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ENTOMON

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Bioecology of the tea thrips, *Scirtothrips bispinosus* Bagnall (Thysanoptera: Thripidae) infesting tea in south India

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ABSTRCT: Investigations were carried out on the life history and seasonal abundance of the thrips, *Scirtothrips bispinosus* infesting tea at Coonoor, The Nilgiris, Tamilnadu. The total developmental duration of females from egg to adult stage was 18.15±0.23, 12.55±0.15 and 10.30±0.23 days at 20, 25 and 30°C respectively. The net reproductive rate (Ro), mean generation time (Tc), intrinsic rate of natural increase (rm), finite rate of increase (λ) and weekly multiplication (Wm) rates were high at 25°C followed by 30°C and 20°C. Multiple regression analysis revealed that population density of *S. bispinosus* showed a negative relationship with rainfall (-0.266), maximum temperature (-38.839) and maximum relative humidity (-3.356) and positive relationship with minimum temperature (63.205), minimum relative humidity (1.686) and sunshine period (2.887). Incidence of thrips was high in the fields recovering from pruning followed by second, third and fourth year in a pruning cycle. The number of thrips per shoot was significantly higher on the plucking table when compared to the shoots present below the plucking table and side branches. In the tea plantations of South India, four species of predatory mites (*Amblyseius cucumeris, A. fallacies, A. degenerans and Balaustium* sp.), two species of predatory thrips *(Franklinothrips vespiformis* and *Leptothrips mali),* an anthocorid predator (*Orius sp.*) and one parasitoid (*Trichogramma* sp.) were found feeding and parasitizing on tea thrips, *S. bispinosus.*

KEY WORDS: Tea plantation, *Scirtothrips bispinosus*, life table, seasonal abundance, natural enemies

INTRODUCTION

Tea (*Camellia sinensis* (L.)) is grown in three states of southern India *viz*., Tamilnadu, Kerala and Karnataka, covering an area of around 106850 ha. It is estimated that more than one thousand species of arthropods and 80 species of nematodes infest tea (Muraleedharan, 1992). Members of Acarina, Hemiptera, Thysanoptera, Lepidoptera, Coleoptera and Isoptera are the most important orders among the arthropod pests of tea. The tea thrips, *Scirtothrips bispinosus* (Bagnall) is endemic to south India. Unlike the polyphagus *Scirtothrips dorsalis*, *S. bispinosus* feeds only on the leaves of tea and coffee plants (Mound and Palmer, 1981). The infested tea leaves become uneven, curly and matty, exhibiting parallel lines of feeding marks on either side of the mid rib. Heavily infested fields sometimes acquire a bronze colour. Damaged terminal shoot may be discoloured, stunted, and deformed. Selvasundaram *et al.* (2004) studied the seasonal abundance in Idukki district, Kerala and reported 11 to 17% crop loss in tea due to infestation of *S. bispinosus*. Ananthakrishnan (1963) reported

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a brief account on developmental duration of *S. bispinosus*. Other than this, no reports are available describing the complete bio-ecology of this species. Most of the research on tea thrips had focused mainly on pest-management aspects and very little is known about its reproductive biology, mating behaviour, population dynamics and the influence of biotic and abiotic factors. Understanding the population dynamics and data on thrips biology are important to evolve control strategies against a pest in the long term. Pure scientific research, for instance in insect reproduction, helps to find unexpected solutions for crop protection problems in current agriculture. The paper enumerates complete aspects of biology, population dynamics, vertical distribution and the influence of biotic and abiotic factors on *S. bispinosus* population.

MATERIALS AND METHODS

Stock Culture: Stock culture was maintained by rearing *S. bispinosus* on the potted tea plants of the clone UPASI-9. The populations of *S. bispinosus* were collected from one of the organic tea estate in Nilgiris.

Rearing of nymphal instars: In the laboratory, *Scirtothrips bispinosus* was reared on leaf discs (ca. 5 cm^2) prepared from the tea clone UPASI-9. The leaf discs were placed on agar (0.5%, 10 mm thick) poured on plastic cups of size 65mm ϕ top X 60 mm ϕ bottom X 30 mm height. Five gravid adult females were introduced per cup and covered with transparent fine nylon mesh. These leaf cups were maintained in an incubator at 25°C under 12L:12D photoperiod and 75±2% RH. Females laid eggs singly in an incision made in the leaf tissue with their ovipositor. The newly emerged larvae were reared on the same leaf disc until they reached the late second instar stage. The second instar larvae failed to pupate on the leaf surface hence the late second instar larvae (two days old) were transferred to a special pupal rearing container for pupation. The special pupal rearing container was standardized after conducting several experiments.

Rearing of pupae: Plastic container of 20 cm ϕ top x 15 cm ϕ bottom x 25 cm height with a closable lid was chosen for the present study. A circle of 5 cm dia was cut on the centre of the container lid and closed with fine transparent nylon mesh to allow ventilation. Rich soil collected from tea field was sieved using No.8 mesh (2057 μ) and mixed with 15 per cent each of vermicompost and dried tea leaf litter. One third of the plastic container was filled by this mixture and dried tea leaves were crushed and again spread over the soil. This acted as a pupal emergence media. For the initial survival of the late second instars fresh green tea leaves were added to ensure adequate supply of food. It is important to ensure that the food is supplied *ad libitum* before it reaches the non-feeding pupal stage. Pupation took place in the soil and leaf litter and the adult emerged within two days after pupation. The emerged adults were transferred to leaf cups to culture subsequent generations.

Life table studies: Life table of *S. bispinosus* was studied under laboratory conditions at three different temperatures *viz.,*20±2°C, 25±2°C and 30±2°C under 12L:12D photoperiod and 75±2% RH. A gravid adult female was introduced on leaf discs of approximately 5 cm^2 placed on agar (0.5%, 10mm thick) in a plastic cup (65 mm ϕ top X 60 mm ϕ bottom X 30 mm height) and allowed to lay eggs. The number of eggs laid by thrips was counted at every 12 h by observing under binocular stereo microscope. Leaf discs containing eggs were removed, labeled and kept separately for further observations on hatching and development. Fecundity of females was recorded till their death. Larvae emerged from the eggs were immediately transferred to leaf discs in plastic containers and reared individually to record the developmental duration. When the larvae entered the late second instar stage, they were transferred individually to pupal rearing containers of size 80 mm ϕ top & bottom x 70 mm height which was prepared as explained above to record the duration of prepupa and pupa. Once the adult emergence was noticed, they were checked under binocular stereo microscope to determine the sex. Accordingly the data were recorded and the developmental duration of both male and female were analysed. Number of female progenies (mx), was calculated using the number of eggs laid per female and female:male ratio. Observations were made daily from hatching

of eggs to emergence and death of adults, which provided the values for life table (lx). Life tables were constructed as per the method of Birch (1948) and Atwal and Bains (1974).

Studies on Morphometrics: For morphometric study, semi-permanent slides were prepared and measurements were taken using calibrated ocular and stage micrometers in a research microscope (ZEISS-Jeneval GF-PA).

Seasonal abundance and vertical distribution: Field studies were carried out between January 2007 and December 2009 to determine the population trends and vertical distribution of *S.bispinosus* on tea bushes. For this purpose, four experimental blocks, designated A, B, C and D each consisting 100 bushes were laid out in UPASI Glysdale Experimental Farm, Coonoor, The Nilgiris, Tamil Nadu, located at an altitude of 1760 m above mean sea level (MSL) and 11.35^{o} N (latitude) 76.82^{o} E (longitude). The bushes in the experimental area were planted in 1982 with the clone UPASI-9 and spaced at 4.0 x 2.5 x 2.5 feet. They were pruned in August 2006 at a height of 60 cm above ground level and the field was not subjected to any pesticide application since pruning.

For assessing thrips populations, fresh tea shoots consisting of three leaves and a bud were collected at fortnightly intervals. From each block, 10 bushes were selected at random and from each bush six shoots were taken, two each from the top, bottom and middle levels. In the present study, shoots from the upper 10 to 15 cm of the bushes (plucking table), were considered "top level shoots" and those present on the bush up to a height of 4 to 40 cm above ground level were treated as "bottom level shoots". The foliage present in between the top and bottom levels was considered as "middle level shoots". The samples from each level of the bush were collected and the number of adults and nymphal instars in the abaxial and adaxial leaf surfaces and predators were counted using a hand lens and data recorded. The shoot samples were then collected in separate polythene bags, labelled and their mouths were tied to prevent the escape of thrips and their predators.

Influence of abiotic factors: Data on abiotic factors such as rainfall, maximum & minimum temperature, relative humidity and sunshine hours were collected from the crop weather observatory at UPASI Glysdale farm, Coonoor.

Influence of age of the field since pruning: To understand the vulnerability of field's age since pruning to thrips infestation, seven experimental plots were laid out in each I, II, III & IV year fields (4 treatments with 7 replications) after pruning and sampling of thrips was done by collecting 25 shoots at random from each block. Leaves were sampled at fortnight intervals on the 14th and 28th of every month. The total number of thrips on each shoot was counted in the field and recorded. Simple correlations were made to study the influence of age of the field since pruning on the incidence of thrips.

Survey on natural enemies: The areas surveyed for natural enemies of tea thrips include Anamallais (Coimbatore Dist., Tamil Nadu), Ooty, Coonoor, Kotagiri and Gudalur (Nilgiris Dist., Tamil Nadu), Vandiperiyar, Peermade and Munnar (Idukki Dist., Kerala), Nelliampathy (Palghat Dist., Kerala) and Wynadd (Wynaad Dist., Kerala). Estates which follow a regular plant protection schedule and also those which neglect this aspect of crop husbandry were included in the survey. One hundred tea shoots (three leaves and a bud) were collected from the fields which were severely infested by tea thrips. The shoots were examined in the field using hand lens for predators and parasitoids and collected in polythene bags for further observation in the lab after proper labeling. Few numbers were preserved in 70% ethyl alcohol for identification and the rest were cultured in the laboratory for further studies.

Statistical Analysis: The data were statistically analysed using IBM SPSS Statistics software version 20 (IBM Corp., USA). Arithmetic mean and standard error (SE) were calculated wherever necessary. Data on developmental duration, vertical distribution were subjected to one way ANOVA with post hoc Tukey's Honestly Significant difference (HSD) test. Data on effect of age of the field since pruning on thrips incidence was analysed using one way ANOVA with Duncan's

post hoc multiple range test. The influence of weather parameters on seasonal abundance was ascertained using multiple regression analysis.

RESULTS AND DISCUSSION

Observations on Life stages

Scirtothrips bispinosus had five life stages *viz*., egg, larva I, larva II, prepupa, pupa and adult (Plate 1). Data on morphometrics of different life stages of *S. bispinosus* are given in Table.1. Tea thrips deposited eggs within the soft tissues of tea leaf using its hook like ovipositor. Eggs were bean shaped and about 0.3 mm long and 0.15 mm wide; at first, they were small, hyaline in nature which could be partially seen as white patches on leaf surface when observed under a stereo microscope. The freshly hatched larva (Larva I) was pale white and turned yellow during development. It measured about 0.50±0.15 mm long and 0.12±0.00 mm wide. Larva II was yellowish and about 0.77±0.01 mm in length and 0.14±0.00 mm in breadth. The prepupa was whitish and was 0.76±0.01 mm long and 0.14±0.00 mm wide. Wing buds did not exceed the third abdominal segment and measured 0.14±0.00 mm. Pupa was white or pale yellow with the eye spots developed. Pupa measured 0.80±0.01 mm long and 0.15±0.0 mm wide. Wing buds exceeded the fourth abdominal segment and measured 0.46±0.01 mm. The newly emerged adults were brown with dark terminal segments. Females were bigger than the males and measured 0.89±0.01 mm long and 0.16±0.00 mm wide. Ovipositor was present on 9th abdominal segment and was about 0.08±0.00 mm long (Plate 2). Male was small, slender and about 0.78±0.00 mm long and 0.15±0.00 mm wide (Plate 3). Recently, Ng *et al.* (2014) re-described *S. dorsalis* from Malaysia based on both morphological and molecular approach. They reported that mean body lengths of first and second instar larvae measured about 370µm and 700µm respectively. The body lengths

Table 1. Morphometrics of different life stages of *S.bispinosus* (*Mean±SE of 10 replications*)

Life stages	Length (mm)	Breadth (mm)
I Instar	0.50±0.01	0.12±0.00
II Instar	0.77±0.01	0.14±0.00
Prepupa	0.76±0.01	0.14±0.00
Prepupa-wing buds	0.14±0.00	0.00±0.00
Pupa	0.80±0.01	0.15±0.00
Pupa-wing buds	0.46±0.01	0.00±0.00
Female	0.89±0.01	0.16±0.00
Antenna	0.18±0.00	0.00±0.00
Wings	0.60±0.00	0.06±0.00
Head	0.06±0.00	0.05±0.00
Pronotum	0.07±0.00	0.06±0.00
Thorax	0.12±0.00	0.12±0.00
Abdomen	0.60±0.00	0.10±0.00
Ovipositor	0.08±0.00	0.03±0.00
Male	0.78±0.00	0.15±0.00
Antenna	0.16±0.00	0.00±0.00
Wings	0.55±0.01	0.05±0.00
Head	0.05±0.00	0.05±0.00
Pronotum	0.10±0.00	0.06±0.00
Thorax	0.17±0.00	0.12±0.00
Abdomen	0.42±0.00	0.09±0.00

of adults ranged from 950 to 1100 µm. Males were similar to females but smaller (<1000 µm).

Influence of Temperature on Life History

Temperature had a direct influence on the life history. Duration of development for different life stages of *S. bispinosus* decreased with increasing temperatures. First and second instar larvae foraged on tea leaf as actively as adults at different temperatures. But, when the second instar larva about to moult into prepupa it stopped feeding and reached the prepupal stage. Both prepupa and pupa remained quiescent and did not feed until it emerged as adult. The developmental time of *S. bispinosus* from egg to adult was found to be relatively similar as reported by Ananthakrishnan (1963) at 25°C. In the present study, *S. bispinosus* reached adulthood faster at higher temperature *i.e.,* 30°C followed by 25°C and 20°C. Total developmental period of male was a little shorter than that of the females (Table 2).

Pre-oviposition, oviposition and post oviposition periods of *S. bispinosus* was comparatively short at 30°C followed by 25°C and 20°C (Table 3). Although oviposition period was longer at lower temperature (20°C), *S. bispinosus* laid less number of eggs when compared to 25°C and 30°C. The other parameters such as oviposition rate, percentage hatchability and survival rates of immature stages were high at 25°C than 20 and 30°C. This is in agreement with the results given for *Heliothrips haemorrhoidalis* with different temperature regimes (Rivnay, 1935; Chhagan and Stevens, 2007). Adult females of *S. bisbinosus* survived longer (20.13±0.19 days) at lower temperature (20°C) but laid less numbers of eggs (16.07±0.43). However, the longevity of *S. bispinosus* decreased with increase in temperature (17.20±0.24 days at 25°C and 14.67±0.23 days at 30°C) and laid 35.80±0.72 and 27.13±0.83 eggs at 25°C and 30°C respectively (Table 3; Fig. 1). Similarly, Teulon and Penman (1991) reported a

Table 2. Developmental duration of the thrips, *S. bispinosus*

Temp. (°C)	Developmental duration in days (Mean±SE)*					
	Egg incubation	I instar	II instar	Prepupa	Pupa	Total
Female						
20	5.30±0.15c	2.40±0.11c	4.25±0.10b	2.35±0.11b	3.85±0.13c	18.15±0.23c
25	3.90±0.12b	1.95±0.05b	2.55±0.11a	1.15±0.08a	3.00±0.07b	12.55±0.15b
30	3.10±0.10a	1.60±0.11a	2.30±0.11a	1.10±0.07a	2.20±0.09a	10.30±0.23a
Male						
20	5.25±0.14c	2.25±0.10c	3.95±0.09b	2.15±0.08b	3.75±0.10c	17.35±0.21c
25	3.85±0.08b	1.90±0.07b	2.40±0.11a	1.10±0.07a	2.90±0.07b	12.15±0.11b
30	2.80±0.09a	1.45±0.11a	2.10±0.07a	1.10±0.07a	2.10±0.07a	9.55±0.15a

*Values followed by same alphabets are not significantly different at 0.05 level (Tukey's HSD)

Table 3. Fecundity, oviposition period and adult longevity of females of *S. bispinosus* at different temperatures

Temp (°C)	N[a]	Total no. of eggs/female[b]	Pre-oviposition period[b]	Oviposition period[b]	Post oviposition period[b]	Total adult longevity[b]
20	15	16.07±0.43c	3.93±0.12b	12.53±0.17c	3.67±0.13b	20.13±0.19c
25	15	35.80±0.72b	2.87±0.17a	11.47±0.22b	2.87±0.17a	17.20±0.24b
30	15	27.13±0.83a	2.40±0.13a	9.07±0.18a	3.20±0.17ab	14.67±0.23a

[a]Number of females tested; [b] values shown are mean±SE and the values followed by same alphabets are not significantly different at 0.05 level (Tukey HSD)

decrease in longevity of *Thrips obscuratus* (Crawford) as temperature increases. Ekesi *et al.* (1999) also found a reduction in adult longevity at high temperatures in addition to reduction in egg production at high photophase in *Megalurothrips sjostedti*. Dev (1964) reported a female biased sex ratio in *S. dorsalis.* However, in the present study, although sex ratio was in favour of females, the ratio of males increased with increasing temperatures. The female: male ratio was observed as 1:0.18, 1:0.24 and 1:0.37 at 20, 25 and 30°C respectively.

Studies on life table parameters revealed that net reproductive rate (Ro), intrinsic rate of natural increase (rm), finite rate of increase (λ) and weekly multiplication (Wm) rates were high at 25°C followed by 30 and 20°C. Similarly, the doubling time was short at 25°C (5.245 days) followed by 30 (6.083 days) and 20°C (10.017) (Table 4). It clearly shows that *S. bispinosus* could multiply faster between 25 and 30°C. *Scirtothrips aurantii* and *S. dorsalis* are reported to cause economic damage to crops over summer when temperatures are high (Bedford, 1943; Shibao, 1996). However, the present study indicates that temperatures below 20°C and above 30°C will slow down the population buildup of *S. bispinosus.*

Seasonal Abundance

The population of tea thrips started increasing from April and reached the peak between May and July and declined after August in Nilgiris (Fig.2). Selvasundaram *et al.* (2004) studied the seasonal abundance of *S. bispinosus* in Vandiperiyar (Idukki district, Kerala) and reported that the population of thrips was more between March and May and decreased during wet months. However, in the present study the population of *S. bispinosus* was high up to the month of July. This is mainly due to the difference in the rainfall and its distribution pattern between Nilgiris and Idukki districts. Moreover, the first phase pruning cycle is normally carried out during the month of April/May in Nilgiris. These pruned fields recover during July and ensures the presence of young succulent foliage which in turn favours the buildup of thrips population. The nymphs mostly occupied the lower surfaces of tea leaves and therefore they escaped from small drizzles and thus available even during wet season. However, heavy rainfall reduced the thrips population considerably as reported by Selvasundaram *et al.* (2004). Multiple regression analysis revealed that population density of *S. bispinosus* showed a negative relationship with rainfall (-0.266), maximum temperature (-38.839) and maximum relative humidity (-3.356) and positive relationship with minimum temperature (63.205), minimum relative humidity (1.686) and sunshine period (2.887) (Table 5). Rattan (1992) reported that in Kenyan tea fields, populations of thrips gradually declined with the onset of monsoon, however prolonged droughts were associated with outbreaks of black tea thrips, *Heliothrips haemorrhoidalis*. Similarly, Sing *et al.* (1999) and Sathyan *et al.* (2017) reported that populations of cardamom thrips, *Sciothrips cardamomi* were high during dry periods and showed positive relationship with sunshine hours and negative relationship with rainfall.

