hoechst 33258, a DNA binding fluorochrome was used to detect the SSD phytoplasma and found that the

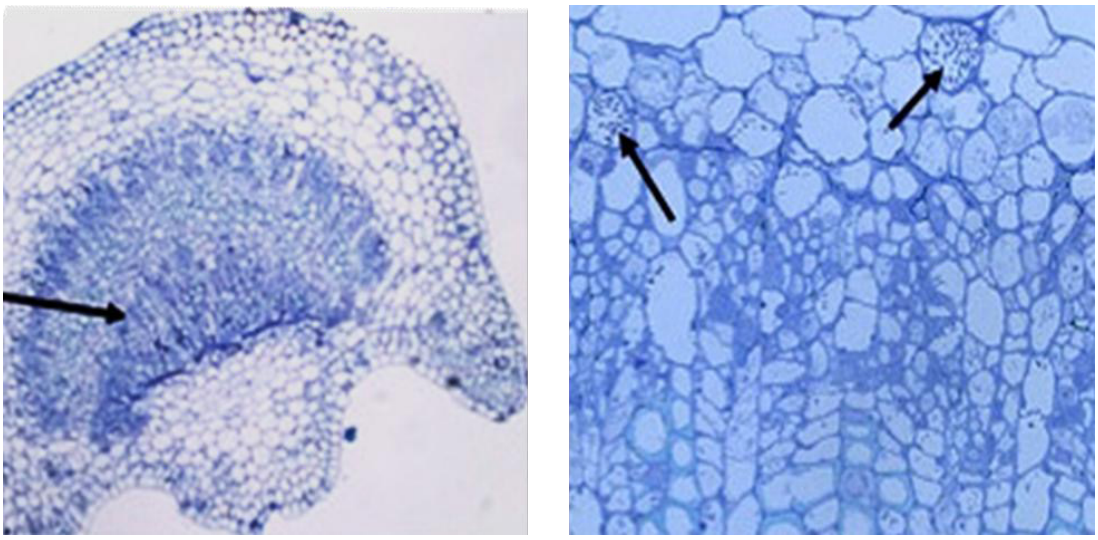


Fig. 2. Diene's staining of sandal stalk showing phytoplasma bodies in phloem cells

fluorescence intensity was higher in stem and petiole compared to root tissues. These studies conclude that fluorescent microscopy is more sensitive than light microscopy for detection of SSD phytoplasma (Ghosh et al., 1985, Rangaswamy, 1995).

Electron microscopy

Transmission Electron Microscopic (TEM) studies led to the discovery of phytoplasma, a new class of pathogen causing plant diseases, which were previously suspected to be caused by viruses. Phytoplasma was first observed in the ultra-thin sections of samples from different plant diseases such as yellows disease of aster, witches' broom of potato, paulownia, and mulberry dwarf, which were referred at that time as mycoplasma-like organisms (MLO) (Doi et al., 1967; Maramorosch, 2011). Phytoplasmas resemble animal or human mycoplasmas morphologically and share several characteristics. In 1994, the MLO associated with plant was referred as phytoplasma, (Sears and Kirkpatrick, 1994). The structural characteristics of phytoplasma can be studied only by electron microscopy (Marcone and Ragozzino, 1996).The phytoplasma characteristics such as pleomorphic nature, unicellular, cells delimited by thin membrane and absence of cell wall can be observed by TEM. TEM is routinely used as a confirmatory technique to identify many phytoplasma since 1967.In the early stage of study, SSD was thought to be caused due to the physiological disorder of the roots caused by the imbalance of sap movement within the plant (Hole, 1917, Venkatarama, 1918) or suspected to be caused by fungi (Latham, 1918), ultramicroscopic bacteria (Fischer, 1918) or virus (Coleman, 1917, 1923). Later, in 1969, mycoplasma like bodies was found in sandal spike phloem tissues by three different groups using TEM (Dijkstra and Le, 1969; Hull et al., 1969; Varma et al., 1969). Therefore, the usage of TEM disproved the association of other pathogen concepts and confirmed that SSD is caused by a phytoplasma.

Scanning electron microscope (SEM) uses a beam of focussed electrons to scan the sample surface and records the interaction between electron and sample to create a three-dimensional magnified image. SEM offers three dimensional topographical forms of phytoplasma structure with reduced resolution compared to two-dimensional, higher resolution TEM. SEM permits rapid examinations of large areas of plant tissues, whereas TEM can be employed to view at the cellular level (Marcone and Ragozzino, 1996).SEM was introduced for studying mollicutes by Biberfeld and Biberfeld (1970) and subsequently used to observe many phytoplasmas (Bove, 1984). Thomas and Balasundaran, 2001, observed the purified SSD phytoplasma using SEM. The phytoplasma showed spherical/elliptical structure with an average size of 1.0 mm diameter in phloem tissues of diseased tissue. The significant feature of mollicute, the attachment of phytoplasma to inner surface of phloem tissues was also observed using SEM.

Protein based methods

Immuno-assays techniques are highly specific for the identification and quantitation of pathogen. Phytoplasma was purified using differential centrifugation (Clark et al., 1983; 1989), Celite pad filtration (Sinha and Benhamou, 1983) and percoll density gradient

centrifugation (Jiang and Chen, 1987) techniques were used to prepare antigen to raise polyclonal antisera. Production of high titre antisera requires extremely purified antigen. The efficiency of purified antigen is reduced due to several rounds of centrifugation. In 1975, Nayar and Ananthapadmanabha used ammonium sulphate purification method to purify SSD phytoplasma. The polyclonal antiserum was produced in rabbit using the purified SSD phytoplasma antigen. Gel diffusion and agglutination tests were employed to detect the phytoplasma with a low titre (1:250) antiserum. The antiserum successfully detected the phytoplasma in SSD infected sandal and *Catharanthus roseus*. Later, the SSD phytoplasma purified by the differential centrifugation method was used to raise a better quality antiserum (Rangaswamy, 1995). The antiserum was tested by indirect ELISA to detect the SSD phytoplasma from the infected sandal and *Catharanthus roseus* at 1:1000 dilution. The antibody could detect the phytoplasma in infected sap at a dilution of 1:200.Thomas and Balasundaran (2001) used differential filtration technique for the purification of phytoplasma in order to produce high quality antigen. The antisera detected the SSD phytoplasma at a titre of 1: 2000.

Nucleic acid-based method

Polymerase chain reaction (PCR) is the most versatile tool for detecting phytoplasmas in plant and insect hosts (Smart, 1996). Nested PCR followed by restriction fragment length Polymorphism (RFLP) is routinely employed for detection and identification of phytoplasma (Saeed and Cousin, 1995). The molecular techniques are more sensitive, specific and reliable for routine detection, quantification and differentiation of phytoplasma at very low titer compared to immunological and microscopic methods. PCR followed by sequencing formed the basis for classification and identification of phytoplasma and strain differentiation.

RFLP

RFLP has been extensively used as a tool for identification and classification of phytoplasma since 1990s. In this method, a set of 17 restriction enzymes are used to generate a banding pattern over 1.25-kb DNA segment amplified through nested PCR from the 16S rRNA sequence (Lee et al., 1998). The new group and subgroup are assigned when the similarity coefficient of the polymorphic banding pattern is less than 0.85 and 0.97, respectively with that of previously established members (Zhao and Davis, 2016). The 16S rRNA based RFLP was further improved with an easy-to-use web-based tool *iPhyClassifier,* where 16SrDNA sequence (Fig. 3) is used to generate a computer simulated RFLP pattern (Zhao et al., 2009).This method is convenient to use as RFLP pattern similarity coefficients and overall sequence similarity scores instantly suggest the group and/or subgroup position of the candidate to be identified.

Initially, restriction analysis of 16S rRNA PCR product with A*lu*I restriction enzyme differentiated phytoplasmas into four groups, with SSD being classified in the first group (Ahrens and Seemuller, 1992). Seemuller et al. (1998) classified phytoplasmas into 20 groups and placed sandal spike phytoplasma in the Aster yellows group (Group I), and unclear subgroup. The later study with RFLP analysis of 16S rRNA nested PCR products and the sequence identity showed that the SSD phytoplasma is closely related to aster yellows subgroup 16SrI-B (Khan et al., 2008). The restriction pattern of the virtual RFLP gel of the available sequence of SSD phytoplasma 16S rRNA gene using *iPhyClassifier* confirmed the identity of 16SrI-Bsubgroup. One isolate, SW03 (MK627527) SSD

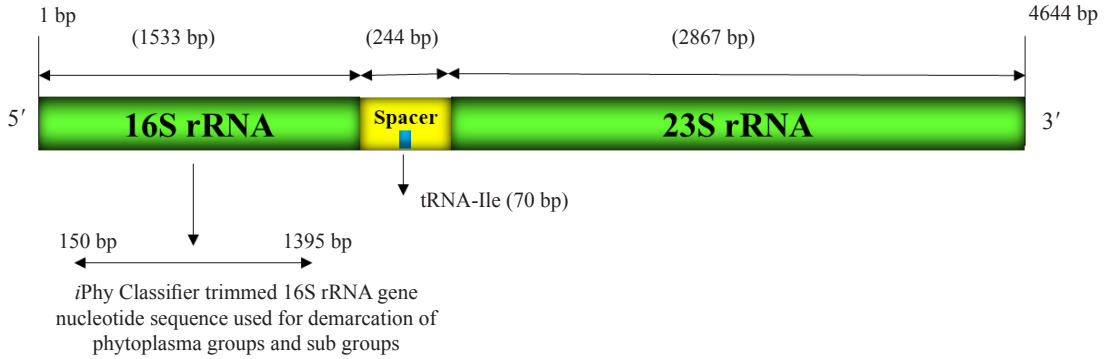


Fig. 3. Diagrammatic representation of Aster yellows witches broom phytoplasma (NC_007716) phytoplasma rRNA operon with 16S rRNA gene, intergenic spacer and 23S rRNA gene.

phytoplasma isolate, however showed different virtual restriction banding pattern compared to the other SSD phytoplasma isolates (Fig. 4).

PCR

Phytoplasma is routinely detected by PCR (Bertaccini et al., 2019). Several primers were developed based on the sequence in the 16S rRNA, internal transcribed spacer and 23S rRNA region for the detection of different phytoplasma belonging to the ribosomal groups and subgroups. The most commonly used PCR method is nested PCR, where two rounds of PCR are performed with the two pairs of universal primers. The first PCR is performed on the total plant DNA isolated from the phytoplasma infected plant with the forward primer P1on16SrRNA: aagagtttgatcctggctcaggatt and the reverse primer P7on23SrRNA: cgtccttcatcggctctt, which generates 1.8 kb amplicon. In the second PCR, the product of first PCR is used as template with the nested primers on 16SrRNA, forward primer R16mF2: catgcaagtcgaacga and reverse primer R16mR1cttaaccccaatcatcgac, which generates 1.2 kb amplicon (Deng and Hiruki, 1991; Gundersen and Lee, 1996). The first PCR based detection of SSD phytoplasma was reported in 1999 by Thomas and Balasundaran, where they were successful in amplification of 558 bp amplicon from SSD affected leaf samples by using *Taq* DNA polymerase as well as Dynazyme II polymerase. Khan et al. 2004found that the direct PCR or southern hybridization in the total DNA isolated from the infected sandal leaves showed neither any positive amplification nor any signal with the specific probes. The reason of the failure of detection was attributed to the low phytoplasma concentration in the total DNA samples. However, Khan et al. 2004 developed a sensitive nested PCR using the above universal primers (P1 & P7 and R16mF2&R16mR), which could efficiently detect the SSD phytoplasma in the highly diluted (60,000 times) first PCR product. The nested PCR is highly sensitive and reliable diagnostic method for the screening of sandal trees and insect vector.

Real time PCR

Phytoplasma occur in low concentration and subjected to seasonal titre variation and uneven distribution in the different parts of plant tissues. Although, nested PCR is a convenient diagnostic method for the routine usage, it is not suitable for quantification of phytoplasma load in plant tissues. Real time PCR (qPCR) is a versatile quantitative PCR technique, which utilises TaqMAN probe specific to the 16S rRNA gene for the detection of phytoplasma with higher sensitivity and specificity. The 16S rRNA qPCR is ten times more sensitive than the conventional nested PCR (Christensen et al., 2004). Universal and group specific qPCR primers and probes targeting 16S rRNA have been established for several phytoplasmas (Galetto and Marzachi, 2010).However, the usefulness of qPCR is yet to be exploited for the SSD phytoplasma studies.

Loop-mediated isothermal amplification (LAMP)

The PCR targeting 16S ribosomal genes is the principal molecular technique for the detection of phytoplasma. PCR can be easily performed, but it requires a well-equipped laboratory. LAMP was invented for the isothermal amplification of DNA during 2000 (Notomiet al., 2000). In this method, *Bst* DNA polymerase and a set of four primers that recognise six distinct sequences on the target DNA are key reagents.

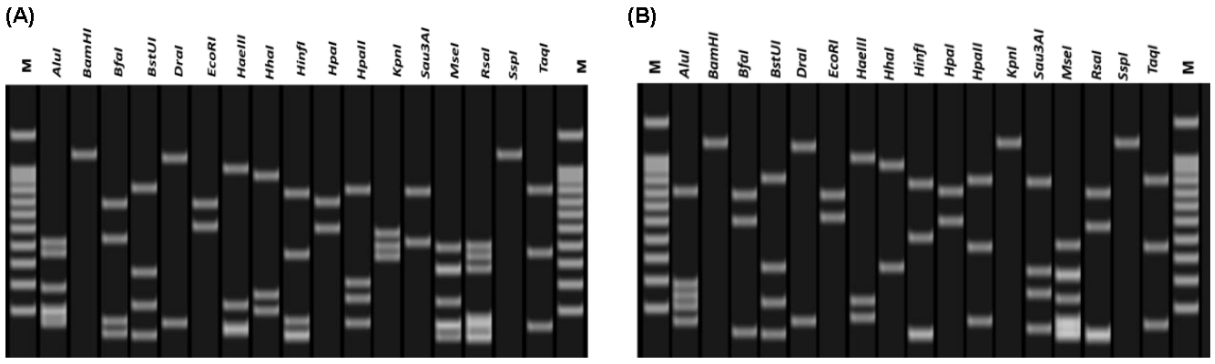


Fig. 4. Virtual gel restriction fragment length polymorphism patterns of 16S rDNA of sandal spike phytoplasma isolates. a. SW04 (accession no. MK627541) isolate showed 99.8% similarity with *Candidatus* Phytoplasma asteris (accession no. M30790) and found to be *Candidatus* Phytoplasma asteris related strain; b. SW03 (accession no. MK627527) isolate showed 98.0% similarity with *Candidatus* Phytoplasma cynodontis, GenBank accession: AJ550984 and found to be *Candidatus* Phytoplasma cynodontis related strain

The Bst DNA polymerase amplifies the target DNA at 60 to 65^{0}C by 30 to 60 min. The amplified targets in LAMP reaction can visualize through a colorimetric assay by using hydroxyl napthol blue that detect the magnesium pyrophosphate by-product in successful LAMP amplification (Goto et al., 2009).LAMP is an easy, rapid, and sensitive amplification technique, which can be performed for the diagnosis of phytoplasma diseases in laboratories that lack the advanced facilities (Bekele et al., 2011; Obura et al., 2011; Sugawara et al., 2012). LAMP assay has been used to detect the aster yellows group phytoplasma in different crops (Tomlinsona et al., 2010), but so far it has not been utilized for the detection SSD phytoplasma. Development and evaluation of a one hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas.

Genome sequence-based identification

The molecular techniques are more **se**nsitive, specific and reliable for routine detection, quantification and differentiation of phytoplasma at very low titer compared to immunological and microscopic methods. Sequencing of 16S ribosomal gene followed by RFLP formed the basis for identification and classification of phytoplasma. However, a single gene-based classification is sometimes unreliable and adoption of multilocus gene sequence is suggested for better classification of phytoplasma (Glaeser and Kampfer, 2015). Several protein coded genes have been proposed as molecular markers for the classification of phytoplasma, for example, rpl22 and rps3 (Lim and Sears, 1992, Martini et al., 2007), preprote intranslocase SecY (Lee et al., 2006), chaperonin 60 (groEL/cpn60) (Mitrovic et al., 2011, Dumonceaux et al., 2014), elongation factor Tu (Schneider and Gibb, 1997), replication initiation protein DnaD, DegV family protein, TIGR00282 family metallo phospho esterase, preprotein translocase SecY and RluA family pseudouridine synthase (Cho et al., 2020).

Identification and classification of SSD phytoplasma is limited by the lack of adequate genome sequence resource. So far, only 16 sequences of SSD phytoplasma from 16S rRNA gene have been submitted in NCBI database (Table 1). Out of 16 sequences, only 11 sequences cover the 1.2 kb region of 16S rRNA gene. The evolutionary analysis of 1.2 kb gene of SSD phytoplasma and other known phytoplasma sequences available at USDA phytoplasma classification database showed that the SSD phytoplasma isolates are found closely related to *Ca.* P. asteris and *Ca.* P. lycopersici and they are clustered in a single clade except one isolate, SW03 (MK627527), which is found to be closely related to monocot infecting phytoplasma, *Cynodon dactylon* and *Oryza sativa (?)* (Fig. 5). The SSD phytoplasma and other known phytoplasma 16S rRNA gene pair wise similarity scores were obtained and the isolates were clustered by rooted neighbour joining phylogenetic tree to get the colour coded matrix by species demarcation tool. The nucleotide sequence analysis of the 16S rDNA gene of SSD phytoplasma and other known phytoplasma groups varied between 86 to 100% nucleotide sequence identities. SW03

Table 1. Sandal spike disease phytoplasma sequence of 16S ribosomal RNA submitted in NCBI GenBank database.

Sl. No.	Accession	No of Bases	Location	Author
1	MK627527	1248	Marayoor, Kerala	Kirdat et al. (2019)
2	MK627528	1246	Marayoor, Kerala	
3	MK627529	1246	Marayoor, Kerala	
4	MK627536	1247	Marayoor, Kerala	
5	MK627537	1246	Marayoor, Kerala	
6	MK627541	1246	Marayoor, Kerala	
7	MK627542	1246	Marayoor, Kerala	
8	MK627543	1245	Marayoor, Kerala	
9	MK627544	1247	Marayoor, Kerala	
10	EF198362	1427	Karnataka	Khan et al. (2009)
11	EF050071	1427	Karnataka	
12	DQ092357	1149	Mysore, Karnataka	Khan et al. (2006)
13	DQ078260	590	Mysore, Karnataka	Khan and Srivastava (2005)
14	DQ078261	542	Mysore, Karnataka	
15	DQ078262	526	Mysore, Karnataka	
16	DQ078263	411	Mysore, Karnataka	

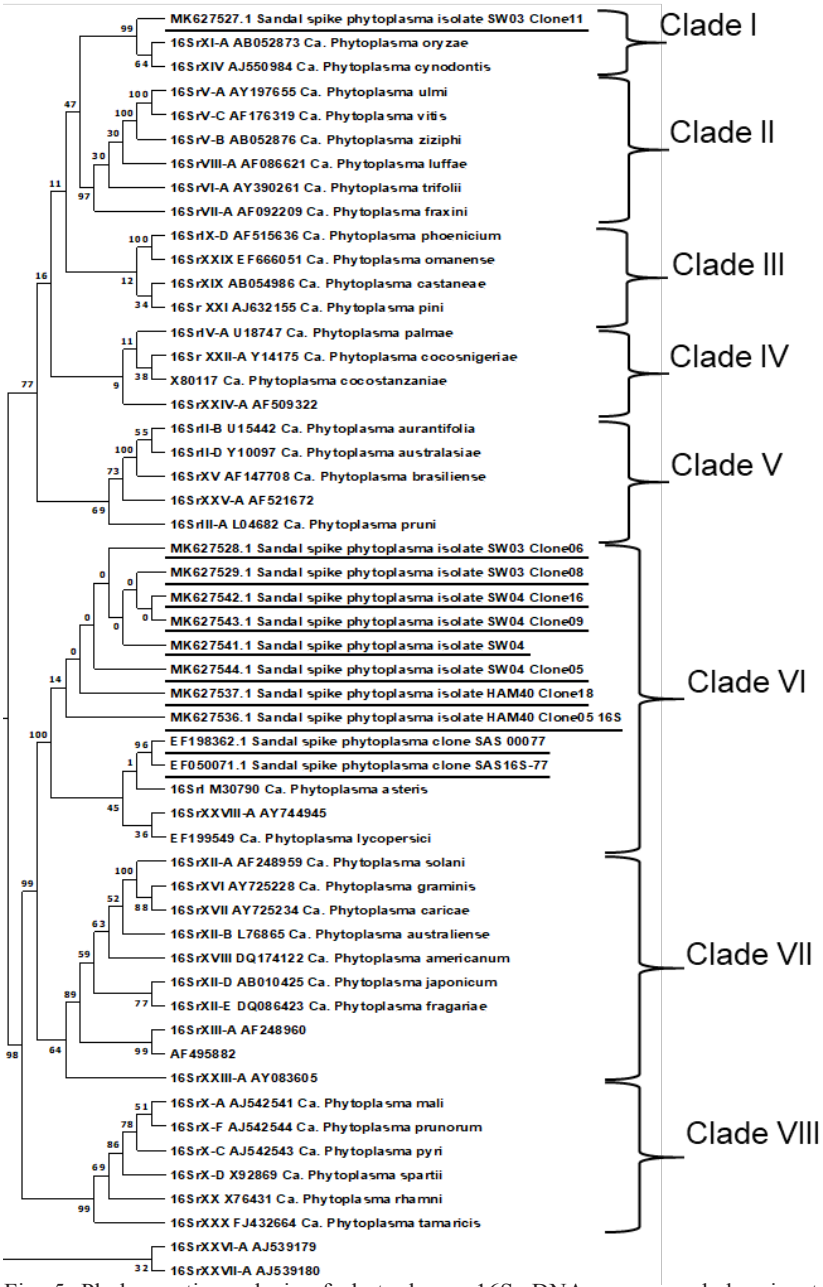


Fig. 5. Phylogenetic analysis of phytoplasma 16S rDNA sequences belonging to different groups. The 16S rDNA sequences were trimmed using the *i*PhyClassifier web tool available online at usda.gov website. The sandal wood 16S rDNA sequences were underlined

isolate showed close nucleotide sequence identity with monocot infecting phytoplasmas, whereas, other SSD isolates showed close sequence identity to *Ca.* P. asteris (Fig. 6).

CONCLUSION

Reliable and sensitive diagnosis is required for the studies leading to development of management options of SSD phytoplasma. Several conventional and molecular techniques have been used to study the nature of SSD phytoplasma. Biological or histopathological assays are time consuming and often not effective for precise identification. Lateral flow and LAMP assays based visual detection of SSD phytoplasma is required for the rapid and large scale screening of plant as well as vector samples in the forest and nurseries. Nucleic acid-based detection like nested PCR and qPCR are more reliable approach for the detection of SSD phytoplasma.

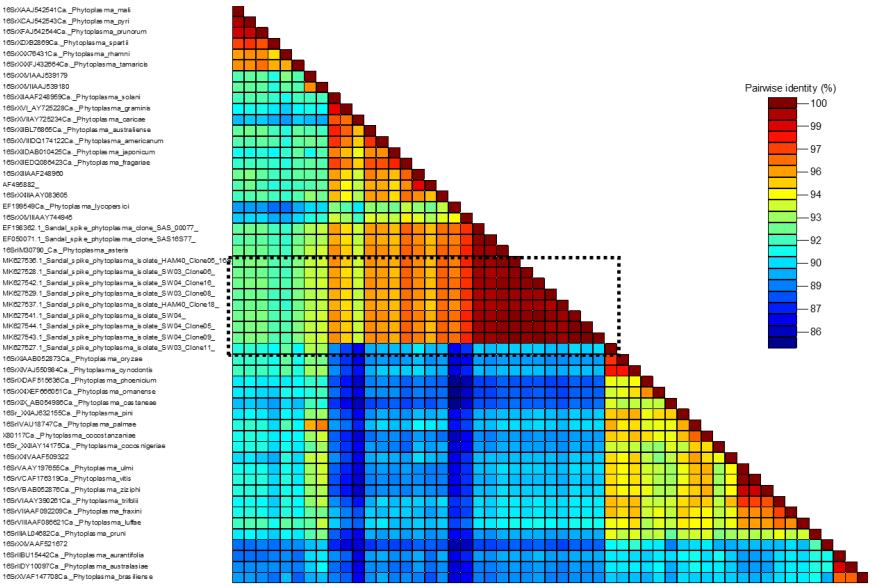


Fig. 6. Pairwise identity of Sandal wood phytoplasma 16S rDNA gene nucleotide sequences (indicated with dotted box) with other known phytoplasma groups. 16S rDNA gene (~1200 bp) nucleotide sequences were trimmed using *i*PhyClassifier web tool available at usda.gov. The matrix was generated using sequence demarcation tool version 1.2.

Molecular diagnosis relies on the availability of genome sequence data. Very limited sequence resource i.e., only 16S rRNA sequence is available for SSD phytoplasma. Sequence identity and phylogeny-based identification provide the finest and measurable difference between the closely related phytoplasma isolates, which are otherwise difficult to resolve even by the molecular tests like PCR or RFLP. Recent studies on SSD phytoplasma indicate the possibility of association of diverse phytoplasma in SSD. Generation of full genome sequence resource of SSD phytoplasma isolates from different geographical regions is essential for better understanding of the pathogen and development of proper diagnosis.

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MOLECULAR MECHANISM UNDERLYING SYMPTOM DEVELOPMENT IN PHYTOPLASMA ASSOCIATED DISEASES - THE KEY PLAYERS AND THEIR ROLE#

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ABSTRACT

Phytoplasma, plant pathogenic Mollicutes that have a trans-kingdom life cycle, are insect transmitted and have been found to be associated with yield affecting traits in a large number of taxa. Many peculiar symptoms observed in the host taxa are often results of dramatic alterations in the normal development program that are generally controlled at the meristematic regions located in the shoot apices. Phytoplasma being vasculature limited are thus able to bring about genetic reprogramming in the regions of host plant that are far removed from their natural niche namely sieve elements of the phloem. Several proteins secreted by phytoplasma in the host plants have been identified and termed as effector molecules namely SAP54, SAP11, TENGU, SAP21 etc. that enable the colonisation, survival, spread of phytoplasma and also bring about dramatic alterations in the host plant. Nevertheless, the mechanisms underlying these peculiar phenomena are far from understood and remain a challenging area for the phytoplasma biologists. A thorough understanding of the processes involved is needed to provide platforms for developing control measures for phytoplasma associated diseases that will also enhance basic understanding on the plant developmental programs affected.

Key words: Pathogens, effector molecules, phyllogeny, miRNA

Phytoplasmas are insect-transmitted bacterial plant pathogens belonging to the class Mollicutes that infect broad range of plants including an array of annual crop plants, several perennials and their associated wild and weedy taxa. Presence of phytoplasma in plants is associated with a wide variety of symptoms viz., stunting, development of numerous tiny branched shoots having small leaves (witches' broom), yellowing, greening of floral organs (virescence), phyllody, reddening of leaves and stems (purple top), stem flattening (fasciation), phloem necrosis etc. (Maejima et al., 2014).

This wall-less bacteria cause considerable yield loss in different crops worldwide. Symptoms induced in infected plants suggest that phytoplasma interfere with the developmental processes within the plant host via a variety of effector molecules to manipulate the host phenotype for their own benefit (Hogenhout et al., 2008; Hogenhout and Loria, 2008; Bai et al., 2009; Sugio et al., 2011). Phytoplasma also modulate immune response of the sap-feeding hemipteran (including plant hoppers, leafhoppers and psyllids) insect vectors in such a way that they spread these bacteria to other plants.

Since phytoplasmas have no cell wall and reside inside the host cells, their membrane proteins and secreted proteins (effectors) can function in the cytoplasm of the host plant or insect cells, and are predicted to have important roles in the interplay between pathogen and host (Oshima et al., 2013; Sugio et al., 2011, 2014).

Further, vasculature limited niche of phytoplasma necessitates the secretion of low molecular weight substances and their movement across the cells to access the meristmatic tissue localised in the apices in order to alter the normal developmental program of the host plant.

As phytoplasmas are obligate parasites, the movement of phytoplasmas is dependent upon their phloem sap-sucking insect vectors which get attracted to plants aggressively producing young and green vegetative tissues. Production of these effectors may stimulate leafhopper feeding, and thereby increase the frequency of phytoplasma acquisition by its vector. Prolonged availability of sap due to reversion of flowering phase to vegetative phase is yet another prospect particularly in case of annual plants.

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The precise mechanism by which these alterations take place has become an active area of research during the last two decades. Several effector molecules have been identified by various workers that have been proposed to genetically reprogram the host plants enabling the colonisation, survival and spread of phytoplasma.

SAP54

One such novel AY-WB (aster yellow phytoplasma strain witches Broom) effector protein, SAP54, that causes symptoms characteristic to phytoplasma infection, alters floral development and persuades production of leaf-like flowers was first identified from an array of 52 genes encoding Secreted AY-WB Proteins (SAPs) (Maclean et al., 2011).

Maclean et al. (2014) for the first time elucidated the mechanism by which phytoplasma alters floral development to convert flowers into vegetative tissues by producing a novel effector protein (SAP54) that interacts with members of the MADS-domain transcription factor (MTF) family, such as SEPALLATA3 and APETALA1 and occupy central positions in the regulation of floral development. SAP54 mediates degradation of MTFs by interacting with proteins of the RADIATION SENSITIVE23 (RAD23) family- eukaryotic proteins that transport substrates to the proteasome as the conversion of flowers into leaf-like tissues diminished in Arabidopsis rad23 mutants in the presence of SAP54. Remarkably, plants with SAP54- induced leaf-like flowers are more attractive for colonization by phytoplasma leafhopper vectors and this colonization preference is dependent on RAD23. Thus, SAP54 has been proposed to act as a link between two key pathways of the host to alter development resulting in sterile flowers/inflorescences (MacLean et al., 2014; Maejima et al., 2014).

The interaction between SAP54 and the K-domain is not well understood, however it depends on the sequence and structural similarities ascribed to convergent evolution during which SAP54 achieved molecular mimicry of K-domain of MIKC-TYPE MTFs. (Rumpler et al., 2015).

More studies on the sequence and structural aspects of SAP54-like proteins in a phylogenetic perspective are required in the future to clarify the case, but are potentially loaded by frequent horizontal transfer of SAP54 genes (Rumpler et al., 2015).

Replacement of leucine or isoleucine residues near the centre of α-helices by proline, α-helical content got massively reduced and mutant protein was unable to interact with any investigated MIKC-type MTFs and did not result in disease phenotype. However, when same residues were substituted with alanines, which did not break helices, SAP54 proteins were still able to interact with their targets and were capable of causing disease symptoms (Aurin et al., 2020). These findings suggest a strong link between the α-helical structures of SAP54 and its ability to interact with some MIKC-type MTFs. Further, removing either of the alpha-helices, SAP54 completely lost its ability to interaction specificity. Deletion of a part of C-terminal alpha helix was sufficient to abolish this interaction whereas deletion of turn of the protein did not alter any sort of interaction suggesting that the distance between the helices is of less importance for interaction (Aurin et al., 2020).

According to the results of dynamic light scattering and electrophoretic mobility shift assay (EMSA), it now seems likely that SAP54 preferentially targets multisets of MIKC-type MTFs (Aurin et al., 2020). SAP54 targets SEP1, SEP2, SEP3 and SEP4 which forms DNA-bound homotetramers (Jetha et al., 2014; Melzer et al., 2009; Rumpler et al., 2018), whereas not with AP3 and PI that do not form homotetramers (Melzer and Theißen, 2009; MacLean et al., 2014;Rumpler et al., 2018).

Furthermore, SAP54 binds to RAD23C and RAD23D (Iwabuchi et al., 2019; MacLean et al., 2014). RAD23 is known to transfer ubiquitylated substrates to the 26S proteasome (Farmer et al., 2010), thus SAP54 could be functioning as an adapter that connects MIKC-type MTFs with RAD23 proteins and delivers them to the proteasome (MacLean et al., 2014). The exact place and mode of RAD23 binding to SAP54 is unknown, however several models have been proposed in which MIKC-type MTFs and RAD23 bind to SAP54 (MacLean et al., 2014).

While it was shown that phyllogens target some MADS TFs, including SEP3 for degradation, it remains largely unknown whether the other SEPs (SEP1, SEP2, and SEP4) of *Arabidopsis* are also degraded by them. Maejima et al., (2015) examined whether phyllogen (PHYL1) of OY-W phytoplasma degrades SEP1, SEP2, and SEP4 of *Arabidopsis* which are highly conserved among various angiosperms. They transiently expressed SEP1, SEP2, SEP3, SEP4, or bZIP63, in *Nicotiana benthamiana* and reported that all SEP proteins of *Arabidopsis* were degraded in the presence of phyllogens *in-vivo*.

Induction of phyllody by phyllogen is not just restricted to *Arabidopsis* as phyllogen causes phyllody phenotypes in several eudicots. Moreover, phyllogen can interact with MTFs of not only angiosperm species including eudicots and monocots but also gymnosperms and a fern, and can induce their degradation (Kitazawa et al., 2017).

Initially *in-silico* predictions had shown that phytoplasma effector protein SAP54, an ortholog of PHYL1 comprises two α-helices connected by a short inter helical region in which a conserved proline breaks the α-helix (Rumpler et al., 2015). Crystal structures and 3D structure predictions further confirmed that SAP54 folds into at least two coiled-coils that are separated by inter helical region. Liao et al. (2019) published the crystal structure $PHYL1_{PnWB}$ from '*Candidatus* phytoplasma strain Peanut Witches' Broom. Similarly, Iwabuchi et al. (2020) for the first time determined the crystal structure of $PHYL1_{OY}$, a phyllogen from OY phytoplasma. The crystal structures of both proteins are very similar, comprising two α-helices connected by a central linker. The structure of $PHYL1_{OY}$ resembled a well-studied bacterial effector AvrRps4C as both of them have a highlighted coiled coil structure that is important for protein-protein interaction.

Using dynamic light scattering, Aurin et al. (2020) found complexes with an apparent molecular mass of ~20 kDa suggesting that SAP54 predominantly formed homodimers under experimental conditions.

Detailed analyses indicated that both α-helices of $PHYL1_{OY}$ contain a repeating series of hydrophobic residues that are highly conserved (Aurin et al., 2020). Remarkably, similar repeating series are found in K-domain of MTFs required for oligomerization.

The N64 residue exposed on the conserved surface of the phyllogen plays a key role in α-helix formation as N64R substitution disrupts the structural integrity supported by the fact that substitutions with glutamine, having similar properties to asparagines did not induce phyllody as glutamine generates more steric hindrance due to its larger size compared to asparagine (Bogan and Thorn, 1998). Thus the steric property of N64 may be crucial for the stabilization of phyllogen- MTF interaction (Iwabuchi et al., 2020). These results suggest that surface structures of phyllogens formed by these hydrophobic and hydrophilic residues are important for phyllogen- MTF interactions.

Recently, for detection of phyllogens in diverse phytoplasma groups' pair of degenerate primers were developed and were used to identify phyllogens from 25 different phytoplasma strains related to nine species (Iwabuchi et al., 2020). The phyllogen family was categorized in four groups: phyl-A,-B,-C and -D. Members of phyl-A,-C,-D, induced phyllody, decreased the amount of SEP1-4 and AP1*in planta*, and interacted with floral MTFs and RAD23C/D. In contrast, phyl-B group phyllogens lacking the ability to induce phyllody still interacted with host factors, but the interaction specificity was different from that of phyllody-inducing phyllogens.

The loss of phyllody inducing activity in the phyl-B group was ascribed to a polymorphic residue at position 64 that regulates the MTF binding and degrading activity of the effector in cooperation with another polymorphic residue at position 30. Auxiliary functional elucidation of the non-phyllody-inducing phyl-B group may reveal unresolved roles of the effector family that improve phytoplasma fitness.

Singh and Lakhanpaul (2020) identified an orthologs of SAP54 as S54LP of SP (SAP54 like Protein of Sesame Phyllody), from *Sesamum indicum* (L.) infected with phytoplasma. Non synonymous substitutions were detected in the SAP54 ortholog sequences from phytoplasmas belonging to same (sub) group using Fixed Effects Likelihood (FEL) approach. The ortholog was found to be under purifying (negative) selection, a total of three amino acid sites were found to be under pervasive purifying (negative) selection and one under pervasive diversifying (positive) selection. Asparagine residues at position 68 and 84 were inferred to be of much importance in the effector protein as they were under pervasive purifying selection. It was also concluded that the signal peptide evolved at a rate higher than mature protein.

The phylogenetic analyses have revealed that of phyllogen family evolved independently in '*Ca.* phytoplasma' species as compared to 16SrRNA phylogeny. Closely related phytoplasmas ('*Ca.* P. asteris' and *'Ca*. P. pruni') were separated into several different phylogenetic groups. It is reasonable to hypothesize that phyllogens were transferred horizontally among phylogenetically distinct phytoplasmas (Iwabuchi et al., 2020). Most of the phyllogens were found to be associated with potential mobile units (PMUs) (Jomantiene et al., 2007; Sugio and Hogenhout, 2012; Maejima et al., 2014). These findings suggest that horizontal gene transfer of phyllogens by PMUs has contributed to the acquisition and sharing of phyllody-

inducing activity among phytoplasmas. Interestingly, it is still unclear how these phyllogens originated as they do not have a potential homologous ancestral protein sequences.

Iwabuchi et al. (2019) found tetramers of SAP54 in the crystals but since SAP54 did not interact with itself in Y2H experiments, they concluded that SAP54 exists as monomer. However, well known bias of Y2H experiments against the detection of homodimer formation (Smirnova et al., 1999; Newman et al., 2000), this conclusion equivocal. Retaining dynamic light scattering, Aurin et al. (2020) found complexes with an apparent molecular mass of ~20 kDa suggesting that SAP54 predominantly formed homodimers under experimental conditions.

Detailed analyses indicated that both α-helices of $PHYL1_{OY}$ contain a repeating series of hydrophobic residues that are highly conserved (Aurin et al., 2020). Remarkably, similar repeating series are found in K-domain of MTFs required for oligomerization.

SAP11

The phytoplasma effector SAP11 results in witches' broom symptoms by inducing degradation of *A. thaliana* TCP (TEOSINTE-BRANCHED, CYCLOIDEA, PROLIFERATION FACTOR 1 and 2) transcription factors (TFs). The TCP TFs are known to repress branching and mediate leaf development. Thus, SAP11 results in induction of axillary branches with crinkled leaves (Sugio et al., 2014). Homologs of SAP11 have also been reported to interact with TCP homologs in other economically important plants such as apple, maize, wheat and coconut where phytoplasma leads to symptoms of witches' broom and alterations in leaf morphology (Janik et al., 2017; Pecher et al., 2019).

AY-WB SAP11 homologs have been reported from '*Ca.* P. solani' (strain SA-1) (Music et al., 2019), '*Ca.* P. asteris' OY-M strain (Chang et al., 2018), Maize bushy stunt phytoplasma, MBSP (strain M3) (Orlovskis et al., 2017), PnWB (Peanut witches' broom) phytoplasma (Chang et al., 2018) and '*Ca.* P. mali' strains AT, STAA and PM19 (Siewart et al., 2014, Janik et al., 2017, Strohmayer et al., 2020), but not from '*Ca.* P. australiense'.