Vertical and Spatial Distribution

Mean number of thrips occupied per shoot was significantly more on the plucking table than on the leaves situated below the plucking table and side branches (Table 6). However there was no

Table 4. Life table parameters of *S.bispinosus* at different temperatures

Temp (°C)	Net reproductive rate (Ro)	Mean generation time (Tc)	Intrinsic rate of natural increase (rm)	Finite rate of increase (λ)	Weekly multiplication (Wm)	Doubling time (DT)
20	6.178	26.316	0.069	1.072	1.623	10.017
25	15.613	20.794	0.132	1.141	2.522	5.245
30	6.331	16.196	0.114	1.121	2.22	6.083

Table 5. Multiple regression analysis among weather factors and *S. bispinosus*

Variable	Partial regression Coefficient	SE	T	P	R^2	F
X_1 Temperature minimum (°C)	63.205	52.457	1.205	0.238	0.512	5.074
X_2 Temperature maximum (°C)	-38.839	56.075	-0.693	0.494		
X_3 Relative humidity minimum (%)	1.686	2.901	0.581	0.566		
X_4 Relative humidity maximum (%)	-3.356	5.527	-0.607	0.548		
X_5 Rainfall (mm)	-0.266	0.100	-2.647	0.013		
X_6 Sunshine (h)	2.887	29.229	0.099	0.922		

Y = 307.968 + 63.205, * X_1 - 38.839, * X_2 +1.686, * X_3 -3.356, * X_4 -0.266, * X_5 + 2.887, * X_6, *Significant at $P < 0.001$

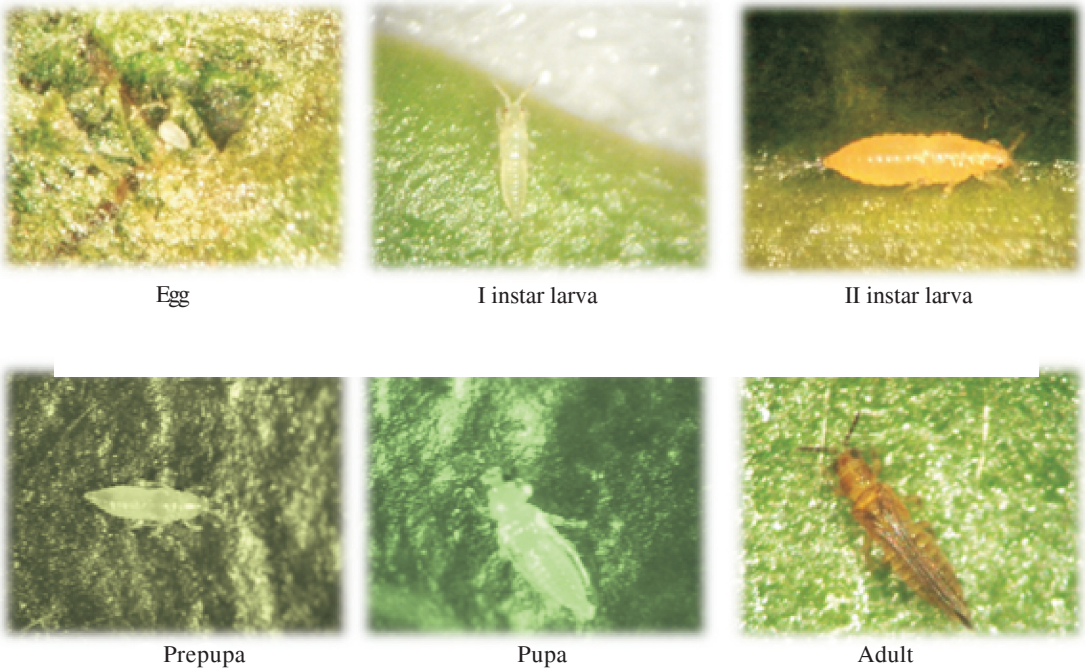

Plate 1. Life stages of *S. bispinosus*

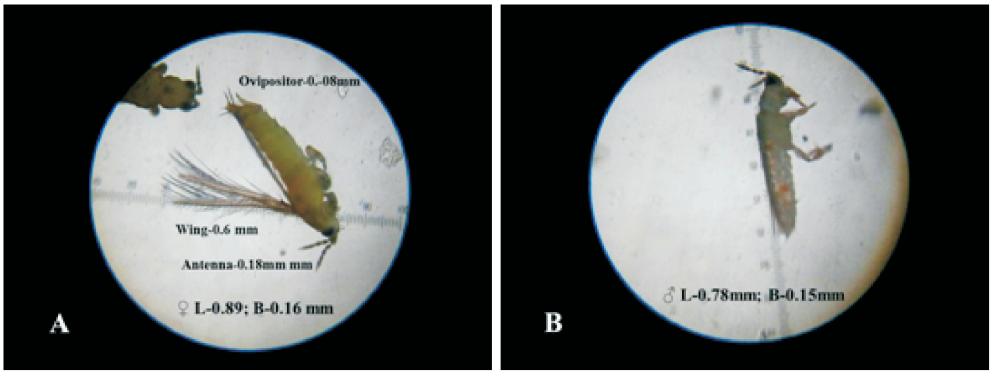

Plate 2. Morphometry of female (A) and male (B), *S. bispinosus*

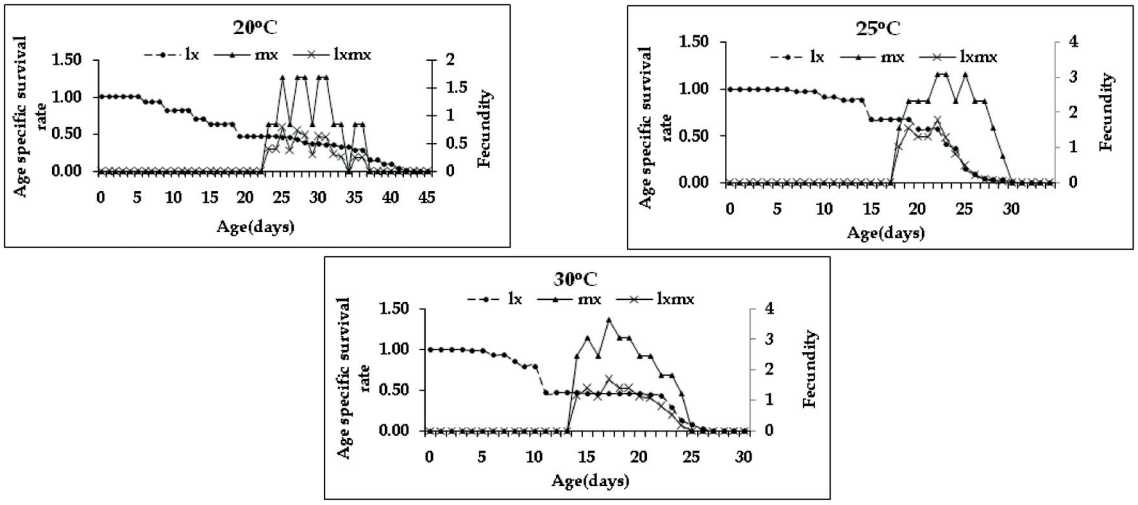

Fig. 1. Age specific survival rate (lx) age-specific fecundity rate (mx) and lxmx curves in *S.bispinosus*[lx=(eclosion of eggs) x (proportion of females alive at age x); mx= (proportion of females) x (age specific oviposition)]

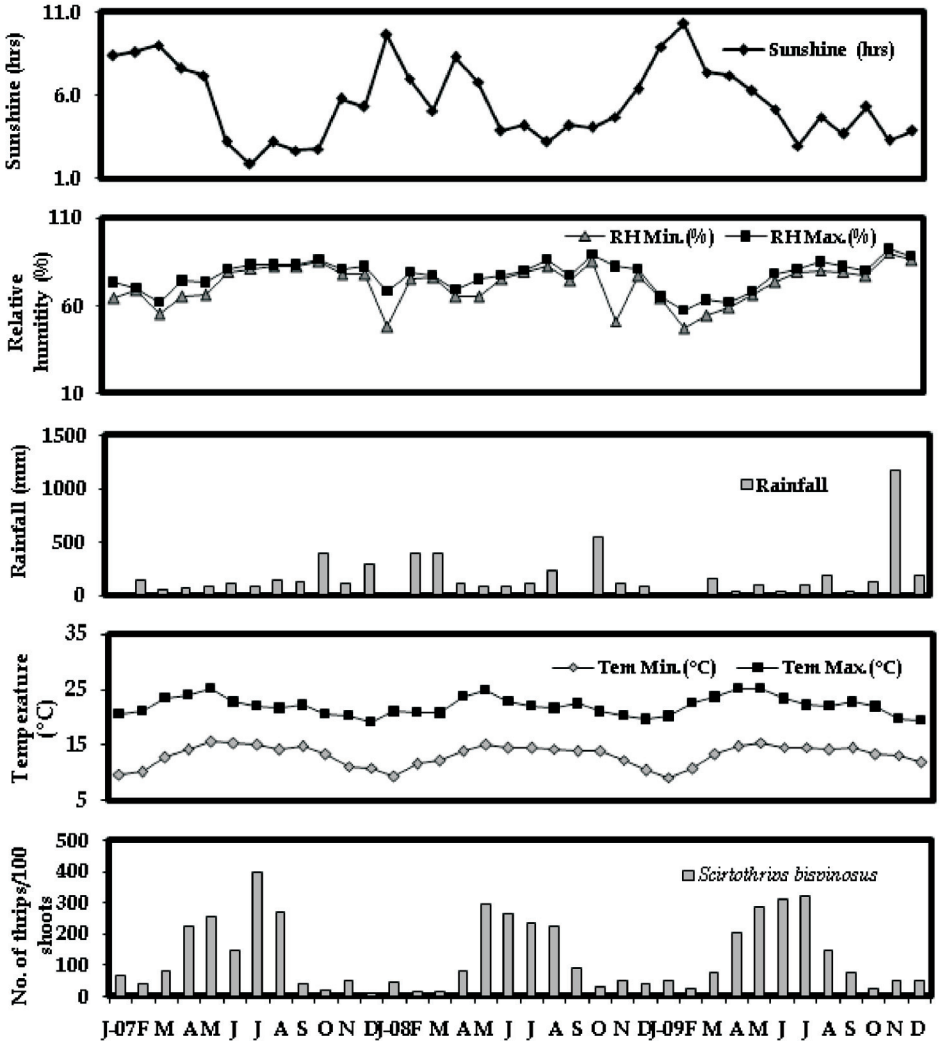

Fig. 2. Seasonal abundance of *S.bispinosus* and weather factors at Coonoor, The Nilgiris

Table 6. Vertical distribution of *S. bispinosus* on tea bushes

Area of bush	Mean No. of thrips*
Plucking table	13.8±1.40b
Below the plucking table	5.92±0.59a
Side branches	3.84±0.58a

*the values shown are Mean ± SE and the values followed by the same alphabets are not significantly different at 0.05 level (Tukey HSD)

Table 7. Influence of age of the field (since pruning) on the incidence of *S. bispinosus**

Year from Pruning	Mean No. of thrips/shoot
I Year	5.40c
II Year	2.96b
III Year	0.70a
IV Year	0.44a

*values followed by the same alphabets are not significantly different at 0.05 level (Duncan Multiple Range test)

significant difference in the number of thrips on the leaves present below the plucking table and side branches. About 72% of the active population (nymphs and adults) was observed on the lower surface of tea leaves (Fig. 3).

Age of the bushes since pruning on thrips incidence

The age of the bush since pruning had a definite influence on the incidence of *S. bispinosus*. The mean number of thrips per shoot was significantly high in first year after pruning followed by second, third and fourth year fields (Table 7). The closeness of fit between thrips population and age of the bushes from pruning was measured and the correlation was positively significant (P =0.05) (Fig.4). The main reason for the higher incidence of tea thrips in the first year after pruning was due to the presence of succulent tea shoots in the bushes. Similarly, Rahman *et al.,* (2014) reported that *S. dorsalis* prefer buds and tender leaves and show less preference to the mother leaf *i.e.* 4th leaf from the apical bud. Radhakrishanan and Mahendran (2010) reported an 'edge effect' which had direct influence on the population density of *S. bispinosus.* They reported that number of thrips per shoot was more on the edges *i.e.,* near the roads and foot paths than the bushes in the center of the field.

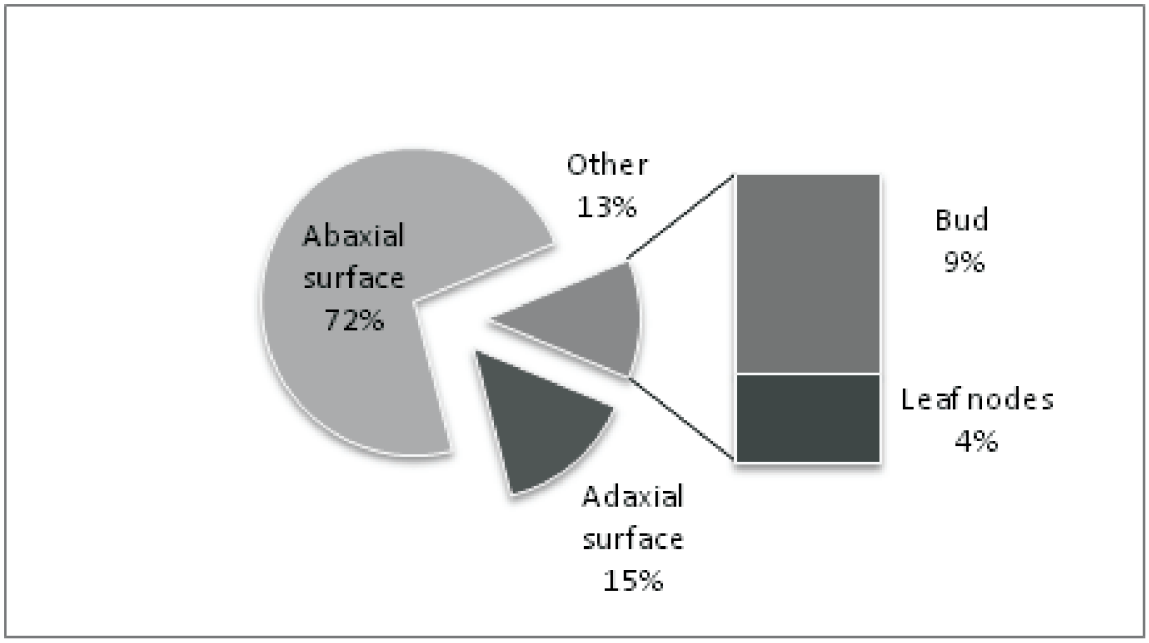

Fig. 3. Distribution of tea thrips (adults and nymphs) on tea shoots

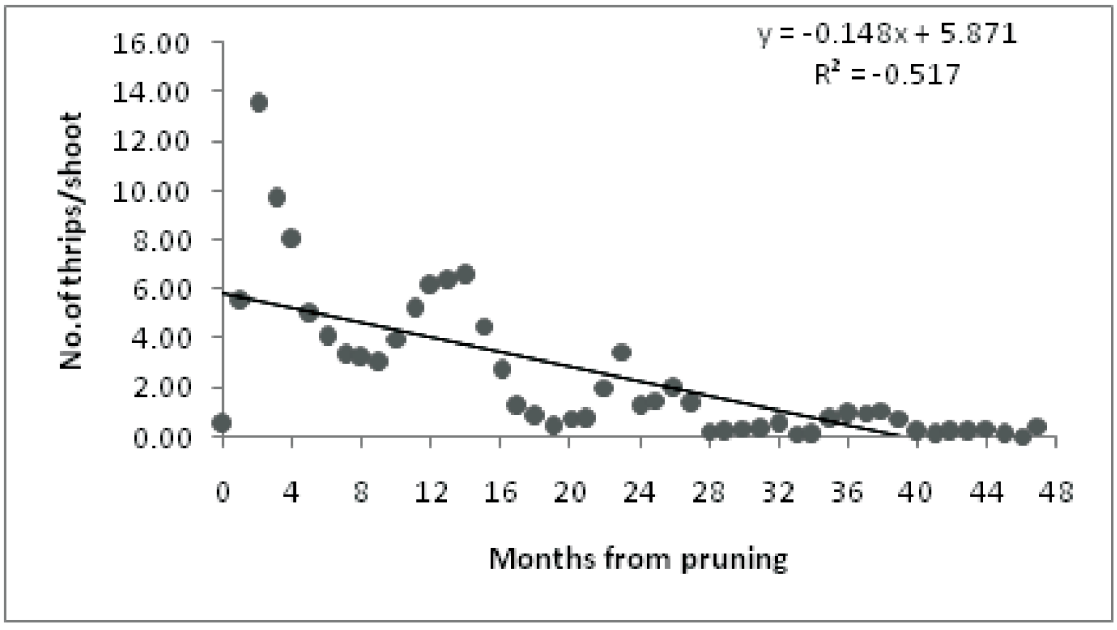

Fig. 4. Relationship between the age of the field since pruning and the population density of *S.bispinosus*

Table 8. Natural enemies of tea thrips, *S. bispinosus*

Sl. No	Species	Family	Order	Period of the peak activity	Distribution
Predatory Mites					
1	*Amblyseius cucumeris*	Phytoseiidae	Acarina	April-Sep	Coonoor, Vandiperiyar, Munnar
2	*Amblyseius fallacies*	Phytoseiidae	Acarina	April-Sep	Coonoor, Vandiperiyar, Munnar
3	*Amblyseius degenerans*	Phytoseiidae	Acarina	April-Sep	Vandiperiyar, Munnar
4	*Balaustium* sp.	Erythraieidae	Acarina	April-Sep	Munnar
Predatory Thrips					
5	*Franklinothrips vespiformis*	Aeolothripidae	Thysanoptera	April-Sep	Coonoor, Vandiperiyar, Munnar
6	*Leptothrips mali*	Aeolothripidae	Thysanoptera	April-Jul	Coonoor
Anthocorid Bug					
7	*Orius* sp.	Anthocoridae	Coleoptera	Apr-Jul	Coonoor, Munnar
Nymphal parasitoid					
8	*Trichogramma* sp.	Trichogrammatidae	Hymenoptera	Apr-Jul	Coonoor

Studies on natural enemies

Extensive survey's carried out in the tea plantations of south India revealed the presence of eight species of natural enemies which were found preying & parasitizing on *Scirtothrips bispinosus.* Among them, four were predatory mites (*Amblyseius cucumeris, A. fallacis, A. degenerans* and *Balaustium* sp.); one anthocorid predator (*Orius* sp.), two predatory thrips (*Franklinothrips vepiformis* and *Leptothrips mali*) and a nymphal parasitoid, *Trichogramma* sp. Peak activity of predatory mites and *F. vepiformis* were seen between April and September and *L. mali, Orius* sp. and *Trichogramma* sp. between April and July (Table 8). Among the natural enemies identified, predatory potential of *F. vespiformis* on *S. bispinosus* was already reported by the authors (Mahendran and Radhakrishnan, 2019).

It is established that the development of *S. bispinosus* could multiply faster between 25 and 30°C. It is also understood that high rainfall, maximum temperature and relative humidity affected the population of tea thrips in the field and minimum temperature, minimum relative humidity and long sunshine hours favoured the buildup of thrips population to certain extend. The studies conducted on vertical and spatial distribution of thrips on tea bushes revealed that major population of thrips occupied the top layer of the tea bush *i.e.,* the plucking table and more particularly on the abaxial (lower) surface of young tea leaves. The present study has also ascertained that fields recovering from pruning harboured more numbers of thrips followed by second, third and fourth year fields in a pruning cycle. There are eight species of natural enemies reported in the present study which helps the scientists to explore its potential in controlling *S. bispinosus.*

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Comparative studies of mymarid diversity from three different zones of paddy ecosystem in Tamil Nadu, India

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ABSTRACT: Surveys were conducted to explore the mymarid fauna from three different rice growing zones *viz.,* western zone, Cauvery delta zone and high rainfall zone in Tamil Nadu during 2015-16. In the present study, 92 mymarid parasitoids comprising of 8 species under 7 genera *viz., Anagrus* sp., *Anaphes* sp., *Camptoptera* sp., *Dicopus longipes* (Subba Rao), *Lymaenon delhiensis* Narayanan and Subba Rao, *Lymaenon munnarus* Mani and Saraswat, *Mymar pulchellum* Curtis and *Ptilomymar dictyon* Hayat and Anis were collected. Alpha and beta diversity were computed for the three zones and the diversity indices (Simpson's index, Shannon-Wiener index, Pielou's index) revealed high rainfall zone as the most diverse zone, while Cauvery delta zone being the least diverse. *Dicopus longipes* is found to the predominant species in rice ecosystem**.** Jaccard's index of species similarity comparison revealed 42.5 per cent similarity between western and Cauvery delta zones and 62.5 per cent similarity between high rainfall and Cauvery delta zones and 62.5 per cent similarity between high rainfall and western zones. Correspondence analysis and Bray-curtis cluster analysis were also done to understand the diversity assemblage of the mymarids that were collected.