The first report for the presence of the gene for SAP11 in a PMU region along with other genes such as *sigF*, *ssb*, *himA* and *tra5* and a sequence repeat of 331-bp was given by Bai et al. (2009). It was further suggested that SAP11 is a part of a larger transcript of ~ 2,800 bp which contains genes for four other SAPs and one membrane protein, thus indicating their simultaneous transcription *in planta*. Thus, SAP11 has been suggested to exist in an operon and it may function alone or in association with other secreted proteins present in the PMU-like pathogenicity island (Bai et al., 2009).

SAP11 was reported to contain a eukaryotic nuclear localization signal (NLS) which is crucial for its localization exclusively in nuclei of plants infected with AY-WB as confirmed by deletion experiments. Apart from NLS, a host factor, namely import in α assists in nuclear targeting of SAP11 in *Nicotiana benthamiana* confirmed by Virus Induced Gene Silencing (VIGS) assay. SAP11 has also been suggested to alter gene regulation at the transcriptional level in tomato plants (Bai et al., 2009).

SAP11 gets unloaded from the phloem tissues to access nuclei of mesophyll cells and other tissues as confirmed by immunofluorescence microscopic experiments. Moreover, most of the SAPs (51 out of 56) are smaller than 40 kDa. Since, the size exclusion limits (SELs) of plasmodesmata in the sink tissue varies between 10-40 kDa, the majority of the SAPs can unload from the phloem to target other developing tissues in plants (Bai et al., 2009). Initial reports on the presence of SAP11 in the leaf hopper *Macrosteles quadrilineatus* showed its accumulation in their midgut and salivary glands suggesting its transfer into plant cells when AY-WB infected insects feed upon plants (Bai et al., 2007).

SAP11 has a modular structure with distinct amino acids in different regions involved in its targeting to nucleus, binding with TCP and degradation of TCP (Sugio et al., 2014). The N-terminal of SAP11 has been reported to be crucial for its nuclear localization in the plant cell. The coiled-coil domain (91- 106 amino acids) at the C-terminal of SAP11 is required for its interaction with TCP13 (Sugio et al., 2014).

Alteration in the root structure has also been reported in *Arabidopsis* plants expressing AY-WB SAP11 leading to the formation of hairy roots, along with accumulation of cellular phosphate. The transgenic plants also showed upregulation of starvation-induced genes and microRNAs and downregulation of salicylic acid-mediated defense responses (Lu et al., 2014) leading to enhanced susceptibility to bacterial pathogens. In plantaproteolytic cleavage and *in vitro* self-interaction of SAP11 have also been reported (Lu et al., 2014).

SAP11 homolog from '*Candidatus* phytoplasma

mali' has also been reported to induce morphological alterations in the development of glandular trichomes and downregulate the expression of *NbOMT1* (encodes for *O*-methyl transferase) in transgenic *N. benthamiana* leading to reduced 3-isobutyl-2-methoxypyrazine (IBMP) synthesis. These changes result in alteration of aroma phenotype in transgenic *N. benthamiana* (Tan et al., 2016).

Chang et al. (2018) characterized the homologs of SAP11 from '*Candidatus* phytoplasma mali' (*Ca* PM), peanut witches' broom (PnWB) phytoplasma, AY-WB phytoplasma and OY-M phytoplasma ($SAP11_{CaPM}$, $SAP11_{PnWB}$, $SAP11_{AYWB}$ and $SAP11_{OYM}$). Alterations in the architecture of leaf and shoot and changes in flowering time were observed in *Arabidopsis* expressing these SAP11 homologs. SAP11 effectors are also involved in destabilization of class II TB/CYC-TCP TFs, thus inducing witches' broom (Chang et al., 2018). Alteration in miR156/SPLs module by $SAP11_{AYWB}$ has been reported to delay flowering (Chang et al., 2018).

SAP11 homologs from Maize bushy-stunt phytoplasma (MBSP) and AYWB differ in their interaction specificities with class II TCP TFs. Both $SAP11_{AYWB}$ and $SAP11_{MBSP}$ interact with CYC/TB1 BRC1 and BRC2 transcription factors, but only AYWB SAP11 interacts with CIN-TCP TFs. Thus, a smaller subset of class II TCP TFs has been reported to be destabilized by $SAP11_{MBSP}$ as compared to $SAP11_{AYWB}$ (Pecher et al., 2019).

A homolog of SAP11 has been identified from *Malusxdomestica* infected with '*Candidatus* phytoplasma mali' strain STAA (Janik et al., 2017). This effector, namely ATP_00189 is 41 per cent homologous to AY-WB SAP11 at the amino acid level and interacts with CIN-TCP group II proteins MdTCP25 (homologue of *Arabidopsis* TCP4) and MdTCP24 (homologue of TCP13/PTF1). Binding of ATP_00189 to MdTCP25 speculates its role in the downregulation of jasmonic acid (JA) biosynthesis, thus resulting in enhanced ABA response. The interaction of ATP_00189 with MdTCP24 is suggestive of its role in regulating plastid genes expression. Altered root growth and reddening are the symptoms commonly observed in apple proliferation which is postulated to be induced by SAP-11 mediated degradation of TCP13/PTF1 in '*Candidatus* phytoplasma mali' infected apple tree (Janik et al., 2017).

Anabestani et al. (2017) identified SAP11 homologs from three different strains of 16SrII group phytoplasma, namely Crotolaria Phyllody (CrP), Witches' Broom Disease of Lime (WBDL) and Faba Bean Phyllody (FBP).

A total of 37 putative effectors identified in Wheat Blue Dwarf (WBD) phytoplasma (Chen et al., 2014) were expressed in *N. benthamiana.* A SAP11 like protein, SWP1 was observed to induce distinct symptoms of witches' broom and was speculated to be the virulence factor. Wang et al. (2018) reported that apart from inducing witches' broom symptoms SWP1 also induces typical proliferation phenotype when expressed in *Arabidopsis.* On expressing deletion mutants of SWP1 in *N. benthamiana*, it was found that nuclear localization and the presence of typical coiled-coil domain are the two important requirements for SWP1 induction of witches' broom. Y2H and BiFC assays confirmed the interaction of SWP1 with BRC1 and BRC2, which are shoot-branching integrators. Further, *in planta* co-expression showed SWP1 mediated degradation of BRC1 involving a proteasome system. SWP11 induced HR like cell death, H_2O_2 accumulation and deposition of callose in *N. benthamiana.* SWP12 and SWP21 were reported to suppress defense related PCD in *N. benthamiana*.

Recently, Strohmayer et al. (2020) reported that the SAP 11- like protein of '*Ca.* P. mali' strain PM19, which is 41 per cent homologous to AY-WB SAP11 at amino acid level, results in the production of crinkled leaves and siliques along with symptoms of witches' broom when expressed in *Arabidopsis thaliana.* Yeast two-hybrid interaction studies revealed that Apple Proliferation (AP) SAP 11 -like protein interacts with a total of six members belonging to class 1 TCP TFs and all the members of class II TCP TFs in *A. thaliana.* It was further demonstrated that the AP_SAP11- like protein does not require the Nuclear Localization Signal (NLS) for its targeting or localization into the nucleus. In fact, the 17 amino acid long NLS, which is involved in targeting AY-WB SAP11 into the nucleus, has been reported to bind with few TCPs in case of '*Ca.*P.mali' SAP 11-like protein leading to crinkled leaf and silique phenotype in *A.thaliana.* Moreover, the presence of AP_SAP 11-like protein in the cytoplasm itself is sufficient for the induction of symptoms.

TENGU

TENGU is a 70 amino acid protein (4.5kDa) known to cause witches' broom and dwarfism in the infected plant. It also induces plant sterility. TENGU gene is restricted to 16SrI group and shows high level

of sequence conservation. It is located near ABC transporter genes in OY-M and MBSP and near PMU-like genome elements in AY-WB genome. (Sugio et al., 2012)

Historically, Paulwonia witches' broom disease caused by phytoplasma was first described more than 140 years ago in Japan. The symptoms include witches' broom and dwarfism. Infected plants produce large number of auxiliary shoots and bear small flowers and leaves with shortened internodes (dwarfism) resulting in broom like appearance and is referred to as Tengu-su (Tengu's nest) disease in Japan because of its resemblance to the nest of Tengu (mythical Japanese goblin).

Hoshi et al. (2009) first characterized virulence factor, tengu-su inducer (TENGU) by expressing it in *N. Benthamiana* plants. They screened 30 putative secretory proteins from OY and each protein was expressed in *N. benthamiana*. PAM765 (one of the secreted protein) gene was transformed into *N. Benthamiana* using *A. tumefaciens*. These transgenic plants expressing PAM765 developed clear symptoms of witches' broom and dwarfism. Apart from this, these plants also had malformed phyllotaxis. This was further confirmed by inoculating *A. thaliana* with OY phytoplasma strain which exhibited the same symptoms. Therefore, PAM765 was termed as Tengu, a virulence factor that induces phytoplasma related witches' broom, dwarfism and abnormal reproductive organogenesis.

TENGU protein travels to the plant phloem via Sec dependent pathway where N terminal signal peptide (32aa) is cleaved. This produces the mature protein (secretory protein) that is only 38 amino acids long. The 11 amino acids of the N-terminal in mature protein make up the functional domain of TENGU and are sufficient to cause symptom development and are conserved. On the other hand, C-terminal is not necessary for symptom induction but it is still conserved. It was found that TENGU is processed by host plant factor to generate functional peptides. The leucine residue at the 11th position is significant for TENGU function. (Sugawara et al., 2013)

Minato et al. (2014) demonstrated that transgenic expression of TENGU induces abnormalities in flower development in *A. thaliana* and that both ARF6 and ARF8 transcripts are significantly diminished in *tengu*-transgenic and OY-infected plants. Other floral maturation genes like LOX2, MYB21 and MYB24 levels were also significantly lower in *tengu*-transgenic plants in comparison to wild-type plants. A significant decrease in total levels of cis-JA and active JA-Ile in tengu-transgenic buds shows that TENGU expression reduces JA biosynthesis. Microarray analysis showed that phytoplasma infection in grapevine induces production of *pathogenesis-related proteins 1* (PR-1) which is a marker gene for SA signalling pathway. This suggests that SA is involved in phytoplasmal infection response but PR-1 expression was not seen in *tengu*-transgenic plants. This indicates that antagonism between SA and JA does not lead to repression of JA synthesis in *tengu*-transgenic flowers.

Transcription profiles of *tengu*-transgenic and GUS-transgenic *Arabidopsis* plants showed 373 genes to be upregulated and 575 genes to be downregulated in tengu-transgenic plants. A large number of auxin related genes downregulated due to expression of TENGU belonged to early auxin responsive genes i.e *AUX/IAA* family genes, *small auxin induced RNA* (*SAUR*) family genes and *GH3* family genes. Moreover, expression of *Dormancy associated protein* (*DRM1*) was found many folds lower in tengu-transgenic plants, along with *2-pin formed* (*PIN*) genes, when compared with GUS-transgenic plants. These results suggest that TENGU interferes with signalling and biosynthesis pathways of auxin (Hoshi et al., 2009).

Thus, TENGU is an effector protein with pleiotropic effect on at least two phytohormones, namely auxin and JA and eventually leads to sterility in plants.

In addition to the proteinaceous effector molecules, role of small RNAs such as microRNA has been proposed in the phytoplasma associated dramatic alterations in the plants.

miRNA

microRNAs (miRNAs) are one of the category of small noncoding RNAs which in case of plants are predominantly 21 nucleotides in length (Chen et al., 2010). They are derived from genomic segments via transcription of RNA polymerase II. This primary miRNA after processing yields mature miRNA that is a duplex which is a result of RNase-III type enzyme DICER-LIKE1 activity (Bartel, 2004). The miRNAs show the feature of perfect complementarity with their target sequences and show transcript repression by processes such as mRNA slicing through ARGONAUTE endonuclease activity. Fifteen different miRNA families are reported in plants (Bartel and Bartel, 2003) which are known to play regulatory role both at transcriptional and post transcriptional level (Yu et al., 2017).

Studies on phytoplasma host plant interaction have revealed that the modulation of miRNAs is one of the factors behind disease development. Following is a brief overview of such reports.

Alterations of leaf morphology in phytoplasma diseased plants involve symptoms like size reduction, leaf yellowing, leaf curling and development of new leaves with short plastochron lengths. In phytoplasma infected paulownia plants such symptoms are shown to be associated with miR156-*SPL9* (Squamosa-promoter-binding-like-protein) interaction (Fan et al., 2015; Cao et al., 2018). Similar studies where over expression of miR156 and its subsequent regulation of SBP-box transcripts has been observed is other phytoplasma infected plants (Ehya et al., 2013; Gai et al., 2014; Shao et al., 2016). RNA sequencing studies have indicated that miR156 might function by degrading long non-coding RNAs (Fan et al., 2018). Symptoms such as internode shortening, smaller inflorescences have also been linked to miR156/*SPL* in *flavescence doree* (FDp) infected grapevine (Chitarra et al., 2018). In addition to miR156, role of miR172 has also been indicated with respect to flower abortion in aster yellows infected grapevine (Synman et al., 2017) and flower sterility in jujube witches' broom disease (Shao et al., 2016).

Deformation of leaves in grapevine seems to involve modulation of multiple miRNAs as altered expression of miR159 and miR319 has also been detected (Synman et al., 2017). These miRNAs target MYB transcriptional factors (R2R3 family) that are responsible for several key functions in plants (Dubos et al., 2010). Symptoms such as leaf roll and shortened petioles in phytoplasma infected jujube plants has been associated with downregulated miR159 and coincident over expression of its target gene *MYB33* (Rubio-Somoza and Weigel, 2013).

During phytoplasma infection of host plant, regulation of miRNAs involved in hormone signalling is shown to play role in development of few disease symptoms. Expression analysis of miRNAs in yellowing disease affected mulberry and grapevine showed high levels of miR160, which is a miRNA that targets ARF (Auxin Response factor) mRNAs for degradation (Gai et al., 2014; Synman et al., 2017). miR393 that targets auxin receptor mRNAs was also upregulated in phytoplama affected lime trees showing symptoms of witches' broom (Ehya et al., 2013). Studies on phytoplasma infected mulberry have shown that regulation by miR393 is more streamlined by production of secondary siRNAs (Gai et al., 2014). Further, studies on phytoplasma infected mulberry phloem sap detected altered miRNAs (miR1233e, miR529b, miR157a, miR849) to function in different hormonal pathways (Gai et al., 2018). *TCP*/miR319 was shown to control biosynthesis of jasmonic acid via regulation of lipoxygenase genes in FDp infected grapevine (Chitarra et al., 2018). Increased level of auxin and alteration in several iso-miRNAs involved in auxin signaling (of miR160, miR166 and miR167) was observed in lime trees suffering from phytoplasma caused witches' broom (Synman et al., 2017). In contrast, jujube plants with phyllody symptoms showed lower auxin accumulation during flower development phase (Ma et al., 2020). In continuation, significant downregulation of *ARF4* was observed before and during floral development in phytoplasma-infected jujube plants, indicating role of hormone signalling in onset of phytoplasma disease symptoms.

Some phytoplasma responsive miRNAs have also been associated with nutrient homeostasis, which can influence appearance of the disease symptoms. Increase of miR395 has been observed in phytoplasma infected grapevine and lime trees (Ehya et al., 2013). miR395 targets the *ATP-sulphurylase* (*ATPS*) gene family and a low-affinity sulphate transporter gene, that play important role in regulating sulphate homeostasis (Mattewman et al., 2012). Its relation to sulphur accumulation and allocation has been well documented (Liang et al., 2010). Significant downregulation of miR399, a miRNA known for maintaining phosphate homoeostasis was also reported in phytoplasma infected host plants (Gai et al., 2014; Synman et al., 2017). The target gene of miR399 is *PHO2* (code for an ubiquitin-conjugating E2 enzyme, UBC24) that positively regulate Pi uptake, translocation (Chiou et al., 2006; Hseih et al., 2009). It is suggested that such fluctuations in nutrient might prove favourable for pathogen growth or lead to some physiological imbalance causing symptom production.

Downregulation of miR166 that targets class III HD-Zip protein was associated with defects in meristem and dwarfing in phytoplasma infected mulberry (Gai et al., 2014). In FDp Infected grapevine, the existence of a regulatory system of AGO10-miR166-HD-ZIPIII was proposed as *AGO10* was found to be over expressed in infected plants and suggested to be responsible for reduced post transcriptional activity of miR166. This also supported the up regulation observed for class III HD-Zip genes *Phabulosa* and *Revoluta* genes in

affected grapevine (Chitarra et al., 2018). Interestingly, reduced lignification observed in phytoplasma infected grapevine was associated with up regulation of a novel miRNA (vvi_mi64) which coincided with down regulation of its target, the polyphenol oxidase II transcript (Chitarra et al., 2018).

Excessive branching and reduction in leaf size was associated with over expression of miR157 in infected lime trees (Ehya et al., 2013), as previously observed in case of torenia plants over expressing *Arabidopsis* miR157 (Shikata et al., 2012). Changes in plant architecture in phytoplasma infected paulownia plants was associated with the miR160-miR172-miR397 cluster and their target genes, which function by regulating the cytokinin pathway (Cao et al., 2018).

Yellowing symptoms in phytoplasma affected mulberry plants has shown accumulation of miRNA29b and miR160a-5p which are involved in downregulation of chlorophyll synthase gene and beta amylase gene, respectively (Gai et al., 2014). miRNA and degradome sequencing in phytoplasma infected paulownia plants showed role of the miR156-miR160-miR5368 cluster in leaf yellowing (Cao et al., 2018). miR156 targets PIF3 (a helix-loop-helix transcriptional factor) that interacts with phyA, phyB, phytochrome responsive genes and CHL1 that synthesizes an enzyme for chlorophyll synthesis. Downregulation of *PIF3* and *CHL1* in phytoplasma infected plants indicates reduced chlorophyll production. Downregulation of miR160a and upregulation of its target gene *RAV* was also observed in such plants (Cao et al., 2018). Over expresssion of RAV (a transcriptional factor with AP2/ERF and B3 domain) in transgenic tobacco showed reduction in chlorophyll content and photosynthesis rate (Zhao et al., 2008). Target of miR5368, the *ZEP*gene which is responsible for accumulation of violazanthin and antheraxanthin was shown to be upregulated in phytoplasma infected plants (Cao et al., 2018). Further, decreased abundance of chlorophyll a-b binding protein and photosystem II 10kDa polypeptide was observed in paulownia plants infected with phytoplasma (Mou et al., 2013; Wang et al., 2017). This reduction has been associated with long non-coding RNAs in addition to miRNAs (Fan et al., 2018).

Overall, the host miRNAs modulation by phytoplasma plays an important role in development of disease symptoms ranging from yellowing, leaf deformation, flower abortion, sterility to changes in plant architecture such as dwarfing and meristem defects including loss of apical dominance, excessive branching.

In summary, targeted research on phytoplasma effector proteins such as TENGU, SAP54, SAP11 and host plant microRNAs have thrown light on the intricate mechanisms underlying symptom development in phytoplasma infection. TENGU induced dwarfism and sterility in plants has been linked with alteration in auxin and jasmonic acid biosynthesis. Analysis of structural motifs and interacting partners of a phyllody causing effector, SAP 54 has revealed its role in degradation of transcriptional factors involved in flower development. Role of another effector molecule SAP11 has emerged in axillary shoot proliferation and hairy root symptoms. Differential interaction of SAP11 with transcriptional factors responsible for cell proliferation is known to mediate such morphological changes. In addition, alteration in microRNAs mediated gene regulation has been proposed for appearance of varied symptoms. Further progress with respect to various aspects of molecular studies on phytoplasma effector molecules and phytoplasma responsive miRNAs of host plant pertaining to different phytoplasma groups and their host taxa presented here will provide effective platform for comprehensive understanding of symptom development and their control in case of phytoplasma associated diseases.

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PHYTOPLASMAS, THE FAST SPREADING VECTOR BORNE PATHOGENS OF FLOWER CROPS: INDIAN SCENARIO#

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ABSTRACT

Floriculture trade in India is one of the most lucrative fields, earning valuable forex to the national exchequer. Exchange of planting material via different modes plays a major role in spreading of the pathogen. Floriculture being an international industry, the transmission of pests or pathogens through live plants, seeds, cuttings and flowers etc., is very high. In recent years, the occurrence of phytoplasmal diseases in flower crops is increasing due to extensive exchange amongst many groups. The prominent symptoms of phytoplasma infection are the flowers turning into leafy structures called phyllody and the formation of sterile green flowers called virescence. In floricultural crops, these two symptoms are of major disadvantages as the flowers are the main economic product. The phyllody symptoms are most often mistaken for novel plant type and are multiplied unwittingly. Phytoplasmas are switch between plant and animal kingdoms. Phytoplasma spread in the field through leaf hoppers by inhabiting in their gut, haemolymph, salivary glands etc. Among the ornamental plants and flower crops, various groups of phytoplasma have been reported from India. More than 40 per cent of the infections are reported from the members of the Asteraceae family with 16SrI-B group, '*Candidatus* Phytoplasma asteris'.

Key words: Floriculture, disease, diagnosis, genomics, management

The floriculture sector is making rapid progress in India with 3.13 lakh ha of land under floriculture, producing 2059000 tonnes of cut flowers. Although, the mainstay of Indian floriculture is cultivating traditional flowers in an open field, the production of cut flowers under polyhouses, production of potted plants, plug plants, landscape plants and lawn grass is increasing. Due to lack of awareness among the stakeholders, often the affected plants are further propagated with a premise that the unique visible symptoms are that of novel variants. This practice is responsible for the spread of phytoplasmas among floricultural crops. Though the efforts to identify phytoplasmal diseases have been evident over the past three decades, their accurate detection and etiology became possible only after the advent of molecular biology tools. The remedial measures advocated so far focused mostly on vector management and prophylactic measures rather than curative.

In an attempt to introduce new plant types and cultivars from other nations or continents, there is greater threat for the entry of invasive plants, weeds, pests and pathogens. The plants infected with viruses, viroids and fastidious prokaryotes are of major concern as they are easily vectored by insect pests and are very difficult to manage once they are established. Ornamentals can act as reservoirs of potential pathogens that spread to other crops. While awareness on virus infection has increased among farmers, the knowledge about the vascular inhabiting insect vectored fastidious prokaryotes is meager. Due to lack of awareness about pathogen and propagation of infected mother plants rapidly spread the phytoplasma diseases.

Symptomatology

Phenotypic expression of phytoplasma infections is the result of alterations in plant morphology due to the imbalance in plant hormones. The common morphological symptoms due to phytoplasma infections can be classified as those occurring during the vegetative phase and flowering phase. Vegetative parts of the phytoplasma infected plants show little leaves or witches' broom symptoms. "Witches broom' symptoms occur as a result of proliferation of axillary buds, short internodes and loss of apical dominance. The reduced size of leaves and their occurrence in clusters is called "little leaf". Other non-specific symptoms include abnormal internode elongation, generalized stunting, abnormal yellowing of the plant called as yellows, excessive pigmentation, loss of the pinnation in leaves, leaf malformations, necrosis of vascular

bundles, dieback of twigs/ branches and decline in trees. During flowering, infected plants develop "phyllody" in which green leaf-like structures are produced instead of flowers, as the phytoplasma infections deregulate the genes involved in flowering (Fig. 1). Virescence is another symptom where the coloured parts in flowers turn green due to loss of pigments. Inhibition of photosystem II and biosynthesis of chlorophyll and carotenoid is reported in phytoplasma infected plants. Sterility of flowers, flower malformations, rudimentary reproductive structures, lack of differentiation of sexual organs are the other symptoms that are observed. The symptom expression varies with respect to host plant and the strain of phytoplasma.

Phytoplasma disease diagnosis

Japanese group of plant pathologists and entomologists discovered that phytoplasma cause dwarf disease in mulberry through Transmission Electron Microscopy (TEM) (Doi et al., 1967). Phytoplasma formally called mycoplasma-like organisms (MLOs) are plant pathogenic bacteria in the class mollicutes, lack rigid cell walls, surrounded by single membrane and are pleomorphic with a size of 80 to 800nm. Until the early 1980s, phytoplasma diseases were diagnosed by transmission electron microscopy (TEM) because of their sub-cellular nature and the difficulty in culturing them on artificial media. In the 1980s, fluorescent microscopy techniques such as direct fluorescence detection (DFD) and DAPI staining were developed where DFD detects auto fluorescence of necrotic phloem cells and DAPI detect phytoplasmal DNA. ELISA, the popular diagnostic technique for viruses couldn't become popular for phytoplasma diagnostics as antibody preparation was difficult. Around 1990, advances in molecular biology enabled



Fig. 1. Typical symptoms of phytoplasma infection. a. Phyllody (Chrysanthemum); b. Virescence (Gerbera); c. Little leaf (Periwinkle); and d. Yellows (Aster)

direct detection of phytoplasmal DNA by nucleic acid hybridization and the polymerase chain reaction (PCR). The 16S rRNA gene based PCR has become the gold standard in phytoplasma diagnostics (Gundersen et al., 1996). Recently, Loop-mediated Isothermal Amplification (LAMP) technique gained importance in diagnosing phytoplasma diseases (Dickinson, 2015). Invention of next generation sequencing enabled in drawing the picture of complete genome of phytoplasma. Recently two draft full genomes of phytoplasma strains associated with sugarcane grassy shoot (SCGS) and bermuda grass white leaf (BGWL) diseases are available from India (Kirdat et al., 2020).

Genome size and diversity

The genome sizes of many phytoplasma are larger than many culturable mollicutes as determined through pulse field gel electrophoresis. A wide range of genome variation from 530kb to 1350kb has been observed among phytoplasma (Fig. 2 & 3). Similarly, in Aster yellows group itself the genome size is widely variable from 660kb to 1130kb and in the X

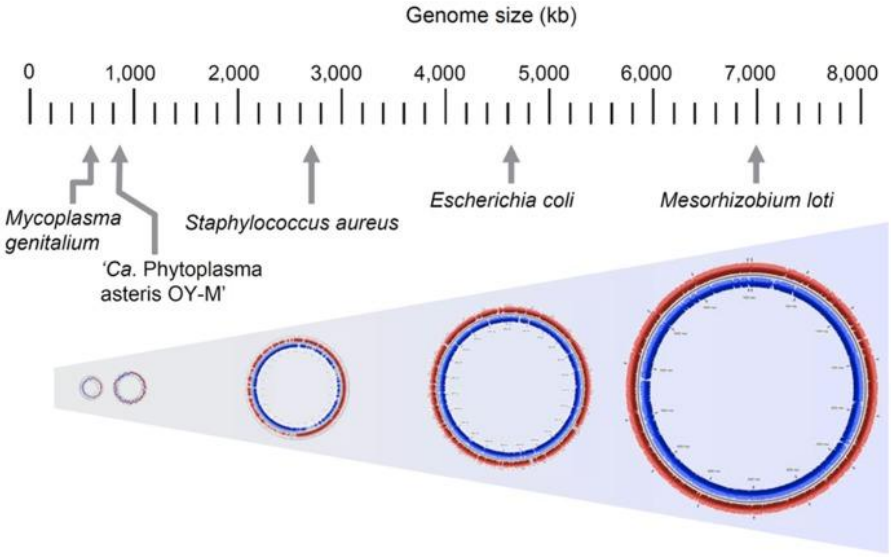


Fig. 2. Size of phytoplasma genome in comparison to other bacteria (Source: Oshima et al., 2013)

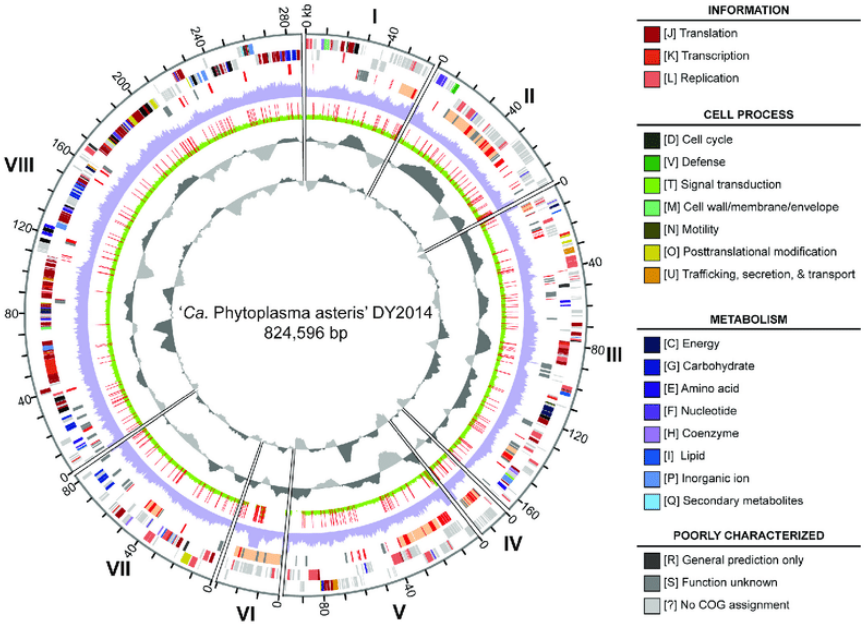


Fig. 3. Genome map of *Ca.* Phytoplasma asteris causing periwinkle yellowing (Source: Cho et al., 2019)

disease group the chromosome size varied from 670 to 1075kbs. The smallest genome so far is of bermuda grass white leaf phytoplasma *i.e.* '*Ca*. P. cynodontis' which is about 530kb. The complete genome sequence of four phytoplasmas and one mutant is available so far. This includes onion yellows mutant, Aster yellows-witches broom strain, '*Ca*. P. australiense' an Australian strain and a New Zealand strain and apple proliferation phytoplasma. '*Ca*. P. australiense'. A number of draft genome sequences of X disease, stolbur phytoplasma, Pigeon pea witches broom etc. are also available. *Ca*. P. asteris and *Ca*. P. australiense have circular chromosomes while *Ca*. P. mali has a linear chromosome. *Ca*. P. asteris and *Ca*. P. australiense reported to contain extra-chromosomal elements (Plasmids) which may be playing an essential role in insect vector transmission.

Among the sequenced genomes, *Ca*. P. australiense has the largest chromosome (960kb) and *Ca*. P. mali is the smallest genome with 602kb. All the genomes have GC content in the range 27-28 per cent with *Ca*. P. mali, 22 per cent. The number of protein coding genes predicted from genomes ranges from 497 to 1126. Phytoplasma genomes have reported to harbor repeated sequences in large clusters approximately of 20kb size that are termed as Potential Mobile Units (PMUs), which appear to be unique to phytoplasma. In genome, different islands of PMU like genetic elements but lacking many segments of the PMUs are also observed and are termed as Sequence Variable Mosaics (SVMs). These PMUs, SVMs and plasmids are responsible for the adaptation of phytoplasma to diverse plant hosts, environments and insect hosts. Phytoplasmas have limited number of genes, thus the biosynthetic capabilities are also limited. In phytoplasma genomes, many genes coding for amino acid, fatty acids and nucleotides biosynthesis are missing. The genome lacks the genes involved in oxidative phosphorylation, tricarboxylic acid cycle, pentose phosphate pathway and the phosphotransferase system. Phytoplasma genomes lack genes coding for F-type ATPases responsible for transmembrane potential and in turn ATP synthesis (Oshima et al., 2013). The genomes largely encode for efficient transporter systems for the uptake of nutrients, which include ATP binding cassette indicating the phytoplasma is dependent on host for most of its metabolism. An exploration into the ability of phytoplasmas to adapt to two diverse environments that of plant and insects through microarray analysis of OY-M strain revealed that approximately 33 per cent of gene expression changes while shifting the host and the PMUs are highly expressed in insects than plants. Phytoplasma membrane proteins and secreted proteins play an important role in host cell interaction and its virulence (Bai et al., 2006). An altered level of oxygen and carbon dioxide has been observed to affect the population of phytoplasma in Oenothera leaf tip cultures.

Phytoplasma vector transmission

Vector-host plant interaction plays a crucial role in limiting or expanding the spread of phytoplasma. Polyphagous vector species are potential to inoculate the phytoplasma among wide range of plant species, depending on susceptibility of host plant. Phytoplasma, like plant pathogenic viruses and symbiotic prokaryotes can be trans-ovarially transmitted. When phytoplasma infected *Scaphoideus titanus* Ball (vector of grape yellows in Europe) females were allowed to lay eggs on healthy host plants, the hatched nymphs of all developmental stages including adults were found to be infected and capable of transmitting phytoplasma (Alma et al., 1997). Kawakita et al. (2000) used electron microscopy to observe phytoplasma in the ovaries and other tissues of *Hishimonoides sellatiformis.* The phytoplasma-insect relationship can be beneficial, deleterious, or neutral in terms of its impact on the fitness of the insect host. Beanland et al. (2000) determined that exposure to one strain of AY increases both the lifespan and fecundity of female *Macrosteles quadrilineatus*; however, exposure to another strain of AY increases the lifespan of test insects but not the number of offspring produced. The corn leafhopper, *Dalbulus maidis*, a specialist, cannot live on unrelated hosts such as healthy aster (*Callistephus chinensis* Nees) when reared on several strains of AY-infected aster, its lifespan is increased and it fed and survived on healthy aster as well (Purcell, 1988). The effects of phytoplasma infection on the insect hosts have implications for the incidence and spread of disease. Vectors that live longer have the opportunity to infect more plants and produce more offspring.

Disease management strategies

Phytoplasmas are obligate parasites which solely depend on the planting material or insect to spread from one location to another. Thus the right intervention at these two epidemiological factors can contain the disease spread to newer locations, but it is very difficult to achieve. Strategies to cure the already infected plant are very rare and with those available, success

rate is very low. The possible solutions to manage phytoplasmal diseases are as follows.

- Clean planting material is the preventive or prophylactic measure where the elimination of phytoplasma and vectors in planting material.
- Insect control can manage the phytoplasma diseases to some extent.

Introduction of infected planting material to a new area where the potential vectors are present can damage the entire plantation of the crop in the area as exemplified in the case of Limonium by Weintraub et al. (2004). To achieve clean propagating material, certification of mother stocks that are phytoplasma free and maintaining the mother plant under insect proof net throughout the year is a must. Production of phytoplasma free planting materials through meristem tip culture and their certification is inevitable to maintain the propagating material disease free. In case of grafting or budding buds/scions and root stock has to be phytoplasma free. In case phytoplasma free mother stock is not available, clean propagation material can be obtained through thermotherapy and meristem tip culture. Since these treatments also do not completely eliminate the pathogen from the whole lot, further certification of the planting material through molecular technique is required to certify it as disease free. Thermotherapy of cuttings and scions followed by the maintenance of grafts in insect proof conditions has been found a good option to produce phytoplasma free grafts. Hot water treatment of propagating material is also found promising. In grapevine hot water treatment of scions at 50^0C for 40 minutes was found effective in reducing phytoplasmal load. Such strategies can be adopted in woody ornamentals (Linck et al., 2019).

Indian Scenario

Sandal spike disease was the first phytoplasmal disease reported in India (Nayar, 1977). Thereafter a large number of phytoplasma diseases have been described, which included brinjal little leaf disease, grassy shoot disease of sugarcane, rice yellow dwarf disease, sesamum phyllody, white leaf disease of *Cynodon dactylon*, little leaf disease of *Acanthospermum hispidium* and yellowing disease of *Urtochloapa nicoides*). More than 129 phytoplasma diseases are attributed to one of six '*Ca*. Phytoplasma' species in India (Rao et al., 2017). The number has been increasing as new reports arise and that species is *Ca*. P. asteris.

Phytoplasma diseases of ornamental plants and flower crops

Due to the development of molecular tools, phytoplasmas have been characterized from as many as 60 plant species including ornamentals in India (Table 1). Aster yellows phytoplasma group (16SrI) are the most common group of phytoplasmas associated with more than 31 diseases of ornamentals, tree species, vegetables, sugarcane, fruit crops and pulses in India. Even though phytoplasma diseases are of common occurrence in India, only few of them have been fully characterized, particularly in North-Eastern and Southern parts of the country. Further studies of these phytoplasma diseases are prompted including their characterization, incidence, transmission and vector identification. Since 2011 there are reports of occurrence of phytoplasma in more than 10 ornamental species. Association of '*Candidatus* P. asteris' with leaf yellows and witches' broom symptoms of brachycome species in India, association of rigeon pea witches' broom phytoplasma (16Sr IX) infecting *Phlox drummondii* in India, rice yellow dwarf phytoplasma (16Sr XI-B subgroup) infecting *Jasminum sambac*, association of pigeon pea witches' broom phytoplasma infecting *Carpobrotus edulis* (L.) N.E. Br, association of '*Ca.* P. asteris' (16SrI group) with flattened stem and witches' broom symptoms of *Petunia hybrida*, characterization of an isolate of '*Ca.* P. asteris' infecting *Zinnia elegans*, detection and identification of phytoplasmas associated with little leaf and witches' broom disease of marigold (*Tagetes erecta* L.) in India are some of the recent reports of phytoplasma in India (Rao et al., 2017).

At ICAR-DFR, an attempt has been made to map the distribution of phytoplasma infecting ornamental plants and flower crops (Fig. 4 and Table 1). Phytoplasma infecting major flower crops has been diagnosed and characterized based on 16SrRNA from the following crops (Table 2).

At ICAR-DFR, 16SrRNA based identification of phytoplasma infecting various members of *Asteraceae* family has been undertaken and two groups of phytoplasma 16Sr-IB and 16Sr-IID have been found to infect marigold (16Sr-I), chrysanthemum and aster (16SrIID). Germplasm of aster and chrysanthemum have been screened for incidence of phytoplasma. In vegetative propagated marigold locally known as Calcutta, incidence of *Ca*. P. asteris has been observed and has become a major concern of spread through the cuttings to different parts of the country. The year

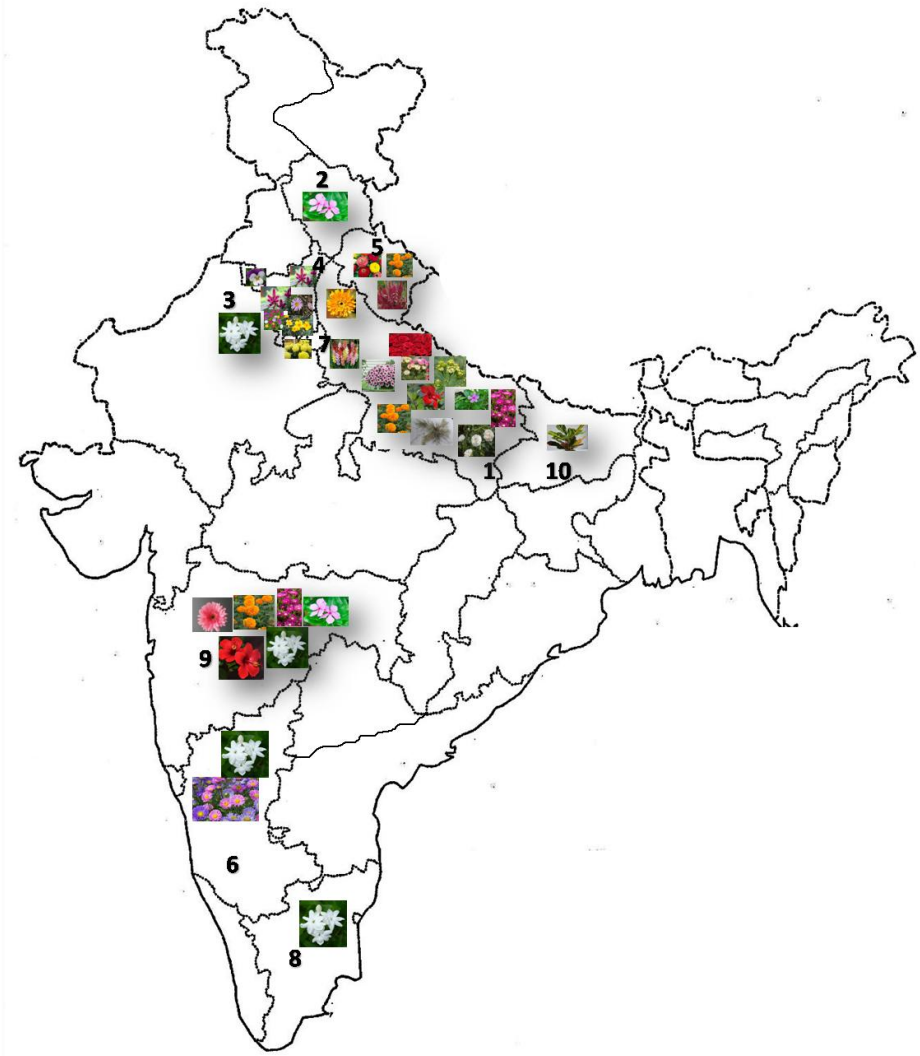


Fig. 4. State wise disease distribution map of phytoplasma on ornamental and flower crops in India (Map not to scale). Based on the surveys and reports from various geographical regions.

round dynamics of leafhopper population and incidence of phytoplasma has been evaluated. The putative leafhopper vectors have been identified. Multi locus genotyping for the right identification of phytoplasma up to species level has been initiated. Parthenium has been identified as a major collateral host in field for year round occurrence of phytoplasma. Various chemicals, botanicals and physical methods for management are being studied to reduce phytoplasmal incidence. Programmes for right identification of phytoplasma infections and management have been organized from time to time for the stakeholders.