KEY WORDS: Parasitoids, Mymaridae, rice, ecosystem

INTRODUCTION

Rice (*Oryza sativa* L.), is an annual grass native to Asia. Rice fields, together with the associated irrigation ponds, ditches and ridges often constitute the traditional landscape in rural environments and are a key ecosystem of Asia (Kiritani, 2009). Tamil Nadu is one of the leading rice growing states in India, has been cultivating rice from time immemorial as this state is endowed with all favourable climatic conditions for paddy crop. Rice fields harbour a rich and varied fauna than any other agricultural crop (Heckman, 1979; Fritz *et al.,* 2011). The fauna is dominated by micro, meso and macro arthropods inhabiting the soil, water and vegetation sub-habitats of the rice fields. The different communities of terrestrial arthropods in the rice field include pests, their natural enemies (predators and parasitoids) and other neutral insects that inhabit or visit the vegetation as tourists (Heong *et al*., 1991). Insect pests are reported as the major threat to its production. More than 800 species of insects are known to infest rice, of which about 20 species are of economic importance. The overall losses due to insect pest damage in rice are estimated as 25 per cent (Pathak and Dhaliwal, 1981; Dale, 1994).

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Farmers generally rely on insecticides to combat pest problems of rice. Indiscriminate use of insecticides resulted in the loss of biodiversity of beneficial organisms like parasitic hymenopterans (Dudley *et al.,* 2005). Reducing the mortality of parasitic hymenopterans caused by insecticides is essential for greater sustainability in rice pest management (Heong and Hardy, 2009; Gurr *et al*., 2011). If the use of insecticides is to be reduced through Integrated Pest Management, then the consequent reduction in pest control has to be augmented in some way and no doubt, parasitic hymenopterans especially mymarids are the best alternatives to pesticides. They show greater stability to ecosystem than any group of natural enemies of insect pests because they are capable of living and interacting at lower host population level. To aid of pest control, it is essential that the diversity of mymarids needs to be studied (Yoshimoto, 1975).

Mymaridae are internal primary parasitoids of insect eggs (Huber, 1986), particularly Auchenorrhyncha whose eggs are concealed/ embedded within plant tissues, under bark, and in soil. Pupation occurs inside the host egg. Some species have been successful in biological control programs (Clausen, 1978). Diversity of mymarids associated with rice ecosystem is inadequately studied in Tamil Nadu. Hence, the present study was undertaken to explore the diversity of mymarid fauna in rice ecosystems of Tamil Nadu.

MATERIALS AND METHODS

Sites of collection: The survey was carried out in the paddy fields during 2015-16 in three different agro climatic zones of Tamil Nadu *viz.,* western zone (District representation: Coimbatore at, Paddy Breeding Station, Coimbatore, 427 m, 10° 59' 43.24'' N 76° 54' 59.22'' E), Cauvery delta zone (District representation: Thiruvarur at, Krishi Vigyan Kendra, Needamangalam, 26 m, 10° 46' 23.93'' N 79° 25' 0.96'' E) and high rainfall zone (District representation: Kanyakumari at Agricultural Research Station, Thirupathisaram, 17 m, 8° 12' 16.70'' N 77° 26' 57.84'' E). Collections were made for 20 consecutive days in each zone to provide the same weightage as well as minimize chances of variations in collection. The sampling time is decided based on the rice growing season and the stages of the crop *i.e.,* 20 days during August-September, 2015 in western zone, October-November, 2015 in high rainfall zone and December, 2015 – January 2016, in Cauvery delta zone.

Methods of collection: A total of three different gadgets *viz.,* sweep net, yellow pan trap kept at ground level and yellow pan trap erected at canopy levels were used. All the three gadgets were employed continuously for 20 days.

Sweep Net: The portable round sweep net (673 mm mouth diameter and a 1076 mm long aluminum handle) is employed in the present study. Sweeping of vegetation was as random as possible from ground level to the height of the crop. One to and fro motion of the sweep net was considered as one sweep. Thirty sweeps were made half an hour per day in the early morning and late evening hours.

Yellow pan traps kept at ground level: Yellow pan traps are shallow trays of 133 mm × 195mm and 48 mm deep with bright yellow colour. The traps were filled (3/4) with water, a few drops of liquid detergent (to break the surface tension) and a pinch of salt (to reduce the rate of evaporation and to prevent rotting of trapped insects). Twenty yellow pan traps were placed at ground level in each site on the bunds with 1.5 m distance each. The traps were serviced after 24 hours.

Yellow pan traps erected canopy level: Ten erected yellow pan traps per site were installed at the crop canopy with a help of polyvinyl chloride pipes. Rests of the protocol were followed as mentioned previously.

Preservation and identification of the specimens: The collected parasitoids were preserved in 70% ethyl alcohol. The dried specimens were mounted on pointed triangular cards and studied under a Stemi (Zeiss) 2000-C and Photographed under Leica M 205-A stereo zoom microscopes and identified through appropriate keys.

Measurement of diversity:

Relative Density: Relative density of the species was calculated by the formula, Relative Density (%) = (Number of individuals of one species / Number of individuals of all species) X 100.

Alpha Diversity: Alpha diversity of the zones was quantified using Simpson's diversity Index (*SDI*) Shannon-Wiener index (*H'*), Margalef Index (α) and Pielou's Evenness Index (*E1*).

Simpson's Index: Simpson's diversity index is a measure of diversity which takes into account the number of species present, as well as the relative abundance of each species. It is calculated using the formula, $D = \Sigma n\ (n-1)/ N(N-1)$ where n = total number of organisms of a particular species and N = total number of organisms of all species (Simpson 1949). Subtracting the value of Simpson's diversity index from 1, gives Simpson's Index of Diversity (SID). The value of the index ranges from 0 to 1, the greater the value the greater the sample diversity.

Shannon-Wiener Index: Shannon-Wiener index (*H'*) is another diversity index and is as follows: $H' = -\Sigma\ Pi\ \ln(Pi)$, where $Pi = S / N$; S = number of individuals of one species, N = total number of all individuals in the sample, *ln* = logarithm to base e (Shannon & Wiener 1949). The higher the value of *H'*, the higher the diversity.

Margalef Index: Species richness was calculated for the three zones using the Margalef index which is given as Margalef Index, $\alpha = (S - 1) / \ln (N)$; S = total number of species,

N = total number of individuals in the sample (Margalef 1958).

Pielou's Evenness Index: Species evenness was calculated using the Pielou's Evenness Index (*E1*). Pielou's Evenness Index, $E1=H'/ \ln(S)$; H' = Shannon-Wiener diversity index, S = total number of species in the sample (Pielou 1966). As species richness and evenness increase, diversity also increases (Magurran 1988).

Beta Diversity: Beta diversity is a measure of how different (or similar) ranges of habitats are in terms of the variety of species found in them. The most widely used index for assessment of Beta diversity is Jaccard Index (JI) (Jaccard 1912), which is calculated using the equation: JI (for two sites) = $j / (a+b-j)$, where j = the number of species common to both sites A and B,

a = the number of species in site A and b = the number of species in site B. We assumed the data to be normally distributed and adopted parametric statistics for comparing the sites.

Statistical analysis: The statistical test ANOVA was also used to check whether there was any significant difference in the collections from three zones. The data on population number were transformed into X+0.5 square root before statistical analysis. The mean individuals caught from three different zones were analyzed by adopting Randomized block design (RBD) to find least significant difference (LSD). Critical difference (CD) values were calculated at five per cent probability level. All these statistical analyses were done using Microsoft Excel 2016 version and Agres software version 3.01. The multivariate analyses were carried out using PRIMER 7 (Clarke and Gorley, 2015)

RESULTS

In the present study, a total of 92 mymarid individuals comprising of 8 species under 7 genera *viz., Anagrus* sp., *Anaphes* sp., *Camptoptera* sp., *Dicopus longipes* (Subba Rao), *Lymaenon delhiensis* Narayanan and Subba Rao, *Lymaenon munnarus* Mani and Saraswat, *Mymar pulchellum* Curtis and *Ptilomymar dictyon* Hayat and Anis were collected. The host details of the 08 species of mymarids are tabulated (Table. 1).

Dicopus longipes (Subba Rao) is found to the predominant species in paddy ecosystem with a relative abundance of 33.7 per cent. Five species of mymarids were collected from western zone and Cauvery delta zone each whereas 8 species were collected from high rainfall zone. *Anagrus* sp., *Anaphes* sp., and *M. pulchellum* were found in all the three zones. *Lymaenon munnarus* was collected only from high rainfall zone, whereas *D.*

Table 1. Host details of Mymaridae collected in the present study

Parasitoid	Host	Reference
Anagrus sp.	Eggs of Cercopidae, Tingidae, and Odonata (Zygoptera), Cicadellidae and Delphacidae	Lin *et al.,* 2007
Anaphes sp.	Eggs of Curculionidae and Chrysomelidae; also from Argidae, Brenthidae, Byrrhidae, phydridae, Gerridae, Miridae, Tephritidae and Tipulidae	Lin *et al.,* 2007
Camptoptera sp.	Eggs of Scolytidae and Buprestidae, and possibly Cicadellidae, Aleyrodidae and Thripidae	Lin *et al.,* 2007
Dicopus longipes (Subba Rao)	Hemiptera: Diaspididae (*Fiorinia fioriniae* and *Unaspis euonymi*)	Lin *et al.,* 2007
Lymaenon delhiensis Narayanan & Subba Rao	Unknown	-
Lymaenon munnarus (Mani & Saraswat)	Eggs of *Cofana spectra* (Distant), *Nephotettix nigropictus* (Stal); *N. virescens* (Distant)	Zeya and Hayat, 1995
Mymar pulchellum Curtis	Unknown	–
Ptilomymar dictyon Hayat and Anis	Unknown. Probably an aquatic insect because *Ptilomymar* spp. are almost collected with yellow pan traps set on or beside running or standing water	Lin *et al.,* 2007

longipes, and *Comptoptera* sp., were collected from both Cauvery delta and high rainfall zones. *Lymaenon delhinensis* and *P. dictyon* were common to western and high rainfall zones. A significant difference is observed via ANOVA in the occurrence of *D. longipes*, *L. munnarus* and *M. pulchellum* between the zones (Table 2).

Table 2. Comparison of Mymaridae collected from three paddy growing zones of Tamil Nadu

Species	Zones						Total			
	Western		Cauvery Delta		High Rainfall					
	No.	%	No.	%	No.	%	No.	%	F	P
Anagrus sp.	1	6.6	2	4.8	1	2.7	4	4.4	1.66	0.84
Anaphes sp.	1	6.6	3	7.3	6	16.7	10	10.8	1.86	0.16
Camptoptera sp.	0	0.0	5	12.1	1	2.7	6	6.5	2.97	0.05
Dicopus longipes	0	0.0	22	53.6	9	25.0	31	33.7	9.48	0.00
Lymaenon delhiensis	4	26.7	0	0.0	4	11.1	8	8.7	1.40	0.24
Lymaenon munnarus	0	0.0	0	0.0	8	22.2	8	8.7	2.92	0.00
Mymar pulchellum	2	13.3	9	21.9	5	13.8	16	17.4	3.34	0.04
Ptilomymar dictyon	7	46.7	0	0.0	2	5.5	9	9.8	2.58	0.08
Total No. collected	15	-	41	-	36	-	92	-	-	
Species Number	05	-	05	-	08	-	06	-		

%- Relative Density, No.- Total number of individuals collected, F-Value, P-Value

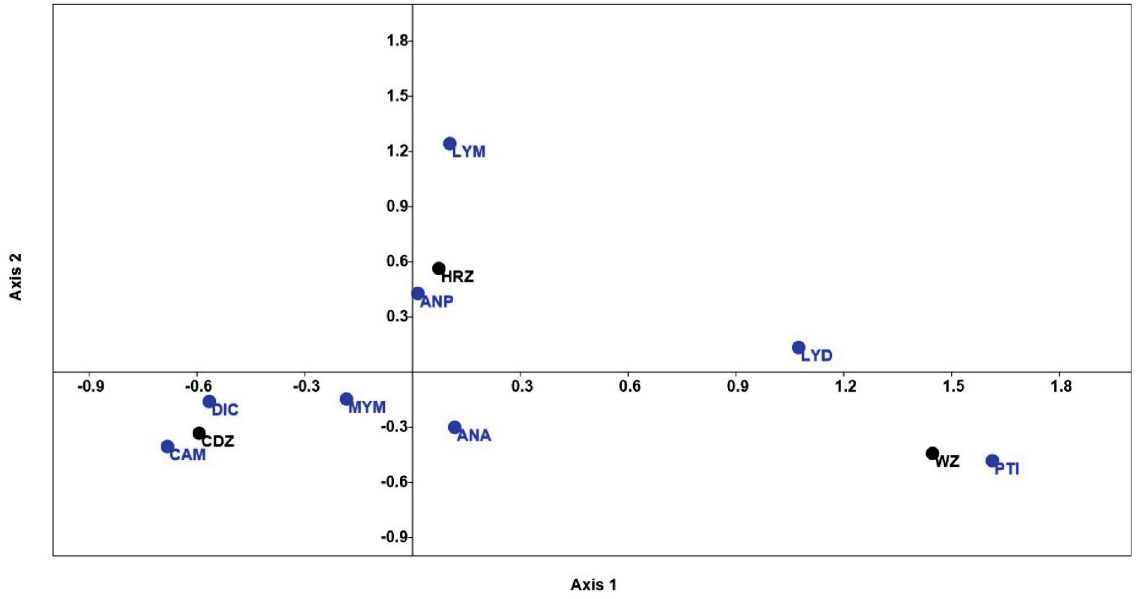


Fig. 1. Correspondence Analysis of mymarid species from three different zones in Tamil Nadu [WZ – Western Zone; CDZ – Cauvery Delta Zone; HRZ – High Rainfall Zone; ANA - *Anagrus*; ANP - *Anaphes*; CAM - *Camptoptera*; DIC - *Dicopus longipes*; LYD - *Lymaenon delhiensis*; LYM - *Lymaenon munnarus*; MYM - *Mymar pulchellum*; PTI - *Ptilomymar dictyon*]

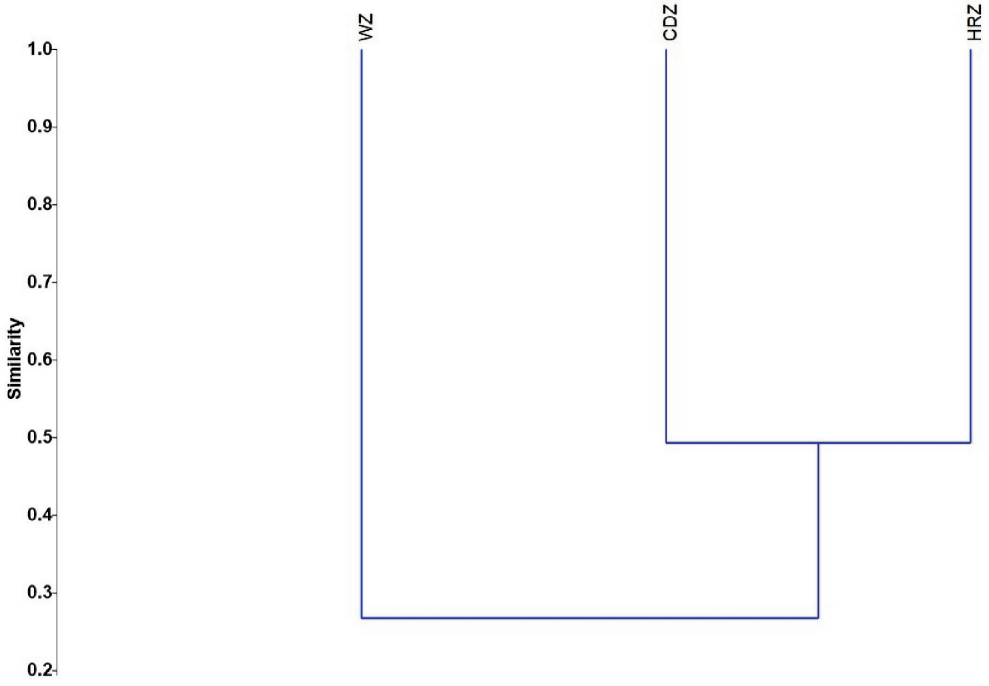


Fig. 2. Bray-curtis cluster anlaysis [WZ – Western Zone; CDZ – Cauvery Delta Zone; HRZ – High Rainfall Zone

Anagrus sp.

Anaphes sp.

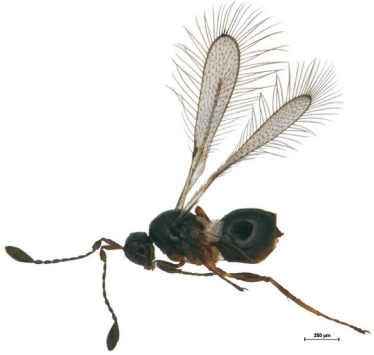
Camptoptera sp.

Dicopus longipes

Lymaenon delhiensis

Lymaenon munnarus

Mymar pulchellum



Ptilomymar dictyon

Plate 1. Eight species of Mymaridae collected from three paddy growing zones of Tamil Nadu

Table 3. Diversity indices of Mymaridae from three paddy growing zones of Tamil Nadu

Zones	Mean No. of Mymaridae collected/day	Std. Error	SID	*H'*	α	*E1*	β %
Western	0.75 (1.06)[b]	± 0.20	0.73	0.58	1.47	0.36	W and C – 42.8
Cauvery Delta	2.05 (1.47)[a]	± 0.46	0.65	0.54	1.07	0.33	C and H - 62.5
High Rainfall	1.80 (1.40)[ab]	± 0.42	0.84	0.80	1.95	0.38	H and W – 62.5
S.ED	0.17	-	-	-	-	-	-
CD (p=0.05)	0.35	-	-	-	-	-	-

Figures in parentheses are square root transformed values; In a column, means followed by a common letter(s) are not significantly different by LSD (p=0.05).
SID- Simpson's Index of Diversity, *H'*- Shannon-Wiener Index, α- Margalef index,
E1- Pielou's index, β-Beta diversity (Jaccard Index).
W- Western Zone, C- Cauvery Delta Zone, H- High Rainfall Zone

A mean of 2.05 ± 0.46 mymarids was collected per day from Cauvery delta zone whereas, and from western and high rainfall zones it was 0.75 ± 0.20 and 1.80 ± 0.83, respectively (Table 3). The Simpson's index of diversity was the highest for high rainfall zone (0.84), followed by western zone (0.73) and Cauvery delta zone (0.65). Similar trend was observed in Shannon-Wiener index also, for Western, Cauvery delta and High rainfall zones with values of 0.58, 0.54 and 0.80, respectively. Maximum species richness (1.95) was found in High rainfall zone followed by western zone (1.47) and Cauvery delta zone (1.07), as revealed by Margalef index. The species evenness was recorded maximum in High rainfall zone (0.38) followed by Western zone (0.36) and Cauvery delta zone (0.33).

DISCUSSION

Daniel *et al.* (2017) obtained similar results by conducting experiments to assess the diversity of pteromalids of paddy ecosystems in Tamil Nadu. The species composition among elevation zones can indicate how community structure changes with biotic and abiotic environmental pressures (Shmida and Wilson 1985; Condit *et al.,* 2002). Studies on the effect of elevation on species diversity of taxa such as spiders (Sebastian *et al.,* 2005), moths (Axmacher and Fiedler 2008), paper wasps (Kumar *et al.,* 2008) and ants (Smith *et al.,* 2014) reported that species diversity decreased with increase in altitude. However, according to Janzen (1976), diversity of parasitic Hymenoptera is not as proportionately reduced by elevation as in other insect groups, a fact that is in supports to results of present study. A similar study conducted by Shweta and Rajmohana (2016) to assess the diversity of members belonging to the subfamily Scelioninae also declared that the elevation did not have any major effect on the overall diversity patterns. Similar trend was observed for Shannon- Wiener index also.

Jaccard's index of species similarity comparison revealed that 42.8 per cent similarity between Western and Cauvery delta zones and 62.5 per cent similarity between High rainfall and Cauvery delta zones and 62.5 per cent similarity between High rainfall and Western zones. The elevational diversity gradient (EDG) in ecology proposes that species richness tends to increase as elevation increases, up to a certain point creating "diversity bulge" at moderate elevations (McCain and Grytnes, 2010). The elevation dealt with the present study is ranged from 17-427 m, in this range, species diversity and richness is not showed any correlation *i.e.,* species diversity and richness were not proportional with elevation. Daniel and Ramaraju (2017) were assessed the diversity of Chalcididae among three paddy growing tracts of Tamil Nadu and concluded that there was no correlation between elevation and species richness.

Studies on the altitudinal variation of parasitic Hymenoptera assemblages in an Australian sub-

tropical rainforest by Hall *et al.* (2015), didn't find any distinct assemblage at each altitude, at the morphospecies level, whereas, clear separation between 'upland' and 'lowland' assemblages. The area under cultivation turns out to be a very important factor with respect to abundance and species density in paddy fields (Wilby *et al.* 2006).

The correspondence analysis of species of mymarids were done for three different paddy growing zones (Western Zone, Cauvery Delta Zone and High rainfall Zone) of Tamil Nadu. The contribution of *Dicopus longipes* (DIC), *Camptoptera* (CAM) and *Mymar pulchellum* (MYM) were observed more towards CD zone whereas the contribution of *Anaphes* (ANP) and *Lymaenon munnarus* (LYM) were observed more towards HR zone. The western zone got outlier due to the contribution of *Ptilomymar dictyon* (PTI) and *Lymaenon delhiensis* (LYD). Similarly, the western zone got outlier in Bray-curtis cluster anlaysis (Fig. 2) where the similarity of the site is less than 30% with the remaining selected study sites. In contrast, a cluster was formed between CDZ and HRZ with 50% similarity based on the observed species abundance (Fig.1).

This study reveals the diversity of mymarids of three different paddy ecosystems of Tamil Nadu, where the High rainfall zone is the most diverse and the Cauvery delta zone being the least. The reasons for the significant changes in diversity of parasitoids and their host insects are to be further studied.

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Volatile metabolites of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and their toxicity to brinjal mealybug *Coccidohystrix insolita* (G)

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ABSTRACT: *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno is a versatile indigenous entomopathogenic fungus with high speed of kill on hemipteran insects. Investigations were carried out to explore the volatile metabolites of *L. saksenae* and bioefficacy of its crude toxin to different life stages of brinjal mealybug, *Coccidohystrix insolita.* GCMS spectrum of crude toxin extracted from cultures grown in potato dextrose broth and Czapak Dox medium revealed the presence of 25 compounds each. The major secondary metabolites identified were 2,6 pyridine dicarboxylic acid (dipicolinic acid), n-hexadecanoic acid, octadecanoic acid, harmine, dl- mevalonic acid lactone, 2-piperidinone, 4H-pyran-4-one 2,3-dihydro-3,5dihydroxy-6methyl, acetamide,N-(2-phenylethyl), pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro. The biological properties of these compounds include insecticidal to nematicidal and antimicrobial activities. Bioefficacy studies with crude toxins revealed the toxicity of secondary metabolities to *C. insolita.* The dose dependent bioassay revealed 100 per cent moratality at a higher concentration of 1000 ppm at 72 and 96 h after treatment on nymphs and adults respectively. Results highlighted the role of secondary metabolites in the pathogenicity of *L. saksenae* and pave way to the utilization of its biocide molecules in safer pest management.

KEY WORDS: Dipicolinic acid, volatile metabolites, *Lecanicillium saksenae* , GCMS

INTRODUCTION

Entomopathogenic fungi constitute the largest taxa of insect pathogens with approximately 750 to 1000 species distributed over 100 genera placed under the order Hypocreales and Entomophthorales (St. Legar and Wang, 2010). They are known to synthesize different secondary metabolites that act as toxins in insects resulting in a series of symptoms such as convulsions, lack of coordination, behaviour alteration, feeding cessation, paralysis and death. The first systematic study of toxin production by fungal entomopathogens *in vitro* was conducted on *Metarhizium anisopliae* which lead to the discovery of two novel insecticidal substances destruxin A and destruxin B (Kodaira, 1961). The genera *Beauveria* and *Lecanicillium* are also known to produce toxic metabolites *in vitro* and

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few of them have been characterized for their metabolites.

The genus *Lecanicillium* includes various species which are pathogenic to a broad range of pests such as insects, phytophagous mites, plant parasitic nematodes, and plant pathogens as well. *L. saksenae* ITCC - LsVs 1-7714 is an indigenous isolate from Kerala, India (Rani *et al*., 2015). It is pathogenic to sucking pests including the true bugs of Heteroptera (Sankar and Rani, 2018). *L. saksenae* which is speculated to be a potent toxin producer, as evidenced by the knock down action in rice bug *Leptocorisa acuta* Thunberg warrants investigation on its metabolite profile. An insight into the identification and bioassay of extracellular metabolites produced by *L. saksenae* would pave way for the development of novel biomolecules with potent insecticidal activity. Recent advances in the analytical techniques facilitate easier separation, identification and structural determination of biomolecules. Among different analytical techniques, GCMS is an authoritative tool for identification and quantification of volatile molecules. In this study, we attempted to profile the volatile secondary metabolites produced by *L. saksenae* by GCMS analysis and assessed their bioefficacy on one of its homopteran host *viz*. brinjal mealybug, *C.insolita.*

MATERIALS AND METHODS

Fungal culture and extraction of metabolites

Culturing was carried out in 250 ml Erlenmayer flasks containing 75 ml of growth media. *L. saksenae* (ITCC Accession No: LsVs1 -7714) was inoculated into two different media *viz.* potato dextrose broth (P^H 5.6) and Czapak Dox medium (P^H 7) with conidial suspension @ $1x10^7 mL^{-1}$. These cultures were incubated at 27°C in an incubator shaker with 150 rpm for 12 days. They were then centrifuged at 4°C for 10 min at 10000 rpm, to remove the mycelia and spores and the supernatants were filtered through millipore filters of pore size 0.45 µm. The filtrates were then divided into 100 mL aliquots and then extracted thrice with equal quantity of ethyl acetate for 10 min at 250 rpm. Ethyl acetate fractions from two different growth media were collected separately and concentrated under vacuum rotary evaporator at 40°C. Dried extracts were reconstituted with methanol and subjected to chromatographic analysis.

Gas chromatography - mass spectrometry (GCMS) analysis

The ethyl acetate extracts of *L. saksenae* cultured in PDB and Czapak Dox media were analysed separately in a Perkin Elmer (CLARUS SQ8C) system equipped DB-5 MS capilary standard non – polar column (dimension: 30mts, Id: 0.25 mm, film: 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}; split ratio of 10 : 1; mass scan 50-600 Da; ionization energy,70 eV; ion source temperature, 240^{0}C and injector temperature, 250^{0}C. The oven temperature was programmed initially at 60^0 C for 2 min, raising by 10^0 C min^{-1} to 300 ^{0}C and then held isothermally for 6 min at 300^{0}C, with a total run time of 35 min. The chemical compounds were identified and characterized based on their retention time (RT). The mass spectral data retrieved from GC-MS was computer matched with those of the standards available in the the database of National Institute of Standards and Technology (NIST) 08 mass spectrum library, having more than 62,000 patterns.

Bioassay

Bioassay was carried out in different life stages of *C. insolita*. The test insects were reared on sprouted potato tubers under controlled conditions of 27°C (Mani and Shivaraju, 2016). The crude toxin extracted from PDB was dissolved in sterile distilled water and centrifuged at 10000 rpm for 5 minutes. The supernatant were made up to the test concentrations of 10, 50, 100, 250, 500 and 1000 ppm, by mixing it with distilled water containing 0.03 per cent Tween 80. Third instar nymphs and adults of *C. insolita* (30 each) of uniform age were transferred separately using a fine camel brush on to brinjal leaves kept inside Petri dishes lined with moist tissue paper. The tests insects were sprayed uniformly with different test concentrations using an atomizer. The treatment with extract of blank medium in distilled water containing 0.03 per cent

tween 80 served as control. The treated insects were air dried and the Petri plates were sealed with parafilm and kept at 27°C. Mortality was recorded at 24 h interval for a period of 96 h. The mortality was corrected using Abbott's formula and subjected to statistical analysis.

RESULTS AND DISCUSSION

GCMS chromatogram of crude toxin extracted from PDB and Czapak Dox cultures are depicted in Fig.1 and 2 respectively. Chromatogram 1 represents the extracellular metabolites produced in PDB. It revealed the presence of 25 compounds of varying chemical groups. Table 1, section A reveals the dominating bioactive compounds including two fatty acid compounds, octadecanoic acid, n-Hexadecanoic acid and an organic acid 2,6 pyridine dicarboxylic acid (dipicolinic acid- DPA). Chromatogram II depicts 25 compounds detected in Czpak dox medium which included alkaloids, terpenoides, fatty acids, cyclic esters and ketones. Chemical names of eight major bioactive metabolites with RT values are detailed in Table 1, section B. Biological activity of the metabolites isolated from *L. saksenae* is illustrated in Table 2, with reference to Dr. Duke's phytochemical and ethanobotanical database (Duke, 2013).

The present study is the first report of the volatile insecticidal metabolites of *L. saksenae*. The paralysis and death of insects treated with the culture filtrates on the same day of treatment as observed in the preliminary studies by Rani *et al*. (2015), Jasmy (2016) and Sankar (2017) was the real instinct to investigate its metabolite profile. As

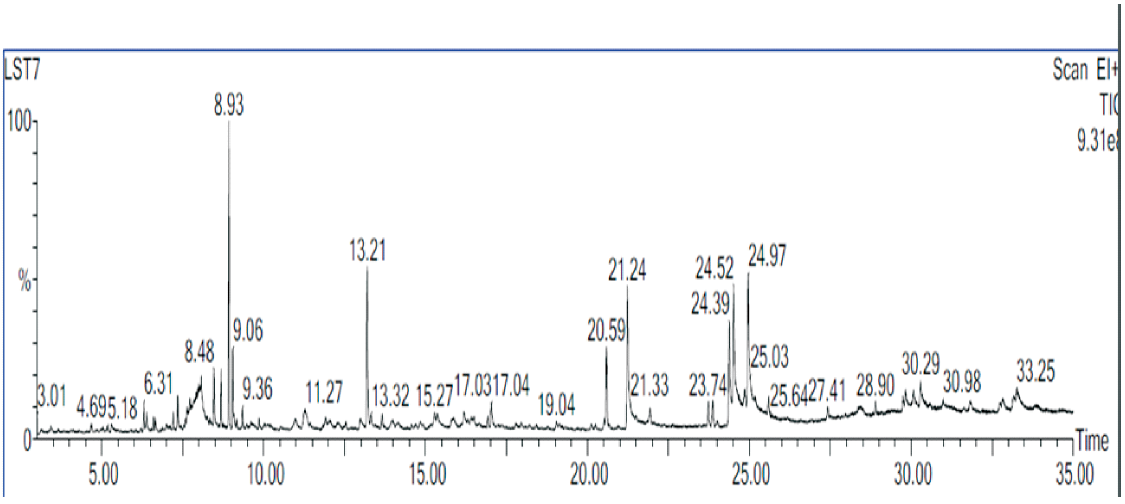

Fig. 1. Chromatogram of ethyl acetate fraction of *L. saksenae* in PDB medium

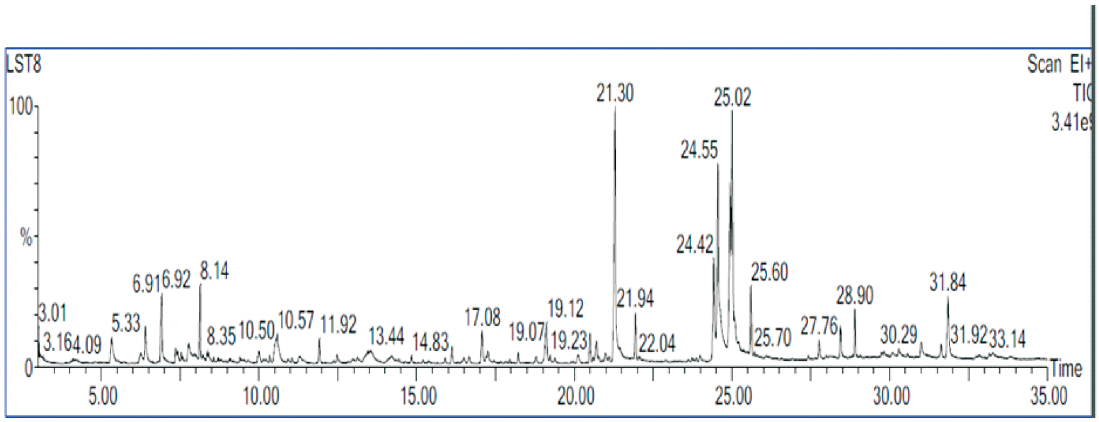

Fig. 2. Chromatogram of ethyl acetate fraction of *L. saksenae* in Czapak Dox medium

Table1. Bioactive volatile compounds detected from *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno

Sl No	RetentionTime	Chemical Group	Name of compound	Molecular Formula
A			Potato Dextrose Broth	
1	13.208	Organic acid	2,6 pyridine dicarboxylic acid	$C_7H_5NO_4$
2	20.591	Fatty acid	N-hexadecanoic acid	$C_{16}H_{32}O_2$
3	24.967	Fatty acid	Octadecanoic acid	$C_{18}H_{36}O_2$
B			Czapek Dox medium	
1	6.400	Ketone	4Hpyran-4 –one,2,3 dihydro3, 5-dihydroxyl- 6- methyl	$C_6H_8O_4$
2	6.925	Alkaloid	2- piperidinone	$C_9H_{19}BrNO$
3	8.140	Terpenoid	Dl- mevalonic acid lactone	$C_6H_{10}O_3$
4	11.917	Amide	Acetamide,N-(2-phenylethyl)	$C_{10}H_{13}NO$
5	13.462	Cyclic ester	3- deoxy-d- mannoic lactone	$C_6H_{10}O_5$
6	17.079	Diketopiperazine	Pyrrolo[1,2a] pyrazine1, 4-dione, hexahydro	$C_7H_{10}N_2O_2$
7	21.301	Fatty acid	N-.hexadecanoic acid	$C_{16}H_{32}O_2$
8	24.942	Alkaloid	Harmine	$C_{13}H_{12}N_2O$
9	25.017	Fatty acid	Octa decanoic acid	$C_{18}H_{36}O_2$

Table 2. Bioactivity* of volatile compounds detected from *L. saksenae*

Sl. No	Insecticidal compounds	Nematicidal compounds	Antimicrobial compounds
1	Dipicolinic acid	Octadecanoic acid	Acetamide,N-(2-phenylethyl), Harmine,
2	2-piperidinone	Harmine	Dl- Mevalonic acid lactone
3	Harmine	N-hexadecanoic acid	4H-pyran-4- one-2,3- dihydro- 3,5 di hydroxy-6 methyl
4	Dl- mevalonic acid lactone		3- Deoxy-d- mannoic lactone
5	Hexadecanoic acid		

* Dr.Dukes Phytochemical and Ethnobotanical Database (Duke, 2013)

per the Duke's database, the metabolites detected from PDB *viz* 2, 6 pyridine dicarboxylic acid (Dipicolinic acid-DPA) and octadecanoic acid are with insecticidal and nematicidal properties respectively. The fatty acid compound, n-hexadecanoic acid possesses both insecticidal and nematicidal properties. Among the metabolites detected from Czapak Dox medium 2-piperidinone and n-hexadecanoic acid are insecticidal, acetamide,N-(2 phenylethyl) and 3-deoxy-d-mannoic lactone, 4H-pyran-4-one 2,3- dihydro-3,5-di hydroxy 6-methyl are antimicrobial in nature. The metabolite harmine possesses insecticidal, antimicrobial and nematicidal properties, whereas dl- mevalonic acid lactone exhibits both insecticidal and antimicrobial properties.

Reports of toxins produced by *Lecanicillium* dates back to those by Suzuki *et al.* (1977) and Kanaoka *et al*. (1978) who detected the bioactive compound, bassianolide from *L.lecanii* . Claydon and Grove (1982) detected DPA and Soman *et al*. (2001),

detected the presence of vertilecanin-A, decenedioic acid and 10-hydroxy-8-decenoic acid. Jasmy (2016) detected the presence of DPA in *L. saksenae* through High Performance Thin Layer Chromatography (HPTLC) to the extent of 0.044 per cent.

Many authors have reported secondary metabolites similar to those identified from *L. saksenae* from different species of entomopathogenic fungi (Moragae and Vey, 2004, Asaff *et al.,* 2005). The major bioactive compounds identified in the ethyl acetate fraction of mycelia of *Beauveria bassiana* were n- hexadecanoic acid, 9,12, octadecadienoic acid, squalene, and octadecanoic acid (Ragavendran *et al* ., 2017). Vivekanandhan *et al.* (2018) reported hexa decanoic acid from mycelia of *B. bassiana* 28 as the major compound responsible for its pathogenicity. Recently, Ragavendran *et al*. (2019) identified mosquitocidal compounds *viz* 1-octadecene, 1-nonadecene, 9-octadecenoic acid and cyclobutane through GCMS analysis of *Penicillium* sp.

Bioactive metabolites are also reported from microbes other than fungi. The insecticidal alkaloid compound, harmine detected from *L. saksenae* is widely distributed among different medicinal plants (Siddiqui *et al.,* 1987). Similarly Pyrrolo (1,2) pyrazine1,4- dione hexahydro with antibiotic property was isolated from a marine bacterium *B. teqquilensis (*Kiran *et al.,* 2018*)*

Results of bioefficacy studies presented in Table 3, revealed the insecticidal potential of secondary metabolites of *L. saksenae*. A higher mortality of 95.98 per cent was observed with 1000 ppm toxin in the third instar nymphs, 48 HAT (Hours after treatment). The corresponding mortality in adults was 85.51 per cent. It took 72h to cause 100 per cent mortality of nymphs while in adults, it was 96 h. Lower concentrations of 100 ppm resulted in 62.83 per cent mortality, after 72 h exposure. In adults, it did not bring about significant mortality (2.5 to 31.08 per cent).

Bioactivity of secondary metabolites isolated from *L. saksenae* in the present study such as 2,6, pyridine dicarboxylic acid and hexadecanoic acid were previously reported from *L. lecanii* and *B. bassiana* respectively. Clydon and Grove (1982) observed insecticidal property of 2,6,pyridine

Table 3. Insecticidal activity of crude extract of *L. saksenae* on *C. insolita*

Concentration (ppm)	*Mean mortality of nymphs at 24 h interval (% ± SE)			
	24	48	72	96
10	2.63 ± 3.04	3.95 ± 7.89	5.56 ± 6.42	6.25 ± 0.00
50	25.44 ± 11.30	37.56 ± 11.60	43.06 ± 10.79	46.88 ± 10.83
100	41.74 ± 8.12	54.02 ± 7.72	62.83 ± 7.32	73.44 ± 10.67
250	60.82 ± 5.03	72.26 ± 4.12	84.40 ± 4.98	95.31 ± 5.98
500	78.29 ± 7.82	95.75 ± 5.35	100.00 ± 0.00	100.00 ± 0.00
1000	85.23 ± 4.74	95.98 ± 5.07	100.00 ± 0.00	100.00 ± 0.00
*Mean mortality of adults at 24 h interval (% ± SE)				
10	2.50 ± 2.89	2.50 ± 5.00	4.02 ± 5.07	5.56 ± 4.54
50	7.57 ± 6.42	7.63 ± 8.61	8.19 ± 3.05	9.90 ± 2.43
100	22.96 ± 6.02	26.29 ± 3.80	27.34 ± 3.85	31.08 ± 8.62
250	67.89 ± 5.21	73.79 ± 5.34	75.22 ± 7.52	79.86 ± 4.17
500	71.84 ± 6.30	76.29 ± 3.14	86.26 ± 3.38	100.00 ± 0.00
1000	83.36 ± 4.86	85.51 ± 5.04	89.04 ± 4.55	100.0 ± 0.00

* Mean of 4 replications

dicarboxylic acid (dipicolinic acid) from seven different strains of *L. lecanii.* Gindin *et al.* (1994) reported the toxicity of methanolic extract of mycelia of *L. lecanii* on the nymphs of *Bemisia tabaci* Gennadius and recorded mortality of 33.6 and 90 per cent at 0.1 and 0.5 per cent concentration respectively. The insecticidal activity of the extract was attributed to the phospholipid entity. Asaff *et al.* (2005) isolated the most abundant insecticidal metabolite of *Paecilomyces fumosoroseus* and characterized it as dipicolinic acid. LD_{50} value was 44.5 +_2.5, mgL^{-1} on brine shrimp *Artemia salina* Linnaeus. They suggested DPA, as the main active compound along with some other compounds might have contributed to the insecticidal activity. Crude toxins of *L. lecanii* were also reported to have ovicidal, repellant and antifeedant activities on *B. tabaci* (Wang *et al*., 2007). N. hexa decanoic acid from *B. bassiana* had a promising larvicidal and pupicidal activity on *Culex quinquefasciatus* Say (Raghavendran *et al.,* 2017).

Role of secondary metabolites in insect pathogenesis and mortality had been documented in many studies. Hunt and Ginsburg (1981) suggested the role of dipicolinic acid as an enzyme inhibitor responsible for the removal of essential ions from metalloenzymes especially Zn. Paterson (2008) suggested that DPA inhibits prophenoloxidase system during melanin synthesis in insects and interfere in the innate immune system. Involvement of secondary metabolites in various biological reactions such as pathogenicity, competition and defence as well as their antibiotic, antifungal, insecticidal and nematicidal properties have been reported by Xu *et al*. (2009).