Phytoplasma diagnostics

The most common method used for diagnosis of phytoplasma in India is nested PCR based on universal primers pairs P1 and P7 and R16F2n/R16R2 (Table 3). In the recent times, international concern on accuracy of using single gene i.e. 16SrRNA for identification has lead to initiation of identification based on Multiple Loci like the genes SecA, Tuf and Immunomembrane proteins (Imp) has been initiated. MLST has been employed for phytoplasma characterization in Marigold and Impatiens recently (Panda et al., 2021a; Panda et al., 2021b).

Genomics and taxonomy

Phytoplasma are categorically placed into 33 ribosomal groups with a number of sub-groups each (Bertaccini and Lee, 2018). The taxon '*Candidatus* Phytoplasma' species has been identified based on unique 16S rRNA gene sequence (>1200 bp), if the 16S rRNA gene sequence has more than 97.5 per cent similarity to that of any of the previously described species, it has

Table 1. Distribution of phytoplasmal diseases in ornamental and flower crops with respect to various locations in different states based on the reports. (Source: Rao et al., 2017)

Place /State	Plant Host	Botanical name	Associated disease symptoms	Phytoplasma species
Gorakhpur /UP	Ice Plant	*Carpobrotus edulis*	Little leaf and leaf yellow	'*Ca*. P. phoenicium'
	Rose Periwinkle	*Catharanthus roseus*	Phyllody	'*Ca*. P. asteris'
	Golden Dew Drop	*Duranta erecta*	Yellows	'*Ca*. P. asteris'
	Hibiscus	*Hibiscus rosa-sinensis*	Yellow leaf	'*Ca*. P. asteris'
	White rose	*Rosa alba*	Little leaf phyllody, witches' broom, virescence, bud proliferation	'*Ca*. P. asteris'
	Bermuda grass	*Cynodon dactylon*	White leaf	'*Ca* Phytoplasma
Lucknow /UP	Chrysanthemum	*Chrysanthemum* sp.	Little leaf, Virescence,	'*Ca*. P. asteris'
	Gladiolus	*Gladiolus* sp.	Yellowing and malformation of flowers	'*Ca*. P. asteris'
	Damask rose	*Rosa damascena*	Rose witches broom	-
	Moss rose	*Portulacagrandiflora*	Portulaca Little Leaf	-
	Petunia	*Petunia* sp.	Witches' broom	'*Ca*. P. asteris'
	Mexican Marigold	*Tageteserecta*	Little leaf	'*Ca*. P. asteris'
	Southern Cone Marigold	*Tagetesminuta*	Phyllody	'*Ca*. P. cynodontis'
Shahajahanpur /UP	Madagascar Periwinkle	*Catharanthus roseus*	Little leaf, Phyllody, Yellows	-
Sitamadhi/ BR	Garden Croton	*Codiaeum variegatum*	Leaf yellow, witches' broom	'*Ca*. P. asteris'
Himachal Pradesh	Indian Periwinkle	*Catharanthus roseus*	Little leaf, Phyllody	'*Ca*. P. asteris'
Mukteshwar /UK	New Zealand Christmas Bell	*Alstroemeria psittacina*	Little leaf	'*Ca*. P. asteris'
	Zinnia	*Zinnia elegans* Jacq.	Phyllody	'*Ca*. P. asteris'
Pantnagar /UK	Amaranthus	*Amaranthus* spp.	Phyllody	'*Ca*. P. aurantifolia'
	Mexican Marigold	*Tagetes erecta*	Witches' broom	'*Ca*. P. aurantifolia'
New Delhi /DL	Yellow Allamanda	*Allamanda cathartica*	Little leaf	'*Ca*. P. trifoli'
	Daisy	*Brachyscome* sp.	Little leaf, witches' broom	'*Ca*. P. asteris'
	Silver Cock's Comb	*Celosia argentea*	Flat stem, witches' broom	'*Ca*. P. asteris'
	Chrysanthemum	*Chrysanthemum morifolium*	Phyllody	'*Ca*. P. aurantifolia'
	Four o' clock Flower	*Mirabilis jalapa*	Little leaf	'*Ca*. P. aurantifolia'
	Garden Petunia	*Petunia hybrida*	Flattened stem, witches'	'*Ca*. P. asteris'
	Phlox	*Phlox drummondii Hook.*	Witches' broom	'*Ca*. P. phoenicium'
	Hybrid Tea Rose	*Rosa hybrid*	Little leaf, Multiple buds	'*Ca*. P. aurantifolia'
	Chrysanthemum	*Chrysanthemum* sp.	Little Leaf	*'Ca*. Phytoplasma'
	Saponaria	*Saponaria* sp.	Little leaf	'*Ca*. P. trifoli'
	Wild Pansy	*Viola tricolor*	Witches' broom	'*Ca*. P. asteris'
Jaipur /RJ	Jasmine	*Jasminum sambac*	Witches' broom	'*Ca*. P. cynodontis'
Karnal /HR	Silver Cock's Comb	*Celosia argentea*	Fasciation	'*Ca*. P. australasia'
Punjab	China Pink	*Dianthus chinensis*	Leaf Yellowing	-
Pune /MH	Hibiscus	*Hibiscus rosa-sinensis*	Yellow leaf	'*Ca*. P. trifoli'
	Annual Chrysanthemum	*Chrysanthemum* sp.	Phyllody, Little leaf	'*Ca*. P. asteris'
	Gerbera	*Gerbera jamesonii*	Phyllody	-
	Marigold	*Tagetes erecta*	Phyllody, Stunting	-
	Periwinkle	*Catharanthus roseus*	Little leaf, Virescence	-
	Aster	*Callistephus chinensis*	Phyllody, Yellows	'*Ca.* P. australasiae'
	Jasmine	*Jasminum sambac* L. Aiton	Phyllody, Little leaf	-
Devanahalli/ Bengaluru/ KA	Jasmine	*Jasminum sambac* L.	Little leaf	-
Mysuru/ KA	Aster	*Callistephus chinensis*	Virescence and phyllody	'*Ca*. P. aurantifolia'
Tamil Nadu	Jasmine	*Jasminum* sp.	Phyllody	-

Table 2. Flower crops exhibiting natural incidence of phytoplasma symptoms

Sl. No.	Crop	Varieties showing natural incidence of phytoplasma
1.	Chrysanthemum	Pusa Anmol, Pusa Chitraksha, PAU-D-11, PAU-35, Preet Shringar, White Prolific, Aprajita, Mahatma Gandhi, Naughty White, Gulmohar, Fireball, Royal Princess, Bidhan Gold
2.	Aster	Phule Ganesh Pink and White, Arka Kamini, Arka Archana
3.	Marigold	Calcutta, Arka Bangara

Table 3. *Candidatus* phytoplasma species associated with ornamental plants of India based on PCR diagnosis

'*Candidatus* Phytoplasma' species	Primers used	Acronym/group-sub-group	GeneBank accession number	References
'*Ca*. P. asteris'	P1/ P6 and R16F2n/ R16R2	16SrI-B	EU694109	Chaturvedi et al. (2009a)
	P1/ P6 and R16F2n/ R16R2	16SrI	HM230686	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI	DQ431842	Raj et al. (2007)
	P1/ P6 and R16F2n/ R16R2	16SrI	HM230688	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-I	FJ491455	Raj et al. (2009)
	P1/ P7 and R16F2n/ R2	16SrI-F	FJ939287	Chaturvedi et al. (2010)
	P1/ P6 and R16F2n/ R16R2	16SrI	GU594056	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-D	FJ429364, KP096315	Chaturvedi et al. (2009b)
	P1/ P6 and R16F2n/ R16R2	16SrI-B	HM230685	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-B	HM230685	Raj et al. (2011)
	P1/ P7 and R16F2n/ R16R2	16SrI-B	MN832584	Rihne et al.2020)
'*Ca*. P. aurantifolia'	R16mF2/ R1 and fU5/ rU3	PWB/16SrII	EU362627	Arocha- Rosete et al. (2008)
		PWB/16SrII-D	KX013259	Kumar et al. (2016)
	R16mF2/R1 and fU5/rU3	PWB/16SrII-G	EU362631	Arocha-Rosete et al. (2008)
Ca. P. australasia	P1/ P7 and R16F2n/ R16R2	16SrII-D	-	Sumashri et al. 2020 Prabha K et al. 2019
'*Ca*. P. cynodontis'	P1/ P7 and 3Far/ 3Rev and R16F2n/ R16R2	RYD/ 16SrXI-B	KF728950	Madhupriya et al. (2015)
'*Ca*. P. phoenicium'	P1/ P7 and R16F2n/ R16R2	AlmWB/ 16SrIX-C	KJ782631	Shukla et al. (2014)
'*Ca*. P. trifoli'	P1/ P7 and 3Far/ 3Rev	CP/ 16SrVI-D	KX641023	Khasa et al. (2016)
	P1/ P7 and R16F2n/ R16R2	16SrVI-D	-	Rihne et al. 2019

been placed under a novel '*Ca*. Phytoplasma' species (IRPCM, 2004). Diversity analysis was undertaken based on the 16SrRNA sequences reported from India. The sequences of 16SrRNA region were retrieved from public database and aligned using clustalW and phylogenetic and diversity analysis was undertaken based on Maximum likelihood method using MEGA 6 software. Results indicate that they belong to three different taxonomic groups and sub-groups like 16Sr I (*Ca.* Phytoplasma asteris), 16SrVI (Clover proliferation group), 16Sr XI-B (Rice yellow dwarf phytoplasma), 16SrIX-C sub-group (*Ca.* P. phoenicium -pigeon pea witches broom), dominated by 16Sr I group. Almost all the phytoplasma species reported exhibited close relation to those reported in countries from North America and East Asia. An elaborative analysis in this direction is required to reach any particular conclusion. Two draft genomes of phytoplasmas associated with sugarcane grassy shoot (SCGS) and Bermuda grass white leaf diseases '*Ca.* P. cynodontis' are submitted from India. SCGS genome is 95.43 per cent complete with 333.98× coverage 404 protein-coding genes, 12 transfer RNA (tRNA), and two rRNA genes. Bermuda grass white leaf phytoplasma genome annotated is 91.32 per cent complete with 373.6× coverage and have 425 protein-coding genes, 13 tRNA, and three rRNA genes (Kirdat et al., 2020).

Phytoplasma transmission

Phytoplasma transmission occurs through sap-sucking insect vectors belonging to families Cicadellidae (leafhoppers) and Fulgoridae (plant hoppers). The study conducted by Tiwari et al. (2016a) shows *Pyrilla perpusilla* (Walker) (Lophopidae: Hemiptera) as a potential vector in transmission of 16SrXI-B sub-group phytoplasmas in sugarcane crops infected with sugarcane grassy shoot (SCGS) disease showing white leaf symptoms at Shahjahanpur district of Uttar Pradesh. In the transmission study conducted by Tiwari et al. (2016b), three leafhopper species *Cofanauni maculata*

(Signoret) (Cicadellidae: Hemiptera), *Exitianus indicus* (Distant) (Cicadellidae: Hemiptera) and *Maiestas portica* (Melichar) (Cicadellidae: Hemiptera) were found to be putative vectors of Sugarcane grassy stunt phytoplasma caused by *Ca.* P. oryzae (16SrXI group). The identification of new vectors of Sugarcane grassy stunt phytoplasma suggested that these leafhopper species may be responsible for secondary spread of SCGS phytoplasma. By PCR detection of sugarcane grassy stunt phytoplasma molecules Srivastava et al. (2006) confirmed the presence of sugarcane grassy stunt phytoplasma molecules in *Deltocephalus vulgaris* (Cicadellidae: Hemiptera) a potential vector of the phytoplasma. PCR assay has successfully detected the presence of sugarcane grassy shoot phytoplasma (16Sr XI) in the leafhopper vector *E. indicus* tested positive for the phytoplasma molecule 16Sr XI (Rao et al., 2014). *E. indicus* was a putative vector for '*Ca.* P. cynodontis' and play a role in transmitting 16SrXIV group phytoplasmas in *Cynodon dactylon*. *Ca*. P. cynodontis causes chlorotic little leaves, witches' broom and shortened stolons /rhizomes on *Cynodon dactylon* observed in Shahjahanpur, Uttar Pradesh, India. In the study conducted by Taloh et al., 2020, the leafhopper, *Hishimonus phycitis* (Distant) (Cicadellidae: Hemiptera) was found positive to 16SrVI group phytoplasmas infecting chrysanthemum, which confirms *H. phycitis* as a putative vector for the transmission of 16SrVI group of phytoplasma strain in chrysanthemum. In the molecular detection of phytoplasma molecules in leafhopper vectors, *H. phycitis* was identified positive for 16SrI-B and 16SrII-D sub-groups of phytoplasmas from chrysanthemum fields at Delhi and jasmine fields at Bengaluru, respectively. This study suggested that *H. phycitis* may acts as potential natural source for secondary spread of the identified phytoplasma strains (Gopala, 2018). In the transmission studies conducted by Pathak et al. (2012), *Orosius albicinctus* (Distant) (Cicadellidae: Hemiptera) successfully transmitted the phytoplasma molecules from infected plants to the healthy plants. In the transmission study conducted by Sajad un Nabiet al. (2015), *H. phycitis* (Distant) was found to be positively associated with the transmission of sesame phyllody caused by *Ca* .P. asteris' sub-group I-B. In the transmission study conducted by Manish Kumar et al. (2017), *H. phycitis* was identified as carrier and natural vector of 16SrVI-D sub-group of phytoplasmas causing brinjal little leaf (Fig. 5-6).

Survey and field evaluation of flower growing areas have found the incidence of leaf hopper vector *Hishimonas phycitis* in marigold and aster. Year round study of seasonal dynamics of leafhopper vector and phytoplasma incidence has been studied. It has been observed that increase in phytoplasma incidence percentage was directly correlated to increase in leaf hopper population in the field (Data not shown). Evaluation of plant germplasms have been undertaken for the natural incidence of phytoplasma symptoms in the field. In case of aster no significant effect of varieties have been observed as both white and purple varieties have been found to be susceptible to phytoplasma. In chrysanthemum percentage incidence varied from 1 to 2 maximum 10%.

Phytoplasma management

As phytoplasmas are very difficult to cultivate on artificial media very limited studies have been done in India to manage them. Tetracycline treatment has been found to reduce the load of phytoplasmas in the affected plants but did not completely eliminate the disease symptoms. As early as in 1978, Verma and Dubey demonstrated the influence of treatment method, duration and concentration of tetracycline on little leaf symptoms in brinjal. Treatment with tetracycline antibiotics at weekly intervals reduced the symptoms of phytoplasma in petunia (Ajayakumar et al., 2007). *In vitro* treatment with oxytetracycline (75 mg/l) was found to be effective in eliminating phytoplasma in plant tissues with 50 per cent efficiency (Singh et al., 2007) Production of disease free planting material of sugarcane by eliminating Sugarcane grassy shoot (SGGS) phytoplasma through apical meristem (2-3 mm) culture has been achieved (Tiwari et al., 2011). Various immune priming chemicals have been evaluated for the recovery of phytoplasma infections in chrysanthemum at ICAR-DFR. The following chemicals, benzoic acid 0.006g/ L, salicylic acid 0.001mM,BTH0.1mM (Acibenzolar S Methyl), IBA100ppm,$GA_3$150mg/ l, chitosan 1.5ml/ l, tetracycline 500ppm, oxytetracycline500ppm, grape seed extract 1g/ l and Alliette 0.1 per cent have been evaluated and there has been no promising effect of these treatments for symptom reversal. To prevent the transmission and spread of the pathogen through nursery plants, creating awareness about phytoplasma is imperative. As a part of awareness about phytoplasma diseases among the nurserymen and farmers, workshops have been organized at Kadiyam, Andhra Pradesh, Junnar Taluka, Pune district etc. by DFR. An integrated approach employing cultural, chemical, biological and nutrient management is essential for reducing the advance of phytoplasmal diseases.

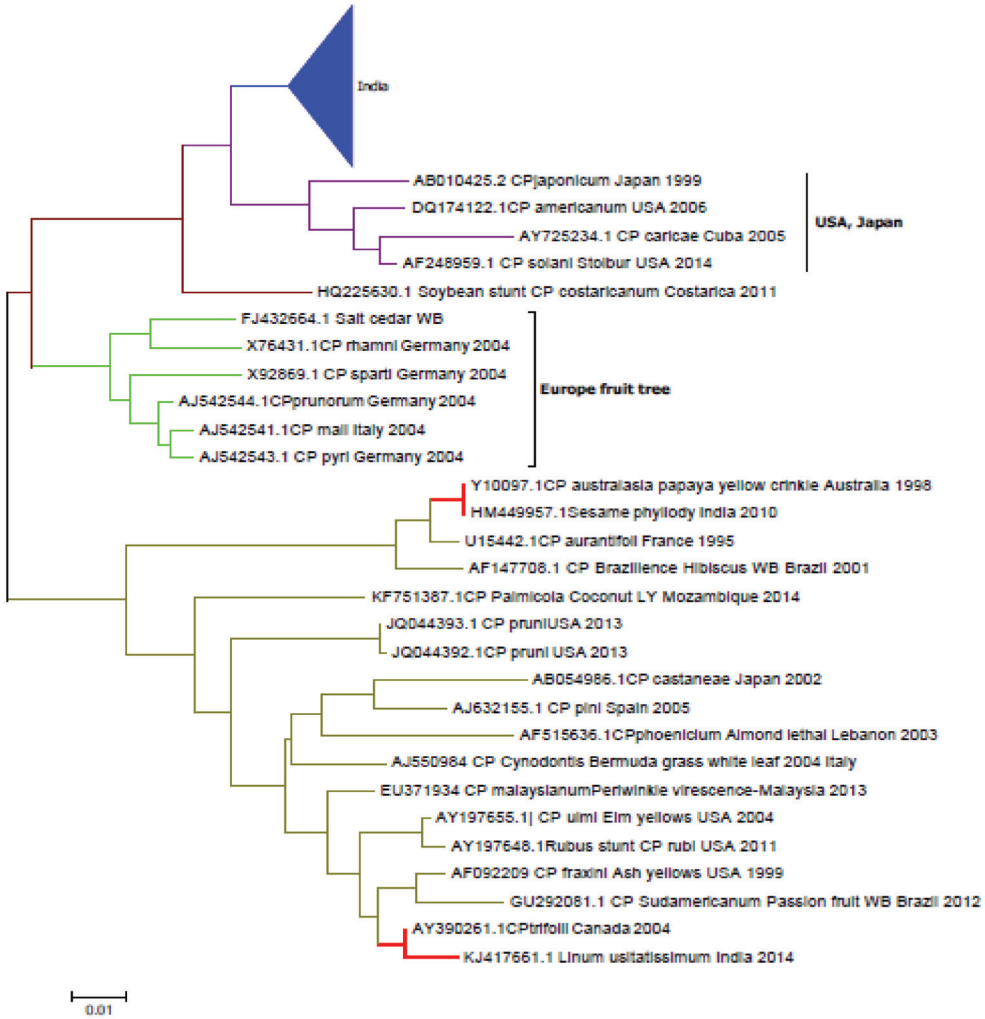


Fig. 5. Phylogenetic tree depicting the relationship of Indian phytoplasma strains with phytoplasmas of other parts of the world based on 16SrRNA gene sequences

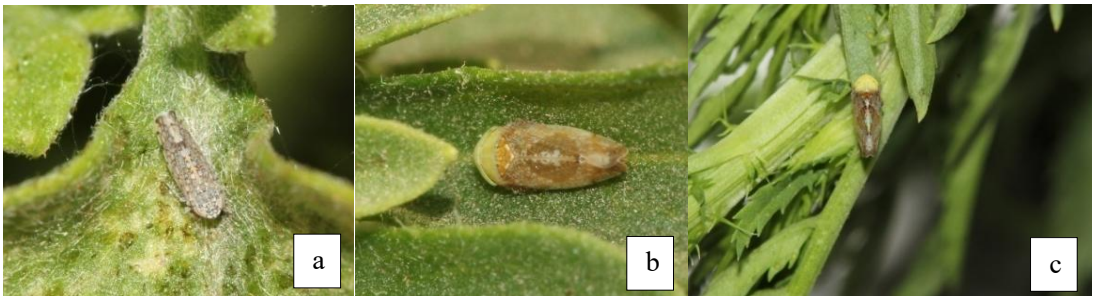


Fig. 6. Leaf hoppers vectors of phytoplasma. a. *Orosius albicinctus* on chrysanthemum; *Hishimonus phycitis* on b. Aster; c. Marigold

Phytoplasma and biosecurity concerns

Indian floriculture industry has grown widely and has established itself across the continents through import and exports. Exporting 4308 MT of floriculture products worth Rs.11200 lakhs are imported in our country every year with fresh cut flowers, foliages and branches, live plants and bulbs having a major share. The boom in the nursery industry along with the real estate, has augmented the import of exotic plants and variants from across the globe. The entry of plants bring along the microbial parasites of the host plants as well and if the new location is favorable, they flourish quickly and start conquering the other members of the family. Even though unknowingly, such imports

have introduced and established many weeds and pathogens in our country in the past like parthenium, eichhornia, michania, lantana, salvinia etc and many pests and diseases like Banana bunchy top, African cassava mosaic disease and a long list of introduced insect pests. Phytoplasma cannot be an exception. Some of the morphological manifestations of phytoplasma infections like greening in gerbera, chrysanthemum, colour breaking in tulip are of ornamental value and are subjected for rapid propagation purely due to lack of awareness. This practice is posing a major threat to our country's biosecurity and this is going to affect the global floriculture trade in future.

The prevalence of a pathogen in our country can be a trade barrier for the export of that planting material to another country. Countries like Australia, New Zealand, Canada, Europe and USA have already developed a system to screen invasion of pathogens. Our major trading partners with respect to export of floricultural products are USA, Germany, Netherland, UK and UAE and top five countries from where India imports are Thailand, Netherland, China, Italy and USA (APEDA Import export statistics 2019-20). The Plant quarantine order 2003 and its amendments in India also regulate and restrict imports of phytoplasma infected plants and planting materials and other flower products imported from other countries. Even though a system is in place, it is not foolproof and lot of escapes occur in India which is evident from the variety of symptom expressions of phytoplasma infections in wide variety of plants and the increasing number of reports of new species of phytoplasma from our country (Prabha et al., 2017). Towards ensuring quality planting material National Horticulture Board has made an initiative by developing guidelines for recognizing horticultural nurseries with a slogan 'Our quality planting material is our Property. We care' where nurseries are ranked from satisfactory to excellent by evaluating source of parent material, propagation in disease free condition by adopting technically prescribed method, adoption of good nursery management practices, reliable record keeping and training of staff etc. If the evaluation is strictly followed and every nursery tries to achieve excellent ranking, issues related to the disease spread will be automatically resolved. Awareness on phyto-biosecurity threats posed by phytoplasma is a must among the first hand users or stakeholders involved in import, inspection, propagation, cultivation, export and certification to secure the floral wealth of our country from exotic strains of phytoplasma and other pathogens. A major hurdle with respect to the floriculture and phytoplasma in India is the ineffective domestic quarantine due to free flow of planting materials from one location to another. India is blessed with wide range of agroclimatic conditions making it favourable for the establishment and spread of phytoplasma. A sense of responsibility and sincere effort from each and every one associated directly or indirectly with floriculture or horticulture as a whole is required to conserve our biosecuirty.

CONCLUSIONS

Phytoplasma is a major threat to the quality and quantity of flower production due to phyllody and virescence. Its occurrence in flower crops poses a major threat to food crops as well. Leaf hoppers in the field contribute to the spread of the phytoplasma from infected plants to healthy ones. Presence of collateral host weeds like parthenium helps year round survival of the phytoplasma in Indian fields. Recent trends in vegetative propagation of seed propagated flower crops like Marigold have resulted in high rate of incidence of phytoplasma in flower crops. This necessitates the need for certification of mother plants and cuttings of vegetative propagated flower crops and ornamentals as phytoplasma free to prevent further spread to the different parts of the country. So far tetracycline is the only effective bacteriostat found effective against phytoplasma but due to its rampant use in the poulty industry, which is posing a threat to the environment, is being considered for its ban. Management of this pathogen is a priority and alternatives to antibiotics and eco-friendly strategies for management of insect vectors are imperative to keep a check on the ever expanding pathogen.

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GLOBAL SCENARIO ON PHYTOPLASMAL DISEASES IN PALMS

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ABSTRACT

Worldwide phytoplasma diseases pose great threat to the sustainability of the palm family members viz., coconut, arecanut, date palm, oil palm and ornamental palms causing setbacks in economic and livelihood security. So far, at least eight *Candidatus* Phytoplasma species belonging to different 16Sr groups have been reported to be associated with coconut. This includes *Ca.* P. asteris (lethal wilt disease in India; 16Sr IB), *Ca.* P. oryzae (Coconut root (wilt) disease in India, Weligama leaf wilt disease in Sri Lanka; 16Sr XIB), *Ca.* P. cynodontis (Coconut yellow decline in Malaysia; 16Sr XIV), *Ca.* P. malaysianum (Malayan yellow dwarf phytoplasma in Malaysia; 16Sr XXXIIB), *Ca.* P. palmicola (Cape St. Paul wilt in Ghana, Awka wilt in Nigeria; 16Sr XXIIB), *Ca.* P. cocostanzaniae (lethal yellowing in East Africa; 16Sr IVC), *Ca.* P. noviguineense (Coconut bogia syndrome in Papua new Guinea) and *Ca.* P. palmae (Coconut lethal yellowing in Americas and Caribbean region; 16Sr IVA,B,E). Though there are several reports on insects transmitting coconut phytoplasmal diseases, except for LY in Florida and RWD in India, the vectors remain elusive. Susceptibility of date palms to phytoplasmal diseases viz., lethal yellowing, Al-wijam, White Tip Dieback (WTD) and Slow Decline (SD) or El Arkish' are emerging concerns in Arabian countries and North Africa. Oil palm is a potential host of diverse groups (16SrI, 16SrXI, 16Sr XIV and 16SrXXXII) of phytoplasma in many parts of the world. Yellow leaf disease caused by 16SrI, 16SrXI and16SrXIV group phytoplasmas is one of the major diseases limiting the productivity of arecanut in south India. Many ornamental palm species harbour a wide array of phytoplasma groups that could serve as an intermediary mode of transmission to cultivated palms. Large scale movement of ornamental palms through international trade warrants the need for strengthening the international quarantine networks and bio-security measures to contain the spread of these phytopathogenic mollicutes. Despite rapid progress accomplished in taxonomy using molecular approaches many palm phytoplasma isolates are yet to be characterized for the assignment of specific taxonomic status. Due to its confinement to phloem, non-uniform distribution and sub-minimal titres in palms, molecular detection is many atimes intriguing. Palm-phytoplasmal interaction and transmission mechanism by auchenorrhynchan fauna are poorly understood. As a victim of climate change, phytoplasma diseases in synergy with survival superiority of insect vectors are extremely challenging to comprehend and evolve effective suppression mechanism. Management approaches involving surveillance, eradication and containment of the disease in newly emerging areas along with resistance breeding to ensure sustainable income to the farmers are discussed in this chapter.

Key words: Arecaceae, mollicutes, vectors, management, diagnosis

Palms (Family: Arecaceae) are monocotyledonous flowering plants widely distributed across the world. This family consists of 181 genera and approximately 2600 species (Baker and Dransfield, 2016). Palms occupy a unique position in tropical and sub-tropical ecosystems with their high species diversity and adaptation to grow in a wide range of habitats. Palms also form an integral part of the culture, cuisine, customs and traditions of several rural communities. They serve as the primary source of income for farmers in many resource-poor countries and are acknowledged as livelihood service providers. Biotic and abiotic stresses limit the growth and productivity of palms and are reported worldwide. Diseases caused by phytoplasmas are a real threat to palm cultivation calling for global solidarity to tackle such menace. The presence of phytoplasmal diseases in palms has a long history of more than 100 years upsetting the economy of several countries (Gurr et al., 2016). They were found pathogenic to more than 50 different palm species growing in different continents

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(Yankey et al., 2018). The recent increase in reports of palm phytoplasmal diseases from different geographical locations and expansion of the host range of existing phytoplasmas to new palm species are viewed very seriously by the researchers. Being fatal on several instances, phytoplasma diseases in palms and the insect vectors associated have drawn international attention to evolve holistic solutions in the multilateral system. This review outlines the global scenario of phytoplasmal diseases in palms.

Phytoplasmal diseases of palms- concern across continents

The discovery of Mycoplasma-Like Organisms (MLOs) by Doi et al. (1967) unveiled the etiology of many palm diseases. Phytoplasmal diseases have been reported from several economically important palm species in America, Africa, Asia and Oceania. The devastating losses of coconut plantations due to phytoplasmal diseases have been recorded since the late 19th century. Some of them are lethal and widespread, whereas others are only debilitating affecting palm health, or are limited to certain geographic regions. The most well-known phytoplasmal disease of coconut is the Lethal Yellowing (LY) in the Americas. After its first scientific report from Jamaica in 1891 (Fawcett, 1891), the disease was recorded from Cuba (De La Torre, 1906), Haiti (Llauger et al., 2002), Florida (Martinez and Roberts, 1967), Mexico (Romney and Harries, 1978; Harrison et al., 2002a), Dominican Republic (Carter and Suah, 1964), Bahamas (Leach, 1946) and Honduras (Ashburner et al., 1996). The disease caused the death of five million Jamaica Tall palms in Jamaica, 6.5 lakh palms in the Yucatan peninsula and one lakh coconut palms in Florida (Mora 2002; Romney, 1983). The coconut production in Haiti declined from 60 million to 30 million nuts a year due to LY. The destructive disease killed about 90% of the palms in the Honduran Atlantic coast and about 80% of palms in the western region of Haiti (Donis, 2002; Doyle, 2001; Myrie, 2002).

Phytoplasmal diseases have annihilated millions of palms namely coconut (*Cocos nucifera* L.), arecanut (*Areca catechu* L.), date palm (*Phoenix dactylifera* L.) oil palm (*Elaeis guineensis* Jacq.) and other ornamental palms across several countries in Africa. Kaincope disease (Maladie de Kaincope) in Togo (Nienhaus and Steiner, 1976), Awkawilt disease (AWD) or bronze leaf wilt in Nigeria (Bull, 1955), Kribi disease in Cameroon (Dollet et al., 1977), Cape St. Paul Wilt Disease (CSPWD) in Ghana (Dabek et al., 1976), Cote d'Ivoire lethal yellowing (CILY) in Cote d'Ivoire (Konan Konan et al., 2013) and lethal decline (LD) reported from Tanzania (Schuilling and Mpunami, 1990), Mozambique (Mpunami et al., 1999) and Kenya are the major phytoplasmal diseases of coconut palms occurring in Africa. The Bogia Coconut Syndrome (BCS) reported from Papua New Guinea (Kelly et al., 2011) is an emerging threat to coconut cultivation in Oceania. CSPWD has wiped out about one million coconut trees in Ghana (Nipah et al., 2007). In Nigeria, 'Awka wilt' killed over 98% of the West African Tall (WAT) and LD destroyed about 56% of palms in southern Tanzania (Mpunami et al.,1999; Odewale et al., 2010) highlighting the seriousness of the disease in the international coconut sector.

In Asia, phytoplasmal diseases of coconut palms have been reported from India, Sri Lanka, Indonesia and Malaysia. This includes Root (Wilt) Disease (RWD), Lethal Wilt Disease (LWD) and Tatipaka from India, Weligama Coconut Leaf Wilt Disease (WCLWD) from Sri Lanka, Kalimantan Wilt (KW) of Indonesia and Coconut Yellow Decline (CYD) from Malaysia (Butler, 1908; Rao et al., 1956; Sitepuet al., 1988; Wijesekara et al., 2008; Nejat et al., 2009a; Babu et al., 2021). Among these RWD, WCLWD and Tatipaka are non-lethal whereas LWD, KW and CYD are fatal to palms. WCLWD causes about 40 to 60% reduction in yield (Weerakkody, 2010) and the crop loss due to RWD was estimated as 968 million nuts (Anonymous, 1985).

Another economically important palm species that is vulnerable to phytoplasma infection is the date palm (*Phoenix dactylifera* L.). The crop is considered as an important component of farming systems in dry and semi-arid regions and is suitable for both small and large-scale farming (Khushk et al., 2009). It is an important subsistence crop in most of the world's desert areas which earns a good amount of foreign exchequer as well. The susceptibility of *Phoenix* spp. to lethal yellowing type disease has been reported as early as in the 1970s (Thomas, 1974). McCoy et al. (1980) first reported the LY of date palms from Texas. The disease is also known as lethal bronzing disease (LBD) or Texas Phoenix Palm Decline (TPPD) was later reported from edible date palm in Florida (Harrison et al., 2008) and central Mexico (Padilla et al., 2011). White Tip Dieback (WTD) affecting young date palms of 5-8 years old and Slow Decline (SD) or 'El Arkish' of mature date palms occurs in the Northern Sudan region in North Africa (Cronje et al., 2000a &b). The disease is prevalent along the Nile between Dongola and Merowe-Karema causing

an annual yield loss of about 6%. Phytoplasma diseases limit the productivity of date palms in Middle East countries also. The Al-Wijam disease is a major concern to date palm cultivation in Saudi Arabia (Alhudaib et al., 2007a & b). Furthermore, phytoplasma diseases of date palms have been reported from Kuwait (Al-Awadhi et al., 2002), Egypt (Al-Khazindar, 2014), Iran (Zamharir and Eslahi, 2019) and recently from Oman (Hemmati et al., 2020).

The susceptibility of oil palm to phytoplasma poses a threat to the global vegetable oil industry. Oil palm is grown extensively in South-East Asian, African and South American countries. Among the various phytoplasmal diseases, lethal wilt is a threat to oil palm cultivation. It was recorded as early as 1994 from Colombia (Alvarez et al., 2014). By 2010, the disease killed a total of 97619 palms in about 690 ha area. In India, Spear Rot Disease of oil palm (SRD) was reported in Kerala to the extent of 1.04% with a range of 0.12% to 7.18% in different plantations (Kochubabu, 1993). The presence of phytoplasma has been reported from oil palm in Ecuador (Bolanos et al., 2019), Mozambique (Bila et al., 2015) and Malaysia (Nejat et al., 2013).

Yellow Leaf Disease (YLD) is the most serious phytoplasma malady affecting arecanut cultivation in Asia. The disease has been reported from India (Nambiar, 1949), China and Sri Lanka (Kanatiwela- de Silva et al., 2015). The YLD considerably reduces the production and quality of the betel nut.

Symptoms of phytoplasmal infection in palms-yellowing to wilting

Symptoms due to phytoplasmal infection in palms vary according to the palm species, cultivar and pathogen group. Most of the coconut phytoplasmal diseases are lethal except Root (Wilt) Disease (RWD) and Weligama wilt (WCLWD). For most of the lethal phytoplasmal diseases of coconut, abnormal shedding of nuts followed by inflorescence necrosis, yellowing and drying of leaves and death of bud form the characteristic symptoms (Fig. 1). In some cases, the yellowing may not be pronounced as in the case of Awka Wilt of coconut and lethal bronze disease in date palm. The symptoms of Texas Phoenix Palm Decline (TPPD) (16SrIV-D) and LY (16SrIV-A) on date palm are similar with one exception; root decay has been observed with TPPD early in the disease process (Harrison and Elliott, 2016). Inflorescence necrosis was found to be absent in bogia coconut syndrome reported from Papua New Guinea (Kelly et al., 2011).The characteristic symptoms of RWD and WLCWD include flaccidity, yellowing and marginal necrosis of leaflets (Fig. 2). The date palm phytoplasma diseases in Arab countries are identified by the presence of yellow streaks on leaf petiole and marked reduction in fruit size. The primary symptoms

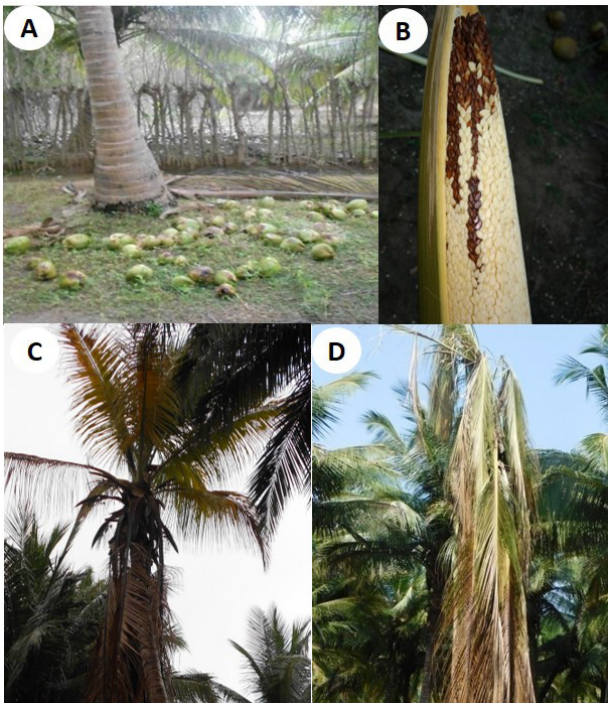


Fig. 1. Impact of root (wilt disease). A, Abnormal shedding of nuts; B, Inflorescence necrosis; C and D, Yellowing and drying of leaves in coconut palm



Fig. 2. Root (wilt) disease affected coconut palm

of the lethal wilt disease of oil palm include vascular discolouration and yellowing of leaves. These leaves dry off, wilt and eventually the palm collapse.