Cao *et al*. (2016) reported that secondary metabolites inhibit multifunctional enzymes *viz.,* glutathione S transferase, α and β esterase that detoxify insecticides and endogenous compounds leading to enhanced death rate in insects. Raghavendran *et al.* (2019) reported the acetyl cholinesterase inhibition activity of the crude toxin containing different secondary metabolites extracted from *Pencillium* sp on fourth instar larvae of *Aedes aegypti* Linnaeus. The activity was attributed to the monoterpenoid fractions in crude extracts which are inhibitory to AchE activity. According to Elbanhawy *et al.* (2019), fungal metabolites are also reported to interfere with the normal physiological functions in insect. The methanol extract of *Purpureocilium lilacinum* caused reduction in the activity of aspartate aminotransferase, alanine aminotransferase, glutathione S. transferase and α and β esterase in *Aphis gossypii* Glover.

GCMS analysis of the ethyl acetate fraction of culture filtrate has thrown light into the chemical nature of the metabolites of *L saksenae*. The insecticidal, antimicrobial and nematicidal compounds detected in the present studies, cleary supported the findings of Goettel (2008) who suggested *Lecanicillium* as a multipurpose microbial agent for the control of arthropods, plant parasitic nematodes and plant pathogenic fungi. Insecticidal property exhibited by crude extract of *L. saksenae* may be due to the activity of more than one metabolite which includes the major metabolites dipicolinic acid, hexadecanoic acid and other nonvolatile compounds. The study established the role of secondary metabolites in the pathogenicity of *L. saksenae.*

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A new genus and a new species of Tropidopolinae (Orthoptera: Acrididae) from India

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ABSTRACT: A new genus *Neooxyrrhepes* gen. n. with a new species *Neooxyrrhepes meghalayensis* sp. n. from Meghalaya, a state of the North Eastern region of India. Description and illustrations of the new genus and species are given. A key to the genera of subfamily Tropidopolinae from North Eastern states of India is also provided. Additonally the characters of male and female genitalia at generic and species level are also given.

KEY WORDS: New species, *Neooxyrrhepes,* Tropidopolinae, Acrididae, Meghalaya, India

INTRODUCTION

All the economically important species belonging to the family Acrididae, are commonly known as grasshoppers and locusts. Sometimes they are called short-horned grasshoppers (Caelifera) in contrast to Ensifera (long-horned grasshoppers). Acrididae possess short antennae, usually shorter than the body, a short ovipositor and three-segmented tarsi. Locusts and grasshoppers constitute an economically important group of Orthopterous pests that infest a number of cultivated and non-cultivated crops. They cause considerable damage to agricultural crops, pastures and forests and are well reputed for their destructiveness all over the world (Joshi *et al*., 1999). Orthoptera comprising 27,200 valid species worldwide (Eades *et al*., 2016) and 8000 species by Acrididae and out of that 136 species under 28 genera of family Acrididae are endemic (Chandra and Gupta, 2013) showing maximum diversity. Stal (1860) was probably the first to initiate the study of Indian Acrididae and a notable taxonomical work on Acrididae was made by Kirby (1914) in the series 'Fauna of British India' followed by series of work by Uvarov (1921, 1927, 1966).

Two species *Oxyrrhepes obtuse and Tristria sp.* have been reported by Chandra *et al*. (2007) from Madhya Pradesh and Chattisgarh. Tropidopola and Tristia have been mentioned from India by Shishodia *et al*. (2010), *Oxyrrhepes obtuse and Tristria pulvinata* have been reported from North Eastern India *by* Usmani and Khan (2010), and only species description given by various authors from different parts of India like Usmani and Nayeem (2012) from Bihar, Nayeem and Usmani from Jharkhand (2012), Kumar and Usmani (2013) from Rajasthan, Nayeem *et al*. (2013) from Bihar and Kumar and Usmani (2013) from Punjab respectively. Only *Tropidopola longicornis* have been reported by Usmani *et al*. (2010) from Western Uttar Pradesh but very recently *Tropidopola longicornis* and *Tristria pulvinata* have been

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reported by Akhtar *et al*. (2016) from Uttar Pradesh, largest state of India.

Bei-Beinko and Mishchenko (1951), Dirsh (1961), Uvarov (1966) studied the Subfamily. This is represented by two genus *viz*. *Tristia* and *Tropidopola* from India. Genus *Tristria* was originally erected by Stal (1873a) to include a new species *lacerta* described from China. Genus *Tropidopola* was erected by Stal (1873b) to include *cylindrica* as a type species. Subfamily Tropidopolinae was erected by Jacobson in 1905 include *Tropidopola* as its type genus. Currently Tropidoplinae is represented by two tribes *viz*. Tristriini, Mishchenko, 1945 and Tropidopolini, Jacobson, 1905 with genus *Tristria* and *Tristriella* under the former tribe and genera *Afroxyrrhepes*, *Chloroxyrrhepes*, *Dabba*, *Homoxyrrhepes*, *Mesopsilla*, *Musimoja*, *Oxyrrhepes*, *Petamella*, *Pseudotristria*, *Tropidopola* grouped under the later one. A survey of the North Eastern states of India revealed a new genus and a new species from Meghalaya state.

MATERIALS AND METHODS

I) Collection and killing

Various agricultural and forest areas of Meghalaya states were surveyed during 2008-2011 to collect the grasshoppers and locusts. Insects were caught by ordinary aerial insect net by sweeping on grasses and crops. Sometimes also captured through hands and foreceps when these are under reach. The collected specimens were killed by putting these in the killing jar having ethyl acetate soaked in cotton. Once killed removed for stretching.

II) Morphological studies

Left wings of the grasshoppers were stretched putting a piece of paper on it, and pinned right of the thorax on stretching board and left for 72 hours to dry. Then with the help of stereoscopic microscope and available literature Grasshoppers were identified up to species level. Permanent collections of pinned specimens were kept in store boxes and cabinets for further studies on their morphological structures.

III) Genitalia studies

For a detailed study of the various components of genitalia, the apical part of the specimen were cut off and boiled in 10% potassium hydroxide to remove unsclerotized and non-chitinous tissues. Thoroughly washed in tap water and examined in 70% ethyl alcohol on a cavity slide and dissected under a binocular microscope with the help of fine needles to separate genetalic structures viz., supra-anal plate, subgenital plate, epiphallus and aedeagus of male, supra-anal plate, subgenital plate, ovipositor and spermatheca of female. The normal process of dehydration was adopted and clearing was done in clove oil. The genital structures were mounted separately on cavity slides in Canada balsam. Drawings of genital structures were made with the help of a camera lucida of the concerned microscope.

IV) Study area

Meghalaya is a small state of North-Eastern India situated at 25.5700° N, 91.8800° E about 300 km long and 100 km wide, with a total area of about 8,660 sq mi (22,429 km^2). The elevation of the plateau ranges between 150 m to 1961 m. The central part of the plateau comprising the Khasi Hills has the highest elevations, followed by the eastern section comprising the Jaintia Hills Region. Meghalaya is the wettest place on earth with average annual rainfall as high as 1200 cm. The maximum temperature not rises beyond 28°C, whereas sub-zero is common during winters. About 70% area of the state are covered with subtropical forests considered to be the richest botanical habitats of the Asia. Nearly 10% of the geographical area of Meghalaya is under cultivation and 80% of the population depends on this tiny area as agriculture. Rice is the dominant crop followed by maize and wheat.

RESULTS

A new genus *Neooxyrrhepes* gen. n. with a new species *Neooxyrrhepes meghalayensis* sp. n. from Meghalaya, a state of the North Eastern region of India. Twelve genus including this new one recoded from the world. *Tropidopola* (representing seven

species), *Tristia* (representing ten species), *oxyrrhepes* (representing three species), *Tristriella* (representing single species), *Afroxyrrhepes* (representing five species), *Chloroxyrrhepes* (representing single species), *Dabba* (representing single species), *Homoxyrrhepes* (representing two species), *Mesopsilla* (representing single species), *Musimoja* (representing single species), *Petamella* (representing two species), three species of *Pseudotristria* (Eades, 2016). Only four genus *Tropidopola, Tristia, oxyrrhepes* and *Neooxyrrhepes* including new one have been reported from India.

Taxonomic account:

Key to the genera from the North Eastern states of India of Tropidopolinae

1. Prosternal process long, nearly or strongly reaching anterior margin of mesosternum; hind femur with knee lobe short or long 2

— Prosternal process short not reaching anterior margin of mesosternum; hind femur with knee lobe short and blunt ..... ***Neooxyrrhepes*** gen. n.

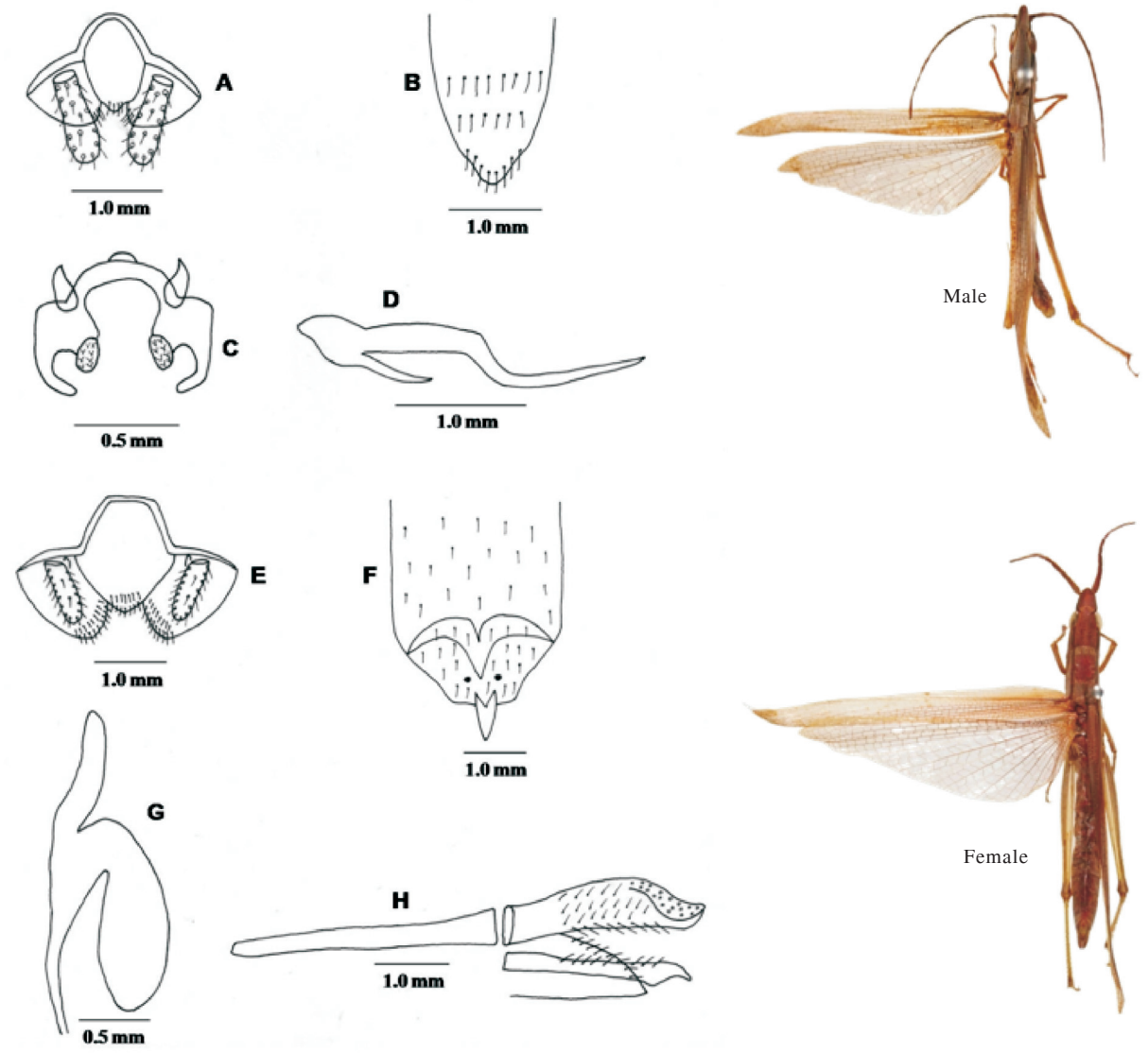

Fig. 1.*Neooxyrrhepesmeghalayensis*gen. n. sp. n.A-D (male); E-H (female) A. Supra anal plate, B. Subgenital plate, C. Epiphallus, D. Aedeagus, E. Supra anal plate, F. Subgenital plate, G. Spermatheca, H. Ovipositor

2. Prosternal process compressed antero-posteriorly, apex rectangular, strongly reaching anterior margin of mesosternum; hind femur with knee lobe short and rounded ……...……..….***Tristria*** Stål, 1873

— Prosternal process compressed laterally, apex conical, nearly reaching anterior margin of mesosternum; hind femur with knee lobe long and acute.…...….. ***Oxyrrhepes*** Stål, 1873

Description

Genus *Neooxyrrhepes* gen. n. (Fig. 1)

Neooxyrrhepes Khan & Usmani, 2019

LSIDurn:lsid:zoobank.org:act:F02E7C0E-0081-4B2C-BF95-AED931A6382D

Type species: *Neooxyrrhepes meghalayensis* sp. n.

Diagnosis: Body smaller in size, elongate. Integument nearly smooth, not shiny. Antennae much longer than head and pronotum together, strongly compressed in basal half while filiform in apical half with elongated segments. Head elongate, longer than pronotum, frons strongly oblique. Frontal ridge narrow, deeply sulcate with lateral carinulae acute, merging anteriorly, slightly constricted below median ocellus, gradually diverging posteriorly. Fastigium of vertex elongate, twice as long as wide, fairly concave with sharp carinulae, fastigial furrow prominent, interocular distance constricted as compared to fastigium width, much narrower than with of frontal ridge and about slightly less than one-third of maximum diameter of eye. Eyes elongate oval with fair convexity. Pronotum of uniform width, metazona tectiform dorsally while slightly rugose laterally, anterior margin straight, posterior margin angular with obtuse apex, lateral margin angulated rather than round, median and lateral carinae sharp, dorsum crossed by two transverse sulci, posterior transverse sulcus crossing median carina, lateral carinae crossed by two sulci and bordered along by post-ocular bands on lateral sides of pronotum. Prosternal process small, prismatic, apex acute not pointed. Mesosternal interspace much constricted or absent, lobes rounded, square or longer than wide, mesosternal pits closely placed. Tegmina lanceolate, sub-hyaline with sparse veination, wings hyaline, margin markedly wavy, wingspan narrow. Hind femur slender, elongate, upper and lower carina smooth, lower genicular lobe smaller and angular, not pointed. Hind tibia straight with two rows of black tipped spines, inner row with thirteen spines while outer row with fifteen spines, inner pair of spurs slightly longer, hind tarsus elongate, arolium small. Male supra-anal plate short, apex bluntly rounded. Cercus very long, broad. Male sub genital plate rounded, apex broadly rounded. Epiphallus bridge shaped, lophi developed and oval in shape. Aedeagus: with apical valve elongate narrow, basal valve moderately broad. Female supra-anal with bluntly rounded apex. Cercus uniformly broad, apex rounded. Female sub genital plate with lateral margin straight and diverging basally, apex blunt. Spermatheca, pre-apical diverticulum moderately broad, sac like. Ovipositor valves broad and robust, much shorter than lateral apodeme.

Remarks: The genus is closely related to *Tristria* Stål, 1873 and *Oxyrrhepes* Stål, 1873 but differs from former in much elongated antennae, basally ensiform while apical half filiform, elongated and concave fastigium of vertex, acutely sulcate and much narrow frontal ridge, two transverse sulci crossing dorsum, posterior margin angular, conical prism-shaped prosternal process with acute apex, medially contiguous mesosternal lobes, angulated lower genicular lobe. The genus differs from *Oxyrrhepes* in much elongated antennae, anteriorly produced fastigium, acutely sulcate and narrow frontal ridge, strongly oblique frons, presence of post-ocular bands, two transverse sulci crossing dorsum, medially contiguous mesosternal lobes having straight anterior and posterior margins rather than diagonal, lower genicular lobe acute angular and shorter than upper.

Etymology: The new genus *Neooxyrrhepes* is given because it is close to genus *Oxyrrhepes*.

***Neooxyrrhepes meghalayensis* gen. n., sp. n.** (Fig. 1)

Neooxyrrhepes meghalayensis Khan & Usmani, 2019

LSIDurn:lsid:zoobank.org:act:674B1E76-5E6A-41FE-AD6A-E97AE0A61C29

Male genitalia: Supra-anal plate short, uniformly broad, longer than wide, apex bluntly rounded.

Cercus very long, broad, three times as long as wide, apex broadly rounded. Sub genital plate rounded, broad basally, narrowing apically, apex broadly rounded. Epiphallus bridge shaped, bridge narrow, uniformly broad, ancorae developed, tip pointed, lophi developed and oval in shape. Aedeagus, apical valve elongate narrow, shorter than basal valve, tip pointed, basal valve moderately broad.

Female genitalia: Supra-anal plate longer than wide, apex bluntly rounded. Cercus uniformly broad, two and half time as long as wide, apex rounded. Sub genital plate with lateral margin straight and diverging basally, straight in the middle, egg-guide short, broad basally, narrowing apically , almost two times as long as wide, apex blunt. Spermatheca, pre-apical diverticulum moderately broad, sac like. Apical diverticulum long, slender, shorter than pre-apical diverticulum. Ovipositor valves broad and robust, dorsal valve broad uniformly, apical tip blunt and much shorter than lateral apodeme, ventral valve elongate, narrow, apical tip bluntly pointed, basal sclerite triangular, pointed apically.

Measurements: (length in mm)

Male: Body 20.1, Tegmina 7.6, Pronotum 4.3, Hind femur 8.5

Female: Body 36.2, Tegmina 20.0, Pronotum 6.3, Hind femur 17.0

Type Material: HOLOTYPE ♂, Meghalaya, Jowai, Thaldskin, 22-X-2008, on grasses. Paratypes 25♀♀, 19♂♂ (same data as holotype).

Depository: Zoological Museum, Department of Zoology, Aligarh Muslim University Aligarh, India (ZDAMU).

Etymology: The name of the new species is given on the name of the state of Meghalaya in India.

Distribution: Meghalaya (India)

DISCUSSION

Earlier studies on the systematics of Indian Acrididae are exclusively based on the external characters like colour, size, texture, number of antennal segments etc. Beside conventional characters, in the present study attempt has also been made to make a comperhensive study on the genitalic structures, viz., supra-anal plate, subgenital plate, epiphallus and aedeagus of male; sub genital plate, supra-anal plate, ovipositor and spermatheca of female. Presence or absence of fastigial furrow; presence or absence of prosternal process; length of lower basal lobe in relation to upper basal lobe of hind femur. shield or bridge-shaped condition of epiphallus; presence or absence of dorso-lateral appendices, oval sclerites and lophi on epiphallus; divided, undivided or flexured condition of aedeagus; presence or absence of gonopore process on aedeagus; condition of apical and pre-apical diverticula of spermatheca; presence or absence of glandular pouches on female subgenital plate; rudimentary or well developed condition of egg-guide are taken as distinct family characters. A comparative study of the genitalic structures particularly epiphallus, aedeagus and spermatheca makes it possible to put forward some suggestions regarding interrelations of families and subfamilies of Acrididae more clearly than the external characters.

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Biochemical changes in *Holotrichia repetita* Sharp (Coleoptera: Melolonthidae) and *Galleria mellonella* L. (Lepidoptera: Pyralidae) due to the biopesticide *Steinernema* sp.