Palms-a haven for phytoplasmas

Palms serve as hosts of diverse groups of phytoplasmas. Worldwide palms have been affected by nine 16Sr groups of phytoplasmas viz., 16SrI, II, IV, VII, XI, XII, XIV, XXII and XXXII (Table 1). Among cultivated palms, coconut harbours the highest number of phytoplasma groups followed by date palm and oil palm. Coconut palm is reported as the host of phytoplasmas belonging to diverse groups viz., *Candidatus* P. asteris (16SrI), '*Ca.* P. palmae' (16SrIV-A,B,E), 16Sr IV D, '*Ca.* P. cocostanzaniae' (16SrIV-C), 16SrVII, '*Ca*. P. palmicola' (16SrXXII-A,B), '*Ca*. P. oryzae' (16SrXI), '*Ca.* P. cynodontis' (16SrXIV), '*Ca*. P. malaysianum' (16SrXXXII-B) and '*Ca.* P. noviguineense' (Babu et al., 2021; Arocha-Rosete et al, 2014; Bertaccini et al., 2014; Harrison et al., 2014; Nejat et al., 2013; Pereraet al., 2012; Wijesekhara et al., 2013; Miyazaki et al., 2018). Several species of ornamental palms are reported as hosts of different groups of phytoplasma. Most of the ornamental palms used for landscaping in America were found to be susceptible to phytoplasma belonging to the 16Sr IV subgroup (Harrison et al., 1999; Bahder et al., 2019a).

Epidemiology of palm phytoplasmal diseases-a grey area

Epidemiological studies play a key role in formulating strategies to prevent the spread of plant diseases. Phytoplasmal diseases are primarily transmitted by auchenorrhychous insects belonging to the order Hemiptera. As soon as a phytoplasmal disease is reported, research on vectors was also initiated. Being a persistent and circulative pathogen, phytoplasma once acquired by the insect needs to cross the gut as well as the salivary gland barrier to become infective and for transmission. Those insects lacking this ability may not become vectors for transmitting phytoplasma diseases. Most of the insect vectors of phytoplasma are non-destructive feeders except the lace bug, *Stephanitis typica* transmitting RWD and brown marmorated stink bug, *Haylomorpha halys*, transmitting witches broom phytoplasma to *Paulownias* sp. (Weintraub and Beanland, 2006).The most appropriate method for determining the vectoral role of an insect species is the cage transmission test. For palm-phytoplasmal pathosystems, this approach has several logistical constraints as it is very difficult to cage and maintain perennial woody palms for a long time. Hence, only very few insects have been identified as phytoplasmal vectors through conventional transmission trials attempted so far. The first successful report was the transmission of LY in coconut by the planthopper, *Haplaxius crudus* in Florida (Howard et al., 1983). Thirty years later, the role of *H. crudus* as a vector of LY in *Pritchardia pacifica* was confirmed by cage transmission experiments in Yucatan, Mexico (Dzido et al., 2020). The role of a lace bug (*S. typica*) and planthopper (*Proutista moesta*) in transmitting RWD phytoplasma has been conclusively established through cage transmission experiments and detection through electron-microscopy (Mathen et al*.,* 1990; Rajan, 2011). Despite the extensive transmission studies conducted in Ghana from 1990s, the vector of CSPWD in Ghana remains unknown (Philippe et al., 2009; Danyo, 2011). Several hemipteran insects have been reported as putative vectors of palm phytoplasmal diseases based on PCR results (Dollet et al., 2011;Kumara et al., 2015; Kwadjo et al., 2018; Alhudaib et al., 2007a, Pilotti et al., 2014), but their vector status has not yet been established by cage transmission trials. Tanne et al. (2001) developed a feeding medium approach as an alternate method to bypass the use of caged palms in the preliminary vector screening trials. The single-insect feeding medium tests coupled with Loop-Mediated Isothermal Amplification (LAMP) developed by Lu et al. (2016) to identify putative vectors of BCS also help in the rapid screening of potential vectors.

Until 1990s, it was thought that there is no possibility of seed transmission as phytoplasmas are phloem-limited pathogens. During the past two decades, evidence for seed transmission of phytoplasma has been reported from different countries. Harrison and Oropeza (1997) detected the presence of lethal yellowing phytoplasma in seeds from LY-affected coconut palms. The presence of LY phytoplasmal DNA was confirmed by PCR in 18.06% of embryos from fruits of diseased Atlantic Tall coconut palms (Cordova et al., 2003). CSPWD phytoplasma was detected in nine out of the 52 embryos from diseased West African Tall (WAT) palms by PCR (Nipah et al., 2007). The presence of phytoplasma DNA in embryos of nuts collected from the root (wilt) diseased coconut palms was reported by Manimekalai et al. (2014a). The detection of LY phytoplasma in coconut plantlets obtained through *in vitro* culturing of the embryo from the seed nuts of diseased palms by Oropeza et al. (2017) indicates the possibility of phytoplasma transmission through seeds. As the embryo lacks a sieve element connection, the

Table 1. Host range and distribution of palm phytoplasmas

16Sr Group	Host	Disease	Distribution	Reference
16SrI	*Cocos nucifera*	Lethal yellowing (LY)	Cuba	Camilo et al. (2019)
		Cote d'Ivoire lethal yellowing (CILY)	Côte d'Ivoire	Kwadjo et al. (2018)
	Phoenix dactylifera	Al-Wijam Date palm yellows	Saudi Arabia	Al hudaib et al.(2008)
		Egypt		Al Khazindar (2014)
	Roystonea regia	Royal palm yellow decline	Malaysia	Naderali et al. (2015)
16Sr IB	*C. nucifera*	Lethal wilt	India	Babu et al. (2021)
	Elaeis guineensis	Lethal wilt	Colombia	Alvarez et al. (2014)
	Areca catechu	Yellow leaf disease (YLD)	India	Chaitra et al. (2014)
	Cyrtostachys renda	Yellow decline	Malaysia	Naderali et al.(2014)
16Sr IID	*P. dactylifera*	LY	Saudi Arabia	Omar et al. (2018)
	Washingtonia robusta			
	P. dactylifera	Streak Yellows	Oman	Hemmati et al. (2020)
16Sr IVA	*C. nucifera*	LY	Florida, Cuba. Guatemala, Jamaica, Honduras, St.Kitts and Nevis, Mexico	Harrison et al. (1994); Howard et al. (1983); Myrie et al. (2006); Brown et al. (2007); Harrison et al. (2008); Myrie et al. (2012); Hernandez et al. (2020)
	P. dactylifera	LY	Florida, Antigua	Harrison et al. (1999); Myrie et al. (2014); Bahder and Helmic (2018)
	Livistona chinensis			
	Pritchardia pacifica			
	Adonidia merrillii			
	Aiphanes lindeniana	LY	Florida	Harrison et al. (1999) Bahder and Helmic (2018)
	Allagoptera arenaria			
	Arenga engleri			
	Borassus flabellifer			
	Caryota mitis			
	Caryota rumphiana			
	Chelyocarpus chuco			
	Corypha taliera			
	Crysophila warsecewiczii			
	Cyphophoenix nucele			
	Dictyosperma album			
	Dypsis cabadae			
	Dypsis decaryi			
	Gaussia attenuata			
	Howea belmoreana			
	Howea forsteriana			
	Hyophorbe verschaffeltii			
	Latania lontaroides			
	Livistona rotundifolia			
	Nannorrhops ritchiana			
	Phoenix canariensis			
	Phoenix reclinata			
	Phoenix rupicola			
	Phoenix sylvestris			
	Pritchardia affinis			
	Pritchardia remota			
	Pritchardia thurstonii			
	Ravenea hildebrandtii			
	Syagrus schizophylla			
	Trachycarpus fortunei			
	Veitchia arecina			

(contd.)

	R. regia	LY	Mexico	Narvaez et al. (2016)
	Acrocomia mexicana			
16SrIVB	*C. nucifera*	Yucatan coconut decline	Yucatan (Mexico), Cuba, Honduras	Tymon et al. (1998); Roca et al. (2006)
	Acrocomia aculeata	Coyol palm decline	Honduras	Roca et al. (2006)
	A. merrillii	LY	Mexico	Hernandez et al. (2020)
16SrIVC	*C. nucifera*	Lethal decline	Tanzania, Kenya	Mpunami et al. (1999)
16SrIV D	*C. nucifera*	Lethal Bronzing disease (LBD)/ Texas Phoenix Palm Decline (TPPD)	Florida	Harrison et al. (2008); Jeyaprakash et al. (2011); Dey et al. (2018); Bahder et al. (2019b)
	Carpentaria acuminata			
	L. chinensis			
	Butia capitata			
	Bismarckia nobilis			
	P. dactylifera			
	P. sylvestris			
	Phoenix roebelenii			
	Syagrus romanzoffiana			
	Sabal palmetto			
	P. canariensis		Florida, Texas, Lousiana, Mexico	Harrison et al.(2002b); Bahder et al. (2019b); Ferguson et al. (2020); Palma et al. (2020)
	Trachycarpus fortunei		Lousiana	Singh and Ferguson, (2017)
	A. merrillii		Mexico, Florida	Lara et al. (2017); Bahder et al. (2019b)
	Sabal mexicana		Mexico	Vazquez-Euan et al.(2011); Narvaez et al.(2017); Poghosyan et al.(2019)
	Pseudophoenix sargentii			
	P. pacifica			
	W. robusta			
	Brahea brandegeei			
16Sr IVE	*C. nucifera*	LY	Dominican republic	Martinez et al.(2008)
16SrIVF	*W. robusta*	LY	Florida	Harrison et al.(2008); Harrison and Elliot (2016)
	P. dactylifera			
16SrVII	*C. nucifera*	LY	Cuba	Camilo et al. (2019)
16SrXIB	*C. nucifera*	Root (wilt) disease	India	Manimekalai et al.(2014b)
	A. catechu	Yellow leaf disease (YLD)	India	Manimekalai et al. (2013)
16Sr XI	*C. nucifera*	Weligama coconut leaf wilt disease (WCLWD)	Sri Lanka Kanatiwela- de Silva et al.(2015)	Perera et al. (2012)
	A. catechu	YLD		
	C. nucifera	Kalimantan wilt	Indonesia	Warroka, (2006); Bertaccini et al. (2014)
16SrXII	*C. nucifera*	LY	Cuba	Camilo et al. (2019)
16Sr XIII	*C. nucifera*	Kalimantan wilt	Indonesia	Sitepu et al. (1988); Warroka (2006); Bertacciniet al. (2014)
16Sr XIV	*C. nucifera*	Coconut yellow decline	Malaysia Naderali et al. (2013)	Nejat et al. (2009a & b)
	Wodyetia bifurcata	Yellow decline		
	P. dactylifera	White tip die back Slow decline	North Africa	Cronje et al. (2000 a & b)
16SrXXIIA	*C. nucifera*	Awka wilt disease Lethal decline	Nigeria Mozambique	Harrison et al. (2014)
	Borassus aethiopum	LY	Mozambique	Bila et al. (2015)
	E. guineensis			
16SrXXIIB	*C. nucifera*	CILY Cape St. Paul Wilt Disease (CSPWD) Ghana	Cote d'Ivoire	Harrison et al. (2014)

(contd.)

(Table 1 contd.)

16Sr XXXII- B	*C. nucifera*	Coconut yellow decline	Malaysia	Nejat et al. (2013)
Palm phytoplasmas not assigned to 16Sr groups				
	C. nucifera	Bogia Coconut Syndrome (BCS)	Papua New Guinea	Miyazaki et al. (2018)
		Kaincope disease	Togo	Nienhaus and Steiner (1976)
		Kribi disease	Cameroon	Dollet et al. (1977)
		Tatipaka Disease	India	Rao et al. (1956)
		Blast	Coted'Iviore	Quillec et al. (1978)

mechanism by which the phytoplasma reaches the embryo remains unknown. The possibility of seed transmission poses a threat to international germplasm exchange. For vegetative propagated palms particularly ornamental palms, the planting material itself may serve as primary inoculums introducing the disease to newer geographical locations.

Diagnosis-a long way to go to the field

Preliminary identifications of phytoplasmal diseases are based on symptoms only. The association of phytoplasma with the disease is usually established by microscopy or molecular detection techniques. The low titre of phytoplasma and their uneven distribution in palms is always a matter of concern for effective diagnosis.

I. Microscopy

i. Light microscopy

Though phytoplasmas could not be directly visualized under a light microscope, accumulation of DNA in extra-nuclear sites indicative of phytoplasmal infection could be demonstrated by staining the tissues with Dienes' reagent (Deeley et al., 1979) or the fluorochrome DAPI (4'- 6'diamidino - 2 phenyl indole) (Russel et al.,1975). Light microscopy has been used to establish the association of phytoplasma with yellow streak disease of date palm (Ammar et al., 2005) RWD (Solomon et al., 1987) and Tatipaka disease of coconut (Rajamannar et al., 994).

ii. Transmission electron microscopy (TEM)

TEM occupies a prime position in the history of plant-pathogen diagnosis as it enabled Doi et al., (1967) to identify and describe a new class of phloem limited phytopathogens-the phytoplasma associated with yellows disease. TEM remained the most reliable diagnostic tool for establishing phytoplasmal etiology till the late 1990s. This technique played a pivotal role in elucidating the association of phytoplasma with several diseases of palms particularly LY and RWD of coconut and YLD in arecanut (Beakbane et al*.,* 1972; Plavsic et al., 1972; Solomon et al*.,* 1983; Nayar and Seliskar 1978). Even though TEM is not preferred as a routine diagnostic technique as it is expensive and time-consuming, still it is considered as a valid tool to study host-phytoplasma interaction.

II. Serology

Sero-diagnostic techniques have been employed in the detection of phytoplasma since 1980s. Due to the difficulty in developing polyclonal antisera with high titre value, the application of serodiagnostics is limited in the detection of phytoplasmal diseases of palms. Enzyme-linked immunosorbent assay (ELISA) has been developed and standardized for the detection of RWD (Sasikala et al., 2010) and WCLWD of coconut (Kanatiwela-de Silva et al., 2019) and YLD of arecanut (Rajeev et al., 2011).

III. Nucleic acid-based detection techniques

The progress in the field of molecular biology imparted momentum in the development of PCR-based methods for the detection of phytoplasma. By 1990s, the 16S rRNA-based taxonomy of phytoplasma was evolved (Lee et al., 1998) and PCR-RFLP has helped in the identification and differentiation of several groups of phytoplasmas. The era of nucleic acid-based detection of palm phytoplasmas initiated the development of DNA probes for the detection of LY phytoplasma of Caribbean origin (Harrison et al*.,* 1992). This was followed by the design of an oligonucleotide primer for selective amplification of LY phytoplasma DNA by polymerase chain reaction (Harrison et al*.,* 1994; Rhode et al*.,* 1993).

Real-time PCR assays using 16S rDNA-based TaqMan primer-probe have been standardized for sensitive, quantitative and rapid detection of LY (Cordova et al., 2014), WCLWD (Wijesekara et al., 2020) and CYD (Nejat et al., 2010) of coconut and YLD of arecanut (Nair et al., 2014). Myrie et al. (2011) developed a multiplex direct-PCR system and real-time

PCR using TaqMan probes based on the 16S rDNA and *GroEL*gene for detection of LY phytoplasma affected palms in Jamaica. For the detection of CSPWD, *secA* gene-based PCR assay was developed by Yankey et al. (2014). Ramjegathesh et al. (2019) developed a qPCR assay conjugated with TaqMan® probe to detect RWD phytoplasma in coconut. The nested PCR (Manimekalai et al., 2010), real-time PCR (Manimekalai et al., 2011) and LAMP (Nair et al., 2016) developed for coconut RWD diagnosis, lack consistency in detection when a large number of root (wilt) affected coconut samples were tested and further refinement of these techniques are necessary for reliable and rapid detection of RWD in the early stage of infection (Hegde et al., 2016). LAMP colorimetric assay for the detection of phytoplasma associated with WCLWD developed by Siriwardhana et al. (2012) also needs further refinement before field application. Bahder et al. (2019a) developed digital PCR Technology (dPCR) for the detection of palm-infecting 16SrIV group phytoplasmas in Florida.

Management-amalgamating surveillance, resistance and sustainability

The non-availability of technologies for the curative treatment of phytoplasmal diseases accentuates the need for developing multipronged strategies to de-risk farmers and to contain the disease in newly emerging areas.

i. Surveillance, eradication and containment-the 'slow down' approach

This may be considered as a prime strategy to be adopted upon the emergence of a phytoplasmal disease in a new area. Measures must be taken to confine the disease in a limited area and create a buffer zone around the affected area to slow down the spread of the disease. Periodic surveillance, diagnosis and eradication of phytoplasma infected palms have to be continued systematically in the diseased zone as well as the buffer zone. This helps to slow down the disease spread. WLCWD is contained to the Southern Province of Sri Lanka by adopting a three km wide and 80 km long buffer zone demarcated around the diseased area. This buffer zone is being periodically inspected for the occurrence of the disease and diseased palms are removed to curtail further spread (Nainanayake et al*.,* 2013). Though the surveillance, eradication and quarantine measures helped to restrict the spread of the disease, isolated incidences in few places quite far from the boundary are being reported (Nainanayake et al*.,* 2016). The systematic implementation of the surveillance and removal programme successfully contained Tatipaka disease in India (Rajamannar et al., 1994). The removal of infected palms slowed down the disease spread in the Dominican Republic and Ghana (Martinez et al., 2008, 2010, Nkansah et al., 2009)

ii. Breeding for disease resistance- for replanting and replacement

An enduring solution to manage phytoplasmal diseases lies in breeding varieties resistant to the disease. Resistant varieties form the most viable and cost-effective option in managing phytoplasmal diseases especially for perennials likes palms. Coconut breeders around the world have identified some palm varieties/ accessions with varying degrees of resistance/tolerance to phytoplasmal diseases. Breeding for phytoplasmal disease resistance is being circumscribed by the long generation time, low multiplication rate and ineffective clonal propagation of coconut palms (Cardena et al., 2003). In Sri Lanka, Green dwarf and *Nana* coconut were identified as promising sources for resistance to WCLWD (Perera et al., 2015). In India also green dwarfs and their hybrids were found promising against RWD. Systematic evaluation trials at ICAR-CPCRI has led to the release of two coconut varieties (Kalpasree (Chowghat Green Dwarf), Kalparaksha (Malayan Green Dwarf) and one hybrid (Kalpasankara (Chowghat Green Dwarf x West Coast Tall) for cultivation in the root (wilt) disease prevalent areas (Thomas et al., 2012; Krishna Kumar et al., 2015). In the resistance trials initiated in Jamaica during 1960-1970, tall cultivars appeared susceptible with about 90% mortality whereas in Sri Lanka and India, Malayan Dwarfs and the King Coconut recorded less than 5% mortality only. But, in certain geographical locations in Florida and Jamaica, Malayan Dwarfs showed a high degree of susceptibility over a period of time (Howard et al., 1987). Though Malayan Dwarfs possessed a high degree of resistance to the disease, the cultivar was not preferred by farmers due to its poor productivity under marginal conditions. Breeding programs initiated during the 1970s resulted in the production of Maypan hybrid (Malayan Dwarf x Panama Tall), combining the advantage of higher resistance of the dwarf cultivar with large size and adaptability of the tall. Initially, the hybrid was found to be only 10% susceptible to LY and became the primary foci of coconut replanting programmes, which led to a recovery of the coconut industry in Jamaica (Been, 1995; Ashburner and Been,1997). In the field trials conducted in Florida during 1982-2001, MYD and Maypan hybrids showed high mortality due to LY

which indicates that these cultivars can no longer be considered as resistant to the disease (Broschat et al., 2002). Resistance breakdown of Malayan dwarfs and Maypan hybrid by LY triggered research on exploring the possibility of the genetic contamination of parents, change in pathogen/vector that altered the palm-vector-phytoplasma interactions. Though research on this line provided evidence on genetic contamination in Panama Tall and MYD in Jamaica it was insufficient to explain a massive outbreak of the disease (Baudouin et al., 2008; Lebrun et al., 2008). Resistance trials conducted on the northern coast of Yucatan, indicated that coconut populations from the Pacific coasts of Mexico are also promising resistance sources of germplasm to deal with L Y (Zizumbo-Villarreal et al., 2008). The Sri Lankan Green Dwarf (SGD) × Vanuatu Tall (VTT) hybrid which shows CSPWD resistance is being used for replanting in Ghana (Dery et al., 1997; Mariau et al., 1996; Quaicoe et al., 2009; Dare et al., 2010). In Nigeria, green dwarfs were reported to have field resistance to AWD (Odewale et al., 2006).

Majority of the field resistance trials against coconut phytoplasma conducted around the world are projecting dwarfs especially green dwarf cultivars from Asian countries as sources of resistance. In order to broaden the genetic base, more diverse populations from different geographical locations need to be explored.

iii. Integrated management approaches ensure sustainable income to farmers

Attempts to control phytoplasmal diseases by foliar and soil application of various chemicals, antibiotics and vector management did not yield any encouraging results (Howard and Oropeza, 1998; McCoy et al., 1976). Integration of on-farm quarantine, surveillance, removal and destruction of symptomatic palms and replanting with disease-resistant high yielding varieties as well as systematic farm management significantly reduced the lethal yellowing disease incidence over years in the coconut plantation of a Jamaican farmer Michael Black (Serju, 2012; Myrie et al., 2011; Gurr et al., 2016). Black's integrated management approach will help the farmers to keep the disease outbreaks in individual farms at a manageable level.

\For non-lethal phytoplasmal diseases like RWD in India and WCLWD in Sri Lanka, coconut farmers are advised to adopt integrated management practices viz., removal of disease advanced palms, replanting with resistant/tolerant cultivars, integrated nutrient management, intercropping and mixed farming along with scientific cultivation practices to ensure a satisfactory and sustainable yield even in a disease prevalent area (Sahasranaman et al.,1983; Amma et al.,1983; Bavappa et al.,1986; Muralidharan et al., 1991; Krishnakumar et al., 2015).

CONCLUSION

During recent years tremendous progress has been made in identifying and characterizing the phytoplasmal pathogens of palms. Despite great advancements in molecular biology, the development of inexpensive, sensitive, reliable, farmer-friendly on-farm diagnostic kits still remains a distant dream. A ready-to-use diagnostic kit is a prerequisite for the implementation of surveillance programmes in disease emerging areas. The epidemiological factors especially the phytoplasmal vectors remain unresolved in the majority of palm diseases and need greater impetus. More detailed studies on vector-host-pathogen-environment interactions are crucial to elucidate the dynamics of disease progression. Accurate identification of insect vectors, characterization of phytoplasma harboured by them and effective transmission trials to establish vectoral ability are still a long way ahead. The application of advanced biotechnological tools like CRISPR may help in accelerating disease resistance breeding programmes. There is ample scope for research in developing alternate management strategies to improve the health of diseased palms. Intense research is required to design and validate location-specific sustainable palm-based cropping systems to de-risk the farmers.

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IN SEARCH OF INSECT VECTORS OF PHYTOPLASMAS WITH A NOTE ON VECTORS OF *XYLELLA FASTIDIOSA*#

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ABSTRACT

Phytoplasmas are phloem restricted, Gram positive bacteria without cell walls and are transmitted by phloem-sap feeding Hemiptera families such as Cicadellidae, Delphacidae, Cixiidae, Derbidae, Psyllidae and rarely by Pentatomidae and Tingidae. In the first part, this review briefly presents information available on the interaction among the phytoplasmas, their insect vectors, host plants and also very briefly the environment. In the second part, it deals with the xylem restricted, vector transmitted, Gram negative, *Xylella fastidiosa* (Wells et al.) and most likely candidate vectors if the organism gets accidentally introduced into India. Two proven vectors of *X. fastidiosa* in Taiwan namely, *Kolla paulula* (Walker) and *Bothrogonia ferruginea* (Fabricius) occur in India. In the third part, ways to search for the promising vectors of both the pathogens is dealt with. Emphasis is laid on for a close cooperation amongst insect taxonomists, molecular biologists and plant pathologists to look into investigations dealing with these organisms right from the initial stages. Brief account of confirmed and putative vectors of phytoplasmas in India and most likely groups of Auchecnorrhyncha that may vector *X. fastidiosa* are given and the species dealt with are illustrated for ease of preliminary identification. Use of recent advances in tissue culture, vector biology, artificial feeding of Hemiptera and molecular biology are suggested for fast tracking the search for vectors of these pathogens.

Key words: Aphrophoridae, Auchenorrhyncha, Cercopidae, Cicadellidae, Delphacidae, Derbidae, *Hishimonus*, *Orosius*, phytoplasma, vector-pathogen interaction, *Xylella fastidiosa*

Ever increasing number of plant diseases caused by phytoplasmas both in cultivated and wild plants are being reported every year. There are more than 700 phytoplasma diseases so far reported, many of them lethal in hundreds of plant species in the world (Weintraub and Beanland, 2006). Phytoplasmas being confined to a particular plant tissue depend largely on insects for transmission from infected to a healthy plant. Though insects remain the most important vectors of these organisms, unfortunately, information on them as vectors of phytopathogens in general and phytoplasmas in particular is very scanty. For many phytoplasma diseases present in India and known for more than a century such as sandal spike disease, coconut root (wilt) and areca yellow leaf diseases, information on their confirmed vectors is not available and only putative vectors are known.

There are several recent reviews on phytoplasma vectors by several workers namely Purcell (1982), Waintraub and Beanland (2006), Bosco and D'Amelo (2010), Alma et al. (2019) and Weintraub et al. (2019) and also on *Xylella fastidiosa* and its vectors by Redak et al. (2004), Almeida et al. (2005), Purcell (2013) and Sicard et al. (2018). This paper heavily draws information from these reviews.

Phytoplasmas

Phytoplasmas are phloem restricted, insect transmitted phytopathogenic, Gram positive prokaryotes (bacteria without cell wall), closely related to mycoplasmas and spiroplasmas. They are obligate parasites and techniques available so far have failed to grow them in culture in artificial media. They are however, capable of multiplying both in their plant hosts and insects that transmit them. They interfere with the physiology of plants and modify plant morphology by stunting their growth, reducing leaf size, shortening internodal length and converting floral parts into either vegetative parts or make them sterile. Phytoplasmas can also kill the infected plants, as in the case of sandal spike, in a few years after infection. Both monocotyledonous and dicotyledonous plants, wild or cultivated, are affected

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by phytoplasmas and some infected plant species may serve as symptomless carriers of these organisms and others as dead-end hosts.

Being phloem tissue bound, they depend on either procedures used in vegetative propagation of plants such as cuttings, grafting etc., or through stolon, rhizomes and bulbs for their spread. A few phytoplasmas are transmitted through seeds as in the case of phytoplasmas affecting alfalfa, carnation, tomato, and citrus (Khan et al., 2002; Botti and Bertaccini, 2006; Satta et al., 2019) and coconut lethal yellowing (Cordova et al., 2003). Phytoplasmas can also be transmitted through the parasitic plants of the genus *Cuscuta.*

Phytoplasmas move in host plant through sieve tube elements in the phloem (Christensen et al., 2004). Often they are distributed unevenly in the host plant tissues. There is also seasonal fluctuation in the titre of phytoplasma in woody plants. Generally, level of phytoplasmas is very low in the roots (sink) and moderate in stems. The highest titre was found in source organs (mature leaves, sometimes almost 40 times higher than that in roots). In sink leaves (very young leaves) the titre remains low or below detectable levels. In deciduous plants the phytoplasma might disappear from the aerial parts of trees during winter, and survive in the root system to recolonize the aerial parts during spring. However, there are several exceptions for this phenomenon and may depend on the phytoplasma and the plant species involved. In some cultivated fruit trees it may be found in scions, and also in aerial parts in winter (Bertaccini and Duduk, 2009).

Taxonomy of leafhopper vectors

Hemiptera are the fifth most speciose order of insects with more than 103590 valid species known from the world. Three of the four suborders of Hemiptera, Auchenorrhyncha, Heteroptera and Sternorrhyncha host the confirmed vectors. The families Cicadellidae (81% of the known phytoplasma vector species), Cixiidae (6%), Delphacidae (4%), Derbidae (1%) Flatidae (1%), Issidae (1%) are among Auchenorrhyncha; the families Pentatomidae (1%) and Tingidae (1%) are among Heteroptera and Psyllidae (7%) among Sternorrhyncha contain phytoplasma vectors.

Among these groups the family Cicadellidae (leafhoppers) contain most of the vectors. Many leafhoppers are phloem feeders thus forming a pool of potential vectors for phloem limited phytoplasmas (Weintraub et al., 2019). Classification of this family based on phylogenetic analysis using morphological and molecular characters recognises 19-20 subfamilies (Dietrich et al., 2001; Zahniser and Dietrich, 2008). The most highly derived lineage of the leafhopper subfamily Deltocephalinae contains more than 75% of all confirmed phytoplasma leafhopper vectors in the world. The feeding habits of Deltocephaline leafhoppers range from monophagous to polyphagous and members can transmit one or more phytoplasma taxa. The tribe Macropsini of the subfamily Eurymelinae contains the second largest number of confirmed phytoplasma vectors and is also fairly highly derived lineage. Macropsini are also either monophagous or polyphagous and feed primarily on woody plants with a few exceptions feeding on annual herbs. Membracidae are phylogenetically closely related to Cicadellidae, however none of them are at present known as phytoplasma vectors. They feed on woody plants gregariously and exhibit sub-social behaviour. It would not be surprising to find that they transmit phytoplsmas primarily associated with woody plants.

Mouthparts and feeding behaviour of phytophagous Hemiptera

For efficient transmission phytoplasmas need a vector that picks them up from the phloem tissue of the infected plant and introduces into phloem tissue of a healthy susceptible plant without causing much tissue damage. This needs highly specialized organisms equipped with structures which can extract nutrients from vascular bundles with least destruction to the host tissue.

Hemiptera have highly specialized suctorial mouthparts to extract sap from different tissues without harming the plant tissue. The suctorial proboscis consists of a stylet bundle capable of piercing intact tissue of plants such as leaf lamina to hard seeds, roots to bark. The stylet bundle consists of four very fine highly chitinized yet supple and innervated needles. The outer pair, derived from mandibles, can move independently and are adorned with concentric ridges on outer surface of each stylet near apex to anchor the stylet bundle. The inner pair, derived from maxillae are held together tightly by interlocking ridges and grooves and are channelled to house dorsal food canal and ventral much narrower salivary duct and both meet near the tip of the stylet into one canal. The labium is segmented, flexible and surrounds the stylet bundle. A group of highly specialized chemoreceptors present at the tip of the labium help to select the host plant and locate the surface to probe. The labium does not take

part in piercing which is done by the stylet bundle. The labrum is a short, triangular lobe at the base on dorsum of the labium and is ventrally indented to direct and position the stylet bundle. At the base of each stylet there are levers on which the muscles are attached which effect movement of each stylet. As the leafhoppers and planthoppers feed, their stylet bundle is surrounded by a sheath formed by viscous saliva secreted by the anterior part of the salivary glands which solidifies on exposure to outer environment and protects the stylets. The phytoplasmas and other circulatory pathogens are introduced into the phloem probably along with watery saliva (secreted by the posterior part of the salivary gland) as the leafhopper stylets penetrate sieve element membranes (Lett et al., 2001).

The food canal posteriorly leads to preciberium which also has chemoreceptors and also a preciberial valve that probably regulates the flow of the fluid and probably also prevents unwanted outward flow of ingested sap. The preciberium leads to the ciberium which has very strong musculature to pump the sap into the oesophagus (ciberial pump) (Backus, 1985). This pump is very strongly developed in leafhoppers that feed on xylem vessels (where the xylem sap is under negative pressure) compared to those feeding on phloem sap (which is under 2-3 times the atmospheric pressure).The strong muscles required to pump the xylem sap need to be housed on the head region and hence the xylem feeding leafhoppers have greatly swollen faces as in the case of Cicadellinae, Evacanthinae and Signoretiinae.

Among the Hemiptera only those that feed on phloem tissues can efficiently transmit the phytoplasmas. These include the members of the families Cicadellidae, Delphacidae, Cixiidae, Issidae, Pentatomidae and Tingidae. The insect vectors (both suspected and proven) reported from India are listed in Table 1 and their photographs are also given for ease of identification (Figs. 1-36).

Hemiptera show some plasticity in feeding on specific host plant tissue. Phloem, xylem and mesophyll feeding guilds are not strict categories, and especially among vascular feeding leafhoppers the distinction between the phloem feeding and xylem feeding guilds is blurred (Wayanade, 1994) and feeding preference could depend on the host plant (resistant and susceptible) and gender (Chuche et al., 2017). Therefore, although phloem feeding behaviour is a pre-requisite for transmission of phytoplasmas and other phloem-limited phytopathogens, one cannot exclude the possibility that species feeding primarily on xylem transmit phytoplasmas. Typhlocybinae leafhoppers are mesophyll feeders but some species may also feed on phloem sap and may be less efficient transmitters of phytoplasmas compared to specialist phloem feeding leafhoppers.

A vector may be able to acquire specific phytoplasma from one plant species but not from another or may acquire a phytoplasma from one plant species but may not be able to transmit to a different species (Bosco et al., 1997). *Empoasca decipiens* Paoli could acquire Chrysanthemum Yellows Phytoplasma (*Ca*. P. asteris, 16SrI-B) (CYP) and transmit to *Ismelia carinata* (Schousb.) Sch.Bip. (as *Chrysanthemum carinatum* Schousb.) but is not able to acquire from or transmit the same phytoplasma to broad bean (*Vicia faba* L.) (Galetto et al., 2011).

Phytoplasma insect vector interactions

The terminologies used to describe various interactions between phytoplasmas and their vectors are same as those used for interaction between the phytopathogenic viruses and their insect vectors.

Acquisition and transmission

Phloem feeding insects acquire phytoplasmas passively during feeding in the phloem of the infected plant. The Acquisition Access Period (AAP) is the feeding duration necessary to acquire a sufficient titre of phytoplasma. The AAP could vary from a few minutes to several hours and longer the AAP, the greater the chance of acquisition (Purcell, 1982). The AAP may depend on several factors such as titre of the phytoplasma in the infected plant, gender, age of the vector etc.

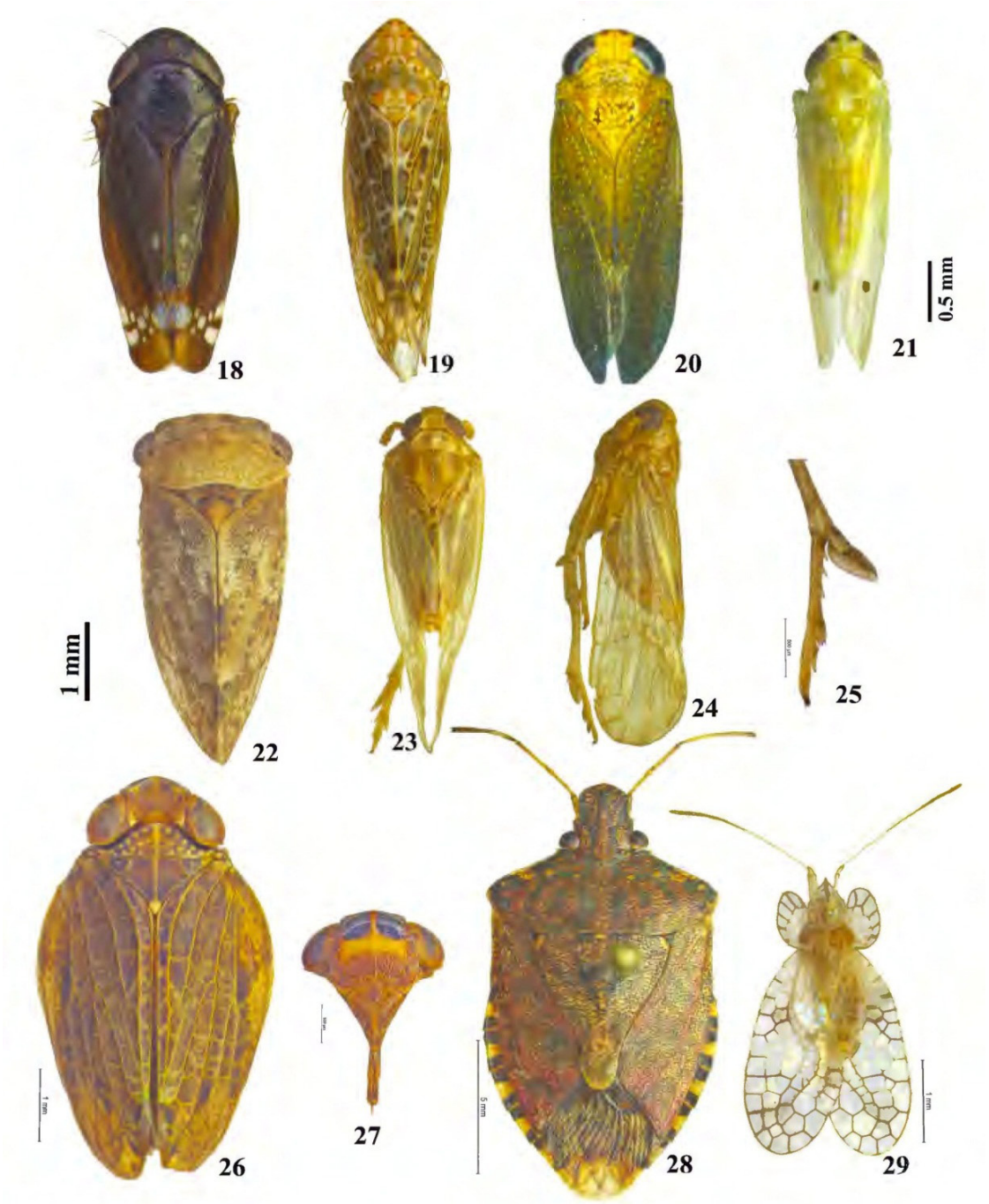
Time taken by the vector from an initial acquisition to be able to transmit the phytoplasma is known as Latent Period (LP) or Incubation Period (IC). The LP varies with the phytoplasma, vector species involved, age and temperature etc. It usually ranges from a few days to more than 80 days (Nagaich et al., 1974; Murral et al., 1996). During the latent period the phytoplasmas pass through midgut, circulate in the haemolymph and infect malpighian tubules, fat bodies, brain (Lherminier et al., 1990; Lefol et al., 1994; Nakashima and Hayashi, 1995) and even the reproductive organs (Kawakita, 2000). The flavescence doree phytoplasma binds strongly to the tissues of alimentary canal and salivary glands but not to the muscles or genital organs of its vector, *Scaphoideus titanus* Ball (Lefol et al., (1993).

Table 1. Leafhoppers, planthopper and other insects reported from India as either proven or suspected phytoplasma vectors

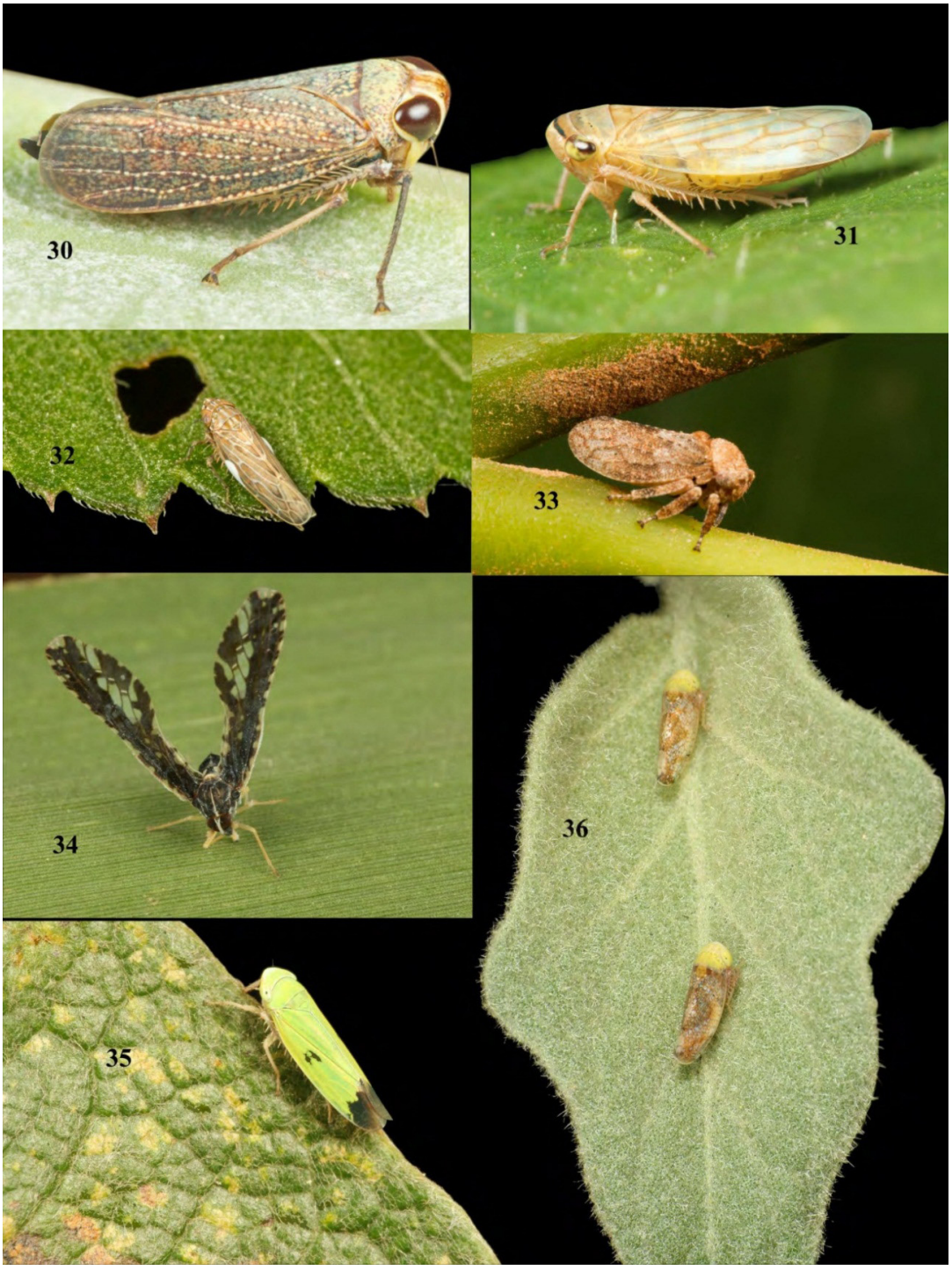
Vector species	Disease association/ phytoplasma group	Host plant	Reference
Nephotettix malayanus Ishihara & Kuwase	Rice yellow dwarf/ 16SrXI-B	Rice	Wilson and Turner (2010)
Nephotettix nigropictus (Stål)	Rice yellow dwarf/ 16SrXi-B	Rice	Nasu et al. (1967)
Nephotettix virescens (Distant)	Rice yellow dwarf/ 16SrXI-B	Rice	Nasu et al. (1967)
	Sandal spike	Sandal tree	Shivaramakrishnan and Sen-Sarma (1978)
Exitianus indicus (Distant)	Sugarcane grassy shoot	Sugarcane	Rao et al. (2014)
Deltocephalus vulgaris Dash and Viraktamath	Sugarcane grassy shoot disease	Sugarcane	Singh et al. (2002)
Maiestas dorsalis (Motschulsky)	Rice oramge leaf disease	Coconut	Kumara et al. (2015)
	Weligama coconut leaf wilt disease		
Maiestas portica (Melichar)	Sugarcane grassy shoot	Sugarcane	Tiwari et al. (2017)
Matsumuratettix hiroglyphicus (Matsumura)	White leaf hytoplasma / 16SrXI-B	Sugarcane	Matsumoto et al. (1970)
Goniagnathus punctifer (Walker)	Weligama coconut leaf wilt disease	Coconut	Kumara et al. (2015)
Cofana unimaculata (Signoret)	Weligama coconut leaf wilt disease	Coconut	Kumara et al. (2015)
Kolla cyelonica (Melichar)	Weligama coconut leaf wilt disease	Coconut	Kumara et al. (2015)
Thomsonia porrecta (Walker)			Wilson and Turner (2010)
Neoaliturus fenestratus (Herrich-Schäffer)	Phyllody	Compositae	Klein (1970)
Neoaliturus tenellus (Baker)	Beet leafhopper transmitted virescence disease	Vegetables	Oldfield et al. (1977) Shaw et al.(1993)
	Tomato big bud/ 16SrVI-A, 16Sr-IX		Weintraub et al. (2004)
Hishimonus phycitis (Distant)	Eggplant little leaf	Eggplant	Maraarosch et al. (1970)
	16SrI-B, 16SrII-D	Parthenium.	Gopalaand Rao (2018)
		Cannabis, Jasmine, Chrysanthemum Sesame	Nabi et al. (2015)
Orosius albicinctus Distant	Sesamum phyllody	Sesame	Kersing and Baspinar (1997)
	Leucern Witches'-broom	Alfalfa	Salehi et al.(1995)
	Purple top	Potato	Nagaich et al. (1974)
	16Sr-V, 16Sr-IX	Solanaceae	Weintraub et al.(2004)
	Limonium phytoplasma	*Limonium*	
Idioscopus clypealis (Lethierry)	Weligama coconut leaf wilt disease	Coconut	Kumara et al. (2015)
Yamatotettix fulvivittatus Matsumura	Sugarcane White leaf phytoplasma	Sugaracane	Hanboonsong et al. (2006)
Olidiana kirkaldyi (Nielson) misidentified as *Jassus indicus* (Walker)	Sandal spike	Sandal tree	Rangaswami and Griffith (1941)
Alebroides nigroscutellatus (Distant)	Potato purple top-roll/ 16SrIII-B	Potato	Singh and Nagaich, (1977)
Amrasca devastans (Distant)	Eggplant little leaf	Solanaceae	Maramarosch et al. (1970)
Mesargus albomaculatus (Distant)	Sandal spike	Sandal tree	Dover and Appanna (1934)
Nilaparvata lugens (Stål)	Un-named phytoplasma	Rice	Cook and Perfect (1989)
Redarator bimaculatus Distant	Sandal spike	Sandal tree	Ghosh et al. (1983), Balasundaran et al.(1988)
Proutista moesta (Westwood)	Coconut root wilt disease	Coconut	Ponnamma and Soloman (1998)
	Arecanut yellow leaf	Arecanut	Ponnamma et al. (1991)
	Spear rot disease of oil plam	Oil palm	Kochu Babu (1993)
	Wilegama coconut leaf wild disease	Coconut	Kumara et al.(2015)
Halyomorpha halys Stål	Paulownia Witches'-broom	Paulownia sp.	Hiruki (1999)
Stephanitis typica (Distant)	Coconut Root wilt	Coconut	Mathen et al. (1990)
	Wilagama coconut leaf wilt disease		Kumara et al. (2015)



Figs. 1-17. Leafhopper vectors of phytoplasmas in India and other countries. 1. *Nephotettix malayanus* Ishihara and Kawase; 2-3. *N. nigropictus* (Stål), male and female, respectively; 4-5. *N. virescens* (Distant), male and female, respectively; 6. *Exitianus indicus* (Distant); 7. *Deltocephalus vulgaris* Dash and Viraktamath; 8. *Maiestas dorsalis* (Motschulsky); 9. *M. portica* (Melichar); 10. *Goniagnathus punctifer* (Walker); 11. *Cofana unimaculata* (Signoret); 12. *Kolla ceylonica* (Melichar); 13. *Idioscopus clypealis* (Lethierry); 14. *Thomsonia porrecta* (Walker); 15. *Neoaliturus tenellus* (Baker); 16. *Hishimonus phycitis* (Distant); 17. *Orosius albicinctus* Distant



Figs. 18-29. Hemipteran vectors of phytoplasmas in India and other countries. 18. *Neoaliturus fenestratus* (Herrich-Scaeffer); 19. *Matsumuratettix hieroglyphicus* (Matsumura); 20. *Olidiana kirkaldyi* (Nielson); 21. *Amrasca devastans* (Distant); 22. *Mesargus albomaculatus* (Distant); 23- 25. *Nilaparvata lugens* (Stål), male, female and part of hind leg, respectively; 26-27. *Redarator bimaculatus* Distant, habitus dorsal view and face, respectively; 28. *Halyomorpha picus* (Fabricius); 29. *Stephanitis typica* (Distant)



Figs. 30-36. Hemipteran vectors of phytoplasmas in their natural habitat. 30. *Olidiana kirkaldyi* (Nielson); 31. *Exitianus indicus* (Distant); 32. *Deltocephalus vulgaris* Dash and Viraktamath; 33. *Mesargus albomaculatus* (Distant); 34. *Proutista moesta* (Westwood); 35. *Nephotettix virescens* (Distant); 36. *Hishimonus phycitis* (Distant)

The biochemical and molecular factors involved in the movement of the phytoplasma are unknown. However, a non-insect transmissible strain of onion yellow phytoplasma had much smaller genomic size (870kbp) compared to the insect transmitted wild type (1000kbp) suggesting that the mechanism of binding to insect cells of the strain has been lost (Oshima et al., 2001). Phytoplasmas possess a major antigenic membrane protein (AMP) that make up majority of their cell surface proteins that interact with microfibrils of the intestinal muscles of the insects. The protein is believed to be an important factor for transmission and infection (Suzuki et al., 2006; Hoshi et al.,2007).Once the phytoplasma enters the intestine through the food canal of the insect mouthparts, they pass through the intestine and they are absorbed into the haemolymph, subsequently the salivary glands are colonised (Bertaccini and Duduk, 2009). The phytoplasma may overwinter in insect vectors or in the perennial plants and may interact with host tissues which may improve or affect their fitness (Christensen et al., 2005). Some phytoplasmas are trans-ovarially transmitted for example, *S. titanus* and aster yellows Phytoplasma (AYP) (Danielli et al., 1996; Alma et al., 1997); *Hishimimonoides sellatiformis* Ishihara and mulberry dwarf (Kawakita et al., 2000); *Matsumuratettix hiroglyphicus* (Matsumura) and sugarcane white leaf (Honboonsong et al., 2002); *Yamatotettix flavovittatus* Matsumura and sugarcane white leaf (Hanboonsong et al., 2006); *Cacopsylla melanoneura* Forster and apple proliferation phytoplasma (Tedeschi et al., 2006).

The resistance of the salivary glands to phytoplasma infection may be one of the reasons why some species acquire phytoplasmas but are not vectors. The phytoplasma must pass through specific cells of salivary glands and accumulate in high titre in posterior acinar cells before it can be transmitted. At each point the pathogen has to overcome barriers posed by the host tissue cells, if it fails to overcome these, the phytoplasma cannot be transmitted (Klien et al., 1998; Wallace and Murphy, 1938; Weintraub et al., 2004) and the insect becomes a dead-end host (a plant that can be inoculated and subsequently become infected with the phytoplasma but from which insects cannot acquire the phytoplasma). The barriers posed by the salivary glands are basal lamina, the basal plasmalemma and the apical plasmalemma (Wayadande et al., 1997). Individuals in a population of vector species may vary in their efficiency of transmission of phytoplasmas (Galetto et al., 2009).

Host range of phytoplasmas is strongly dependent on the insect vectors as they depend on their vectors for spread. It is also possible that the host range of phytoplasma may be much wider than the combined host range of its insect vectors. Sometimes an insect can transmit a given phytoplasma under laboratory conditions, but under natural conditions they are not vectors as they do not feed on all the natural host plants of the phytoplasma. *E. variegatus* a laboratory vector of flavescence doree (FDP) on broad bean is not the natural vector of grapevine FDP.

Some phytoplasma vector leafhoppers can also transmit other pathogens like viruses, rickettsia-like organisms and spiroplasmas. For example, *Neoaliturus tenellus* (Baker) transmits beet curly top hybrid gemini virus (Wallace and Murphy 1938), phytoplasma (Weintraub et al., 2004) and *Spiroplasma citri* (Klein et al., 1988) but whether they use same receptor sites in the midgut cells or different ones is not known (Weintraub and Beanland, 2006).

There are complex biological interactions between the phytoplasmas and the insect vector that transmit them. There appears to be high degree of fidelity between the vector species and the phytoplasmas they transmit. However, several phytoplasmas like aster yellows and Western X strains in North America are transmitted by several species of vectors (Ebbert et al., 2001). Similarly, a single vector may transmit two or more phytoplasma strains (Lee et al., 1996).

Vector host plant interactions also play an important role in determining the spread of the phytoplasma. Polyphagous vectors have the potential to infect a wide range of plant species. It is also shown that insects that normally do not feed on certain plant species can acquire from and transmit phytoplasmas to those plants under laboratory conditions.

Bosco et al. (1997) showed that the leafhoppers differ in their ability to acquire the phytoplasmas from different infected plant species. Chrysanthemum yellows (CYP) is successfully transmitted by three leafhoppers, *E. variegatus, Macrosteles quadripunctulatus* Kirschbaum and *Euscelis incises* Kirschbaum. All three species can acquire phytoplasma from infected chrysanthemum and transmit it to uninfected chrysanthemum. However, only *M. quadripunctulatus* and *E. variegatus* could acquire phytoplasma from CYP infected periwinkle and subsequently transmit to healthy plants. On the other hand, none of the leafhoppers could acquire the phytoplasma from CYP infected celery, a dead-end host. Several dead-end hosts are known for the phytoplasmas,

for example for AYP potato and cyclamen; for Western X disease peach.

The mechanisms that prevent phytoplasma acquisition from dead-end plants are not well understood. The absence of phytoplasmas may be one of the factors in some plant parts. The phytoplasmas may not be found in leaves that were mature before infection (Siddique et al., 1998). Leafhopper behaviour also provides explanations as leafhoppers alter feeding behaviour depending on the plant host (Backus et al., 2005) and this change may influence the titre of the ingested phytoplasma or ingestion of phytoplasmas. *Nephotettix virescens* (Distant) feeds principally from phloem in rice but occasionally consumes some xylem sap. However, in virus resistant cultivars, it feeds primarily on xylem (Khan and Saxena, 1984). Leafhoppers do not feed in the phloem readily on non-preferred host plants (Chiyakowski and Sinha, 1988). This may explain why only some plants are phytoplasma acquisition hosts. Phytoplasmas also change the entire physiology of the host thus affecting the normal nutritional and hormonal balance in the plant tissues making them either attractive or unattractive to the feeding vector. This may also affect the acquisition of the phytoplasma by the vector. Phytoplasma may not also be there in symptomatic plant parts (Weintraub and Beanland, 2006).

There are no reports on the vectors selectively acquiring one phytoplasma from a host plant infected with more than one phytoplasma strains. *M. quadrilineatus* could acquire and subsequently transmit a single strain when given short AAP on lettuce. This is however, a result of short AAP rather than selective acquisition or transmission (Zhang et al., 1998).

Factors mediating vectors capacity

Leafhopper gender can influence the acquisition and transmission dynamics of phytoplasmas. Female *M. quadrilineatus* were more efficient at transmitting AYP to lettuce than males (Beanland et al., 1999). However, Lefol et al. (1994) observed that the phytoplasma titre was lower in males at an earlier age compared to at an older age in the salivary glands of *E. variegatus* compared to those in females. Behaviourally also the males are more active and move around more in search of mate compared to the females.

Vector age is another important factor influencing acquisition and transmission of phytoplasmas. Neonate *E. variegatus* do not acquire CYP with the same efficiency as the fifth instar nymphs (Palerno et al., 2001). Efficiency of transmission is increased when they acquire the pathogen as nymphs rather than as adults (Murral et al., 1996).

Effect of phytoplasmas on its leafhopper vectors

Phytoplasmas are obligate endosymbionts with a reduced genome and lacking genes required for major metabolic processes (Oshima et al., 2004). They rely on their insect vectors for dispersal and may influence the vector's biology, behaviour directly once infected and indirectly through the host plant. Phytoplasmas manipulate insect and plant host in their favour by suppressing host immune responses, altering host cell processes and interfering with plant development.

Direct effects

They may directly influence the vector's biology: influence the abundance of their populations, improve fitness of the vector by increasing survival rates, life span and fecundity. Sometimes the phytoplasma may affect the vector adversely. For example, the FDP infected *S. titanus* have reduced survival and reduced fecundity, this may be due to the recent association of these two organisms. FDP infected *S. titanus* disperses less compared to uninfected individuals. On the other hand, AYP infected *M. quadrilinatus* displayed greater mobility compared to the uninfected individuals. Some phytoplasmas may not have any effect on their vectors.

Indirect effects

The phytoplasmas may manipulate insect vector behaviour through infected host plants. They can change the volatile composition of the host plant thus making the host plant more attractive to the vector. They may also change the leaf area reflectiveness. They may alter plant nutrient quality and suppress the herbivore induced defence responses to promote insect feeding and oviposition. *M. quadrilineatus* laid more eggs and produced higher number of nymphs on aster yellows-witches' broom infected *Arabidopsis thaliana* (L.) Heynh. plants.

Epidemiology of phytoplasma diseases

Several phytoplasmas that are there in the host plants may not be of importance in a geographical area because of their inability to spread without an efficient vector. The vector and the phytoplasmas might not have come together in their evolutionary sojourn, but when they come together the phytoplasma disease may cause severe economic loss in plants affected by it. The FDP was there in Europe as a very minor pathogen until

S. titanus got introduced into Europe during 1950s resulting in epidemic spread of FDP in the vine yards of southern Europe. Similarly, *E. variegatus*, a Palaearctic leafhopper introduced into North America, became a vector of Western X-disease and AYP.

Host range of both phytoplasma and its vector greatly influence chances that a phytoplasma and a potential vector will come into contact. Species of *Cacopsylla* are monophagous on pome or stone fruits and hence are the only vectors of '*Candidatus* Phytoplasma mali', '*Ca*. Phytoplasma pyri' and '*Ca* Phytoplasma prunorum' which infect pome or stone fruits (Weintraub and Beanland, 2006). *Orosius albicinctus* Distant, a polyphagous leafhopper on herbaceous hosts (Suryanarayana et al., 1998) transmits different species/strains of phytoplasmas to several plant species (Table 1).

Bosco and D'Amelio (2010) recognised four categories of phytoplasma and vector relationships based on their specificity which profoundly influences the epidemiology of the disease.

Phytoplasma and vector relationships and phytoplasma epidemiology

Plant generalist and vector generalist

The phytoplasmas infect several species of plant hosts and use leafhoppers that feed on several species of plant hosts. Some examples are aster yellows transmitted by several vector species to hundreds of plant species (Weintraub and Beanland, 2006). Western X disease '*Ca*. Phytoplasma pruni' (Lee et al., 2000).

Plant generalist and vector specialist

The beet leafhopper transmitted virescence (BLT) has several host plant species but is transmitted only by *N. tenellus*.

Plant specialist and vector generalist

Some phytoplasmas can be transmitted by a narrow range of vector species but some plant specialists are known to be transmitted by different vector species. Maize bushy stunt is transmitted by *Dalbulus maidis* DeLong, *D. eliminates* (Ball) and *Graminellani grifrons* (Forbes). Similarly, apple proliferation phytoplasma is transmitted by *Cacopsylla costalis* (Flor), *C. melanoneura* and *Fieberiella florii* (Stål).

Plant specialist and vector specialist

Some plant specialist phytoplasmas are transmitted by a specific vector only. Grapevine FDP is transmitted only by *S. titanus* in nature.

Epidemiologically transmission cycle could be grouped either as closed cycle or open cycle. In a closed cycle, the phytoplasma circulates between a main, if not exclusive, host plant and a main, if not exclusive, vector species as in grapevine FDP and its vector *S. titanus*. In an open cycle, the phytoplasma circulates among different host plants as its vector(s) can regularly or accidentally, feed on different plant species (crops). Bois Noir (BN) in grapevines is transmitted by *Hyalesthes obsoletus* Signoret from weeds to grapevine. Peach Western X disease is transmitted by *Paraphlepsius irroratus* (Say) and other species from Chokecherry to peach.

Phytoplasma vectors of the Indian subcontinent

In this section, information on the confirmed as well as putative vectors of phytoplasmas and *Xylella fastidiosa* are given briefly. The confirmed vectors of phytoplasma include two species of *Nephotettix* Ishihara, *O. albicinctus* and *Hishimonus phycitis* (Distant). All others are here considered as putative until further confirmations are published. Several reviews are available on the phytoplasma diseases associated with plants in India namely, sandal spike (Teixeir da Silva et al., 2016), little leaf of brinjal (Rao and Kumar, 2017), sesamum phyllody (Rao et al., 2015), coconut root (wilt) phytoplasma (Rajan, 2011) and sugarcane grassy stunt phytoplasma (Rao et al., 2014).

Species of *Nephotettix*: The genus belongs to the tribe Chiasmini and the genus *Exitianus* Ball is a close relative. They are grass feeders. *N. nigropictus* (Stål) (Figs. 2, 3) and *N. virescens* (Figs. 4, 5, 35) are the species of green leafhoppers on rice which are confirmed vectors of rice yellow dwarf (Table 1) in India and abroad. Both *N. nigropictus* and *N. virescens* breed on species of the genus *Oryza* and nymphs develop on that host and the former species can also breed on grasses. However, adults can be found on a number of hosts both dicots and monocots. Considerable information on their biology, ecology and management has accumulated as they are considered serious pests of rice. *N. virescens* occurs in two morphs namely, green and blue morphs (Muniyappa and Viraktamath, 1981) and both are efficient vectors. The third species *N. malayanus* Ishihara and Kawase (Fig. 1) is listed as a vector of yellow dwarf (Wilson and Turner, 2010).

***Hishimonus phycitis* (Distant):** The genus *Hishimonus* includes very similar looking species

feeding on herbs and shrubs. The genus has been recently revised and includes 23 species from the Indian subcontinent (Viraktamath and Anantha Murthy, 2014; Meshram and Chaubey, 2016). The genus belongs to the tribe Opsiini that includes most important vectors of plant viruses, phytoplsamsas and spiroplasmas (Nielson, 1979). *H. phycitis* (Figs.16, 36) is the most common species on shrubs and herbs both in cultivated and uncultivated areas. This and also *O. albicinctus* can be collected together on *Parthenium hysterophorus* L. and sesame. It is a polyphagous species using several plant species for oviposition and nymphal development. The adults feed on a number of plants other than those on which the nymphs develop (Bindra and Sohi, 1968; Bindra and Singh, 1969). The leafhopper breeds well on brinjal, *Amaranthus mangostanus* L., *A. tricolor* L., *Lepidium sativum* L., Lime (*Citrus aurantifolia* (Christm.) Swingle), desi cotton (*Gossypium arboretum* L.) and sesame (Bindra and Singh, 1969). The species is distributed in the Oriental region and in the Middle East in the Palaearctic region.

***Orosius albicinctus* Distant:** *O. albicinctus* (Fig. 17) also belongs to the tribe Opsiini and is one of the most important leafhopper vectors transmitting a number of phytoplasmas to different crops in India and abroad (Table 1). It is a species distinct from *O. orientalis* (Matsumura) with which Ishihara (1982) had erroneously synonymised it. Fletcher *et al*. (2017) revised the genus based on male genitalia and molecular characters and correctly reinstated *O. albicinctus* as a valid species. They also mentioned that the populations found in India, Pakistan, Middle East and North Africa belong to this species. It is a polyphagous species using a number of herbaceous plants as oviposition and nymphal development hosts (Suryanarayana et al., 1998) whereas the adults feed on a number of plants belonging to several plant families. *Trigonella foenum-graecum* L., *Parthenium* and *Crotolaria juncea* L. are good hosts for rearing this species in the laboratory apart from sesame.

***Neoaliturus tenellus* (Baker) and *N. fenestratus* (Herrich-Schäffer):** The beet leafhopper, *Circulifer tenellus* (Baker) is now placed in the Opsiini genus *Neoaliturus* Distant and the Indian species *Neoaliturus gardineri* (Distant) described from Minicoy, Lakshadweep has been synonymised with the Palaearctic species *N. fesnestratus* (Herrich-Schäffer) (Fig. 18). These are well-known vectors of a number of plant viruses, phytoplasmas and spiroplasmas (Table 1). Ironically, in India none of them have been incriminated as vectors. Bindra et al. (1970) reported the occurrence of *N. tenellus* (Fig. 15), *N. dubiosus* (Matsumura) and *N. opacipennis* (Lethierry) from northwest India (Punjab, Haryana, Himachal Pradesh and Rajasthan). N. *opacipennis* is very common in northwest India including Delhi breeding on Chenopodiaceae plants whereas *N. tenellus* is very rare. Bindra et al. (1970) collected these leafhoppers on several species of plants however; nymphs and adults of *N. opacipennis* were collected on *Heliotropium eichwaldi* Steud. and *Peganum harmala* L. and those of *N. tenellus* on *Eruca sativa* (Miller) Thell. and *Depterigium* sp. *N. fenestatus* occurs in Minicoy (Lakshadweep Islands) and also in Ullal (Karnataka) along the sea coast and breeds on a species of the genus *Launaea* (Asteraceae). These species need to be included in transmission studies on suspected phytoplasma and spiroplasma diseases to evaluate their role as vectors.

***Exitianus indicus* (Distant):** This is a leafhopper of the tribe Chiasmini mainly breeds on grasses. It is very widely distributed in the Indian subcontinent. *E. indicus* (Figs. 6, 31) is suspected as a vector of sugarcane phytoplasma (Table 1). However, it failed to transmit the white leaf sugarcane phytoplasma in Thailand (Hanboonsong et al., 2002, 2006).

Species of the tribe Deltocephalini: Species of the genera *Deltocephalus* Burmeister, *Maiestas* Distant and *Matsumuratettix* Ishihara belong to this tribe and contain either proven or putative vectors (Table 1). *Deltocephalus vulgaris* Dash & Viraktamath (Figs. 7, 32) is reported as a vector of sugarcane grassy shoot phytoplasma (Table 1). It mainly breeds on grasses and attains large populations. It occurs in two forms as far as the male genitalia is concerned (Dash and Viraktamath, 1998) and both the morphs in the same population have been proved to belong to the same species molecularly (Zhang et al., 2019). Zig zag leafhopper, *Maiestas dorsalis* (Motschulsky) (Fig. 8) (earlier placed in the genera *Deltocephalus*, *Inazuma* Ishihara and *Recilia* Edwards) breeds on rice and vectors a number of viruses and phytoplasma affecting rice. However, no reports of these diseases are there in India. *Maiestas portica* (Melichar) is again a polymorphic species with respect to the development of black spot on crown and coloration of head and pronotum varying from golden yellow to dull creamy white without black spot (Fig. 9). It breeds on grasses in moist habitats and has wide distribution in the Oriental region. It has been associated with transmission of sugarcane phytoplasma (Table 1). *M. hieroglyphicus* (Fig. 19) earlier known as

Pruthiorosius maculatus (Pruthi) in India is a proven vector of sugarcane white leaf phytoplasma in Thailand and the phytoplasma is trans-ovarially transmitted (Hanboonsong et al. 2002). It is a very rare species in India and should be searched in areas surrounding sugarcane fields. It breeds on grasses. It can be readily identified by its reticulate forewing venation, however there are couple of species of *Maiestas* resembling this species externally but have entirely different male genitalia.

***Goniagnathus punctifer* (Walker):** This species belongs to the tribe Goniagnathini and breeds on grasses and feeds both on grasses and dicotyledonous herbs. *G. punctifer* (Fig. 10) vectors Weligama coconut leaf wilt phytoplasma in Sri Lanka (Table 1).

***Thomsonia porrecta* (Walker):** This grass feeding leafhopper is common throughout the Oriental region. It is suspected as a vector of phytoplasma disease (Wilson and Turner, 2010). *T. porrecta* (Fig. 14) was earlier placed in the genera *Hecalus* Stal and *Thomsoniella* Signoret. It is a common species on grasses and also on rice.

Cicadelline genera *Cofana* Melichar and *Kolla* Distant: These genera belong to the subfamily Cicadellinae and the tribe Cicadellini. They are mainly xylem feeders. Kumara et al. (2015) incriminated *Cofana unimaculata* (Signoret) and *Kolla ceylonica* (Melichar) as the putative vectors of Wilegama coconut leaf wilt phytoplasma along with *G. punctifer*. *C. unimaculata* (Fig. 11) is commonly found on rice ecosystem and also on seedlings of various Poaceae crops such as wheat, ragi (*Elusine corocana* Gaertn.) etc. *K. ceylonica* (Fig. 12) is found on grasses and also on dicotyledonous herbs and shrubs. It would be of great interest if they prove to be efficient vectors of the phytoplasma.

***Idioscopus clypealis* (Lethierry):** This is one of the most important species of mango leafhoppers belonging to the subfamily Eurymelinae. *I. clypealis* (Fig. 13) has been reported as a phytoplasma vector of Weligama coconut leaf wilt disease in Sri Lanka (Kumara et al., 2015). It is a monophagous species breeding only on the inflorescence of mango. However, adults are found on several plants during non-flowering season of mango (Viraktamath, 1989).

***Yamatotettix fulvivittatus* Matsumura:** This species belongs to the tribe Macrostelini of the subfamily Deltocephalinae. This tribe contains large number of phytoplasma vectors in the world. This species occurs in northeast India and its close relative *Yamatotettix sexnotatus* (Izzard) occurs throughout India on sugarcane (Webb 1986). *Y. fulvivittatus* is known to transmit sugar cane white leaf phytoplasma and the pathogen is also trans-ovarially transmitted (Hanboonsong, 2006).

***Olidiana kirkaldyi* (Nielson):** This Coelidiinae leafhopper was earlier misidentified as *Jassus indicus* (Walker) which was incriminated as the vector of sandal spike phytoplasma (Rangaswamy and Griffith, 1941; Nielson, 1982) and was described as a new species, *Calodia kirkaldyi* Nielson. It is now placed in the genus *Olidiana* Nielson. *C. kirkaldyi* (Figs. 20, 30) is a large species feeding on *Calotropis gigantea* (L.) W.T. Aiton, *Tectona grandis* L. f., *Morinda tinctoria* Roxb., *Bambosa* sp., *Morus alba* L., *Santalam album* L., cotton (*Gossypium* spp.), horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). The nymphs and adults of this species are seen on *Dodonaea viscosa* Jacq. and *Ziziphus oenoplia* (L.) Miller suggesting that these may be the breeding hosts (Subba Rao et al., 1988). Its ability to transmit sandal spike phytoplasma needs to be confirmed.

***Alebroides nigroscutellatus* (Distant):** This is a slender cream coloured leafhopper belonging to the second largest and one of the most advanced clade of leafhopper sub-families Typhlocybinae. It was earlier known as *Alebroides dravidanus* (Ramakrishnan & Menon). This transmits potato purple top roll phytoplasma (Singh and Nagaich, 1977) (Table I). It is widely distributed in India and Singh et al. (1983) report that the phytoplasma caused some adverse effects on the vector. The leafhopper apparently breeds on potato.

***Amrasca devastans* (Distant):** This species (Fig. 21) commonly known as cotton leafhopper [also known as *A. biguttula biguttula* (Ishida)] is a very polyphagous leafhopper breeding on economically important plants across several families including Malvaceae and Solanaceae. Maramarosch et al. (1970) reported it to transmit little leaf of brinjal which however, need to be confirmed.

***Mesargus albomaculatus* (Distant):** This species earlier known as *Moonia albomaculata* Distant (Figs. 22, 33) was suspected as a vector of sandal spike (Table 1). However, its ability to transmit the phytoplasma needs to be confirmed. It breeds on several hosts including sandal and the creeper, *Dolichandra unguis-cati*. (L.) Miers Both nymphs and adults are more

sedentary feeding on shoots and pencil thick stem of these plants.

***Nilaparvata lugens* (Stål):** The brown planthopper (Figs. 23-25) belongs to the family Delphacidae of the superfamily Fulgoroidea. It is a common species in rice ecosystem and monophagous breeding only on the species of *Oryza*. As in the case of adult leafhoppers, these also probe on several plants both monocots and dicots. It is known to transmit several viruses of rice plant however, it is suspected to transmit an unknown phytoplasma affecting rice (Table 1). It has a very complex life cycle and exhibits wing polymorphism based on population density and host plant suitability (Wilson and Claridge, 1985).

***Redarator bimaculatus* Distant:** This Issidae planthopper of the superfamily Fulgoroidea is reported as a vector of sandal spike disease in Kerala (Table 1). *R. bimaculatus* (Figs. 26, 27) is a very distinctive Issid planthopper with prominent pair of facial black spots which sometimes coalesce to form one transverse band on upper part of face. No further confirmation of this has so far been published and hence it needs to be proved that *R. bimaculatus* indeed is also one of the vectors of sandal spike. Information on its host plants and biology is not known.

***Proutista moesta* (Westwood):** *P. moesta* (Fig. 34) is a beautifully coloured derbid planthopper belonging to the family Derbidae and superfamily Fulgoroidea. The nymphs usually breed in decaying vegetable matter feeding on fungi and adults are found on palms and also on crops like maize and sorghum often in large congregations. *P. moesta* is considered as the main vector of coconut root (wilt) phytoplasma and also areca nut yellow leaf phytoplasma found in south India (Table 1).

***Halyomorpha halys* Stal:** This is a medium sized Heteroptera bug belonging to the family Pentatomidae. This species does not occur in India, but its close relative *Halyomoprha picus* (Fabricius) (Fig. 28) is a common species affecting areca nut, pigeon pea, cotton, pomegranate, cowpea, lablab, tamarind, etc. *H. halys* is an invasive pest in the United States of America and is also known to transmit phytoplasma (Table 1).

***Stephanitis typica* (Distant):** This is another suspected Heteroptera bug belonging to the family Tingidae and superfamily Miroidea vectoring phytoplasmas. Both nymphs and adults of *S. typica* (Fig. 29) feed on under surface of leaf and all stages can be found there. They feed on mesophyll and remove cell contents thus the affected leaf shows silvery stippling symptoms which later coalesce and form large grey patches. This species is reported as vector of coconut root (wilt) disease in south India (Table 1). However, this needs to be confirmed.

The bacterium, *Xylella fastidiosa*

The bacterium, *Xylella fastidiosa* is a Gram negative plant xylem restricted gamma-Proteobacterium living as endosymbiont in several plants and a few of its clades causing serious diseases in several economically important plants (Janse and Obradovic, 2010). More than 563 plant species in 82 families harbour this bacterium, some asymptomatic and others showing symptoms of leaf scorching and stunting. The entire genome of this bacterium has been sequenced and usually five subspecies are recognised: (i) *Xylella fastidiosa* subsp. *fastidiosa* Wells et al., Pierce's Disease (PD) and Almond Leaf Scorch (ALS), strains from cultivated grape, alfalfa, almond, and maple; (ii) *X. fastidiosa* subsp. *multiplex* Schaad et al., Phoney Peach Disease (PPD), Plum Leaf Scald (PLS), strains from peach, elm, plum, pigeon grape, sycamore and almond; (iii) *X. fastidiosa* subsp. *pauca* Schaad et al., citrus variegated chlorosis (CVC), strains from citrus and probably those from coffee leaf scorch (CLC); (iv) *X. fastidiosa* subsp. *sandyi*, strains from *Nerium,* Oleander Leaf Scorch (OLS); (v) *X. fastidiosa* subsp. *tashke*, strains from the ornamental tree *Chitalpatash kentensis* T.S. Ellis & Wisura. Pierce's Disease (PD) of grapevine, Citrus Variegated Chlorosis (CVC), Almond Leaf Scorch (ALS), Oleander Leaf Scorch (OLS), and Olive Quick Decline Syndrome (OQDS) are among the most economically significant *X. fastidiosa* related diseases in the world. Leaf scorch symptoms and ultimately plant death are thought to be the outcome of bacterial growth and clogging up of xylem vessels (Hopkins, 1989).

This bacterium is transmitted by the xylem sap feeding Hemiptera that include Cicadidae (Cicadoidea), Cercopidae, Machaerotidae, Aphrophoridae (Cercopoidea) and Cicadellidae (mainly the species of the subfamily Cicadellinae). As a vector-borne pathogen, plant-to-plant spread of *X. fastidiosa* is directly related to the presence, abundance, and behaviour of insect vectors in relation to infected and healthy plants. The relationship of the bacterium with its vector is very unique. The bacterium is transmitted in non-circulative but propagative manner. Both nymphs and adults can transmit the bacterium but the nymphs

lose their infectivity after a moult (non-transstadial). The bacterium is also not trans-ovarially transmitted. The vectors have a very short AAP lasting for a few hours, however, more the AAP better is the transmission and there is no appreciable latent period (Redak et al., 2004). After acquisition, the bacterium is retained on the cuticular lining of the insect foregut, likely in the part of the pre-cibarium proximal to the cibarium (Almeida and Purcell, 2006). It also multiplies in these areas and forms biofilm. A number of species of Cicadellinae and Cercopoidea can transmit the bacterium but with varying transmission efficiency (Almeida, 2016). As far as PD in California is concerned, *Graphocephala atropunctata* (Signoret) is the most efficient transmitter. However, the disease was almost under control after the first outbreak during 1930s. It was shown that the occurrence of PD was much higher in the grapevines that are bordering riparian areas from where the leafhoppers could arrive during spring and infect the vines. However, secondary spread was not very high as the leafhopper *G. atropunctata* and *Draeculacephala minerva* Ball and other native cicadelline vectors feed on new shoots and fresh leaves and the bacterium did not have enough time to invade woody tissue and these parts are pruned during winter pruning thus limiting its spread to woody tissues. However, with the introduction of Glassy-Winged Sharp Sooter (GWSS) *Homalodisca vitripennis* (Germar) [earlier known as *H. coagulata* (Say)] in California changed the entire scenario and there was severe epidemic of the disease threatening the entire grape industry. *H. vitripennis* is considered the most important vector despite its relatively inefficient transmission rates (Almeida and Purcell, 2003). Its high mobility, extreme polyphagy, lack of biological control organisms, high densities in preferred habitats from which they disperse, and wide geographical distribution due to transport of commercial nursery products offset its poor transmission ability (Redak et al., 2004). GWSS feeds both on young shoots and older stem and is also capable of transmitting the bacterium to dormant canes. When the bacterium is in the woody part of the vine it is protected from the low winter temperature and by the spring it can infect the plant. Thus GWSS plays an important role in secondary spread of the bacterium leading to the epidemics. This also led to considerable research on biology and ecology of the vectors, the pathogen and its epidemiology. Various factors influence vector transmission of *X. fastidiosa*, including the distribution and density of bacterial populations in host plants, insect host range and plant preference, season of inoculation, and climatic conditions. The ecology of vectors can affect epidemics, as demonstrated by the large increase in PD of grapevine incidence in California after the introduction of GWSS (Almeida et al., 2005).

The first confirmed report of *X. fastidiosa* in the Old World was from Taiwan during 1980s when pear leaf scorch disease was reported (Leu and Su, 1993) and later PD was reported in grapevines in Taiwan (Su et al., 2013). Search for the vector showed that *Kolla paulula* (Walker) and *Bothrogonia ferruginea* (Fabricius) transmitted PD with transmission rates of 13.3 and 6.7%, respectively, in grapevine (Tuan et al., 2016). The aphrophorid, *Poophilus costalis* (Walker) was also suspected as a vector of PD (Su et al., 2011). These species of insects are also present in India. *X. fastidiosa* has been reported in several Asian countries however, as stated by EFSA (2015), some of the detections appear to be unconfirmed namely, that from India (Jindal and Sharma, 1987) and Turkey (Guldur et al., 2005).