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ABSTRACT: In the present study, *Steinernema*- sp. were isolated from Tirupur soil sample and tested for its antagonistic potential against larvae of *Galleria mellonella* and *Holotrichia repetita* in laboratory condition. 100 per cent mortality was observed after 24 and 48 hours for *G. mellonella* and *H. repetita* respectively. Further biochemical estimations *viz.,* protein, carbohydrates and lipids were carried out after 24 and 48 h of application. The results showed that there was a significant decrease in all biochemical parameters of parasitized larvae compared to control. *Steinernema* sp. can be incorporated in IPM program for the control of *G. mellonella* and *H. repetita.*

KEY WORDS: EPN, *Steinernema* sp*,* protein, carbohydrate, lipid

INTRODUCTION

The objective of biocontrol is to maximize the efficiency of the natural enemy complex in suppressing pests and ultimately enhancing crop yield in agriculture (Denno *et al.,* 2008). The Entomopathogenic nematodes (EPN) consist of genus *Steinernema* and *Heterorhabdus* (Nematoda: Rhabditidae) are symbiotically associated with entomopathogenic bacteria *Xenorhabdus* sp., (Thomas and Poinar, 1979) and *Photorhabdus* sp., (Boemare *et al*., 1993) respectively. These EPNs have been successfully used as biological control agents of various insect pests (Shahina and Mehreen, 2015). Once infective juveniles (IJs) of EPNs entered into host body by natural openings like mouth, anus and spiracle then it releases symbiotic bacteria from the intestine to body cavity of the host body (Kaya and Gaugler, 1993). The bacteria will logarithmically replicate in haemolymph (Hussien and Hanan, 2008) and act as a primary agent for killing the host usually by haemocoel infection such as septicemia (Forst *et al.,* 1997) within 24 to 48 hr. The greater wax moth, *Galleria mellonella* Linnaeus, (Lepidoptera: Pyralidae) is widely distributed throughout the world in temperate, tropical and subtropical beekeeping regions and major economically destructive pest of the honeybee, *Apis mellifera* Linnaeus, and *Apis cerana* Fabricius (Kwadha *et al*., 2017). *G. mellonella* damage only during their larval stages. It obtained nutrients from honey, pollen, wax, castoff pupal skins and other impurities found in

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the beeswax (Mohamed, 2014). *Holotrichia repetita* Sharp (Coleoptera: Melolonthidae), severely damages tuber vegetables such as potato, groundnut and sugarcane in all over India (TNAU, 2018). Adult beetles are 16.0–22.0 mm, dark bronzy green, legs greenish black, and abdomen deep red color (Chandel *et al*., 2015). *H. repetita* grubs are 'C' shaped with orange head. It feeds on roots and tubers. Adults were emerged as soon as temperature starts rising. Adults feeds on foliage during night and damage is more during autumn (TNAU, 2018). Both *G. mellonella* and *H. repetita* considered as a major destructive pest in agriculture. So, the present study was carried out to control *G. mellonella* and *H. repetita* through EPN and their biochemical parameters were studied.

MATERIALS AND METHODS

The larvae of greater wax moth *Galleria mellonella* were used for baiting the nematodes (Bedding and Akhurst, 1975). The larvae were cultured and maintained in a large container at room temperature on an artificial diet. 250 gram of soil samples were collected at a depth of 15 - 20cm from different agricultural location in and around Coimbatore and Tirupur Districts, Tamilnadu. Collected soil samples were brought to laboratory and stored at room temperature for further study.

Entomopathogenic nematodes were recovered from soil sample using standard method as described by Bedding and Akhurst (1975). Five fifth instar larvae of *G. mellonella* were placed in 100ml plastic containers which contained 50 grams of collected moisture soil. Larvae were checked for infection every day and the dead ones were removed and live larvae were placed in the containers. The dead larvae were isolated and thoroughly rinsed in 0.01% formalin and placed in White's trap method (Kaya and Stock, 1997) until the emergence of third-stage infective juveniles of nematodes in another two to three days. EPN genes were identified based on the dead cadaver (parasitized) of *G. mellonella*. Further conform to genes of EPNs, a loopful of haemolymph from parasitized larvae were streaked on NBTA medium (Akhrust, 1980). The plates were incubated at 28^0C for 24 hours. Bacterial colony color noted and morphological studies such as Gram's staining and Motility test were done.

After isolation of nematodes, their pathogenic potential was tested against *G. mellonella* and *H. repetita* at laboratory condition. *H. repetita* larvae were collected from Nilgiri Hills and reared in the laboratory. Five last instar larvae of *G. mellonella and H. repetita* were taken in a sterilized Petridish and *Steinernema* sp. (IJs) was gently applied over the larvae by the help of small camel hair brush then maintained at room temperature with untreated controls were identical to the treatment except that no IJs were added. The larval mortality was observed for 24 and 48 hours and the duration for the death of the larvae was noted. Further biochemical analyses were carried out between parasitized larvae and control (Untreated) for 24 and 48 hrs of after infection. Protein estimation was done by Lowry's method (Lowry *et al*., 1951). Carbohydrates estimation was carried out by Roe's method (Roe, 1995) and Lipids estimation by Folch's Method (Folch, 1957).

Obtained data were subjected to multi-factorial ANOVA by using SPSS v16.0 software. Results with $p<0.05$ was considered as a statistically significant.

RESULTS AND DISCUSSION

Total 10 soil samples were collected from different agro ecosystems in and around Coimbatore and Tirupur, Tamilnadu. Among 10 soil samples only one soil sample tested positive for the presence of EPN. Both biotic factors (vegetation and host availability) and abiotic factors (temperature, soil type, depth, and moisture) are responsible for the presence of EPN in soil (Molyneux, 1985). The infected cadaver of *G. mellonella* was black in color (Woodering and kaya 1988), these shows that the isolated EPN, in this study belongs to *Steinernema* Genus (kaya and Nelson, 1985). Further to conform EPNs genus, a loop of haemolymph were streaked on NBTA medium, color of bacterial colony was maroon which shows the isolated symbiotic bacteria was *Xenorhabdus* sp. (Akhrust, 1980). Morphological studies show that isolated bacteria was gram negative, motile

and showed no bioluminescence. So, in this present study isolated EPNs were *Steinernema* sp. with its symbiont *Xenorhabdus* sp.

The isolated *Steinernema* sp. was checked for their pathogenic potential against *G. mellonella* and *H. repetita*. Larval mortality occurred after 24 hours and 48 hours in laboratory condition at room temperature which shows that the isolated EPNs are effective against *G. mellonella* and *H. repetita* respectively. Larval mortality occurred via an abundance of tissue damage in parasitized larvae because of actin cytoskeletons rearrangements and induced apoptosis in both haemocytes and epithelial tissues due to activity of Mcf toxins which was produced by symbiotic bacteria (Daborn *et al.,* 2002). Members of Enterobacteriaceae such as *Photorhabdus*, *Xenorhabdus*, *Serratia*, and *Yersinia* sp. produce insecticidal toxins (neurotoxins, digestive toxins and cytotoxins) with oral toxicity similar to that of Bt toxins (Castagnola and Stock, 2014).

The protein content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 4.82mg/100mg and 5.73 mg/100mg respectively, in control were 11.88 mg/100mg and 13.41 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 2.78 mg/100mg and 4.18 mg/100mg respectively, in control were 7.53 mg/100mg and 9.25 mg/100mg respectively (Fig. 1).

The carbohydrate content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 16.36mg/100mg and 12.73 mg/100mg respectively, in control were 28.03mg/100mg and 25.03 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 13.21mg/100mg and 10.53 mg/100mg respectively, in control were 24.16mg/100mg and 22.73 mg/100mg respectively (Fig. 1).

The lipid content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 10.23mg/100 mg and 15.4mg/100mg respectively, in control were

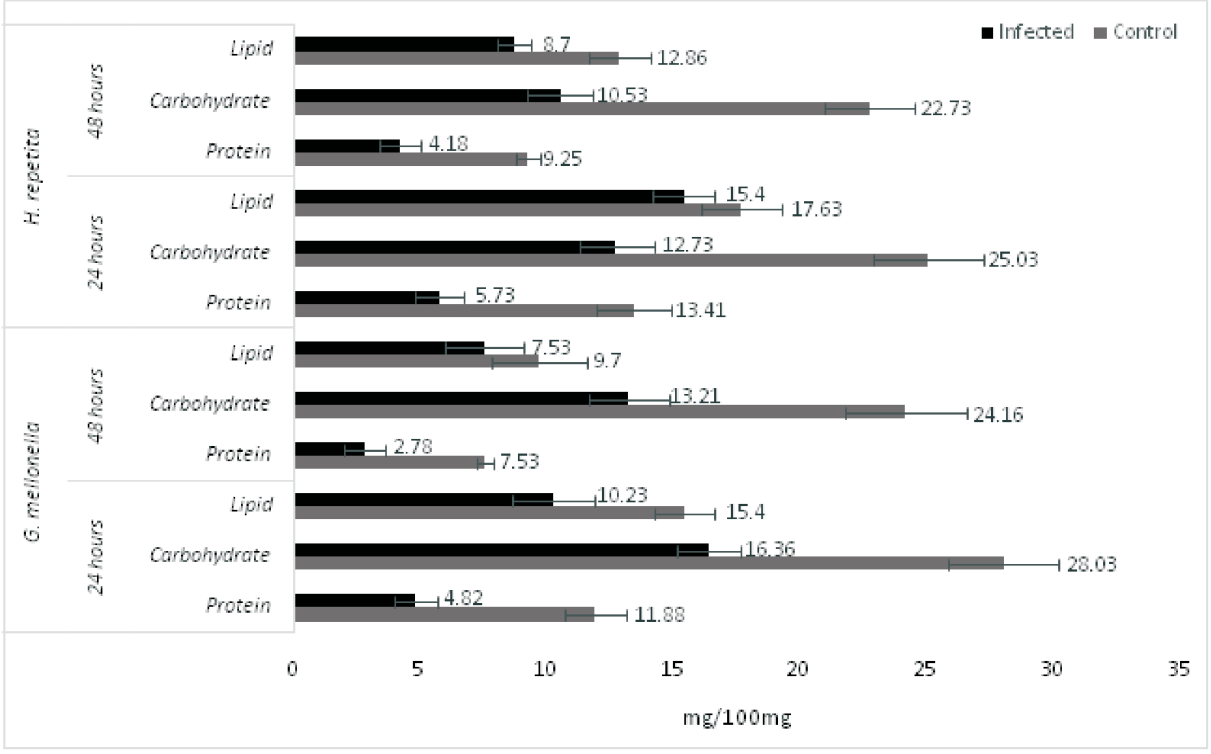

Fig. 1. Estimation of protein, carbohydrate and lipid (mg/10mg) in *G. mellonella* and *H. repetita* infected by *Steinernema* sp. after 24 and 48 h of application (Mean ± SD, n=3)

15.4mg/100mg and 17.63 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 7.53mg/100mg and 8.7 mg/100mg respectively, in control were 9.7mg/100mg and 12.86 mg/100mg respectively (Fig. 1).

Biochemical estimations *viz.,* protein, carbohydrate and lipid showed highly significant (df=2, F=27.17, $p<0.01$) reduction in infected larvae of *G. mellonella* and *H. repetita* when compared with control groups. Significance difference (df=1, F=5.48, $p<0.05$) of biochemicals compounds was noted in between 24 and 48 hours of after estimation. Between *G. mellonella* and *H. repetita,* biochemical reduction was not a significant at $p>0.05$. Control larvae of both *G. mellonella* and *H. repetita* having a high biochemical content of carbohydrate followed by protein and lipid. After the 24 h and 48 h of application, in both *G. mellonella* and *H. repetita*, biochemical reduction percentage was high in protein content (54% to 63%) follwed by carbohydrate (41% to 53%) and lipid content (12% to 33%) (Fig. 2). Eventhough carbohydrate is present as a high quantity in non paeasitized larvae, but symbiotic bacteria relase more proteolytic enzymes and proeolytic toxins towards to host proteins. EPNs and its symbiotic bacteria first targets proteins because proteins might be play an important role for its growth, reproduction and development. It might be reason for high reduction percentage of protein in parasitized larvae. Jaroz (1996) observed that low level of antibiotics are present in cadaver of *G. mellonella* larvae when infected with *S. carpocapsae*. It might be reason that *G. mellonella* more susceptible to EPNs.

Gotz *et al.* (1980) recorded that even EPNs also secretes and release toxic substances against the host. Both *S. carpocapsae* and *H. bacteriophora* release protease secretions which destroys the antibacterial factors of *G. mellonella* larvae. Hanan, (2009) has observed biochemical changes in *Sarcophaga aegyptiaea* and *Argaspersicus* haemolymph infected with EPNs which shows

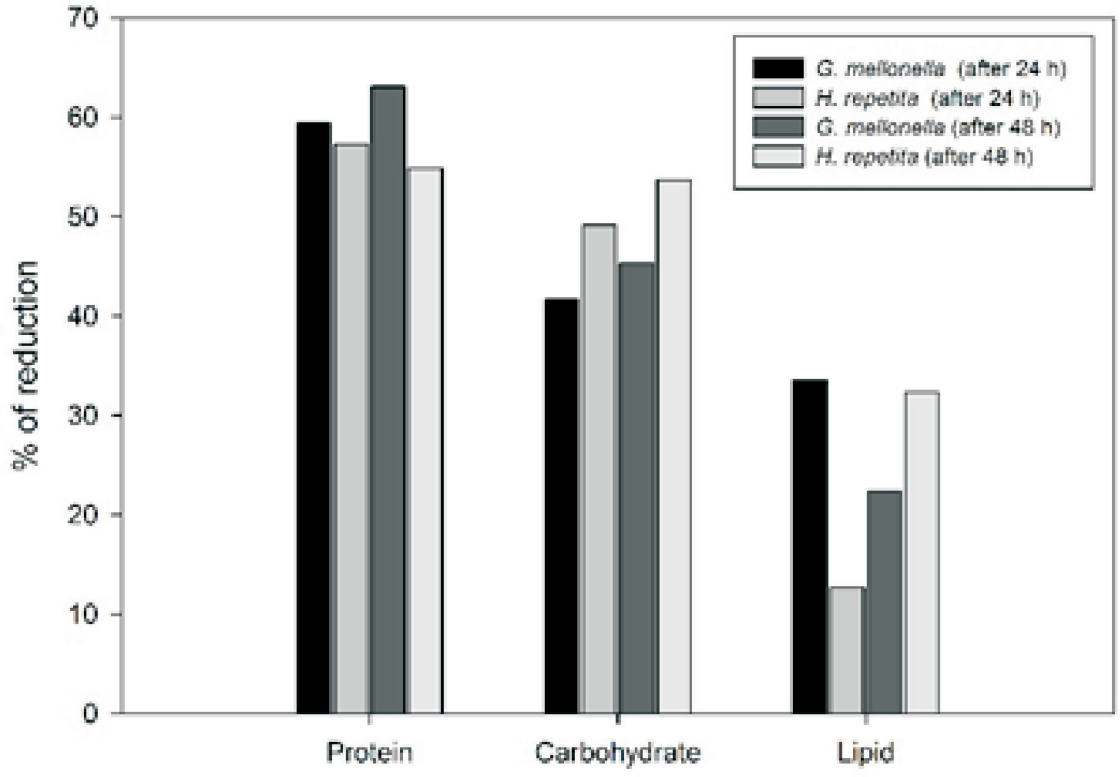

Fig 2. Reduction percentage of biochemicals in *G. mellonella* and *H. repetita* due to infestation of EPNs at 24 and 48 h of after application.

EPNs hydrolysis the host proteins by secreting proteolytic enzymes into haemocoel of host body. Amino acid transport, hormones regulating excretion process and host endocrine balance was disturbed by the EPNs. They were responsible for reduced protein content in haemolymph. The depletion of glycogen and lipid contents in parasitized larvae might be due to utilization of these reserves for energy generation as a result of Cry1Ac-HacCPV- induced stress. Similarly, a study by Santhana bharathi *et al.,* (2016) showed significant decrease in protein and carbohydrate content of *Steinernema* infected *H. armigera* larvae may be due to energy loss for immune reaction against infection. *Steinernema* sp. secretes lipase enzyme into host haemolymph. Lipase has insecticidal toxic activity which would have degraded the lipid content and suppressed the immunity of the pest *H. armigera* and *L. orbonalis* (Chitra *et al.,* 2016). *Helicoverpa armigera* (Santhana bharathi *et al.,* 2016), *Spodoptera litura* (Sindhu, 2016), *Leucinodes orbonalis* (Sujatha, 2017) showed decreased biochemical content in *Xenorhabdus* sp. infected larvae when compared to non-infected larvae (control) due to the utilization of carbohydrate and protein resources by nematode bacterial complex for their growth, multiplication and reproduction (Sindhu, 2016). Txp40 protein and *xpt* gene has been identified in both *Photorhabdus* sp. and *Xenorhabdus* sp. and this is involved in causing damage to the insect midgut and the fat body in dipteran and lepidopteran insects (Castagnola and Stock, 2014), may be true in the study also since the greater wax moth *Galleria mellonella* is also an lepidopteran.

The present study paves way for the control of pests like *G. mellonella* and *H. repetita* through the bio pesticide *Steinernema* sp., isolated from Tirupur soil sample and it is efficient bio pesticide as this does not affect non-target organisms and is environmentally friendly, which can be incorporated in IPM programs.

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Efficacy and biosafety of new generation insecticides for the management of *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) in brinjal and *Earias vitella* Fabricius (Lepidoptera: Noctuidae) in okra

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ABSTRACT: The field trials were conducted at the College of Agriculture, Vellayani to determine the efficacy of eight new generation insecticides *viz.,* emamectin benzoate 5SG @10 g a.i. ha^{-1}, spinosad 45 SC @ 75 g a.i. ha^{-1}, novaluron 10 EC @ 100 g a.i.ha^{-1}, chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}, indoxacarb 14.5 SC @ 60 g a.i. ha^{-1}, fipronil 80 WG @ 50 g a.i. ha^{-1}, thiodicarb 75 WP@ 750 g a.i. ha^{-1} and flubendiamide 480 SC@ 100 g a.i. ha^{-1} against fruit borers of brinjal and okra. Two conventional insecticides (carbaryl 50 WP @ 750 g a.i. ha^{-1} and malathion 50 EC @ 500 g a.i. ha^{-1}) and an untreated control were maintained as check. Damages to brinjal and okra fruits were reduced by 45.96 to 72.21 per cent and 44.34 to 83.26 per cent, respectively by these new generation insecticides. Chlorantraniliprole, indoxacarb, emamectin benzoate and flubendiamide recorded more than 60 per cent reduction in fruit damage in brinjal, and chlorantraniliprole, flubendiamide and indoxacarb with more than 70 per cent reduction in fruit damage in okra were superior. The yield was also significantly high in these treatments in the two crops. All the insecticides were compatible with *Beauveria bassiana* (Blas.) Vuill, and *Metarhizhium anisopliae* (Metsch). Flubendiamide and carbaryl inhibited the growth of *Lecanicillium* (*Verticillium) lecanii* Humber.

KEYWORDS: Fungal entomopathogens, brinjal borer, okra borer

INTRODUCTION

Leucinodes orbonalis Guenee (Lepidoptera: Pyralidae) is the most destructive pest of brinjal, widely distributed in the Indian sub-continent. Yield losses of 85 to 90 per cent have been reported from various states of India by this pest (Patnaik, 2000; Jagginavar *et al.,* 2009). The fruit borer *Earias vitella* Fabricius (Lepidoptera: Noctuidae) is found throughout the year, attacking shoots and fruits of okra. It causes extensive damage resulting in 40 to 53 per cent reduction in yield (Rabindra, 2001).

After hatching, the neonate larvae bore into the shoots or fruits, thus becoming inaccessible to the action of the chemicals applied. This cryptic habit of the pest reduces the chances to kill the fruit borer larvae and has resulted in misuse of pesticides. It

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is not unusual for the vegetable growers to give 10 to 12 sprays in okra and five to six sprays in brinjal in a season. Thus, the fruits which are harvested at short intervals are likely to retain high level of pesticide residues which may be hazardous to the consumers (Sardana *et al*., 2006).

The old chemistries (especially organophosphates and carbamates) attack the nervous systems of insects. Since, insects and other animals have similar tissues, reproductive, hormonal and nervous systems; these compounds have potential for non target effects. This commonality has rendered old insecticides highly toxic to non-target organisms including human. Currently, numerous classes of insecticides with varied modes of action, target selectivity and benign ecological, ecotoxicological and environmental profiles are available which could be exploited for pest management. Fungal pathogens *viz*., *Beauveria bassiana* (Blas.) Vuill*, Lecanicillium* (*Verticillium) lecanii* Humber and *Metarhizhium anisopliae* (Metsch) are generally utilized in vegetable ecosystem on account of their wide host range. Hence, information on the compatibility of the new generation insecticides with these bio agents is a pre requisite for developing suitable IPM strategies for vegetable crops.

In fact, most of the vegetable growers consider these borers as the most serious pests and nearly all of them use only chemical insecticides to combat them. The present investigation was taken up to evaluate the efficacy of the relatively safer new generation insecticides recommended for these borer pests.