In Europe, the first confirmed report of *X. fastidiosa* was from France in coffee plants in containment. However, its field establishment was that from Italy (Saponari et al., 2013) affecting olive trees (*Olea europaea* L.) growing on the west coast of Salento Peninsula, resulting in the decline of the trees because of an unknown disease subsequently named Olive Quick Decline Syndrome (OQDS) (Martelli et al., 2016). The report of the bacterium from Germany is now reported as eradicated. Europe does not have many species of Cicadellinae. One very widely distributed species, *Cicadella viridis* (Linnaeus) (in the Palaearctic region) is considered as the vector. However, Cercopoidea and cicadas, which are considered to be marginally important vectors in other continents, are thought to play a key role in *X. fastidiosa* epidemiology in Europe. So far, the role of cicadas in transmitting *X. fastidiosa* has not been well established and considering their short adult life-cycle they may be of very minor importance as vectors. Among the Cercopoidea, the polyphagous and wide spread meadow spittlebug, *Philaenus spumarius* (Linnaeus) (family Aphrophoridae) is considered the most important vector responsible for the spread of the bacterium in olive orchards (Cornara et al., 2017).

Auchenorrhycha that could serve as vectors of *Xylella fastidiosa* in India

X. fastidiosa does not have strong vector specificity and a number of xylem feeding Hemiptera can transmit them with varying degree of efficiency. As there are no confirmed plant diseases caused by this bacterium

reported from India, here only those genera which are more common and can probably serve as vectors are mentioned.

Species of Cicadellinae and Signoretiinae: As mentioned earlier, the leafhopper subfamilies Cicadellinae and Signoretiinae include those leafhoppers which feed on xylem sap and have greatly enlarged faces to accommodate strong muscles required to pump the xylem sap which is under negative pressure.

The following common species of Cicadellinae may serve as vectors of *Xylella*. *Anagonalia melichari* (Distant) (Fig. 37), *Anatkina helena* (Distant) (Fig. 38), *Atkninsoniella opponens* (Walker) (Fig. 39), *Bothrogonia albidicans* (Walker) (Fig. 40), *Bothrogonia sclerotica* Young (Fig. 41), *Bothrogonia ferruginea* (Fabricius), *Cofana lineata* (Distant) (Fig. 42), *Cofana unimaculata* (Signoret) (Fig. 43), *Cofana spectra* (Distant) (Fig. 44), *Kolla ceylonica* (Melichar) (Fig. 45), *Kolla paulula* (Walker) (Fig. 46) and the species of the subfamily Signoretiinae, *Signoretia* sp. (Fig. 47). The adults of these leafhoppers are very common on different plant species either on the new foliage (*K. ceylonica*, *K. paulula* and *Signoretia* sp.) or on leaves and shoots of different species of plants in moist forests. Both nymphs and adults can act as vectors of



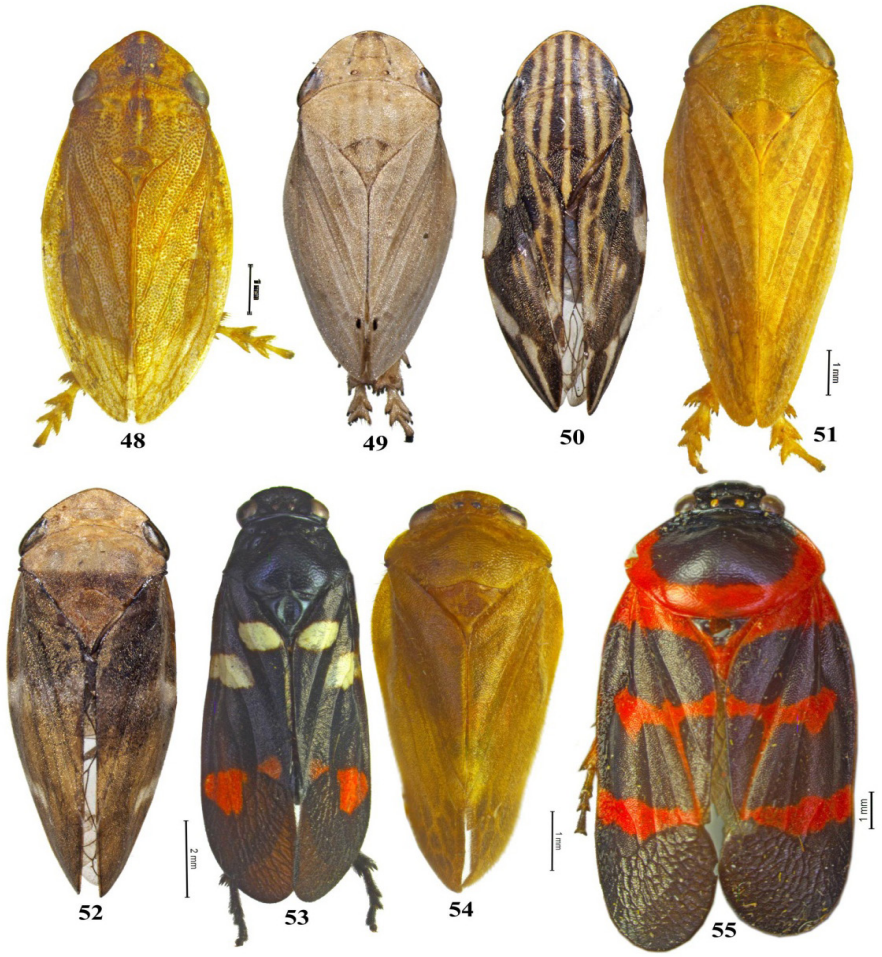
Figs. 37-47. Leafhoppers that probably act as vectors of *Xylella fastidiosa* if it gets accidentally introduced into India. 37. *Anagonalia melichari* (Distant); 38. *Anatkina helena* (Distant); 39. *Atkinsoniell aopponens* (Walker); 40. *Bothrogonia albidicans* (Walker); 41. *B. sclerotica* Young; 42. *Cofana lineata* (Distant); 43. *C. unimaculata* (Signoret); 44. *C. spectra* (Distant); 45. *Kolla ceylonica* (Melichar); 46. *K. paulula* (Walker); 47. *Signoretia* sp.

Xylella. *K. paulua* and *B. ferruginea* have already been demonstrated as vectors of *X. fastidiosa* causing Pierce's Disease of grapevine and Pear leaf scorch disease in Taiwan (Tuan et al., 2016).

Species of Cercopoidea: The species of Cercopoidea belong to three families namely Aphrophoridae, Cercopidae and Macherotidae. Of these the species of the first two families may be important as vectors of *Xylella*. Both nymphs and adults are xylem feeders. The nymphs are in froth (spittle) and are sedentary and my not play any role in transmission of *Xylella*. Only the adults are able to transmit the pathogen with varying degrees of efficiency. Some of the species which can act as vectors of *Xylella* in the Indian subcontinent are as follows: Aphrophoridae: *Aphrophora* sp. (Fig. 48), *Clovia puncta* (Walker) (Fig. 49), *Clovia lineaticollis* (Motschulskey) (Fig. 50), *Poophilus costalis* (Walker) (Fig. 51) and *Ptylinellus praefectus* (Distant) (Fig. 52); Cercopidae: *Callitettix versicolor* (Fabricius) (Fig. 53), *Eoscarta* sp. (Fig. 54) and species of *Cosmoscarta* (Fig. 55). Among these, species of *Aphrophora*, *Poophilus*, *Ptylinellus*, *Callitettix*, *Eoscarta* and *Cosmoscarta* are found on woody trees and shrubs in addition to grasses. *C. lineaticollis* is found breeding on jack fruit saplings and trees and *C. puncta* breeds extensively on grasses and the adults feed both on grasses and herbs and shrubs. The Indian fauna of Cercopoidea is in need of revision as it is difficult to identify the species based on century old descriptions given by Distant (1908, 1916).

Search for the unknown vector(s) of phytoplasmas

The knowledge of the insect vector is crucial for well-timed and efficient control strategies of phytoplasma diseases and to avoid further spread of the pathogen (Alma and Tedeschi, 2011). In the case



Figs. 48-55. Spittlebugs (Cercopoidea) that probably act as vectors of *Xylella fastidiosa* if it gets accidentally introduced into India. 48. *Aphrophora* sp.; 49. *Clovia puncta* (Walker); 50. *C. lineaticollis* (Motshchulsky); 51. *Poophilus costalis* (Walker); 52. *Ptylinellus praefectus* (Distant); 53. *Callitettix versicolor* (Fabricius); 54. *Eoscarta* sp.; 55. *Cosmoscarta* sp.

of new phytoplasma diseases, the vectors are always unknown and intensive studies are required to identify the insects. Similarly, when a new outbreak occurs in new geographical areas, the already known vectors may not have the same role and the presence of other possible vectors need to be explored.

Insect sampling for vector search

Different sampling techniques need to be employed not only on the crop plant but also on associated plants including weeds and shrubs in the diseased area. Sampling also has to be done during different seasons of the year as the life cycle of the insect vectors may differ. Once the putative vector is identified the knowledge of its wild host plants becomes of utmost importance to understand its ecology and behaviour and the epidemiology of the disease.

When nothing is known, our aim should be to sample large number of insect fauna from the diseased area. A number of insect collection methods that are available could be adopted for collecting vector Auchenorrhyncha. However, till the time the vector groups are narrowed down, general collection methods in the area of prevalence of the phytoplasma disease could be used. These could be sweep net collecting on vegetation, light traps, malaise traps, yellow pan traps, beating tray and yellow sticky traps. These would yield large number of insect samples. Details regarding how to set up and use these methods could be found in Triplehorn and Johnson (2005, and the references therein). The sweep net and beating tray will yield live insects, whereas the others are likely to yield only dead insects, unless they are manually attended as in the case of light traps. Some collection equipment like Malaise trap, light traps are useful as they act as random samplers irrespective of the host plants of the insects. Information on place of collection, methods used, date, name of the collector and the host plants if available need to be carefully recorded and documented. However, the material collected need to be sorted out and the Auchenorrhyncha fauna need to be identified and could also be used for testing whether any of them contain phytoplasmas of interest. Sticky traps can be used for trapping insects associated with different plant species and need to be serviced more frequently to get better quality dead insects for further study and identification. To collect live insects for developing cultures and other laboratory studies hand picking through aspirator, sweep nets and beating trays would be most useful. In addition to live insects, these methods can give us information on host plants and natural enemies of the insects collected. The vacuum insect collector (D-VAC) is a very useful instrument to sample live insects from dense vegetation, thorny plants and very low vegetation close to ground to collect surface dwelling vectors which may be missed otherwise.

Ones the groups of Auchenorrhyncha that harbour the phytoplasma of interest are narrowed down either to genus or to species, specific methods could be devised to selectively collect different stages of these based either on the literature or by the advice of the experts or field observations and also their host plants and preferred habitats, ecological conditions, seasonal incidence behaviour etc. could be studied.

Handling collected insects

Dead insect samples thus collected need to be sorted, labelled (locality label including data on place, date of collection, host plants if any, method of collection and collectors name) individually after mounting them appropriately depending on their size (either pinning them directly or mounting them on a triangular paper point or preserving in 75-80% ethanol, labels in alcohol have to be either printed or written on soft paper with pencil) and identified. Triplehorn and Johnson (2005) may be consulted for further details.

Cooperation of well-trained taxonomists need to be sought and can be built up right at the beginning of the project proposals for studies on vector borne plant pathogenic diseases. The technicians involved in the project may be trained in preliminary identification of the species of Auchenorrhyncha and Sternorrhyncha groups of phytoplasma vectors. They also need to be trained in the preparation of wing and male genitalia and should be conversant with the terminologies used. Identification of these groups is mainly based on the detailed study of the male genitalia and any deficiency in that would affect accurate identification. They should also be trained in the identification of immature stages and sexes. It should also be noted that the vectors involved could be un-described taxa and help of a specialist in the group may be needed to address this issue. It is also necessary to preserve the voucher specimens used in the investigation in a recognised insect collection. It is also advised to clearly mark the proven vectors and material used in molecular identification etc. for future reference and verification if needed.

When live samples are collected, they need to be placed in clean vials along with shoots or leaves of their

host plant and brought to the laboratory and maintained on their respective host plants for further studies in the laboratory. Here also species need to be sorted out and reared on their respective host plants.

Transmission studies

Experimental transmission studies are essential to ascertain the vector ability of putative vector species. For this the test insect could be naturally infected or experimentally infected (Alma and Tedeschi, 2011).

Experiments with naturally infected insects

1. Healthy insects (usually laboratory reared) are caged on infected plants for Acquisition Access Period (AAP). The AAP may be for 24, 48, 72 hours or even longer if needed.
2. After AAP the insects are removed to suitable healthy plants to complete their Latency Period (LP). The LP may be for 8, 10, 15, 20, 30 days or even more if required.
3. After LP the insects are transferred to healthy plants for Inoculation Access Period (IAC). This may last for a few days to several weeks. The number of insects used, stage of insect used and also the sexes used may be varied to test their relative efficiency.
4. After IAC, the insects are removed and tested for the presence of phytoplasma using molecular techniques. The plants are maintained in insect proof cages for symptom development which may take a few to several months to appear. The plants may also be tested for the presence of the phytoplasma using molecular techniques. In woody plants, the symptoms may appear very late even after one year or more and phytoplasma titre could be very low, thus not detectable for months.

Field collected insects (putative vector species) could also be used for transmission studies in preliminary experimental stages, if there is difficulty in establishing laboratory culture of putative species.

All these studies need to be done in insect proof cages in greenhouse condition so that the researchers know that the healthy plants are really healthy and the insects specimens used are also healthy. These experiments need to be carefully planned well in advance so that the required number of healthy plants and healthy insects to be tested is always available for the experimentation. It is also necessary to have a good infected source plant with accurately identified phytoplasma.

Tests with experimentally infected insects

Transmission to artificial feeding medium is a good and practical alternative to test the inoculative ability of the candidate insect vector (Tanne et al., 2000; Bressan et al., 2006; Pinzonti et al., 2008). In this procedure the insects after LP (see previous section) are allowed to feed on artificial feeding medium such as sucrose or sucrose plus sorbitol with buffer solution. After IAP the insects are removed and tested for the presence of phytoplasma. The feeding medium is removed and frozen and tested for the presence of the released phytoplasma using molecular procedures.

Vector search on fast track

Search for the insect vectors of a vector transmitted plant pathogens take considerable time and energy. However, with the advancement in the molecular biology, tissue culture and rearing techniques in Hemiptera, it is possible to reduce the quantum of Hemiptera to be tested and the time required to find out putative vectors those can be subjected to more rigorous tests for their role in transmission of the pathogen in nature.

Some of the pre-requisites for fast tracking the search are the following:

(a) Availability of tissue culture techniques for production of large number of young susceptible host plants of the pathogen especially in the case of perennial plants like coconut, arecanut or sandal. If such a technique is not available then, identification of annual plant species if any which can act as laboratory hosts for maintenance and transmission of the pathogen.

(b) Availability of good source plant of the pathogen under study. Ensure that it is not a dead-end host. The pathogen needs to be periodically transferred to young plants so that the pathogen is in active multiplication state for further experimentation.

(c) Developing molecular identification techniques for the pathogen both in the plant and in the vectors.

Procedure

1. Collect live auchenorrhynchan insects from both diseased plants, healthy plants and also from surrounding vegetation and maintain them separately. Pay particular attention to specimens collected on diseased plants.
2. Sort them into species. Allow 5-10 specimens of each species separately to feed on source plant

Infected with the pathogen under investigation and maintained in the laboratory for 24-48 hours (AAP) and transfer them to healthy plants for completing their latent period.

3. After 8-10 days (or more days) of LP, allow a batch of 5 specimens of each species to feed on 3-5 ml of buffered sugar solution for about 24-48 h IAP. Test the fed solution for the presence of pathogen using molecular technique and also test the insects for the same. Those insect species that released the pathogen in the sugar solution when feeding are the vectors. These insects can further be tested rigorously for transmission using the susceptible young host plants.
4. Those insect species which tested positive for the pathogen but the sugar solution on which they fed tested negative may not be the vectors of the pathogen.
5. One can also test the collected specimens for the presence of pathogen individually or species-wise and narrow down the species that carry the pathogen and then use the steps 2 and 3 mentioned above.

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POLLINATORS IN TROPICAL ECOSYSTEMS OF SOUTHERN INDIA WITH EMPHASIS ON THE NATIVE POLLINATORS *APIS CERANA INDICA* AND *TETRAGONULA IRIDIPENNIS*#

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ABSTRACT

Ecosystems are rapidly urbanizing at the global and regional scales, particularly in the tropics, which has deleterious effect on hymenopteran pollinators. Based on the literature spanning multiple disciplines including ecology, pollination, agriculture, agroecology and entomology, this review deliberates on the pollinators and their global decline. Also, it turns the focus on honey bees and their role in agroecosystem. Relevant information from melissopalynology is brought together and the gaps and directions of future research on conservation and management of honey bees in tropical peninsular India are discussed. Focus is on the two species of the hived native *Apis cerana indica* F., and *Tetragonula iridipennis* Smith (Hymenoptera: Apidae), as these play a major role in transforming existing agricultural landscapes into agroecosystems, benefitting the farmers and maintaining ecological balance in tropical peninsular India. This review brings to the fore the fact that there is a tangible gap in reports and long-term studies of many native pollinators and in particular the two hived honey bees. Most studies present in a thorough manner visual observations of pollinators (bees) on plants but rarely combine them with quantifying the resources gathered from the plants, especially pollen. This combined approach is especially important to understand the hymenopteran pollinators from the purview of the pollination service they provide. It can be concluded that there is a pressing requirement for long-term observations along these lines with quantifiable pollen and vegetation data to arrive at meaningful plant-pollinator networks that are essential for conservation and management of the native Asiatic honey bees as pollinators.

Key words: Hymenoptera, Apidae, pollinators, agroecosystems, pollinator decline, pollen, melissopalynology, transformation, landscapes, pollination service, management of honey bees, conservation

Pollinators, including insects, bats, and birds, key for ecosystem functioning and global food security, are in dramatic decline over the past decades (Rhodes, 2018). Even if the extent of this decline is a much-debated topic because of the lack of uniform, long-term observations at the global scale there are estimates that should serve as a wakeup call. For example, there is an estimate of a "dramatic decline in average airborne insect biomass of 76% (up to 82% in midsummer) in just 27 years for protected nature areas" in Germany, which considerably exceeds the estimated decline of other animals (Hallmann et al., 2017). Similarly, in the UK and The Netherlands, over 60% decreases in bees and hover flies have been estimated since 1980 (Potts et al., 2010). As many as 200,000 pollinators serve to fertilize around 308,000 species of flowering plants (Rhodes, 2018). Globally, 87 major food crops depend on animal pollination, accounting for 35% of the world's food production (van der Sluijs and Vaage, 2016). Worldwide, an estimated 35% of crop production is dependent on insect pollination (Klein et al., 2007). Such dependence on animal pollination, especially insect pollination, underscores the key concern on the decline of pollinators. In tropical peninsular India too, much of the crop production – subsistence and commercial - depend even more on pollinators which is why we focus on this region and in particular on two of its native hymenopterans *Apis cerana indica* F., and *Tetragonula iridipennis* Smith because of their relevance and potential to transform existing agricultural landscapes into agroecosystems. With insect pollination as the focus, this review is written in the framework of ongoing studies including a pilot project aimed at Protection of pollinators and agroecological transitions in the Pondicherry region (POLLIN).

#Note: Scientific names given for plants and insects wherever possible, but where the publications have not provided them, it is preferred to retain their common names.

The importance of cross pollination, and specifically by insects, has been stressed since long. Charles Darwin found that the products of cross pollination resulted in more numerous, larger, heavier, more vigorous and fertile offspring, even in species that normally self-pollinate, attributing this result to 'hybrid vigour' (Darwin, 1876). Pollinators recognize flowers mostly by olfaction, followed by vision even if nocturnal pollination in several angiosperm families is well established. In turn, plants sometimes offer attractive, but non-rewarding structures to attract pollinators (Borges et al, 2003, 2016) - a fascinating subject beyond the purview of this paper. "Regardless of the type of pollinator, they become dusted with pollen when actively feeding on the nectar and/or pollen of a flower. Pollinators that do not actively feed on nectar, pollen, or plant exudates also become 'dusted' with pollen by walking around or in the flowers in search of food, mates, prey, shelter, etc. Thus, pollen is a natural marker on and or in any pollinator" says Jones (2014) in a stand-alone manuscript that provides a "detailed, step by step acetolysis technique" to recover that natural marker from any pollinator.

A great decline in pollinating entomofauna is reported worldwide, especially since the advent of industrial agriculture (van der Sluijs and Vaage, 2016). Globally, pollinator limitation has emerged as a potential risk to crop production and socio-economic stability too (Basu et al., 2011). Ollerton (2015) summarizes the evidence base for this decline, categorized taxonomically and geographically. Wild bees, honey bees, hoverflies, moths and butterflies, wasps, birds and mammals in Europe, North America, Tibet, China, Japan, South and Central America are included in this summary. There are some syntheses for Australia too (Klein et al., 2007). Although there has been a marked increase and interest in the scientific community on this topic in recent years, this has not yet caught popular media attention to the extent that (anthropogenic) climate change has. There is very little synthesis carried out in India (Pannure, 2016). A frequent emphasis has been on highlighting the threat, specifically, to native pollinating Hymenoptera in the areas where *Apis mellifera* L. has been introduced on an industrial scale (Anonymous, Status of pollinators in North America 2007; Allen-Wardell et al., 1998; Biesmeijer et al., 2006; Klein et al., 2007; Potts et al., 2010; Nicholls and Altieri, 2013). From an ecological point of view, Rhodes (2018), in a comprehensive review shows that it is most probable that the decline of pollinating Hymenoptera will be an unavoidable ecological calamity that cannot be ignored. The review underlines the need for more empirical longer-term studies and data that are both more 'geographically encompassing and species-inclusive'.

Many drivers, such as population crisis, urbanization and industrialization, deforestation, habitat degradation, poor agricultural practices like mono cropping, applying more pesticides, excessive chemicals and manuring could be the causes of global pollinator decline (Fitch et al., 2019). A recent assessment of pollinator decline for six regions and a global median underlines eight major drivers with land cover and configuration topping the list (Dicks et al., 2021). This paper also highlights as knowledge gap research in Africa, linking human wellbeing and pollinator decline. This could be the case for the Indian subcontinent as well. As several drivers of pollinator decline identified in this paper are likely linked with climate change, this review recommends that a focus on how to mitigate and adapt to it should be central to pollinator research and conservation strategies. These conclusions agree with those of Rhodes (2018), referred earlier. In an earlier paper Solomon Raju et al. (2003) had highlighted another of these eight drivers – the extensive use of genetically modified transgenic crops and other applications like chemicals in agriculture associated with long-term adverse impacts on biodiversity and particularly pollinators, the main risk being 'gene pollution'.

Honey bees are considered to be the most important among the pollinators of agricultural and horticultural crops and there are several reviews of the same (Nicholls and Altieri, 2013; Yousuf et al., 2020). The value of the honey bee as a pollinator is far greater than its value as a honey producer. Of all the insects, honey bees in hives are the most amenable for crop pollination and can be reared in sufficient numbers and placed in orchards as required for effective pollination. It has been found (Abrol, 2011) that the use of hive bees results in a manifold increase in yields and an improvement in the quality of produce: world honey production is worth around £380,000,000, but the value of insect pollinated agriculture is worth somewhere in the region of £800,000,000,000; this includes the contribution of all insect pollinators, not just honey bees. The term 'honey bee' is used widely in both the popular and in serious academic writing referring to *Apis mellifera*, the industrially managed and introduced species. The economic activity of producing honey and bottling it for human use occurs nearly all over the world and the fact that beekeeping has been a part of the social and

scientific consciousness in Europe for several centuries now (Maeterlink, 1901) implies that *A. mellifera* is the most studied pollinating hymenopteran. In addition to *A. mellifera*, nine other different pollinating hymenopteran species, also popularly referred as 'honey bees' are recognized, all of them native to Asia, eight belonging to the genus *Apis* (Apidae), the ninth being the stingless bee *Tetragonula iridipennis* (Hymenoptera: Apidae) (Guerin, 2020). Three species of *Apis*, viz., *A. cerana indica, A. florea* F., and *A. dorsata* F., and *Tetragonula iridipennis* are the native species of honey bees common in southern India (Ramachandran, 1939; Xavier et al., 2014). Honey bee in this review refers to any and/ or all of this larger subset of corbiculate hymenopteran taxa.

Experimentation on introduction of the exotic *Apis mellifera* in several parts of India dates back to the 1880s (Ramachandran, 1939). In the1920s and1930s, large consignments of *A. mellifera* were imported into India, but these first efforts at scientific beekeeping proved unsuccessful. Similarly, the efforts made by private and government agencies in Uttar Pradesh, Punjab, Jammu & Kashmir met the same fate. Later, intensive efforts by the scientists of Punjab Agricultural University (PAU), Ludhiana in 1962 yielded results (Goyal, 1990; Rao and Rao, 1993). In southern India, although *A. mellifera* is still present and continues to be introduced by the government agencies (State Agriculture Departments, National Bank for Agriculture and Rural Development- NABARD), beekeeping with *A. cerana* is more widespread. Beekeeping with the stingless bee *T. iridipennis* is a traditional practice in many parts of Kerala. Wild or feral colonies of all four native species continue to be present in both urban and rural settings and in forests, but as landscapes transform, even these 'generalist' species are losing their habitats rapidly.

It is in a broad global and regional context that this review first briefly addresses the pollinators and their global decline and then turn the focus on honey bees and their role in agroecosystems. It also brings together relevant information from melissopalynology and discuss the gaps and directions of future research on conservation and management of honey bees in tropical peninsular India. The focus of this review is on *A. cerana indica* and *T. iridipennis* because of their current relevance and potential to transform existing agricultural landscapes into agroecosystems incidentally benefitting farmers and maintaining ecological balance. This review also highlights the specificity of southern India and contrasts this region with other places where the introduction of *A. mellifera* has been successful (Koetz, 2013). This review is written within the framework of ongoing studies in the Pondicherry region, that although subject to heavy degradation, shows considerable resilience of the forest patches to anthropogenic pressure (Navya et al., 2017) and hence prompted the ongoing pilot project and a PhD work on pollinators in this landscape and their impact the agrarian system.

Bees in agroecosystems

Pannure (2016) evaluated pollinator decline in India using the TNAU Agritech portal (Tamil Nadu Agricultural University). There is very little integrated information in the public domain on this topic. This portal, with an entire section on "bee flora and pollination of crops" is a ready reckoner that estimates yield increases as high as 112% in *Medicago sativa* (Leguminosae) fields, and 17-19% in *Gossypium* spp. (Malvaceae) due to pollination by bees. The number of colonies/ ha for pollination is given (3 to 9 colonies/ ha) but the portal is ambiguous about the bee species referred to, as common names (Italian bee and the Indian honey bee) or *indica* (*sic*) and *A. mellifera* (*sic*) are all provided. The total acreage of bee-dependent crops in India is c. 50 m ha, but bee colonies are insufficient with only 1.2 million out of the 150 million required, i.e. <1% of the requirement. This underlines the wide scope for expansion of beekeeping for pollination in India. Table 1 lists 41 crops benefited by bee pollination in India, as inferred by us from selected publications, emphasizing the pollinator(s) observed on these crops and clearly underscoring the importance of *A. cerana* and *T. iridipennis* in pollination services.

The agrarian community of India is highly dependent on the production of vegetables such as *Cucumis sativus* (Cucurbitaceae), *Solanum melongena*, *Lycopersicon esculentum*, *Capsicum annuum* (Solanaceae), and *Allium cepa* (Amaryllidaceae) (Table 1). Approximately 70% of the tropical vegetable crop species produced are dependent on the activity of Apidae, which optimize the yield of most of these commodities (Roubik, 1983; Klein et al., 2007). Species of the Apidae are considered the best pollinators due to their speed of work, longer working time, large populations, migratory behaviour and their floral fidelity. Indirect benefits through pollination of certain crops such as coffee with Apidae population have increased the fruit set up to 83% (Deodikar and Suryanarayana, 1973). Among the other native Apidae, the major forage plants of *A. florea* in many parts of tropical peninsular India (Sambasiva

Table 1. Key crops that benefit from bee pollination

No.	Family	Botanical name	Common name	Pollinator(s) observed
Cereals and pulses				
1	Poaceae	*Triticum aestivum* L.	Wheat	(a)*
		Oryza sativa L.	Rice	(b)*⁑
2	Leguminosae	*Vigna radiata* (L.) R . Wilczek	Mung Bean	(c)•
Oil seeds				
3	Asteraceae	*Helianthus annuus* L.	Sunflower	(a)*, (f)*
4	Pedaliaceae	*Sesamum indicum* L.	Gingelly	(b)*⁑; (d) ⁑; (g) •
5	Leguminosae	*Arachis hypogaea* L.	Groundnut	(c)•
Vegetable crops				
6	Amaranthaceae	*Amaranthus sp.*	Amaranthus	(d)
7	Amaryllidaceae	*Allium cepa* L.	Onion	(a)*
8	Leguminosae	*Cyamopsis dentata* (N.E.Br.) Torre	Cluster bean	(a)*
9	Cucurbitaceae	*Cucumis melo* L.	Muskmelon	(a)*
		C. sativus L.	Cucumber	(a)*
		Cucurbita pepo L.	Pumpkin	(a)*
		Benincasa hispida (Thunb.) Cogn.	Ash gourd	(d)⁑
		*Sechium edule (*Jacq.) Sw.	Chayote	(j) ⁑
		Trichosanthes cucumerina L.	Snake gourd	(d)⁑
10	Malvaceae	*Abelmoschus esculentus* (L.) Moench	Okra	(b)*⁑; (c)•
11	Solanaceae	*Solanum melongena* L.	Brinjal	(a)*
		Capsicum annuum L.	Chilli	(b)*⁑
		Lycopersicon esculentum Mill.	Tomato	(b)*⁑
12	Moringaceae	*Moringa oleifera* Lam.	Drumstick	(a)*
Fruit crops				
13	Anacardiaceae	*Mangifera indica* L.	Mango	(a)*
14	Lythraceae	*Punica granatum* L.	Pomegranate	(a)*
15	Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Watermelon	(a)*
16	Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle	lime	(a)*
		C. aurantium L.	Orange	(a)*
		C. limon (L.) Osbeck	Lemon	(b)*⁑; (h)⁑; (e)•; (g)•
17	Musaceae	*Musa* sp	Banana	(b)*⁑; (d)⁑
18	Myrtaceae	*Psidium guajava* L.	Guava	(b)*⁑
19	Rosaceae	*Malus pumila* Mill.	Apple	(i)•
20	Moraceae	*Artocarpus heterophyllus* Lam.	Jack Fruit	(b)*⁑
Flower crops				
21	Rosaceae	*Rosa damascene* Herrm.	Rose	(a)*
Plantation crops				
22	Arecaceae	*Cocos nucifera* L.	Coconut	(b)*⁑; (d) ⁑
		Borassus flabellifer L.	Palmyra palm	(c)•
23	Rubiaceae	*Coffea benghalensis* B. Heyne ex Schult.	Coffee	(b)*⁑; (d) ⁑
24	Myrtaceae	*Eucalyptus* spp.	Eucalyptus	(c)•; (d)⁑
Medicinal plants				
25	Meliaceae	*Azadirachta indica* A.Juss	Neem	(e)•; (d) ⁑ ; (k)*⁑•
26	Phyllanthaceae	*Phyllanthus emblica* L.	Indian Gooseberry	(e)•
27	Rutaceae	*Murraya koenigii* (L.) Spreng.	Curry tree	(e)•
28	Lamiaceae	*Ocimum tenuiflorum* L.	Thulsi	(e)•;(d) ⁑
Cash crops				
29	Anacardiaceae	*Anacardium ocidentale* L.	Cashew nut	(b)*⁑
30	Malvaceae	*Gossypium hirsutum* L.	Cotton	(d)⁑

Pollinator(s) (**Apis cerana*; ⁑ *Tetragonula iridipennis*; • Honey bees) observed and reported by: Bhalchandra et al. (2014) (a); Singh et al. (2016) (b); Chaudhary (2001) (c); Gowda et al. (2020) (d); TNAU Agri-Tech portal (2021) (e); Slaa et al. (2006) (f); Kumar et al., (2015) (g); Abrol (2012) (h); Dulta and Verma (1987) (i); Mukherjee et al., 2019 (j) and Sundararaju (2011) (k), help illustrate the importance of the focal bee species of this review, in pollinating a wide range of plants.

Rao et al., 2015; Vidhya et al., 2019) were trees such as *Pongamia* (Leguminosae)*, Azadirachta indica, Citrus* sp., and *Albizia* sp. (Leguminosae). *Anacardium occidentale* was visited for nectar collection alone (Sundararaju, 2011) by all three native *Apis* spp. (*A. florea*, *A. cerana indica* and *A. dorsata*) while the stingless bee (*T. iridipennis*) collected both nectar and pollen grains. In addition to the focal bee species (Table 1), Sundararaju (2011) found that four other bee species from coastal Tamil Nadu: *Ceratina* (*Pithitis*) *binghami* (Hymenoptera: Apidae), *C. smaragdula* (Hymenoptera: Apidae), *Braunsapis* sp (Hymenoptera: Apidae) and *Pseudapis oxybeloides* (Hymenoptera: Halictidae), also visited the flowers of this important cash crop (cashew) and were found carrying pollen grains on their legs and body hairs.

Apis cerana are desirable pollinators for a range of crops and specifically crops such as *Sesamum indicum* (Pedaliacae) and *Helianthus annuus* (Asteraceae). Managed populations of *A. cerana* maintained in hives start foraging early in the morning until late in the evening for long time durations proving their efficiency in pollination. *Apis cerana* are more effective in pollination only when the colony occurs close to a crop field. They spend more time foraging, with high flight time, to the floral source (Abrol, 2012). Numerous agricultural and medicinal plants are visited by *T. iridipennis* (Roopa et al., 2017), which is also an established pollinator of vegetable crops such as *Sechium edule* (Cucurbitaceae) and is considered a critical pollinator, influencing positively the fruit-to-flower ratio (Mukherjee et al., 2019) (Table 1). While conventionally the Apidae are always correlated with pollination services, a study in New Jersey, USA found that native bees alone provide sufficient pollination at > 90% of farms, since the total pollen deposit in flowers was strongly, significantly correlated with native bee visits and not with the introduced *Apis mellifera* visits (Winfree et al., 2007). At the time of this study, honey bees were undergoing extensive die-offs because of 'colony collapse disorder' (Stokstad, 2007 a, b) and in New Jersey native bees had the potential to buffer the drop in agricultural productivity.

In contrast, in a region such as India with a native population of diverse Apidae, in Aurangabad district, where *A. mellifera* is an introduced species, only a few *A. cerana* were observed, while *A. florea* and *A. dorsata* were more abundant (Waykar and Baviskar, 2015). Given the close proximity in size of *A. cerana* and *A. mellifera*, it seems that the former faces the brunt of competition from the introduction of the latter. Interactions between the Apidae and flowering plants are excellent examples to understand co-evolution and mutualism. There is a need to understand bee –plant relationships to study food preferences of the Apidae and pollination (Shubharani et al., 2013). Bees are also site-specific and repeat their foraging paths (Janzen, 1971) because of which pollination by Apidae were considered to be the most important component of increasing the crop yields in agriculture.

Apis cerana

Smith and Hagen (1996) studied the distribution of the cavity-nesting *A. cerana* that extends from Iran to China and from Japan to southern Indonesia. In this extensive distribution, *A. cerana* occupies a wide range of habitats, such as rock crevices, tree hollows, gulleys. It has also been introduced into Australia, although not to the same extent as *A. mellifera* has been, where it is considered alien and invasive (Clarke et al., 2021). Unravelling the genetic diversity of *A. cerana*, an indigenous strain, is essential since it would unveil some useful elements on breeding strategies to honey bee farmers. Molecular genoming of colonies of *A. cerana* from 10 localities of Nilgiri Biosphere reserve revealed the taxonomy of two subspecies of *A. cerana: A. cerana cerana* (the black strain) and *A. cerana indica* (the yellow strain). The results suggested that mobile beekeeping has resulted in genetic recombination of different strains of the same species (Chalapathy et al., 2014).

Among the Asian honey bee taxa, *A. cerana* is a medium-sized honey bee (11.46± 0.091 to 12.74± 0.070 mm (Baskaran and Thiyagesan, 2013), smaller than the giant Asian honey bees *A. dorsata* and the Himalayan honey bee *A. laboriosa* Smith (Hymenoptera: Apidae)), larger than the dwarf Asian honey bees (*A. florea*). *Apis cerana indica* is the only *Apis* that has been domesticated in India (Ramachandran, 1939). In China and India, while almost all *A. mellifera* are managed and all *A. dorsata* run wild, it is difficult to distinguish between wild and managed *A. cerana* with any degree of certainty (Senapathi et al., 2021). Both *A. cerana* and *A. mellifera* look similar to an unaided eye. But, *A. cerana* is striped in the abdomen with evenly spaced black bands spreading left to right on the abdomen. However, colouration is highly variable in nature, and the most reliable morphological characteristic that distinguishes *A. cerana* is the extension of the radial vein on the hind wing, which is prominently absent in *A. mellifera* (Ruttner, 1988). Both *A. cerana* and *A.*

mellifera have pollen baskets on their hindlegs which allow the bees to transport pollen from flower to the hive which is composed of honey combs for storing nectar and pollen.

Foraging range of *A. cerana* varies in different locations, but generally it will occur within a maximum of 1500-2000 m (Dhaliwali and Sharma, 1974). It has a very wide range pollination of crop plants (Table 1), likely due to its long glossa which allows access to a wide range of plants (Deodikar, 1960). On a single foraging trip, *A. cerana* tend to collect either pollen or nectar (not both); on any given day, during daylight hours, they tend to forage on the same species (Corlett, 2011). Foraging time often depends on weather parameters, available floral resources and competition with other honey bee species. Some studies suggest that the introduction of honey bees (*Apis* spp.) may also have negative impacts, such as competition with the native pollinators for available floral resources, competition for nesting sites, co-introduction of nesting enemies, especially pathogens that infect the native organisms, disruption of pollination services of native plants and pollinating exotic weeds. Thus the ecological impacts of cavity occupancy by introduced honey bees (*Apis* spp.) gone feral is a subject that needs a complete review by itself (Goulson, 2003; Saunders et al., 2021).

Tetragonula iridipennis

Stingless bees (Apidae: Meliponinae) consist of two genera- *Melipona* and *Trigona*. Meliponinae includes eight genera, having 15 subgenera and 500 species (Grüter, 2020; Slaa et al., 2006), and *Melipona* includes 40 species, all of which remain restricted to the Neotropics (Camargo et al., 1988), amongst which *Melipona iridipennis* Smith is the most common species found in India. This species was subsequently renamed, initially as *Trigona iridipennis* and finally *Tetragonula iridipennis*. It was first described from Ceylon (presently Sri Lanka) by Smith (1954). Later, the same species was reported from India (Sakagami, 1978; Swaminathan, 2000; Danaraddi et al., 2009). These bees are commonly known as dammar bees in India (Rasmussen, 2013). Apart from *T. iridipennis*, seven other stingless bee species were also reported from India with their abundance in southern part of India (Rasmussen, 2013). This species is characterized by wings with brilliant iridescence (Swaminathan, 2000). Its body length is 3.5-4.0 mm, with stingers much reduced. This stingless, small honey bee species uses its mandibles as the organ of defence to protect their nests from the intruders.