MATERIALS AND METHODS

The experiments for determining their efficacy against fruit borers were laid out in randomized block design (RBD) with three replications in the Instructional farm, College of Agriculture, Vellayani. One month old brinjal (variety Haritha) seedlings were transplanted to 3 × 3 m^2 plots at a spacing of 75 x 60 cm. Each plot had a density of 20 plants with one plant per pit. The seeds of okra variety Varsha Upahar were sown in plots of 3 × 2 m^2 with a spacing of 60 x 45 cm. The plants were maintained as per the recommended package of practices of Kerala Agricultural University (KAU, 2011). The insecticide sprays were given on need basis. The first spray was given one month after transplanting of brinjal when shoot damage was noticed. This was followed by a second spray 60 days after transplanting and a third spray 80 days after transplanting. The first insecticidal spray for okra was given when shoot damage was noticed 25 days after planting. This was followed by a second spray 45 days after planting and a third spray two weeks after the second spray.

The number of shoots damaged by the fruit borer was recorded three, five, seven, ten and 15 days after spraying from each brinjal plot and five, seven, ten and 15 days after spraying from okra. The damaged shoots were tagged and the count of freshly damaged shoots was taken during each observation. The total number of fruits and the number of damaged fruits were recorded at harvest five, ten and fifteen days after spraying for brinjal and three, five, seven, ten and fifteen days after spraying in the case of okra. The extent of damage was computed as

$$\text{Percent shoot/fruit damage} = \frac{\text{Number of infested shoots / fruits}}{\text{Total number of shoots /fruits}} \times 100$$

The insecticide molecules evaluated against the fruit borers were tested for their safety to *B. bassiana*, *L. lecanii* and *M. anisopliae*, the entomopathogenic fungi commonly used for pest management in vegetable ecosystem following the poison food technique (Nene and Thapliyal, 1993).

Data relating to each aspect were analyzed statistically. Appropriate transformations were made wherever necessary. The F test was done by analysis of variance (Panse and Suhatme, 1985). Significant results were compared on the basis of critical differences. The overall efficacy of the insecticides against the fruit borers was worked out for which the insecticides were ranked based on their performance in each parameter (pest control, yield, waiting period and compatibility with entomopathogens) studied. The mean rank for each crop was worked out and overall efficacy was determined.

RESULTS AND DISCUSSION

The data on the shoot and fruit damages recorded at definite intervals subsequent to the insecticide sprays revealed that all the new generation insecticides *viz.,* emamectin benzoate 5SG @10 g a.i. ha^{-1}, spinosad 45 SC @ 75 g a.i. ha^{-1}, novaluron 10 EC@ 100 g a.i. ha^{-1}, chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}, indoxacarb 14.5 SC @ 60 g a.i. ha^{-1}, fipronil 80 WG @ 50 g a.i. ha^{-1}, thiodicarb 75 WP @ 750 g a.i. ha^{-1} and flubendiamide 480 SC @ 100 g a.i. ha^{-1} reduced the infestation of *L. orbonalis* on brinjal(Table 1,2 and 3) and *E. vitella* in okra (Table 4 and 5) significantly in the

Table 1. Damage of shoots by *Leucinodes orbonalis* in brinjal plots treated with new generation insecticides 30 days after transplanting

Treatments	Dosage(g a.i.ha^{-1})	5 DAS	10 DAS	15 DAS
Emamectin benzoate	10	0.25(1.12)	1.26(1.50)	2.77(1.94)
Spinosad	75	0.43(1.19)	1.14(1.46)	0.66(1.29)
Novaluron	100	0.69(1.30)	1.28(1.51)	1.58(1.61)
Chlorantraniliprole	30	0.12(1.06)	0.43(1.19)	0.43(1.19)
Indoxacarb	60	0.57(1.25)	0.73(1.31)	0.61(1.27)
Fipronil	50	0.22(1.10)	1.17(1.47)	1.57(1.60)
Thiodicarb	750	0.89(1.37)	0.68(1.30)	1.70(1.64)
Flubendiamide	100	0.29(1.14)	0.95(1.40)	0.73(1.32)
Carbaryl	750	0.73(1.31)	0.78(1.33)	1.37(1.54)
Malathion	500	0.67(1.29)	0.85(1.36)	1.08(1.44)
Untreated control		2.23(1.80)	3.20(2.04)	3.31(2.08)
CD(0.05)		(0.29)	(0.40)	(0.37)

Figures in parentheses are square root transformed values. DAS - Days after spraying

Table 2. Damage of shoots and fruits by *Leucinodes orbonalis* in brinjal plots treated with new generation insecticides 60 days after transplanting

Treatments	Dosage (g a.i.ha^{-1})	Shoot damage (%)			Fruit damage (%)		
		5 DAS	10 DAS	15 DAS	5 DAS	10 DAS	15 DAS
Emamectin benzoate	10	0.53(1.24)	1.03(1.43)	1.80(1.67)	6.27(2.70)	16.48	17.35
Spinosad	75	0.43(1.19)	1.02(1.42)	0.68(1.30)	14.03(3.88)	18.65	31.22
Novaluron	100	0.56(1.25)	1.00(1.41)	1.30(1.52)	19.53(4.53)	26.11	25.76
Chlorantraniliprole	30	0.24(1.11)	0.43(1.19)	0.57(1.25)	2.34(1.83)	15.99	18.33
Indoxacarb	60	0.57(1.25)	0.73(1.31)	0.62(1.27)	5.24(2.50)	13. 40	18.60
Fipronil	50	0.61(1.27)	1.17(1.47)	1.11(1.45)	13.08(3.75)	26.95	19.57
Thiodicarb	750	0.72(1.31)	0.86(1.36)	1.70(1.64)	17.37(4.29)	31.79	26.14
Flubendiamide	100	0.30(1.14)	0.95(1.40)	1.09(1.44)	6.82(2.80)	24.66	26.24
Carbaryl	750	0.73(1.31)	0.95(1.40)	1.34(1.53)	19.16(4.49)	37.35	41.11
Malathion	500	0.82(1.35)	1.04(1.43)	1.18(1.48)	18.13(4.37)	39.26	37.91
Untreated control		2.38(1.83)	3.20(2.05)	3.53(2.13)	33.04(5.83)	51.48	46.63
CD(0.05)		(0.33)	(0.40)	(0.26)	(2.06)	17.64	15.60

Figures in parentheses are square root transformed values. DAS - Days after spraying

Table 3. Damage of shoots and fruits by *Leucinodes orbonalis* in brinjal plots treated with new generation insecticides 80 days after transplanting

Treatments	Dosage (g a.i.ha^{-1})	Shoot damage (%)			Fruit damage (%)		
		5 DAS	10 DAS	15 DAS	5 DAS	10 DAS	15 DAS
Emamectin benzoate	10	0.40(1.18)	1.03(1.43)	1.37(1.54)	5.04 (2.46)	13.39	29.81
Spinosad	75	0.46(1.21)	0.57(1.25)	0.91(1.38)	11.26 (3.50)	21.37	31.30
Novaluron	100	0.68(1.30)	0.80(1.34)	1.19(1.48)	14.65 (3.96)	25.36	31.79
Chlorantraniliprole	30	0.24(1.11)	0.45(1.21)	0.70(1.30)	1.98 (1.73)	10.87	23.28
Indoxacarb	60	0.41(1.19)	0.73(1.31)	0.84(1.36)	5.04 (2.46)	13.03	23.54
Fipronil	50	0.73(1.32)	1.06(1.44)	1.11(1.45)	9.78 (3.28)	14.85	21.43
Thiodicarb	750	0.60(1.26)	0.68(1.30)	1.16(1.47)	8.65 (3.11)	22.79	30.53
Flubendiamide	100	0.29(1.14)	0.95(1.40)	1.09(1.44)	4.36 (2.32)	18.23	24.13
Carbaryl	750	0.73(1.31)	0.95(1.40)	1.10(1.45)	15.17 (4.02)	27.50	38.36
Malathion	500	0.63(1.28)	0.91(1.38)	0.95(1.40)	9.13 (3.18)	26.19	43.07
Untreated control		2.15(1.77)	2.79(1.95)	2.38(1.84)	31.09 (5.66)	50.46	46.14
CD(0.05)		(0.28)	(0.30)	(0.23)	(1.35)	13.68	20.80

Figures in parentheses are square root transformed values, DAS - Days after spraying

Table 4. Damage of shoots by *Earias vittella* in okra plots treated with new generation insecticides 25 days after planting

Treatments	Dosage (g a.i.ha^{-1})	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS
Emamectin benzoate	10	0.93	1.25 (1.50)	2.18 (1.78)	4.08 (2.25)	4.01
Spinosad	75	1.91	2.46 (1.86)	2.97 (1.99)	3.42 (2.10)	5.09
Novaluron	100	1.98	1.63 (1.62)	2.59 (1.89)	4.18 (2.28)	4.15
Chlorantraniliprole	30	0.98	0.69 (1.30)	1.63 (1.62)	2.97 (1.99)	3.26
Indoxacarb	60	0.93	0.73 (1.31)	1.57 (1.60)	3.32 (2.08)	4.13
Fipronil	50	1.89	1.66 (1.63)	1.61 (1.62)	2.63 (1.91)	5.03
Thiodicarb	750	1.80	1.62 (1.62)	2.26 (1.81)	3.37 (2.09)	4.92
Flubendiamide	100	0.89	0.75 (1.32)	1.53 (1.59)	3.32 (2.08)	3.29
Carbaryl	750	0.98	1.61 (1.62)	3.42 (2.10)	4.16 (2.27)	5.78
Malathion	500	1.04	1.61 (1.62)	3.40 (2.10)	4.17 (2.27)	5.81
Untreated control	-	5.06	8.05 (3.01)	12.88 (3.73)	12.59 (3.69)	11.14
CD(0.05)	-	-	(1.00)	(0.98)	(0.68)	3.68

Figures in parentheses are square root transformed values, DAS - Days after spraying

field. Treatment of brinjal with the new generation insecticides reduced the damage of shoots by *L. orbonalis* by 62.53 to 84.00 per cent. The reduction in damage in carbaryl and malathion sprayed plots was only 64.72 and 66.36 per cent, respectively. Among the newer molecules, chlorantraniliprole (84.00 per cent), indoxacarb (76.28 per cent), spinosad (75.24 per cent) and flubendiamide (71.64 per cent) registered higher reduction in infestation of shoots. Infestation of fruits were also significantly lower, the reduction in damage ranging from 45.96 to 72.21 per cent in new generation

Table 5. Damage of fruits by *Earias vittella* in okra plots treated with new generation insecticides 45 and 60 days after planting

Treatments	Dosage (g a.i.ha^{-1})	45 DAP					60 DAP				
		3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS
Emamectin benzoate	10	2.56	14.60 (3.95)	12.51 (3.68)	14.52	18.66	0	7.66	13.06	23.89	24.81
Spinosad	75	3.03	10.44 (3.38)	19.03 (4.48)	13.68	22.22	4.17	14.98	19.52	16.98	22.91
Novaluron	100	6.67	18.92 (4.46)	12.59 (3.69)	19.07	41.67	5.56	11.36	13.61	22.84	27.30
Chlorantraniliprole	30	0.00	1.84 (1.69)	4.69 (2.39)	8.40	11.67	0.00	4.88	4.69	7.29	11.81
Indoxacarb	60	0.00	6.09 (2.66)	7.20 (2.86)	7.22	15.86	1.67	8.62	7.26	12.17	18.03
Fipronil	50	0.00	2.22 (2.63)	5.92 (4.44)	18.71 19.24	29.42	4.30	10.04	19.72	19.95	23.23
Thiodicarb	750	0.00	8.51 (3.08)	12.20 (3.63)	15.40	14.35	3.03	13.15	12.33	18.58	25.32
Flubendiamide	100	0.00	3.10 (2.24)	6.59 (2.75)	11.19	12.15	2.78	6.46	6.67	7.78	17.85
Carbary l	750	0.00	2.56 (4.50)	19.29 (4.39)	18.26 22.55	32.22	3.33	12.57	18.28	21.85	24.44
Malathion	500	0.00	16.87 (4.23)	27.65 (5.35)	20.32	24.21	0.95	17.60	27.78	25.45	24.17
Untreated control		11.96	34.32 (5.94)	35.71 (6.06)	37.37	47.78	9.14	34.72	35.83	35.15	44.81
CD(0.05)		-	(2.14)	(1.21)	8.06	15.61	-	9.80	10.17	10.37	13.72

Figures in parentheses are square root transformed values. DAP-Days after planting, DAS - Days after spraying

insecticide treated plots and 31.87 to 33.09 per cent in carbaryl and malathion treatments, respectively. Chlorantraniliprole (72.21 per cent), indoxacarb (70.51 per cent), emamectin benzoate (66.70 per cent) and flubendiamide (71.64 per cent) treated plots recorded higher reduction in the fruit damage. Commensurate with the reduced pest incidence, significantly higher yield was obtained from these plots, being 17.82 (flubendiamide), 17.63 (indoxacarb), 17.52 (chlorantraniliprole) and 16.51 (emamectin benzoate) kg per 9 sqm plot (Table 6). For every one rupee invested in plant protection, the returns from the treatments were Rs 5.55 (chlorantraniliprole), 5.33 (flubendiamide), 5.28 (indoxacarb) and 2.32 (emamectin benzoate). The effectiveness of novaluron 0.01 % against *L. orbonalis* recorded earlier (Chatterjee and Roy, 2004 ; Sawant *et al.,* 2004) , is contrary to the results of the present study. The efficacy of different formulations of flubendiamide against *L. orbonalis* was also documented earlier (Reshma and Behara, 2018; Jagginavar *et al.*, 2009). Similarly, emamectin benzoate 5 SG @ 20 g a.i. ha^{-1} was found effective in reducing fruit damage by *L. orbonalis* in brinjal (Kumar and Devappa, 2006; Anil and Sharma, 2010). The lowest shoot and fruit infestations (7.47 and 9.88 per cent) and highest marketable fruit yield of 143.50 q ha^{-1} were recorded in the plots treated with spinosad 2.5 SC (50 g a.i. ha^{-1}) followed by indoxacarb 14.5 SC 50 g a.i. ha^{-1} (8.89 and 13.13 per cent), emamectin benzoate 5 SG 15 g a.i. ha^{-1} (10.95 and 16.66 per cent), respectively (Patra *et al.,* 2009).

All the new generation insecticides were equally effective in reducing the shoot infestation by *E. vitella* in okra, the reduction in damage ranging from 68.09 to 80.82 per cent in the newer molecules

and 67.89 and 67.73 in carbaryl and malathion treated plots respectively. Chlorantraniliprole (80.82 per cent), flubendiamide (80.34 per cent), indoxacarb (78.53 per cent) and emamectin benzoate (74.95 per cent) were comparatively more effective in preventing shoot damage. The reduction in damage of fruits ranged from 53.53 to 83.26 per cent in the novel insecticide treatments as against 45.82 and 43.97 per cent in carbaryl and malathion treatments, respectively. Among the insecticides, chlorantraniliprole, flubendiamide and indoxacarb with 83.26, 77.39 and 76.59 per cent reduction in fruit damage, respectively were superior. The associative yield obtained from these plots were significantly higher, being 5.80 kg (chlorantraniliprole), 5.61 kg (flubendiamide) and 5.50 kg (indoxacarb)

Table 6. Yield of brinjal and benefit: cost ratio of insecticidal treatments

Treatments	Dosage (g a.i.ha^{-1})	Yield			Monetary benefits (Rs ha^{-1})	Expenses for insecticides (Rs ha^{-1})	B: C ratio
		(kg/9 m² plot)	(t/ha)	Increase over control(%)			
Emamectin benzoate	10	16.51	18.34	33.76	68833.27	2.32	2.32:1
Spinosad	75	15.34	17.04	23.91	168666.67	1.54	1.54:1
Novaluron	100	15.74	17.49	27.14	175333.33	1.63	1.63:1
Chlorantraniliprole	30	17.52	19.47	41.52	205000.00	5.55	5.55:1
Indoxacarb	60	17.63	19.59	42.41	206833.33	5.28	5.28:1
Fipronil	50	15.70	17.44	26.82	174666.67	1.63	1.63:1
Thiodicarb	750	15.24	16.94	23.10	167000.00	-	-
Flubendiamide	100	17.82	19.80	43.94	210000.00	5.35	5.35:1
Carbaryl	750	14.47	16.08	16.88	154166.67	2.27	2.27:1
Malathion	500	14.52	16.13	17.29	155000.00	1.97	1.97:1
Untreated control		12.38	13.76			-	-
CD(0.05)		1.43					

Table 7. Yield of okra and benefit: cost ratio of insecticidal treatments

Treatments	Dosage (g a.i.ha^{-1})	Yield			Monetary benefits (Rs ha^{-1})	Expenses for insecticides (Rs ha^{-1})	B: C ratio
		(kg/6 m² plot)	(t/ha)	Increase over control(%)			
Emamectin benzoate	10	4.48	4.97	27.64	33333.33	5874/-	4.95:1
Spinosad	75	4.13	4.59	17.66	21666.67	5184/-	3.67:1
Novaluron	100	4.26	4.74	21.37	26000.00	8010/-	3.65:1
Chlorantraniliprole	30	5.80	6.45	65.24	77333.33	5625/-	6.23:1
Indoxacarb	60	5.50	6.11	56.70	67333.33	3855/-	6.96:1
Fipronil	50	4.11	4.57	17.09	21000.00	3402/-	4.51:1
Thiodicarb	750	3.95	4.39	12.54	15666.67	7350/-	3.20:1
Flubendiamide	100	5.61	6.23	59.83	71000.00	4620/-	6.93:1
Carbaryl	750	4.31	4.79	22.79	27666.67	2625/-	2.96:1
Malathion	500	4.13	4.59	17.66	21666.67	1013/-	3.34:1
Untreated control		3.51	3.89				-
CD(0.05)		0.80					

Table 8. Growth of *Beauveria bassiana, Lecanicillium lecanii* and *Metarrhizium anisopliae* on PDA media poisoned with different insecticides

Treatment	Dosage (g a.i.ha^{-1})	Mean mycelial growth (cm)														
		Beauveria bassiana					*Lecanicillium lecanii*						*Metarrhizium anisopliae*			
		3 DAI	9 DAI	15 DAI	21 DAI	27 DAI	3 DAI	9 DAI	15 DAI	21 DAI	27 DAI	33DAI	3 DAI	9 DAI	15 DAI	21 DAI
Emamectin benzoate	10	1.20	3.08	4.43	6.03	6.23	1.1	1.90	2.67	3.27	5.43	5.63	1.90	3.70	5.53	6.80
Spinosad	75	1.50	3.17	3.70	3.85	3.33	1.17	1.80	2.77	3.97	5.17	5.37	1.95	3.67	8.80	9.00
Novaluron	100	1.13	2.13	2.73	3.53	3.60	1.13	1.73	2.73	4.10	6.30	6.73	2.23	6.25	7.20	7.73
Chlorantraniliprole	30	1.67	3.87	4.33	5.10	5.23	1.13	2.33	3.57	4.73	7.17	7.50	2.30	4.20	8.33	9.00
Indoxacarb	60	1.70	4.27	4.87	6.40	6.63	1.17	2.30	3.33	4.37	6.03	6.47	2.33	8.33	8.73	9.00
Fipronil	50	1.17	2.57	3.60	4.57	4.73	1.17	1.57	2.73	4.13	6.93	7.13	2.28	4.17	6.83	7.77
Thiodicarb	750	1.33	2.57	3.37	4.40	4.50	1.10	1.27	2.50	3.73	5.77	6.03	1.93	3.80	5.77	6.67
Flubendiamide	100	1.13	2.13	2.60	3.47	3.63	1.07	1.47	2.10	2.33	2.53	2.80	1.50	4.40	6.42	7.67
Carbaryl	750	1.13	2.20	2.80	3.93	4.00	1.13	1.23	1.53	1.80	2.13	2.30	1.97	4.35	6.60	9.00
Malathion	500	1.20	2.90	3.77	4.73	4.83	1.10	1.37	3.10	3.40	4.90	5.10	2.27	5.30	7.47	9.00
Control		1.13	2.10	3.07	4.00	4.07	1.17	1.40	2.57	3.87	6.60	7.23	2.50	3.97	6.87	9.00
CD(0.05)		0.23	0.93	1.22	0.856	0.67	0.144	0.36	0.56	0.55	0.22	0.29	0.42	1.32	1.84	1.38

DAI - Days after inoculation

per six sq. m plot compared to the untreated plot (3.51 kg) (Table 7). The benefit cost ratio indicated that Rs 6.96 (indoxacarb), Rs 6.93 (flubendiamide) and Rs 6.23 (chlorantraniliprole) could be incurred in return for every one rupee spent to control the pest. The results of the study conform to the reports of other workers. Superiority of flubendiamide 480 SC @ 60 g a.i. ha^{-1} and 48 g a.i. ha^{-1} against okra fruit and shoot borer, *E. vitella* was reported by Katti and Surpur (2015). Emamectin benzoate 5 SG @ 11 g a.i. ha^{-1} reduced the larval population of *E. vitella* in okra (Kuttalam *et al.,* 2008). Significantly low fruit infestation was noticed with the application of spinosad 45 SC @ 30 g a.i. ha^{-1} followed by abamectin 1.9 EC @30 g a.i. ha^{-1}. Indoxacarb 0.015 % and fipronil 0.005 % were highly effective in preventing borer damage (Sinha *et al.,* 2009; Gupta *et al.,* 2009). Rynaxypyr 20 SC @ 30 g a.i. ha^{-1} and @ 20 g a.i. ha^{-1} were superior in recording less larval populations, lower fruit damage and higher fruit yield in okra, followed by spinosad @ 56 g a.i. ha^{-1}, emamectin benzoate @ 15 g a.i. ha^{-1} and flubendiamide @ 45 g a.i ha^{-1} (Chowdhary *et al.,* 2010).