Tetragonula iridipennis has the ability to visit a wide range of flowers and plant species, especially of the understorey herbs (Table 1). It uses a field-based communication mechanism to a specific food location. Field-based communication mechanisms include scent trailing, food-site marking, and potentially aerial odour trails (Grüter, 2020). The members live in permanent eusocial colonies usually of thousands of female workers, a reproductive queen, and male drones. The nesting sites of this bee are tree trunks and wall cavities (Danaraddi et al., 2009). Majority of the nests occur in cavities of live trees (Roubik, 1983). The combs of *T. iridipennis* are made of a dark 'cerumen', a mixture of wax and resin which is the material used to build brood, honey and pollen pots. They are efficient pollinators because of their small size and their ability to forage the flowers of different sizes and shapes as listed in Table 1. Earlier studies on stingless bees were conducted on biology, nesting behaviour, morphometric characters, foraging behaviour and melissopalynology (Batista et al., 2003; Roubik, 2006). Vijayakumar and Jeyaraj (2016) have studied its distribution in southern India. However exclusive studies on species diversity of *Tetragonula* in different parts of India are lacking.

Melissopalynology

Studies conducted in Melissopalynology in India have generally been focused on *A. cerana* (Fatima and Ramanujam, 1989; Garg and Nair, 1993; Jhansi et al., 1994; Chakraborti and Bhattacharya, 2015) although there has been considerable interest in *T. iridipennis* too recently (Ponnuchamy, 2014; Vijayakumar and Jeyaraaj, 2016; Bisui et al., 2019). Pollen data, be it from honey or from bee-loads is quantifiable and such an approach can contribute better to understanding the true impact of pollinators especially in agroecosystems, as also the surrounding vegetated lands, for example addressing questions such as specific crops that are visited, in connection with determining the possibility of pollen transport and potential cross pollination with related wild plants in the surrounding environment.

Pollen from honey samples collected by *A. cerana* from some districts of Andhra Pradesh in the southern part of India, showed that the honeys originate from two discrete floristic regimes: deciduous forests (Visakhapatnam and East Godavari) and agricultural tracts (Guntur) (Jhansi et al., 1994). The East Godavari district honey was unifloral and the remaining samples were multifloral which in turn shows that *A. cerana* adapts to different floral regimes. Similar studies were

carried out in Dharmapuri, Tamil Nadu (Agashe and Mary Scinthia, 1995) and in Hyderabad, Telangana (Fatima and Ramanujam, 1989). Another study in the Pondicherry region focussing on understanding the foraging behavior of *A. cerana* using classical melissopalynology (pollen from honey) over a three-year period used a statistical approach to show that pollen spectra were equally comparable between locations and also between months and years. This study demonstrated the complexity of ecological/ environmental phenomena involved in the process of foraging by bees in a heterogeneous and complex landscape (Ponnuchamy et al., 2014). Foraging preferences of *A. cerana* in Madurai district, Tamil Nadu during summer in relation to seasonal and diurnal variations and flower availability revealed that specific groups of worker bees responded differently to the weather parameters. Among all the factors examined, wind velocity bore a negative impact on the foraging activity of bees (Hemalatha et al., 2018)

In Bankura District of West Bengal, *T. iridipennis* foraged on diverse angiosperm flora supporting the broad polylectic foraging of the bee species, and most of the visited plants were trees with flowers coloured yellow, white, and cream; these bees were considered small-sized by Layek and Karmakar (2018). Pollen loads of *T. iridipennis* revealed that the bee preferred trees with small sized flowers as a source of pollen and also that pollen diversity was higher in rural areas in comparison with urban areas (Bisui et al., 2019). Pollen loads from *T. iridipennis* hives nesting in wall crevices (Hyderabad) revealed that a major part of the pollen came from *Prosopis juliflora* followed by *Peltophorum pterocarpum* (Leguminosae) and *Cocos nucifer*a (Ramanujam et al., 1993).

CONCLUSIONS

Most studies present in a thorough manner visual observations of pollinators (bees) on plants but rarely combine them with quantifying the resources gathered from the plants, especially pollen. This combined approach is especially important to understand the honey bees from the purview of the pollination service they provide. Pollen collection by *T. iridipennis* can also be considered antagonistic for the plant and partly beneficial for both the bee and the plant, especially on plants which produce flowers with small amount of pollen as most of the pollen meant for pollination is removed (Sambasiva Rao et al., 2015). Plants can mitigate the fitness costs associated with consumption of their pollen by honey bees, by optimizing pollen release rates. Especially in buzz-pollinated plants, bees apply vibrations to remove pollen from anthers with small pores. These poricidal anthers potentially function as mechanism staggering pollen release (Kemp and Vallejo-Martin, 2021). Wild bees such as the carpenter bee (*Xylocopa* spp (Hymenoptera: Apidae)), blue banded bees (*Amegilla cingulata* (Hymenoptera: Apidae)) and other solitary bees also play a major role in pollination of tropical dry forests. Carpenter bees are more effective pollinators in crops like Solanaceae (tomatoes (*Solanum lycopersicum)* and brinjal (*Solanum melongena*)), that require buzz pollination as do Legumes (eg., *Cassia*). Usually, carpenter bees nest in tunnels bored into wood or hollow stems (bamboo) (Kevan et al., 1990). We found *T. iridipennis* nesting on *Mangifera indica* (Anacardiaceae) and *Ficus benghalensis* (Moraceae) trees in and around Pondicherry. They also inhabit the urban or built environment using niches like doors, windows, walls of the old buildings and also electric switch boxes.

A study on *Abelmoschus esculentus* (Malvaceae) crop ecosystem was conducted in Bangalore (Nandhini et al., 2018). Due to golden yellow flowers and nectaries, this plant is freely visited by honey bees, bumble bees, ants, butterflies and other insects during flowering period. The study revealed that *Apis* spp were the most dominant flower visitors (41.75%) followed by *Lasius niger* (Hymenoptera: Formicidae) (34.23%) and *Solenopsis invicta* (Hymenoptera: Formicidae) (24.02%). This is in agreement with the preliminary pollinator surveys conducted by us in the Pondicherry region on *Abelmoschus esculentus* crop ecosystem. The decline in wild bee faunal diversity has been strongly correlated to decline in wild flowers (Biesmeijer et al., 2006). When a patch of naturally occurring weeds within a managed farmland was removed, simultaneously that created a decrease in yield in the main crop sown within the same cropping season. Conversely, an increase in non-crop flowering species improves pollinator diversity. Field margins or bunds sown with a range of different annual and perennial flowering species result in variable improvements in the diversity and abundance of bees and other pollinators, depending on plant composition, seasonal flowering patterns and bee forage preferences (Carvell et al., 2007). There is also a long-standing theory that co-flowering plants may facilitate pollination rather than competing for it (Sletvold et al., 2016).

In the tropical peninsular Indian scenario, many

of the under-utilized common, mostly herbaceous, weedy plants that are edible, pan-tropical and flowering almost throughout the year, are important for both the pollinators to thrive and for the farmers in the agroecosystem (Sengupta, 2015). The persistence dynamics of most weed species in arable land are largely driven by insect pollination; the frequency of flower visited by insects is a good parameter for classifying a weed's pollination strategy (Loose et al., 2005; Lunau et al., 2006; Mahale, 2019). Pollinator declines can result in loss of pollination services which have important negative ecological and economic impacts that could significantly affect the maintenance of wild plant diversity, wider ecosystem stability, crop production, food security (Abrol, 2011). Annual mass flowering crops temporarily change the floral resource availability which in turn modifies the pollinator preferences and their stability in weed pollinator networks. (Schleuning et al., 2012; Mahale, 2019). Thomas et al. (2009) in the Nilgiri Biosphere Reserve, studied the diversity of social bees at 15 sites along with floristic analyses of local vegetation at each site. A Bee Importance Index (BII) was developed to obtain a measure of the bee diversity at each site. Seventy three plant species were identified as social bee plants and of them 45% were visited by one species of bee (*Apis cerana*), 37% by two bee species (*Apis cerana, Trigona* sp.) and 18% by more than two bee species (*Apis cerana, A. florea, A. dorsata, Trigona* sp.) indicating a certain degree of floral specialization among bees. Much value can be added to such studies by linking them with the analyses of the key resource gathered from the plants, pollen.

Along similar lines, Melissopalynology studies are found in various geographies of India and in different part of the world, but their main focus has been on characterizing the geographic origins as also some physiochemical parameters of honey, all targeting the honey market (Chakraborti and Bhattacharya, 2015). However, as a robust, quantifiable tool, palynology can be used much more effectively in understanding honey bee-plant relationship and can help demonstrate the complexity of ecological and environmental phenomena involved in the process of foraging by bees in heterogeneous and complex landscapes (Ponnuchamy et al., 2014). Given the direction of landcover and land use changes the world over, there is scope for further research from this angle too. Such a synthetic approach has been attempted in our ongoing studies, mentioned at the end of the Introduction. We analyse pollen loads as well as honeycombs: a) corbicular loads and comb samples from the hived bee *A. cerana* and b) pollen collection from the pollen pots in the *T. iridipennis* hives. Plant flowering phenology and plant-pollinator surveys are conducted in parallel to pollen analyses. In the laboratory, the bee pollen loads were examined directly under the microscope and after chemical processing using acetolysis (Jones, 2014).

A comparison of observations from the ongoing studies and some published literature provided a set of 34 pollen/ plant taxa that are important for bee forage, especially the focal species of the review (Table 2). In the Pondicherry region nearly 22 have been stable for well over a decade and regionally, some taxa, over three decades (Ponnuchamy et al., 2014). Taxa such as *Antigonon leptopus* (Polygonaceae) and *Evolvulus alsinoides* (Convolvulaceae), though frequent in the field observations were not widely reported as bee or pollinator friendly; the former is an important nectar source and the latter preferred for the nutritive value of its pollen like other herbaceous plant species like *Euphorbia cyathophora* (Euphorbiaceae) and *Mimosa pudica* (Leguminosae) (Table 2). The list consists of a mix of the native TDEF vegetation and local crops and plantations. One extra regional study at sub temperate Bhimtal in Himachal highlighted common floral resources such as Asteraceae, Lamiaceae and Poaceae. In India, 41 key crops that depend on bees for pollination services have been listed from the literature already (Table 1). These results, particularly the overlap in the bee visited flora and the crops, further validate the win-win situation for agroecosystems that choose to diversify the available floral resources for the focal bee species of this review (*A. cerana* and *T. iridipennis)*.

Observations of the authors on the field of *T. iridipennis* and *A. cerana* visiting plants such as *Cocos nucifera, Sesamum indicum, Talinum portulacifolium* (Talinaceae) were validated by the analyses of the pollen loads they carried. From the diversity of sites in and around Pondicherry, it is clear that several bees are generalist visitors to a range of available plants, though some loyalties to the plants that flower regularly throughout the year or mast flower seasonally are observed. *Cocos nucifera* is a steady source that flowers every month regularly and is available all through the year to the bees (Jay, 1974) and this is in agreement with our findings in the ongoing studies; in contrast, the focal bee species also utilize resources like *Syzygium cumini* (Myrtaceae) and *Peltophorum pterocarpum*, which mast flower (Table 2). At one of the study sites around Pondicherry, a farmer shared his observations of a marked decrease in fruit drop in his coconut trees

Table 2. Flora visited by the focal bee species- field and microscopic observations, ongoing study around Pondicherry and also those reported elsewhere

No.	Plant species/ pollen taxa	Where and when observed in the literature	
		Place	Year(s) of observation
1	*Lannea coromandelica* (Houtt.) Merr.*	Pondicherry	2007-2009
2	Phoenix*	Pondicherry	2007-2009
3	Casuarina**	Pondicherry	2007-2009
4	Commelina*	Pondicherry	2007-2009
5	*Fluggea leucopyrus* Willd.*	Pondicherry	2007-2009
6	*Leucaena leucocephala* (Lam.) de Wit.*	Pondicherry	2007-2009
7	*Peltophorum pterocarpum* DC.Backer ex K. Heyne.**	Pondicherry	2007-2009
8	Liliaceae*	Pondicherry	2007-2009
9	Boerhavia*	Pondicherry	2007-2009
10	*Sesamum indicum* L.**	Pondicherry	2007-2009
11	Ixora *	Pondicherry	2007-2009
12	*Dodonaea viscosa* (L.) Jacq.*	Pondicherry	2007-2009
13	*Tribulus terrestris* L.*	Pondicherry	2007-2009
14	*Borassus flabellifer* L.*	Pondicherrry, Andhra Pradesh	2007-2009, 1982
15	Phyllanthus*	Pondicherry, Andhra Pradesh	2007-2009, 1982
16	*Cocos nucifera* L.**	Pondicherrry, Karnataka, Andhra Pradesh	2007-2009, 2010-2012, 1982
17	*Syzygium cumini* (L.) Skeels**	Pondicherrry, Karnataka, Andhra Pradesh	2007-2009, 2010-2012, 1982
18	*Mimosa pudica* L.*	Pondicherry, Karnataka	2007-2009, 2010-2012
19	Eucalyptus**	Pondicherry, Karnataka	2007-2009, 2010-2012
20	Asteraceae***	Pondicherry, Bhimtal, Karnataka, Madurai	2007-2009, 1994, 2010-2012, 2016-2017
21	Lamiaceae***	Pondicherry, Bhimtal, Karnataka, Madurai	2007-2009, 1994, 2010-2012, 2016-2017
22	Poaceae***	Pondicherry, Bhimtal	2007-2009, 1994
23	*Psidium guajava* L.**	Karnataka	2010-2012
24	Euphorbia*	Karnataka, Madurai	2010-2012, 2016-2017
25	Coffea**	Karnataka, Pondicherry	2010-2012
26	*Canthium coromandelicum* (Burm.f.) Alston*	Pondicherry	2003-2006
27	*Memecylon umbellatum* Burm.f.*	Pondicherry	2003-2007
28	*Solanum melongena* L.**	Coimbatore	2011-2013
29	*Solanum lycopersicum* L.**	Coimbatore	2011-2013
30	*Capsicum annuum* L.**	Coimbatore	2011-2013
31	*Abelmoschus esculentus* (L.) Moench**	Coimbatore	2011-2013
32	*Momordica charantia* L.**	Coimbatore	2011-2013
33	*Trichosanthes cucumerina* L.**	Coimbatore	2011-2013
34	*Anacardium occidentale* L.**	Karnataka	2003-2008

TDEF vegetation (*); local crops and plantations (**); extra regional commonalities (***); first remain stable well over a decade in Pondicherry; the duration of observations varies between 1-5 years with most of them 2-3 years only highlighting the lack of long-term observations.

Out of 34 taxa observed in the ongoing studies the first 22 had been reported by Ponnuchamy et al (2014) from the same study area; 14-17 were recorded elsewhere earlier by Jhansi et al (1994); 16-21 & 23-25 by Subharani et al. (2013); 20-22 also by Garg (1996); 20-21 also by Hemalatha (2018) who recorded 24 as well. Nayak and Davidar (2010) report 26-27, two species that are characteristic of the Coromandel Coast while Davidar et al. (2010) report 28 to 33 from Coimbatore and Sundararaju (2011) 34 from Karnataka; 25 was reported in a review work by Deodikar and Suryanarayanan (1973)

within a few months of introducing both *A. cerana* and *T. iridipennis*. In the same site, where abundant pollinators' populations were observed, it was found that hedges and fences were planted with crop species like *Sesamum indicum* and weed plants like *Spermacoce hispida* (Rubiaceae), *Sida acuta* (Malvaceae), *Croton bonplandianus* (Euphorbiaceae) and *Tribulus terrestris* (Zygophyllaceae) were commonly reported.

Weeds have considerable value in supporting the lives of flower visiting insects, especially pollinators though it has been considered by some that weeds are predominantly self-pollinating (Sutherland et al., 2004). It has been observed in the ongoing studies that many weeds including the ones cited in Sengupta (2015), that occur in the vicinity of the crop lands and forested vegetation, do attract pollinators; these include common weedy plants such as *Tridax procumbens* (Asteraceae), *Asystasia gangetica* (Acanthaceae), *Tribulus terrestris* visited by many insect pollinators including the focal bees species of this review (Table 2). Therefore, it would be worthwhile to identify such plants in the surroundings of the landscapes to "trap" the pollinators inside in order to effectively reap the pollination services rendered by them. In urban settlements, road side plants, often considered weeds, attract pollinators like honey bees. Road side ornamental plants like *Turnera subulata* (Passifloracear), *Tecoma stans* (Bignoniaceae), that are preferred by *A. cerana* and *T. iridipennis* can be planted and managed to enhance the populations of these bees.

In India, managed *Apis cerana* is a better pollinator than *A. mellifera* because of its longer foraging period (Sihag and Mishra, 1995). In Himachal Pradesh, Gupta et al., (1984) observed in *Plectranthus rugosus* (Lamiaceae) flowers that *A. cerana* foraged longer hours, starting early in the morning, than *A. mellifera,* though the latter was more efficient in gathering nectar. But it was found out in Fuzhou, China (Zhang et al., 2019) that *A. cerana* workers make more foraging trips, live longer and consume less sugar, partly due to smaller size, suggesting that *A. cerana* performs better than *A. mellifera* at the community level. This work also indicated that *A. cerana* is better adapted to scattered nectar sources than *A. mellifera*, which will starve at the same location. A study conducted in West Bengal (Bhattacharya et al., 2017) inferred that the age-old tradition of associating bees with honey and *Apis* seems to have strongly influenced the bee knowledge of the farming families whose knowledge on the other native bee species was limited. In India, the knowledge of the right pollinators for most crops is not available. Little is known about the status of bee pollinators in the wild and their population dynamics, life history, habitat requirements, pollinator interactions with other elements of crop and crop associated biodiversity, the ecology of pollinators, or the ultimate consequences of their decline (Pannure, 2016).

The notion of scientific beekeeping in India goes back to at least the beginning of the 20th century (Ramachandran, 1939) and there has been a fascination with the idea that this can serve the farmers as an alternate source of easy income as honey is an important commercial product. However, since the floral sources in tropical peninsular India are generally available round the year, unlike in Europe, there is no necessity for the bees to store excess quantities of honey in their hives, and moreover, *A. cerana* doesn't store as much honey as does *A. mellifera* (Abrol, 2012). Our results highlight the role of the focal (native) honey bees (*A. cerana* and *T. iridipennis*) in pollination and suggest beekeeping with these species; the protection of their floral sources will sustain them in the ecosystem and consequently serve the agroecosystem. To accommodate the various pollinators, weeds, wild herbaceous plants, shrubs and trees, must be available which will allow the wild and managed pollinators to thrive well and adapt. This review senses a tangible gap in reports and long-term studies of many native pollinators and in particular the two hived Asiatic honey bees in South India. This review would like to put on record a pressing requirement for long-term observations comprising visual observations with quantifiable pollen and vegetation data to arrive at meaningful plant-pollinator networks.

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MOBILITY AND DEGRADATION OF PESTICIDES IN SOIL- RISK TO GROUNDWATER CONTAMINATION

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ABSTRACT

Pesticides are used in modern agriculture to prevent crop losses by different pests and allow sustentation of the current crop yields. The pesticides after their use enter the environment viz., soil, water and air, then undergo various interactions and complex transformations resulting in the formation of multiple metabolites. The fate of pesticides in the environment depends on the properties of pesticide, soil and some other miscellaneous factors. Pesticides and their metabolites may move downward and reach groundwater under the effect of gravity, percolation, agricultural runoff or backsiphoning, hence there is risk to groundwater contamination.Chemicals have been noticed in groundwater, drinking water and surface water supplies. Therefore, research on ill-effects of the pesticide use and their fate in the environment is of great concern. The indigenous micro-flora is successful in biodegradation and removal of xenobiotics. So, in order to ensure public safety as well as the environmental protection from the chemical use, the pesticide use must be judicious and biodegradation of such chemicals to reduce their residues in agricultural products needs to be escalated.

Key words: Pesticides, residues, mobility, degradation, groundwater contamination, agrochemicals, environment, biodegradation, bioremediation, microorganisms, enzymes

Total food grain production on global basis has shown a leap from 500 to 700 million tons since the end of 20th century till now (FAO, 2018). International food demand is estimated to rise from 70 to 100% in the coming decades to cope with the rising human population (Foong et al., 2020). India produces about 250 million tons of grain per annum, out of which about 11-15% is lost annually due to pests and other causes (Walter et al., 2016). To avoid/minimize losses, pesticides are being used that deter, discourage, incapacitate or kill certain pest populations viz., insects, fungi, rodents, weeds etc., and are indispensable to modern agricultural production and important for ensuring national food security. Pesticide production worldwide has increased from 0.2 million tons in 1950s to more than 5 million tons by 2000 (11% increment per year). According to an estimate, 5.2 billion pounds of pesticides are used worldwide every year. In India, total pesticide consumption has shown a variable trend from 1980s till 2017, as presented in Fig. 1 (Indiastat, 2019). Under field conditions, only 0.1% of pesticide sprayed reaches the target pests, and the rest 99.9% goes into the environment, thereby adversely affecting and contaminating the ecosystem and its components like air, water and soil in major (Pimentel, 1995). However, according to Parte et al. (2017b), only 10% of pesticide sprayed reaches the target organism. The rest of it is deposited on/in non-target areas, resulting in the pollution of soil, water, air etc. Furthermore, the metabolites formed out of pesticide degradation persist in the environment for a longer period e.g. organochlorines (OC). Szeto and Price (1991) recovered the residues of OC from aquatic bodies at the detection limit even after 20 years of ban. Some metabolites are even more toxic than the parent compounds (Rasool et al., 2021, 2022). Among the OC's, dichlorodiphenyltrichloroethane (DDT) being most persistent has entered into the various food chains operating in an ecosystem and

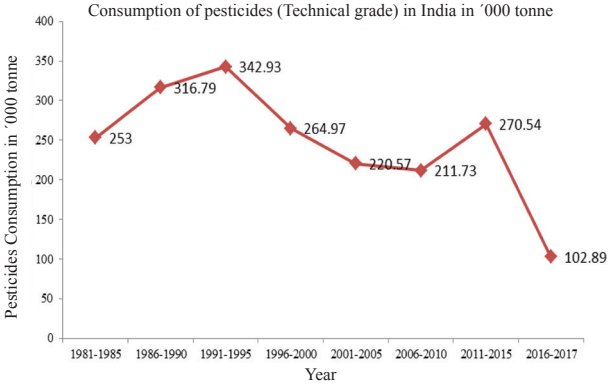


Fig. 1. Trend of pesticide (technical grade) consumption in India

underwent biomagnification due to its indiscriminate use. The use of pesticides above the recommended dose is perilous to the environment, deteriorates the fertility of the soil and also has adverse effects on all living organisms (Parte et al., 2017b). Also, there have been concerns about the pesticide in context to the occupational hazards, residues in food and water above the permissible limit even though these are manufactured under strict regulation to function with certainty and minimal effect on non-target organisms (Damalas and Eleftherohorinos, 2011). There are reports of exposure of non-targent organisms like pollinators to pesticides resulting in economic and ecological losses (Brar et al., 2022). So, it becomes important to have vast knowledge about the nature of pesticide to be applied, their movement in soil and various tactics to prevent their risk to non- target organisms. In this review, information on pesticide interactions, their mobility, degradation and risk of contamination are discussed.

A. Pesticide residues

The pesticides may enter into the given environment through several ways namely; soil, water and air (Fig. 2). Once the soil application of pesticides is done, it enters the various soil medium components after dissolving rapidly (Usman, 2018). When applied to crops, the foliar pesticides also enter the soil through various routes, mainly the drift losses and wash off due to rains, thereby polluting the water bodies. The active ingredient of soil-applied pesticide granules reaches directly into the soil environment through leaching. The movement of the pesticide in soil is further affected by soil structure, irrigation, pesticide formulation, soil moisture/water content, pesticide application time and rainfall (Flury, 1995). The pesticides contaminate the soil and accumulate in the crops sown. Various forms of pesticide residues i.e. free and bound, are formed as a result of pesticide degradation. The free residues

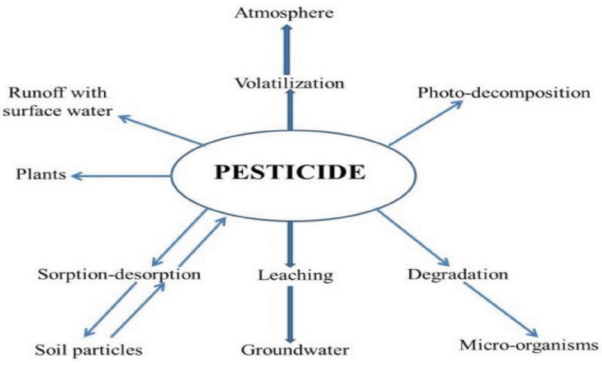


Fig. 2. A flow chart representing the mobilization of pesticide in the environment

are readily extractable from the soil without change in chemical structures. However, bound residues are resistant ones for extraction. The bound residues are defined as "chemical species originating from pesticides, using good agricultural practices and are non-extractable by methods which do not significantly change the chemical nature of these residues" (Roberts, 1984). Because of their non-extractability, these have environmental significance (Khan, 1982). The bound residues are further of two types: available and non-available. The distinction has been made by Khan (1982) as: the available fraction of bound residue is easily available to plants and soil microfauna, while the non-available fraction cannot be tapped by these organisms. Thus, the study of pesticide dynamics i.e. whether the pesticide has degraded, absorbed, adsorbed or transformed, has become important (Lopez-Blanco et al., 2005). According to U.S. Environmental Protection Agency (USEPA), water has more contaminants from organochlorines and organophosphate groups, and their concentration is higher at surface level of water than in groundwater, and humans are at maximum risk of getting exposed to pesticides by means of water contaminated with these toxic compounds (Younes and Galal-Gorchev, 2000).

B. Soil-pesticide interactions

Adsorption is the important phenomenon with which the pesticide interacts with the soil particles. The extent of adsorption is controlled by various factors involving the properties of soil namely; texture, structure and pesticide too i.e. its chemical functions, molecular structure, acid-basic nature, solubility, polarity (Pignatello and Xing, 1996). Adsorption may be physical or may be due to weak interactions like electrostatic or van der Waals forces. The pesticide persistence in soil depends on the stable linkages between unaltered pesticides or their metabolites (Dec and Bollag, 1997). The compounds and their metabolites which are adsorbed onto the soil (through cation exchange or ionic bonding) exist as cations or get protonated to become cationic. The ionic bonding is due to easily ionisable or ionized humic substance containing carboxylic and phenolic hydroxyl groups e.g., the paraquat and diquat (bipyridilium pesticides) form stable bonds with the carboxyl groups through ion exchange. On the other hand, binding of amitrole and triazine herbicide which are less basic in nature are affected pH (Weber et al., 1969). From toxicological point of view, the xenobiotics binded to stable soil compound i.e. humus leads to: (a) decrease of availability of material to the biota

(b) reduced compound toxicity (c) immobilisation of the compound thus reducing transport and leaching properties (Dec and Bollag, 1997).

C. Factors affecting fate/ movement

Adsorption-desorption phenomena strongly affect pesticide behaviour in soil. For accurate prediction of mobility and bioavailability of the chemicals, it is important to know the processes influencing the soil solution composition, so as to reduce or limit their impact on the ecosystem and non-target organisms. Pesticide leaching phenomenon and hence the risk to groundwater contamination depends on factors which are broadly classified into three main categories as pesticide properties, properties of soils and miscellaneous factors (Fig. 3).

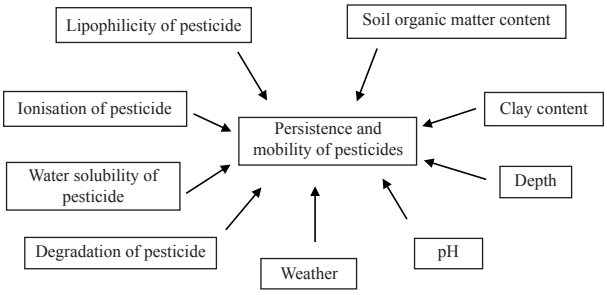


Fig. 3. Environmental factors affecting the pesticide degradation

i. *Pesticide properties*

Water solubility: It pertains to the pesticide amount that will dissolve in a known amount of water, and its units of measurement are milligrams per liter of water (mg L^{-1}) or parts per million (ppm). The water solubility of pesticides affects its potential to leach down the soil as the seeping water carries down the water-soluble chemicals. Therefore, the pesticides having high hydrophilicity are more soluble and likely to be carried away with runoff water and contaminate the groundwater. On the other hand, the fate of poorly soluble pesticides not incorporated into soil is decided by the runoff water or erosion (Geyikci, 2011).

Volatility: The pesticides which remain exposed to atmosphere are likely to be lost in the form of gas and the process is known as volatilization. More the tendency of a pesticide to become a gas, more are the chances of escaping into the environment. Loss of pesticides through volatilization is insignificant for most pesticides compared to leaching or surface losses. Various environmental factors such as temperature, humidity and aerial drift affect the volatilization of pesticides. A highly volatile pesticide has the least water solubility, thus has more possibility for escaping into the atmosphere, and a very low amount is leached to groundwater.

Volatility may account for a significant pesticide dissipation pathway depending upon the environmental conditions, leading to a loss of up to 90% of the applied pesticide. Such losses have been reported in the case of trifluralin, which dissipated to 90% within a week of application by means of volatilization. Further, the gaseous form of pesticides in the air is a potential water contaminant that may get drifted from air. To reduce the volatilization losses, surfactants or carriers can be put into use (Geyikci, 2011).

Adsorption: Literally, adsorption is a phenomenon of binding of a substance onto a solid surface. Here, in the context of the review, it refers to the pesticide binding to soil particles. The amount of pesticide adsorbed depends upon the soil properties such as texture, structure, pH, and the type of pesticide applied. Soils with high clay content or high organic matter adsorb pesticides strongly. This strong bond formation further affects the availability of pesticides to be taken up by plants, to get leached and volatilized (Tiryaki and Aysal, 1999). But this strong bond cannot resist the runoff water, and the soil particles and pesticides adhered are easily eroded away. In contrast, loosely bound pesticides with high water solubility will get easily leached to varying degrees. A term called adsorption partition coefficient (Kd) has been proposed for a pesticide's potential to get adsorbed onto soil particles. The partition coefficient and leaching potential share a reciprocal relationship as the lower the value of partition coefficient, greater is the pesticide leaching potential (Geyikci, 2011).

Degradation: By the process of photodecomposition, microbial degradation and various other chemical and physical processes, the pesticide is broken down into different chemical forms. Pesticide degradation in soil, i.e. post-volatilization and photo-degradation, is considered an important process in determining the fate of pesticide (Hester and Harrison, 2017). The rate of pesticide degradation defines its persistence in the soil, which in turn affects its leaching potential. The advance prediction of both rates and routes of pesticide degradation is difficult. Degradation products are more polar than parent pesticide but less biologically active Hence, they are more prone to leaching. In contrast to this statement, the biological oxidation products

of aldicarb retain their biological activity (Nicholls, 1987). Sharma and Singh (2013), while studying the metabolism of imidacloprid in soil, reported the formation of nitrosamine and 6-chloronicotinic acid. Other metabolites of imidacloprid in soil like imidacloprid-nitrogunidine, olefin, urea have also been reported, out of which olefin and nitrosimine are having more insecticidal activity than parent compound, and guanidine is having more mammalian toxicity (Sahoo and Singh, 2016). Some neonicotinoid pesticides along with their fate in soil are shown in Fig. 4.

ii. *Properties of soils*

Organic matter: The soil organic matter is directly correlated to the chemical adsorption. With increase in soil organic matter, adsorption also increases, thus, very less amount is available for degradation in the soil, and it is usually interrupted by a continuously increasing microbial population that results in increased pesticide degradation (Nicholls, 1987).

Clay content: Clayey soil has more than 40 per cent of clay particles and has large internal reactive surface area, thus facilitating more pesticide adsorption. Data generated from research has shown a direct correlation between the clay content in the soil and their binding ability to retain pesticides (Hermanson and Forbes, 1966). This correlation is a bit complex, because clay is in the form of colloids in the organic matter. Following are the factors influencing pesticide adsorption by soil colloids (Bailey and White, 1970): i) physicochemical configuration of the colloids ii) pesticide physicochemical configuration iii) pesticide dissociation constant iv) pesticide water-solubility v) size of the pesticide molecule vi) hydrogen ion concentration of soil vii) temperature conditions viii) the moisture content of the soil ix) electrical potential of clay surface and x) pesticide formulation.

pH: If the degradation reactions in soil are acid or base catalysed, then pH definitely influences the rate of degradation. The negatively charged soil surface results in a higher concentration of hydrogen-ion near the soil surface than the bulk solution. Further, if the pH reduces, the negative charge on the soil surface decreases and is more capable of adsorbing the anions. For example, sulphonylureas persist in alkaline chalky subsoils as these get degraded by acid-catalysed hydrolysis reactions. However, oxime-carbamates persist in acid subsoils since these get degraded by base-catalysed reactions (Van Raij and Peech, 1972).

Depth: With depth, microbial activity and organic matter in soil decreases, possibly exposes clay but resulting in weaker adsorption. Due to a decrease in microbial population and organic matter in the soil,

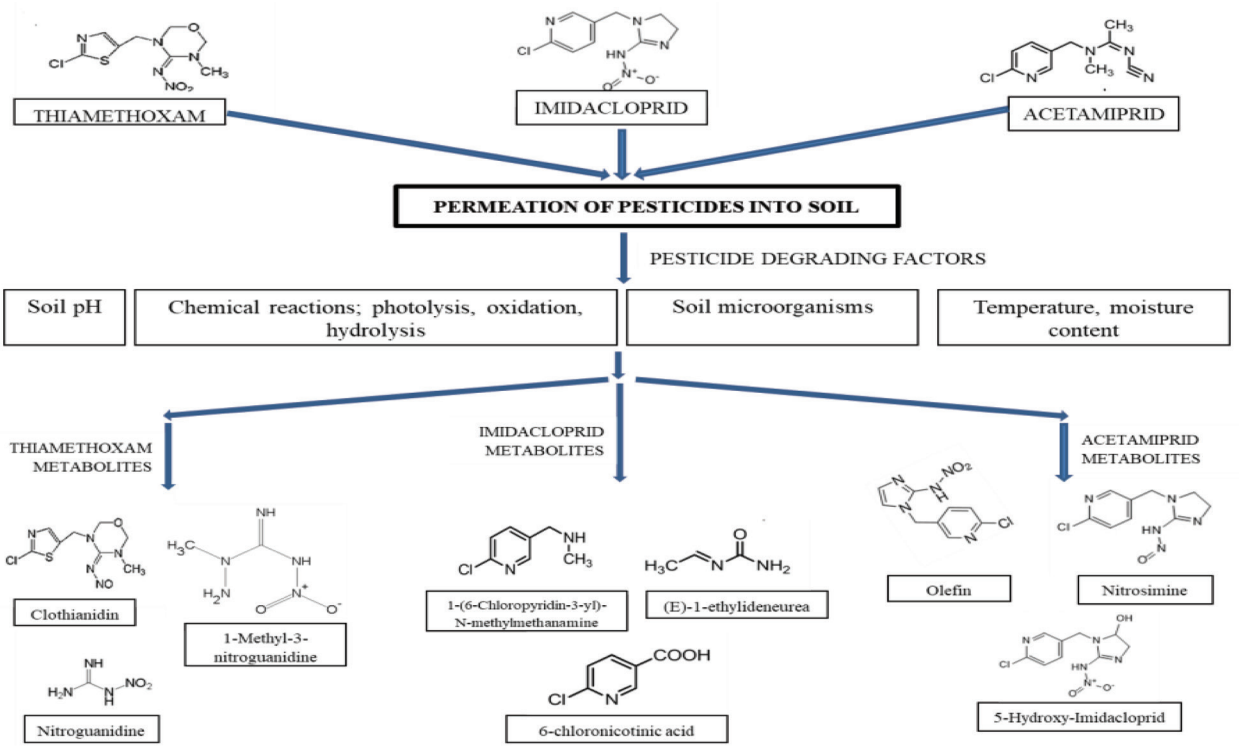


Fig. 4. Degradation pathway of neonicotinoid pesticides and their metabolites formed in the soil

the degradation rate of pesticides decreases. However, special processes may take place with depth e.g. by ferrous ion catalysed anaerobic reduction reaction, oxamyl is exceptionally degraded in acidic sandy subsoils (Leistra, 1986). The soil adsorption coefficient (Kd) also positively correlates with herbicide depth movement. The dynamics (soil movement, adsorption and dissipation) of herbicides namely; simazine, diuron, pendimethalin, oxyfluorfen and flumioxazin was studied in the sandy loam soil in Casablanca Valley, Chile. The movement in soil was 120 cm in simazine, 90 cm in diuron and 30 cm in flumioxazin. Oxyfluorfen and pendimethalin were found in top 5 cm layer (Kogan et al., 2007), and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) being widely used against the broad leaf weeds has posed a risk to ground water. The 2, 4-D and its metabolite, 2, 4-Dichlorophenol (2, 4-DCP) gets adsorbed, desorbed, degraded and displaced in soils having organic matter, iron, aluminium oxide and clay. The distribution coefficients for 2, 4-DCP sorption were linear and more than 2, 4-D. The increased preferential flow due to porous soil and high soil clay content has further increased risk to groundwater contamination by reducing the soil's buffering capacity (Prado et al., 2016).

Soil texture: According to Brown (2012), soil texture affects the movement of water passing through it, thereby affecting the movement of pesticides dissolved in water. In coarser soils, percolating water moves faster, and the chances of adsorption of pesticide to the soil particles is less. Soils with clayey texture have more surface area onto which more pesticides can get adsorbed and hold water for longer period.

iii. *Miscellaneous factors*

Factors like weather, climate and irrigation practices affect the pesticide degradation and leaching. If the soil temperature is warmer, there is an increase in degradation and if cooler then degradation gets retarded (Anonymous, 2019). IF watering is done in large quantity to the field it favours pesticides and fertilizers to leach down and get distributed deep into the soil profile and finally reach the groundwater. According to a study on pesticide accumulation in soil that has been done in Mezquital Valley (Central Mexico) on maize crop, the crop treated with atrazine (weedicide) for over 20 years resulted into two metabolites in the soil namely; hydroxyatrazine and deethylatrazine. Hydroxyatrazine was detected after first and third irrigation in the soil to a concentration of 0.08 mg $kg^{-1,}$ while deethylatrazine got percolated down with second irrigation and was detected in percolation water only (0.03 mg L^{-1}). The mobility potential of 32 pesticides viz., carbaryl, carbofuran, chlorpyriphos, dimethoate, quinalphos, imidacloprid, thiamethoxam, thiodicarb, quinclorac etc. on surface and groundwater was assessed using pesticide risk indicators in the paddy fields of Mahaweli river basin in Sri Lanka. Among all the pesticides, carbofuran, thiamethoxam, and quinclorac were the most leachable pesticides. The most mobile pesticides in the soil are carbofuran and quinclorac as a result of runoff, while thiamethoxam and novaluron are by means of erosion (Aravinna et al., 2017). The studies on mobility of carbofuran and diuron in forty-three different soil surfaces (dry and wet zones) of Sri Lanka revealed that both pesticides have high adsorption coefficient (Kd). Sorption for both was two times higher in dry zone than in wet zone (Liyanage et al., 2006).

D. Degradation

Organic pesticides are major sources of pesticide property, environmental conditions, etc. (Rao et al., 1983). As far as the microbial transformation of various pesticides is concerned, it is expected that the chemical compounds can be present in the soil, water and plant, and toxicity may be more or less than the soil pollution in agricultural lands. Most of these pesticides are persistent and tend to bio accumulate in humans upon consumption of contaminated plants (El-Saeid et al., 2022). Pesticide degradation is a fundamental process for pesticide residue attenuation (Guo et al., 2000) and is governed by biotic and abiotic factors, viz., microbial interactions, soil composition, pesticide property, environmental conditions, etc. (Rao et al., 1983). As far as the microbial transformation of various pesticides is concerned, it is expected that the chemical compounds can be present in the soil, water and plant, and toxicity may be more or less than the parent compound (Chowdhury et al., 2008).

i. *Biodegradation/ microbial degradation*

Biodegradation/microbial degradation is a natural instinct of microbes, a strategy for survival. Efficient and environmentally friendly microbial degradation has been considered as the most promising remediation method (Pang et al., 2022). The microbiota (especially bacteria) in the soil flourish in the presence of available pesticides (Guillen-Garces et al., 2007), which act as electron donors, sources of carbon and other nutrients, thereby causing pesticide degradation (Qiu et al., 2007). Some microbes are modified to degrade pesticides rapidly and thus microbes with such capabilities

can be utilized for removing contaminants from the actual site of application. Pesticide biodegradation is influenced by the factors such as soil organic matter, moisture content, soil texture, aeration, temperature, pH etc. and is highest in well aerated, moist, warm soil with a neutral pH. The soil profile with high organic matter in the surface soil shows a higher rate of microbial degradation whereas the rate of degradation is inversely related with the depth of soil because conditions of temperature, aeration, and moisture are not favorable for activity of soil microbes. To achieve precise bioremediation, it requires an understanding of the genetics and physiology of the desired microbe. The reaction rate of degradation reactions aided by microorganisms is enzyme conciliated, and it doubles for each 10^{o}C rise in temperature with temperatures ranging between 10 and 45^{0}C. Enzymatic activity is colossally reduced above and below these temperatures. The gene encoding the enzymes for degradation has been identified for various pesticides and can be used for a super strain development to accomplish the desired results (Singh, 2007). In the absence of a microbial system for pesticide degradation, it leads to pesticide persistence in soil which is also affected by other factors like pesticide distribution pattern between soil and foliage, temperature, soil characteristics such as composition, soil pH, water pH etc. (Singh and Thakur, 2006).

In recent years, a number of microbial floras viz. bacteria, actinomycetes, fungi etc., have been isolated and screened for degradation of pesticides. The soil-inhabiting bacterial genera namely; *Bacillus*, *Arthrobacter*, *Pseudomonas* and *Micrococcus,* constitute the majority group involved in organochlorine degradation (Langlois et al., 1970). Rana et al. (2015) isolated bacteria species namely; *Pseudomonas putida* strain IMBL 5.2 and *Bacillus aeromonas* strain IMBL 4.1 for thiamethoxam degradation which resulted in 38.23 and 45.28 per cent thiamethoxam (50 µg mL^{-1}) reduction, respectively, in 15 days and proved to be potential thiamethoxam degrading species. *Pseudomonas* sp. has also been reported as dieldrin degrading bacteria (Matsumura et al., 1968). Endosulfan degradation by *Pseudomonas aeruginosa* (88.5%), *Stenotrophomonas maltophilia* (85.5%), *Acinetobacter lowffii* (80.2%), *Bacillus atrophaeus* (64.4%) and *Citrobacter amolonaticus* (56.7%) was reported by Ozdal et al. (2016). For detection of endosulfan degradation, Kafilzadeh et al. (2015) isolated bacteria from water samples and sediments from agricultural land. The bacteria oxidised the endosulfan to carbon dioxide and water, which acted as a source of carbon and energy, thereby supplementing their growth and reproduction. Several bacteria competent of degrading aldicarb, carbofuran, carbaryl or baygon (Larkin and Day, 1986), endosulfan (Hussain et al., 2007), monocrotophos (Subhas and Singh, 2003) etc. have been identified.

The 'Esd' gene from *Arthrobacter* sp. encodes for an enzyme (monooxygenase) that degrades endosulfan α and ß (Sutherland et al., 2002; Weir et al., 2006). The 'lin' gene has been characterized in Sphingomonads namely; *Spingobium indicum, Spingobium francense* and *Spingobium japonicum,* responsible for uptake, pesticide metabolism, and successful degradation of hexachlorocyclohexane (HCH) (Pal et al., 2005). The enzyme HCH dehydrochlorinase encoded by 'lin' gene mediates dehydrochlorination of HCH (Lal et al., 2006). Thus, for the development of bioremediation technology for pesticides like HCH, the diversity, distribution and genetics of lin gene should be deciphered Also, the bacterial degradation of α-endosulfan and ß-endosulfan by *Staphylococcus, Bacillus circulans*-I and *B.circulans* II has also been reported by Kumar and Philip (2006). Some other enzymes responsible for biodegradation of pesticides are enlisted in Table 1.

The fungal species are also potent candidates for pesticide degradation. Kamaei et al. (2011) reported efficient degradation of endosulfan and endosulfan sulphate by white rot fungus, *Trametes hirsuta*. *Trichderma harzianum* and *Rhizopus nodosus* isolated from pesticide contaminated soil degraded 70 to 80 per cent of chlorpyriphos and ethion within 21 days (Harish et al., 2013). For the carbofuran tolerance test, 319 actinomycetes were selected by Jayabarath et al. (2010) from Maharashtra, and they observed that strains of *Streptoverticillium album, Micromonospora chalcea, Streptomyces alanosinicus, Nocardia farcinia, Nocardia vaccini, Streptomyces atratus* and *Nocardia amarae* were potent enough to grow and degrade pesticides. Lin et al. (2011) isolated cypermethrin degrading strain HU-S-01 of *Streptomyces parvulus* from waste water sludge as a potential biological agent for decontamination of cypermethrin contaminated crop, soil or water. Some of the pesticide degrading microbial organisms have been enlisted in the Table 2. In addition to bacteria, fungal cultures are also effective tools in the bioremediation of pesticides. Fungal hyphae secrete extracellular enzymes that can penetrate deeper into the soil matrix with a larger surface area than bacterial isolates (Shi et al. 2018; Guo et al. 2019).

Table 1. Enzymes responsible for bioremediation of pesticides (credit: Ortiz-Hernández et al., 2013)

Enzyme	Organism	Pesticide
Oxidoreductases (Gox)	*Pseudomonas* sp. LBr Agrobacterium strain T10	Glyphosate
Monooxygenases:		
ESd	*Mycobacterium* sp.	Endosulphan and Endosulphate
Ese	*Anthrobacter* sp.	Endosulphan, Aldrin, Malathion, DDDT and Endosulphate
Cyp1A1/1ª2	Rats	Atrazine, Norflurazon and Isoproturon
Cyp76B1	*Helianthus tuberosus*	Linuron, Chlortoluron and Isoproturon
P450	*Pseudomonas putida*	Hexachlorobenzene and Pentachlorobenzene
Dioxygenases (TOD)	*Pseudomonas putida*	Herbicides Trifluralin
E3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester
Phosphotriesterases: OPH/OpdA	*Agrobacterium radiobacter, Pseudomonas diminuta, Flavobacterium* sp.	Insecticides phosphotriester
Haloalkane Dehalogenases: LinB	*Sphingobium* sp., *Shingomonas* sp.	Hexachlorocyclohexane (β and δ isomers)
AtzA	*Pseudomonas* sp.	Herbicides chloro-s-trazina
TrzN	*Nocardioides* sp	Herbicides chloro-s-trazina
LinA	*Sphingobium* sp., *Shingomonas* sp.	Hexachlorocyclohexane (γ isomers)
TfdA	*Ralstonia eutropha*	2,4 - dichlorophenoxyacetic acid and pyridyl-oxyacetic
DMO	*Pseudomonas maltophilia*	Dicamba

Table 2. Enlisting some microorganisms involved in pesticide degradation

Type of microorganism	Pesticide degraded	Scientific name	References
Bacteria	Fenitrothion	*Burkholderia* sp.,	Hong et al., 2007
	Isoproturon	*Sphingomonas* sp.	Bending and Rodríguez-Cruz, 2007
	Methyl parathion	*Ochrobactrum* sp.	Qiu et al., 2007
	Diuron	*Micrococcus* sp.	Sharma et al., 2010.
	Methyl parathion	*Sphingobium* sp.	Yuanfan et al., 2010.
	Endosulphan	*Pseudomonas* sp.	Verma et al., 2014
	Chlorpyrifos	*Bacillus* sp.	Verma et al., 2014, Upadhyay and Dutt, 2017
	Glyphosate	*Flavobacterium*	Upadhyay and Dutt, 2017
	Dichlorovos	*Pseudomonas stutzeri*	Parte et al., 2017a
Fungus	Endosulfan	*Mortierella* sp strain W8 and Cm1-45	Shimizu, 2002
	Pirmicarb	*Trichoderma viridae, Trichoderma harzianum*	Eapen et al., 2007
	β-Cypermethrin	*Aspergillus niger*	Deng et al., 2015
Actinomycetes	Carbofuran	*Streptomyces allanosinicus*	Wu et al., 2004
	Chlorpyrifos	*Streptomyces* sp strain SJRO-06 and SJRO-10	Sunitha and Onkarappa, 2018

Bisht et al. (2019) screened 20 fungal isolates for their ability to degrade endosulfan and chlorpyriphos. Both α- and β-endosulfan isomers were completely degraded by *Trametes hirsuta* and *Cladosporium cladosporioides* resulted in maximum degradation of chlorpyrifos.

ii. *Chemical degradation*

Chemical degradation reaction takes place in the presence of water, oxygen or other soil chemicals, and under extremes of soil pH and microbial activity. However, such conditions may be in favor of chemical degradation at a faster rate (Kerle et al., 2007). The different ways of pesticide degradation include photolysis, reduction, oxidation, hydrolysis, decarboxylation, dehydrogenation, condensation, dehalogenation etc. Photolysis can be enhanced by adding humic materials or other additives like riboflavin, rose bengal, methylene blue, etc. that act as photosensitizers (Miller et al., 1987). Hydrolysis is another chemical process involved in pesticide degradation. Wei et al. (2001) considered it a primary transformation pathway for carbamates in the environment.

iii. *Photodegradation*

Photodegradation has become an important index in evaluating the environmental safety of pesticides. It significantly influences the pesticide fate on crop surface (residue, efficacy and toxicity), in the atmosphere and water (Hong and Zeng, 2002). Almost all chemical pesticides are photodegradable to some extent, influenced by sunlight intensity, exposure period and pesticide properties. Foliage and soil surface applied pesticides are more prone to photodegradation than soil incorporated. Pesticide degradation is at a faster rate inside plastic cladded greenhouses than glasshouses as glass reflects back much of the ultraviolet rays that are having the potential to degrade pesticides (Kerle et al., 2007).

iv. *Mechanism of microbial degradation*

Enzymes are of paramount importance for xenobiotic degradation in the soil and play an important role inpesticide degradation. After getting into the microbes, these pesticides undergo some biochemical and physiological changes aided by enzymatic reactions, thereby getting transformed into some less toxic or non- toxic compounds (Parte et al., 2017b; Tang, 2018). Kim et al. (2004) proposed the metabolic pathway for degradation of carbofuran by bacteria like *Arthrobacter* sp and *Sphingomonas* sp. The first product of carbofuran degradation is carbofuran phenol followed by the formation of 2-hydroxy-3-(3-methylpropan-2-ol) phenol. The bacterial microorganisms are known to combat secondary pollution as they help transform toxic organic molecules into the non-toxic ones (Prabha et al., 2017). Some microbes have enzymes that enable them to degrade the pesticides and convert the organic compounds to inorganic ones by a process called mineralization. These microbes utilize these pesticides as a source of their nutrients. Another process for degradation of pesticides involves co-metabolism in which pesticides could be degraded by adding some organic matter like exo-genous or iso-biomass as the primary energy source (Zhang et al., 2010).

E. Risk to groundwater contamination

Groundwater is a major source of fresh water for the global population and is used for domestic, agricultural and industrial uses. Approximately one third of the global population depends on groundwater for drinking water (International Association of Hyrogeologists 2020). Pesticide use has undoubtedly resulted in improved crop production by preventing the damage by the pests but has increased the risk of groundwater contamination, especially in urban areas. The quality of drinking water has deteriorated in recent times. The reason is, the increasing population pressure, unplanned growth of cities, mixed land use patterns, poor sewage facilities, unplanned or illegal disposal of wastewater from industries and households. Shallow aquifers have been polluted to a greater extent due to industrial and domestic effluents. In India, water pollution is a major concern, as the groundwater reserves and about 70 per cent of its surface water resources are already contaminated by organic, inorganic and biological pollutants (Rao and Mamatha, 2004). Urban areas in India have accessibility to only groundwater, so the quality and quantity of groundwater are equally important. With the increasing awareness regarding groundwater contamination, efforts have been made to prevent or reduce groundwater pollution (Kalinski et al., 1994). The contaminated groundwater limits its use in agricultural areas as a source of crop irrigation and thus has become a global concern. Several problems like human diseases, soil fertility degradation and reduced farmers' income may result from the use of contaminated groundwater (organic and inorganic contaminants) for irrigation. The impacts of this contaminated groundwater are more pronounced in intense agricultural areas where sufficient water is not enough to irrigate crops (Komnitsas et al., 2015).

i. *Sources of groundwater pollution*

Groundwater contamination is a global problem that has a significant impact on human health and ecological services (Li et al., 2021). Risk to groundwater refers to, "the probability that groundwater will suffer unacceptable contamination due to human activities". The concentration and type of groundwater contaminant in the solution depends upon the point source of groundwater, rate of groundwater movement, and surface and subsurface environment (Prasad et al., 1999). It has become important, especially in agricultural areas, to recognize groundwater pollutants and evaluate the risk involved in pesticide use. Pesticides and nitrates are dominant non-point contamination sources in agricultural fields especially for the shallow alluvial aquifers. Factors for the vulnerability of alluvial aquifers include high permeability of alluvial deposits, shallow water table, interconnections between surface water and agricultural related land uses, and sea water intrusion due to over-pumping of groundwater for irrigation (Guler et al., 2012).

Rainwater is relatively free of minerals till it comes in contact with various constituents present in the soil, where pesticides, minerals and salts get dissolved in it, which are carried into the solution as water moves through the aquifer. Various ion concentration, viz., cations and anions, is dependent on mineral solubility, minerals present in the solution, the time duration for which water is in contact with the rocks and the amount of dissolved CO_2 present in the water (Viessman et al., 1989). Various factors affect the chemical alteration of the ground water: solid phase interaction, polluted river water seepage, the residence time of ground water, saline water getting mixed with groundwater, and anthropogenic impacts (Umar and Absar, 2003).

Various pesticides have been reported as groundwater contaminants. Atrazine has been found to bind easily to aliphatic and aromatic compounds that have posed a risk to groundwater contamination (Dutta et al., 2015). Mittal et al. (2014) reported the enhanced leaching capacity of pesticides through silty textured soil into groundwater in the Malwa region of Punjab. For this reason, it has been described as India's 'cancer capital', consuming almost 75 per cent of total pesticide consumption in Punjab, leading to poor groundwater quality. Jayashree and Vasudevan (2007) reported DDT, HCH and endosulfan residues from groundwater of Thiruvallur district of Tamil Nadu, India. Residue of endosulfan sulphate (metabolite of endosulfan) was detected in a range of 15.9 µg L^{-1} in the bore wells, γ-HCH was found to be as high as 9.8 µg L^{-1} in open wells and pp-DDT (DDT isomer) was in the range of 14.3 µg L^{-1}. For land-use planning and management, and protection of groundwater, risk assessment is an imperative tool. Though detection and control of groundwater contamination is a tough task than dealing with surface water as they may persist for many long years or even centuries (Todd, 1980) Even then there are some major methods involved in risk assessment of ground water pollution namely; index method, process-based methods, statistical methods and fuzzy comprehensive evaluation method. There are also other methods for pollution risk evaluation based on measuring solubility, degradation and sorption. Gustafson (1989) developed a leaching risk index based on groundwater contamination. In addition to giving an index/score, it predicts the probability of the chemical being present in groundwater (Worrall et al., 1998).

ii. *Factors affecting groundwater pollution*

Pesticide flux in soil is one of the important factors affecting groundwater contamination. Chemical pesticides rapidly pass to groundwater when they cross the unsaturated soil zone. This movement is controlled by water. The hydraulic gradient and hydraulic conductivity of the soil, govern the rate with which water flows through the soil profile. The size, shape, and interrelation of the soil pores regulate the conductivity, which varies according to the soil type. Gas phase also controls the water flow paths in the unsaturated zone above the water-table (Rao et al., 1988). The pesticide properties like soil dissipation half-life and soil organic-carbon partition coefficient influence pesticide leaching in the soil. Also, the soil properties like structure, clay content, organic matter, and soil hydrological processes influence the magnitude and rate of pesticide transportation in soil. Wauchope et al. (2001) correlated pesticide mobility and its leaching risk into groundwater, especially for hydrophobic ones with weak sorption on the soil matrix. They observed that the ratio of adsorbed pesticide to the solution-phase pesticide got normalized with respect to organic matter content in the soil. However, Elliott et al. (2000) observed pesticides with adsorption constant (Koc) 1000 got transported into groundwater shortly after application due to heavy rainfall. In the tropical regions, animal galleries viz., termite galleries are the preferential routes of flow under rainstorms (McGarry et al., 2000).

In addition to these, some other factors which affect pesticide pollution of groundwater are (Anonymous,

2017): adjuvants (solvents or diluents, emulsifiers, wetting agents, adhesives, extenders, preservatives and buffers) that are mixed with the active ingredient; products formed during chemical, photochemical or microbial degradation of the active ingredient; pesticide mobility in soil, both horizontally and vertically in the soil structure, e.g. residual herbicides are designed such that they bind directly to the soil; contaminants in the active ingredient; half-life of pesticidesPesticides that take longer time to break down have higher stability and persistence; pesticide application rate and solubility in water; microbial activity and soil temperature etc.

CONCLUSIONS

Pesticide retention by soil prevents its short-term admittance to the surface and groundwater. Still, non degraded pesticides and their metabolites result in an interminable risk to the environment and human health. So, an adequate understanding of these processes, i.e. retention, mobility and degradation of pesticides is essential Degradation of pesticides by a complex of methods provides an important tool for decontamination of areas affected by the overuse of pesticides. Biodegradation is an environment-friendly approach for reducing the risk associated with use of hazardous chemicals. Hence, the scrutinization of highly efficient microbial strains for pesticide degradation to reduce environmental pollution is the need of the hour.

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TIME TO RESTART: STUDY OF THE DERMAPTERA OF THE INDIAN SUBCONTINENT

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ABSTRACT

The Dermaptera is a polyneopteran order with > 2,000 described species from mainly tropical, subtropical, and warm temperate regions. More than 310 species of the Dermaptera belonging to nine families have been reported from the Indian subcontinent (India, Pakistan, Nepal, Bhutan, Bangladesh and Sri Lanka) as a result of the extensive work of Gyanendra Kumar Srivastava. Embracing environmental and climatic heterogeneity, the Indian subcontinent is an intersection of multiple faunal regions and includes several dermapteran groups of special interest. To restart the studies on the Dermaptera of this region, which has been stagnant for a decade, this paper includes a beginner's guide for collecting and identifying Dermaptera, together with a brief summary of recent advances in their classification and phylogeny.

Key words: Dermaptera, classification, collection, earwigs, India, ecology, distribution, genitalia, identification, regional fauna characteristics, alpha taxomoy, DNA barcoding, recent advances

Dermaptera is a polyneopteran order with >2,000 described species from mainly tropical, subtropical, and warm temperate regions (Popham 2000; Grimaldi and Engel, 2005; Zhang, 2013; Haas, 2018; Hopkins et al., 2018). The insects of this order, commonly known as earwigs, are characterized by their sclerotized, unsegmented forceps at the caudal end of the abdomen, which are specialized cerci (Fig. 1A-C). Forceps are used in defence to enemies (Eisner, 1960), predation (Jiang and Kajimura, 2020), and opening of wings (Kleinow, 1966; Haas, 2003). Adult forceps are usually sexually dimorphic, and used in male-male competition for mates and courtship displays (Briceño and Eberhard, 1995; Kamimura, 2014). Most of the species of the Dermaptera are either omnivorous or carnivorous insects that live in diverse natural and semi-natural environments (Günther and Herter, 1974; Renz and Kevan, 1991). Several species are considered nuisance organisms or pests in either horticultural or agricultural contexts; for example, *Nala lividipes* (Dufour) (Labiduridae) is known to a pest of beetroot seedlings (Cooper, 1992), and *Euborellia cincticollis* (Gerstaeker) (Anisolabididae) damage seedlings and mature fruit of muskmelons (Knabke and Grigarick, 1971). On the other hand, some other species could be useful as biological control agents. For example, *Labidura riparia* (Pallas), a cosmopolitan species of Labiduridae, is known to be predaceous on many insects including lepidopteran larvae (Schlinger et al., 1959; Tawfik et al., 1972). Nishikawa et al. (2006) reported that *Chelisoches variegatus* (Burr) (Chelisochidae) eat *Brontispa longissima* (Gestro) (coconut leaf beetle) in coconut fields. However, a vast majority of the species of Dermaptera are considered to have no direct relationships with human activities. Accordingly, the basic bionomics have been studied for only a few of the more common species. Little published information on the ecology of most species is currently available (Günther and Herter, 1974; Renz and Kevan, 1991; Costa, 2006; Haas, 2018), irrespective of their potential, hitherto unknown ecosystem functions in natural and agricultural environments: recent studies have revealed that *Doru luteipes* Scudder, a predatory Forficulidae, is attracted to herbivore-induced plant volatiles (Naranjo-Guevara et al., 2017) and termitophily of *Spirolabia kaja* Kočárek (Spongiphoridae) in a dipterocarp rain forest in Borneo (Kočárek and Wahab, 2021).

Engel and Haas (2007) revised the hierarchical classification and family-group names of living and extinct Dermaptera. Their system and subsequent modification by Engel et al. (2017) classify extant Dermaptera species into 12 families in two infraorders: Protodermaptera: Karschiellidae, Diplatyidae, Pygidicranidae and Haplodiplatyidae; Epidermaptera: Hemimeridae, Apachyidae, Anisolabididae

(= Carcinopholidae), Labiduridae, Arixeniidae, Spongiphoridae (= Labiidae), Chelisochidae and Forficulidae. Representatives of nine of these families are known from the Indian subcontinent viz. the Republic of India and adjacent countries (Pakistan, Nepal, Bhutan, Bangladesh, and Sri Lanka) (Srivastava, 2013): exceptions being Karschiellidae that are possible ant predators in the Ethiopian region (Hincks, 1959), Hemimeridae that live phoretically on the giant pouched rats (*Cricetomys* Waterhouse spp.) in Africa (Nakata and Maa, 1974), and Arixeniidae that are associated with bat (*Cheiromeles torquatus* Horsfield) roosts in the Oriental tropics (Nakata and Maa, 1974).

In the extant Dermaptera, males of Apachyidae, Labiduridae, Anisolabididae, Pygidicranidae, Diplatyidae, Haplodiplatyidae and Karschiellidae usually have laterally paired penises (Fig. 1E, E', E''): but the left penis is largely reduced and vestigial in Karschiellidae (Steinmann, 1986), whereas only a single penis is present in Spongiphoridae, Chelisochidae, Forficulidae, Arixeniidae and Hemimeridae (Fig. 1F, F'; Burr, 1915a, b; Popham, 1965; Kamimura, 2014). A single virga, a sclerotised tube with a species-specific shape, which constitutes the terminal part of the ejaculatory duct, is enclosed in the membranous penis lobe (Fig. 1E, F). The phylogenetic relationships among the families are still largely unsettled, possibly due to difficulty in rooting the Dermaptera by a sister order. Several studies based on the morphological and/or molecular datasets suggested that monophyletic or paraphyletic Protodermaptera consists of the basal offshoot(s), with (Haas, 1995; Haas and Kukalová-Peck, 2001) or without (Jarvis et al., 2005; Kočárek et al., 2013; Naegle et al., 2016) monophyly of the families with a single penis (Nanorder Eudermaptera plus Hemimeridae). However, some recent studies suggest that Apachyidae is the sister group to the remaining Dermaptera (Wipfler et al., 2020; Chen, 2022).

Alpha taxonomy provides the basis for all other disciplines of biology, including biogeography and applications in agriculture such as biological control programmes. Most of our knowledge of the α-taxonomy of the Dermaptera of the Indian subcontinent is due to the work of Gyanendra Kumar Srivastava of the

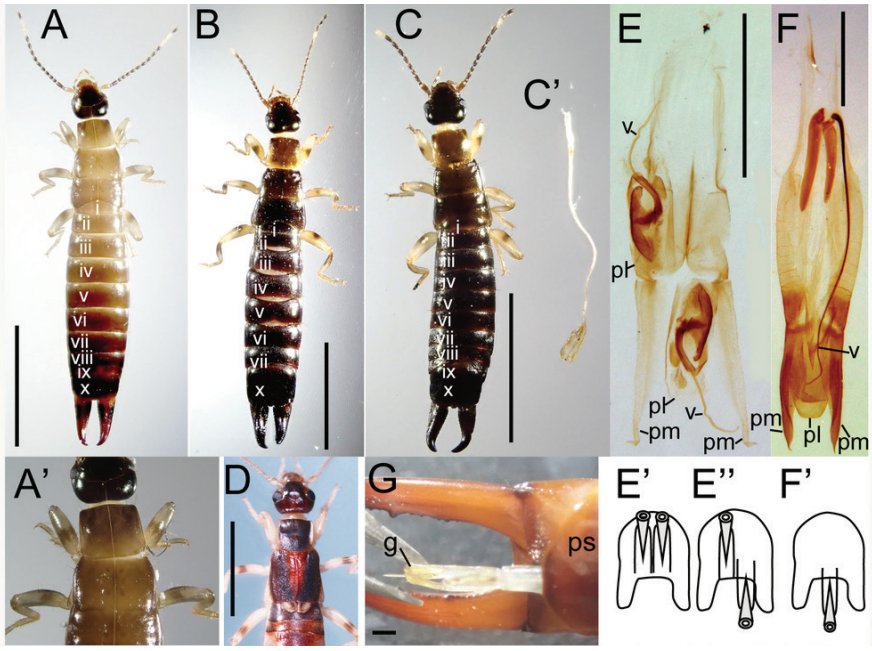


Fig. 1. A last-instar nymph (A), adult female (B), and adult male (C) with his genitalia extracted from the body (C') of *Euborellia annulipes* (Lucas), an apterous species of Anisolabididae. An enlarged view of A shows thoracic ecdysial lines (A'). Head and thorax of male *Labidura riparia* (Pallas) with the fully developed tegmina and wings (D). Male genitalia of *Epilandex burri* (Borelli) (Anisolabididae) (E) and *Adiathella tenebrator* (Kirby) (Chelisochidae) (F) with schematic drawings of male genitalia with two penises (E', E'') and one penis (F'). Male genitalia of *L. riparia* extracted from the genitalia chamber (the space above the penultimate sternite) with fine forceps (G). Roman numerals indicate the abdominal sternites I-X. Abbreviations: g, male genitalia; pl, penis lobe; pm, paramere; ps, penultimate abdominal sternite; v, virga. Scale bars: 1 cm in A-C; 5 mm in D; 1 mm in E-G.

Zoological Survey of India, who published >120 papers and notes on the Dermaptera of India and other countries. In his Fauna of India and the adjacent Countries, Dermaptera, he has reported >310 species (Srivastava, 1988; 2003a; 2013), corresponding to *c*. 16% of the Dermaptera worldwide. Obviously, the extensive studies by Srivastava resulted in the knowledge of stunning species diversity, which is relating to the climatic and environmental heterogeneity of the Indian subcontinent as discussed below. However, the study of the Dermaptera of the Indian subcontinent had stalled in the last decade.

Characteristics of the dermapteran fauna of the Indian subcontinent

The cerci of adult Dermaptera are always present as unsegmented forceps. However, segmented cerci are known in the nymphal stages of Haplodiplatyidae (Bormans and Krauss, 1900), Diplatyidae (Shimizu and Machida, 2011b) and Karschiellidae (at least in the distal part; Haas et al., 2012), supporting the view that these families represent the earliest offshoots of extant Dermaptera (Haas and Klass, 2003). Among them, *Haplodiplatys* Hincks, which comprises the monotypic family Haplodiplatyidae (Engel et al., 2017), is of special interest being characterized by multiple plesiomorphic features, including the laterally symmetrical tegmina and absence of a spiny ridge (a component of the tegmina-locking device) on the dorsal side of the mesothorax (Haas and Kukalová-Peck, 2001). Among the 36 species known in *Haplodiplatys* (Hopkins et al., 2018: accessed 25 July 2022), 16 species (plus another four described by Srivastava in 1988) have been reported from the Indian subcontinent (Srivastava, 2013). Majority of them are more common in forested slopes of Himalaya, but *H. stemmleri* Brindle, is sometimes found at higher elevation up to 3100 masl (Srivastava, 1988). Strikingly contrasting to the distribution of *Haplodiplatys*, the diversity of *Diplatys* Audinet-Serville (Diplatyidae) is the greatest in peninsular India (Karthik et al., 2022). These examples indicate the relevance of environmental heterogeneity in the Indian subcontinent, from alpine to lowland tropical, as the cause of the total diversity in this region. Similarly, many Forficulidae (e.g., *Eudohrnia* Burr, *Liparura* Burr and *Anechura* Scudder) have Palaearctic distributions and are confined to the northern part of the Indian subcontinent, while Chelisochidae is a tropical-subtropical element (Srivastava, 1988; 2013), consistent with their general global trends (Popham, 2000).

The generic diversity of Anisolabididae strongly supports the view that the Indian subcontinent is an intersection of multiple faunal regions. The occurrence of some genera on the Indian subcontinent, such as *Titanolabis* Burr and *Epilandex* Hebard indicates the affinity to the Oriental-Australian regions (Srivastava 2003a). On the other hand, six species of *Aborolabis* Srivastava have been recorded from northeast India, Nepal and Bhutan, while others are known from the Iberian Peninsula to North Africa (Srivastava, 1993b; 2003a). In Anisolabididae of Srivastava (1999; 2003a; 2013), the Isolaboidinae, recorded only from India to Anatolia, is of special interest because of its unique morphology and the controversy about its phylogenetic placement. The males of Isolaboidinae possess unique genitalia with a single, well-developed penis and a conspicuous spirally-coiled virga (Brindle, 1978; Steinmann, 1989a; Srivastava, 1996; 2003a). Although many authors place Isolaboidinae under Spongiphoridae (Steinmann, 1989a, b; Engel and Haas, 2007), males of which possess only a single penis (see above), Srivastava (1996) argued that the right penis lobe (= distal lobe) is atrophied either with or without rudimentary virga, whereas the left penis is developed with a spirally coiled virga in the Isolaboidinae. Accordingly, Srivastava (1996; 1999; 2003a; 2013) placed Isolaboidinae in Anisolabididae, which is characterized by laterally paired penises. Studies indicate that the left penis is atrophied in the ancestor(s) of Eudermaptera (including Spongiphoridae) (Popham, 1965; Kamimura, 2006; 2007). Accordingly, the evolutionary loss of the right penis in Isolaboidinae should have occurred independently to instances of left-side reduction. To resolve this controversy, future studies must examine the phylogenetic placement, development and reproductive biology of Isolaboidinae.

The 'Zoogeography' section in Srivastava (1988) provides additional details on the generic compositions of the Dermaptera of the Indian subcontinent. His contributions go far beyond the α-taxonomy of the Dermaptera and generic and suprageneric classifications proposed by him (Pygidicranidae and Diplatyidae, Srivastava, 1993a; Anisolabididae, Srivastava, 1999; Spongiphoridae, Srivastava, 1995; and Chelisochidae, Srivastava, 2003b) are important references for understanding the evolution of the Dermaptera.

How to study the taxonomy and basic ecology of Dermaptera

The Dermaptera occurs in diverse environments: under barks of living or dead trees in natural and secondary forests; under rocks along streams; associated

with marine litter; associated with dungs (fumicolous); or in agricultural fields or insect-induced galls. Some species of *Forcipula* Boliver and *Nala* Zacher (Labiduridae) are almost semi-aquatic (e.g., Julka and Purohit, 1983). Hand sorting with a shovel (and with an aspirator for smaller taxa) is usually the most efficient way to collect them. For some species, pitfall traps with various baits (Núñez-Bazán et al., 2022) or light traps (Girod and Matzke, 2020) are also effective.

It is usually difficult to identify the Dermaptera to the species level based only on nymphs. As in other hemimetabolous insects lacking a pupal stage, dermapteran nymphs resemble adults (Fig. 1A-C). Adults can be distinguished from nymphs by the presence of tegmina (= forewings) and wings (= hindwings): the latter are folded under the tegmina when in repose (Fig. 1D: Haas et al., 2000; Saito et al., 2020). In some species, both the tegmina and wings are totally absent (Fig. 1 A-C). Even in such species, nymphs can be distinguished from adults by their thin exoskeleton with the ecdysial line (Fig. 1A'). As in many other insect groups, adult female Dermaptera are usually more difficult to identify to species than adult males. Rearing females (and nymphs) in the laboratory is one way of identifying them. Wild-caught females frequently possess sperm in the spermatheca (an internal organ for sperm storage) and lay fertilized eggs under rearing conditions. All the Dermaptera studied to date show varying degrees of maternal care of the offspring [Costa 2006; but see Shimizu and Machida, 2011a for a possible exception in *Apachyus chartaceus* (de Haan) (Apachyidae)]. By this, one can also study their reproductive biology (Matzke and Klass, 2005; Kamimura et al., 2016; Kočárek and Wahab, 2021), and obtain laboratory-reared adults for additional taxonomic, morphological, developmental, and molecular studies. Bhaskara Narasurama Ramamurthi of Loyola College (Tamil Nadu), one of the pioneers of dermapteran taxonomy of India, also studied the development of male genital systems of several groups, and the ovoviviparity of *Marava arachidis* (Yersin) by means of rearing (Ramamurthi, 1956; 1958).

Animal genitalia used for copulation and sperm transfer, especially those of males, are often more complex than is necessary for sperm transfer alone and seem to evolve more rapidly than other structures (Eberhard, 1985; Hosken and Stockley, 2004). Since males voraciously pursue mating while females avoid mating, sexual conflict over mating can result in antagonistic coevolution of genitalia (Arnqvist and Rowe, 1995; 2002; Kokko and Jennions, 2014). For species that are potentially polygamous, post-copulatory sexual selection can also promote genital coevolution between the sexes (Eberhard, 1985; 1996). This is also true for Dermaptera (Kamimura, 2014). For example, male *M. arachidis* possess a pair of sclerites in the penis lobe, which forcibly pinch the genital region of the female mates during copulation, resulting in copulatory wounds (Kamimura, et al., 2016). Male *Euborellia* spp., are known to use the elongated genitalia to remove rival sperm from the tubular spermatheca of the mates (Kamimura, 2000; van Lieshout and Elgar, 2011). Accordingly, the identification of closely related species often requires an examination of the genital morphology by specialist taxonomists. Contemporary descriptions of new species of Dermaptera include detailed illustrations of male genitalia, and the description of a new species based only on nymphal and/or female specimens is not recommended (particularly when intraspecific morphological variations are not clarified).

In adult males, 10 abdominal segments are observable from the dorsal side, as in nymphs of both sexes. The 8th and 9th abdominal segments of adult females are largely reduced and invisible from the dorsal side (Fig. 1 A-C). From fresh or soft alcohol-preserved material (or specimens stored in a freezer at <20°C for a short period before desiccation of the body), it is easy to remove the genitalia from the male dermapteran body compared with other insect groups. By gently lifting the penultimate sternite under a stereomicroscope, one can directly see and grasp the main part of the male genitalia with fine forceps (Fig. 1G). Carefully pulling them from the body, the genitalia can be removed without damaging other body parts. In some species (especially many Anisolabididae), however, the male genitalia are very elongate (Fig. 1C, C') and extra caution is required to not tear them during extraction. To remove the male genitalia from dry or hard specimens, because of storage in >80% ethanol, the whole body must first be softened in hot water. In many Dermaptera, sclerites or denticulated pads also develop on the penis lobe. To observe these structures in detail, the male genitalia can be treated in alkaline solution (usually 10% KOH) for clearing; this is sometimes indispensable for hardened samples preserved in ethanol. The shapes of the parameres (Fig. 1E, F) and those structures associated with penises are important for generic and specific diagnoses, as well as to observe the virgal morphology.

To identify the Dermaptera of the Indian subcontinent,

"The Fauna of India and the Adjacent Countries: Dermaptera, Parts I–III" by Srivastava (1988; 2003a; 2013) is the most useful reference. Steinmann's (1986; 1989a; 1989b; 1990; 1993) monographs and catalogue, bibliographic compilations by Sakai (1985; 1987; 1990-1994; 1995a-d; 1996), and the website Dermaptera Species File (Hopkins et al., 2018) are also helpful. To identify closely related species, DNA barcoding is effective. The universal primer set, LCO1490 and HCO2198, which have been designed to amplify an approximately 700 base-pair region of the mitochondrial cytochrome oxidase subunit I (COI) gene (Folmer et al., 1994), usually works well in most of the extant dermapteran taxa (Stuart et al., 2019; Kalaentzis et al., 2021; Kočárek and Wahab, 2021; Y K, unpublished data). Ethyl acetate vapour is often used to kill insect samples including Dermaptera to avoid subsequent rotting and hardening of the body. However, it causes serious DNA degradation and can hamper subsequent molecular analyses (Dillon et al., 1996). A recent study demonstrated that propylene glycol can be a promising preservative comparable to ethanol for PCR-based studies (Nakamura et al., 2020).

Possibly due to increased freight traffic, some of species have been reported to have expanded their distributions. For example, Nishikawa and Naka (2019) reported *Paradiplatys gladiator* (Burr) (Diplatyidae) from a port in Japan, and considered it was incidentally introduced from India. For researchers outside India, it is sometimes difficult to detect and examine the type material of species described from India and adjacent countries. As recommended in Recommendation 16C of the International Code of Zoological Nomenclature (fourth edition, 1999) "authors should deposit type specimens in an institution that maintains a research collection, with proper facilities for preserving them and making them accessible for study". Photographic databases of museum specimens, especially those of name-bearing types, are also warranted to accelerate studies of Dermaptera, so that we can catch up with accelerating changes in global environments. The fauna of the Indian subcontinent is also changing. In 2018, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) invaded southern India, and a native Dermaptera species (*Forficula* sp.: possibly *F. gravelyi* Burr) is a possible natural enemy of *S. frugiperda* (Shylesha et al., 2018; Sharanabasappa et al., 2019). In addition, Karthik et al. (2022) discovered a new species of Diplatyidae, *Diplatys sahyadriensis* Karthik, Kamimura et Kalleshwaraswamy in a sugarcane field in Kartanaka. Although Srivastava published many faunal monographs for each Indian state or nature reserve, no comprehensive review has been published for southern India (Karnataka, Kerala, Andhra Pradesh, Tamil Nadu and Telangana states). Extensive faunal studies in what might be the most species-rich area of the Indian subcontinent will be important for furthering our understanding of the Dermaptera.

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