All the insecticides were compatible with the white muscardine fungus, *B. bassiana* and in particular profuse growth of the fungus was seen in indoxacarb, emamectin benzoate and chlorantraniliprole indicating a probable synergistic effect on the fungus. Similarly, the various insecticides were safe to *M. anisopliae*, though comparatively lower growth of the green muscardine was noted in media poisoned with emamectin benzoate (24 per cent) and thiodicarb (26 per cent). All the treatments were found compatible with *L. lecanii* except flubendiamide and carbaryl which recorded 61 and 68 per cent reduction in the growth of the fungus, respectively (Table 8).

Considering the efficacy of the insecticides against the pests, associated yield increase, benefit cost ratio of the insecticide treatments and compatibility with bio agents, chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}, indoxacarb 14.5 SC @ 60 g a.i. ha^{-1} and emamectin benzoate 5SG @10 g a.i. ha^{-1} were adjudged as the potential insecticides against the fruit borers of brinjal and okra.

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Detection of dengue virus in *Aedes* mosquitoes in Delhi, India

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ABSTRACT: Detection of viruses in human sera particularly in endemic areas is cumbersome and laborious. Therefore, an alternative approach, Immuno-fluorescence assay (IFA) was performed to determine dengue virus (DENV) positivity in mosquitoes. A total of 1055 adult *Aedes aegypti* female mosquitoes were tested for IFA test against DENV. Minimum infection rate (MIR) for DENV was found higher during August to November 2016 ranging from 10.75 to 20.83. The average yearly MIR was about 6.64. Higher MIR for *Ae. aegypti* was found in Sarfabad, Noida (12.71) and Khoda Colony, Ghaziabad (11.90). Minimum MIR (4.67) was observed in Sanjay colony (Faridabad). The main contribution of this study resides in the development of a more suitable monitoring system for early detection of viral circulation and to prioritize early intervention in the non-transmission season.

KEYWORDS: *Aedes aegypti*, minimum infection rate, immuno-fluorescence assay, DENV

INTRODUCTION

Dengue fever is a disease of the public health importance caused by arbovirus and transmitted by *Aedes* mosquitoes (Diptera: Culicidae) in both rural and urban areas. The dengue viruses consists of an antigenic sub-group of closely related, yet antigenically distinct virus, serotype DENV 1-4, within the genus Flavivirus, Family Flaviviridae (Defoliart *et al*.,1986). Serotypes produce disease ranging from the relatively mild dengue fever (DF), a self-limiting febrile illness to the severe dengue haemorrhagic fever (DHF) characterized by hemorrhaging with or without fatal shock syndrome (Halstead, 2007).

DF has been the most important arboviral disease in the world, responsible for significant morbidity and mortality, especially in tropical countries (Bhattacharya *et al*., 2013; Khan *et al*., 2013; Restrepo *et al*., 2014). The geographic distribution of dengue has increased over decades. In the 1950s, nine countries reported dengue; today over 100 countries are endemic for DF. Mostly deaths are due to lack of early diagnosis of dengue virus infection caused by four serotypes (Anez and Rios, 2013). DF is the most prevalent arboviral infection worldwide, with up to 40% (2.5–3 billion people) of the world's population living in endemic regions. It is estimated that 50–80 million dengue infections occur each year, with 500,000 cases of DHF, and

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at least 12,000–24,000 deaths, mainly among children under 15 years of age (WHO, 2011).

With the ongoing search for an efficient vaccine and an antiviral drug, prevention is still the best way to control the disease, which is possible through vector control in several forms (Black and Lundkvist, 2013; Resende *et al*., 2013). When adult mosquito density is low, direct entomological monitoring is not a sensitive indicator for outbreak prevention. It is in this particular situation that detection of DENV in vector population becomes a particular element as part of an early alert system which allows to position the vector as the primary element in the transmission cycle during epidemiological evaluations (Chow *et al*., 1998). Surveillance of mosquitoes infected with dengue virus can help to monitor the infection rates within vector mosquito population and provide an early warning signal to predict an impending outbreak of DF (Tewari *et al.,* 2004)

Delhi NCR carries high receptivity and vulnerability to *Ae. aegypti* because of high international traffic as well as from the bordering satellite towns of Noida and Ghaziabad (UP) and Faridabad (Haryana) which require a well-organized and coordinated effort to control the incidence of dengue in Delhi and NCR. A major dengue outbreak with more than 10,000 cases and 425 deaths occurred in Delhi in 1996. In 1967, 1968 and 1969, outbreaks of dengue occurred in Delhi when a number of strains of DENV-2 were isolated from humans. Dengue outbreaks were reported from Delhi in 1970, 1982, 1996, 2003, 2006, 2010, 2013 and 2015 (Kumari Roop *et al*., 2011; Sharma *et al.,* 2014; Chaturvedi and Nagar, 2018). In 2015, the city witnessed 1587 cases, the worst crisis in 20 years with the disease claiming 60 lives. Though Vazeille *et al*. (2003) reported that *Ae*. *aegypti* has a relatively low receptivity for DENV-2 as compared to *Ae*. *albopictus,* Arm strong and Ricco-Hesse (2003) proved that *Ae*. *aegypti* has significantly more receptivity to DENV-2 than *Ae*. *albopictus*. In this study, we report the results of dengue virus detected in *Ae. Aegypti* collected from August 2016 to July2017 from selected localities of NCR, Delhi with the help of IFA. Reliable estimation of natural mosquito infection with arbovirus forms a key element in any surveillance system and is essential for vector incrimination and also for developing appropriate preventive measures.

MATERIALS AND METHODS

The study was conducted in six localities viz., Nithari Sector 30 and Sarfabad (Noida), Khoda colony and Railway Colony (Ghaziabad), Sanjay Colony and Sehatpur (Faridabad), of North Central Railway (NCR). They were selected on the basis of confirmed dengue cases during 2015. NCR has witnessed indiscriminate construction activities causing stagnation of water in containers lying in and around the construction sites. *Aedes* larvae were collected from different localities of study areas by inspecting the water holding containers in domestic and peri-domestic environment. The larvae were bought in the laboratory and reared up to adults. Individual *Aedes* mosquitoes were screened for the dengue virus from each locality. The mosquitoes were sorted out as males and females from each locality. IFA was performed on female *Ae. aegypti* mosquitoes to determine the positivity percentage and dengue virus Minimum Infection Rate (MIR). MIR is estimated from the number of virus-positive female mosquitoes/total number of female mosquitoes tested multiplied by 1000.

Head of each mosquito was squashed by pressing it on the glass slide through 12mm^2 coverslip. The cover slip was then lifted gently and discarded in decontaminating pan. The slides were air-dried and the tissues were fixed with chilled acetone at 4°c for 10 minutes. After washing these slides for 10-15 minutes with PBS (phosphate buffered saline) and mounted in glycerol, the bound were detected by addition of a drop of Florecin isothiocynate (FITC) conjugated goat anti-mouse lgG (procured from M/s sigma, USA). The slides were again incubated for 40 minutes at 37c and counter stained with drop of Evan's blue solution for 5 minutes at room temperature. 5-6 mosquito squash were made in each glass slide for screening. The virus infection rate was expressed as minimum infection rate (MIR) calculated per thousand mosquitoes as described by Gajanana *et al*. (1995).

RESULTS AND DISCUSSION

A total of 1055 adult *Ae. aegypti* females were subjected individually to IFA test against DEN virus. Our results on F1 generation showed 6.64 MIR which seems to be high vertical transmission in generation. Monthly mosquito positivity showed that F1 reared mosquitoes were found positive during the months of August to November 2016. Though in January 2017 more number of mosquitoes were tested but none of the mosquitoes was found positive. The minimum infection rate (MIR) for DENV was found to be higher during the months of August to November 2016 ranging from 10.75 to 20.83 which seem to be very high. The average yearly MIR was 6.64 (Table 1, Fig. 1).

The urban system of Delhi is complex as it is an amalgamation of different socio cultural and socio economic groups as well as populations visiting on daily basis for their day today life processes including jobs, purchase, treatment etc. As per the results of the present study, overall combined MIR of all six localities of NCR was found to be 6.64, which is much lower than the MIR calculated in earlier observations (Ilkal *et al*., 1991). Our results demonstrated high MIR for *Ae. aegypti* in Sarfabad (Noida) and Khoda colony (Ghaziabad) i.e., 12.71 and 11.90 respectively which are consistent with infection rate 9.1 and 9.2 reported in a study carried out in Delhi by Kumar *et al*. (2015). Trasovarial transmission of DENV in *Aedes* mosquitoes is considered an important mechanism for the maintenance of the virus in nature and may be implicated in the occurrence of outbreaks and epidemics of the disease (Arunachalam *et al.,* 2008). Source reduction activities i.e. removal of all water holding and dry containers lying in the urban system of Delhi/NCR during non-transmission season i.e. January to June should be carried out to prevent dengue outbreaks.

The MIR in our study was higher in transmission months (Aug-Nov). It is known in DENV that if MIR reaches 10, suggesting a high risk to the surrounding community. If human herd immunity is high, the probability of transmission will be lower in an area regardless of the magnitude of measures of entomological risk. Conversely, if herd immunity is low, relatively low population densities of *Ae. aegypti* could precipitate an epidemic (Scott and Morrison, 2003). Domestic containers play a crucial role in *Aedes* breeding especially during non-transmission season. Therefore, community should take initiative to clean their own breeding sources otherwise those will act as key containers in transmission season (Nagpal *et al*., 2016a,b)). Such water storage practices promote *Aedes* mosquito breeding throughout the year (Sharma *et al*. 2008; Samuel *et al*., 2019).Such areas with persistent of *Aedes* breeding can act as foci for the next dengue outbreak. Entomological surveillance and vector control measures are essential to prevent devastating disease outbreaks.

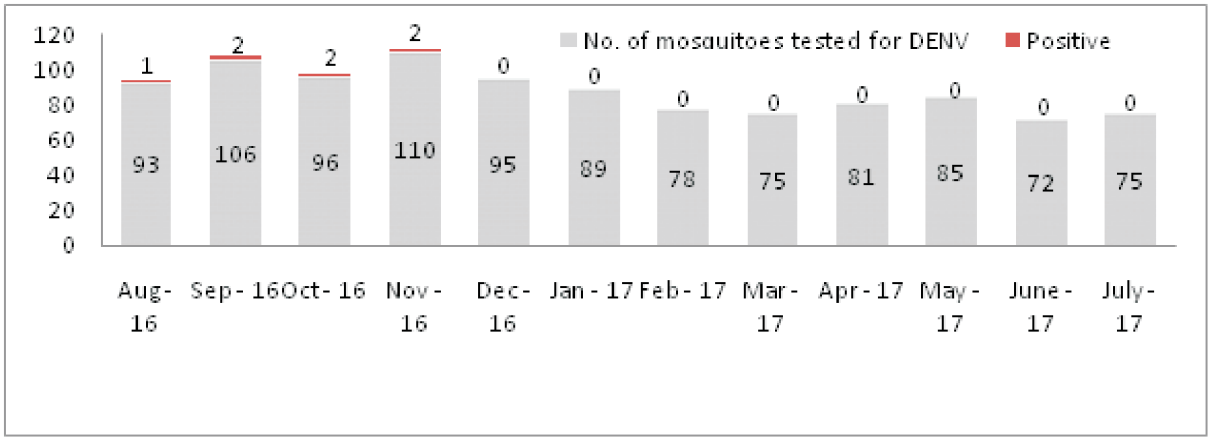

Fig. 1. Number of *Aedes aegypti* mosquitoes tested for DENV in different months (August 2016 to July 2017)

Table 1. Monthly positivity of *Ae. aegypti* for DENV collected from selected localities in NCR, DELHI

Sl. No.	Locality		Aug 16	Sept 16	Oct 16	Nov 16	Dec 16	Jan 17	Feb 17	Mar 17	Apr 17	May 17	Jun 17	Jul 17	Total	MIR
1.	Nithari Sector 30(Noida)	No. of Mosquito	16	17	23	17	14	4	8	13	12	15	14	16	169	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
2.	Sarfabad (Noida)	No. of Mosquito	20	25	15	18	28	27	16	14	18	20	18	17	236	12.71
		Positive	1	0	1	1	0	0	0	0	0	0	0	0	3	
3.	Khoda colony (Ghaziabad)	No. of Mosquito	22	26	16	17	25	30	24	18	18	20	19	17	252	11.90
		Positive	0	1	1	1	0	0	0	0	0	0	0	0	3	
4.	Sanjay Colony (Faridabad)	No. of Mosquito	18	20	24	37	15	16	15	18	15	15	8	13	214	4.67
		Positive	0	1	0	0	0	0	0	0	0	0	0	0	1	
5.	Railway Colony (Ghaziabad)	No. of Mosquito	10	12	14	16	8	8	9	8	12	8	7	7	119	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
6.	Sehatpur Village (Faridabad)	No. of Mosquito	7	6	4	5	5	4	6	4	6	7	6	5	65	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total		No. of Mosquito	93	106	96	110	95	89	78	75	81	85	72	75	1055	6.64
		Positive	1	2	2	2	0	0	0	0	0	0	0	0	7	
		Monthly MIR	10.75	18.87	21	18	0	0	0	0	0	0	0	0	6.64	

The study carried out by Kumari Roop *et al*. (2011) in Delhi showed high mosquito positivity in F1 generation mosquitoes. Joshi *et al*. (2002) reported persistence of DENV-3 through transovarial transmission passage in successive generations in *Ae. aegypti* mosquitoes. It is thus important to highlight the implications for future studies on vectorial competence and virus-vector interaction, as well as the mechanism involved in the maintenance of DENV in nature. In this context, it is clear that the main contribution of the study resides in the development of a more suitable monitoring system for the early detection of viral circulation and the risk of epidemics and severe forms of the disease. Our study implies that Noida and Ghaziabad in NCR region having higher MIR and at higher risk of dengue transmission in which better virological and entomological surveillance are required for effective dengue vector control. Such studies should also be integral part of routine surveillance of the city which can provide early evidence of transmission potential area to prioritize early intervention in the non-transmission season.

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Evaluation of entomopathogenic fungi against *Raoiella indica* Hirst (Acari: Prostigmata: Tenuipalpidae)

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ABSTRACT: Entomopathogenic fungi *Metarrhizium anisopliae, Beauveria bassiana* and *Lecanicillium lecanii* tested against immature and adults of *Raoiella indica* under laboratory condition with five different concentrations of each sprayed on leaf discs containing larvae, nymphs, and adults, indicated that all life stages were susceptible. Larval and nymphal stages were generally less susceptible than adults. Based on probit analysis, *L. lecanii* was the most virulent with LC_{50} of 8.15×10^5 conidia ml^{-1} and 1.30×10^5 conidia ml^{-1} followed by *M. anisopliae* 18.05 x 10^5 conidia ml^{-1} and 2.70×10^5 conidia/ml and *B. bassiana* (27.13×10^5 conidia ml^{-1} and 4.80×10^5 conidia ml^{-1}) for immature and adults, respectively. However the efficacy of the fungal pathogens evaluated clearly differs from that of the controls. These entomopathogenic fungi could be considered as an environmentally friendly alternative for biocontrol of *R. indica.*

KEYWORDS: Red palm mite; *Metarrhizium anisopliae, Beauveria bassiana, Lecanicillium lecanii*; pathogenicity

INTRODUCTION

Arecanut is an important commercial crop and it is attacked by an array of insect and non-insect pests. The pests infest all parts of the palm *viz.,* stem, leaves, inflorescence, root and nuts. As many as 102 insect and non-insect pests have been reported to be associated with arecanut palm (Nair and Daniel, 1982). Among these, the red palm mite, *Raoiella indica* Hirst. (Acarina: Tenuipalpidae) is the most serious pest mainly in young areca plantations and active infestation of leaves occurs after the onset of hot weather. The mite feeds on the underside of palm fronds of various hosts in the orders Arecales and Zingiberales. The mite attained economic significance when it was first reported as an invasive species in the Caribbeans in 2004 (Flechtmann and Etienne, 2004). It was reported as a serious pest of economically important fruit-producing trees like the coconut, *Cocos nucifera* and banana, *Musa* spp (Nagesha-Chandra and Channabasavanna, 1984; Welbourn, 2006) and it formed the first mite species in which feeding was observed through the stomata of its host plants (Ochoa *et al.,* 2011). Through this specialized feeding habit, *R. indica* interferes with the photosynthesis and respiration processes of its host plants. Mite infested palms display stunted growth and withering of leaves. *R. indica* is primarily controlled by acaricides in India. Long-term reli-ance on chemical acaricides results in pest resistance and residue problems. It is necessary to

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search for alternatives such as biopesticides to control *R. indica*. Advantages of biopesticides are their low mammalian toxicity, short environmental persistence, safety to beneficial and non-target organisms, as well as minimum risk of resistance development. Biological control, including the use of entomopathogenic fungi as part of an inte-grated pest management (IPM) strategy, is expected to reduce the dependence on synthetic acaricides. Most reports on the subject deal with insects and only few reports are available on Acari. However, research reports on the use of *Metarrhizium anisolpliae* (Metsch)*, Beaveria bassiana* (Bal.) and *Lecanicillium lecanii* (Zimm.) against *R .indica* are limited. In this respect, this study focused on examining the effectiveness of these entomopathogens against *R. indica* populations under laboratory conditions. The objective was to evaluate their virulence against this important mite pest and facilitate progress in microbial control with fungal pathogens. The experiment was conducted under laboratory conditions at Organic Farming Research Centre (OFRC), Organic Farming Research Centre, University of agricultural and horticultural Sciences (UAHS), Shivamogga.

MATERIALS AND METHODS

Preparation of fungal pathogen suspension{ Entomopathogenic fungi (*Beauveria bassiana Metarhizium anisopliae* and *Lecanicillium lecanii*) were used for evaluation against *Raoiella indica*. All the three fungi were cultured in standard Potato Dextrose Agar medium. After ten days of incubation, the spores were harvested, spore suspension prepared with distilled water and filtered through a double layered muslin cloth to get a clear spore suspension. Tween 80 (0.02%) was used to disperse the conidia uniformly in the solution. One ml of the spore suspension was poured on to haemocytometer to count the fungal spores and adjusted to required level. Serial dilutions were made from the stock spore suspensionto obtain the required concentrations for bioassay studies.

Determination of concentration range and pathogenicity: Before conducting bioassay in the laboratory, the procedure suggested by Daoust and Roome (1974) for bracketing of pathogens was followed. Accordingly**,** the serial dilutions were prepared to arrive at approximate range of concentrations inflicting mortality of mites between 10 and 90 per cent. Five different concentrations (10^4, 10^5, 10^6, 10^7, 10^8) were selected within each range and used for the determination of median lethal concentration (LC_{50}).

Two square centimeter leaf discs were cut from the areca palm, sterilized with 0.1 per cent sodium hypochlorite solution, later transferred to 5 ml distilled water blanks to remove excess solution**,** and dried (Gerson *et al.,* 1982). Such leaf discs were placed on water saturated cotton contained in petri dishes. In each leaf discs 50 immatures (larvae and nymphs)/ adults were transferred carefully using sterilized brush. Ten ml spore suspensions of the different entomopathogenic fungi were prepared by using 0.05 per cent Tween 80 solution to get uniform spray solution .The spore suspension of different concentrations were sprayed on mites contained on leaf discs with a hand atomizer spray. Such three replications and a control was maintained for each entomopathogenic fungi. The plates were incubated at 24±1^{0}C, 90 to 92 per cent RH in BOD incubators. Mortality of the mites was recorded 2, 4 and 6 days after spraying.

Lethal effects of entomopathogenic fungi were evaluated as per cent corrected mortality in the control variant according to Abbott's (1925) formula. For each concentration, mortality data from all the replicates were pooled and subjected to probit analysis. These data were analysed by IBM SPSS 23.

RESULTS AND DISCUSSION

Pathogenicity of Lecanicillum lecanii:

Maximum mortality of mites at two days after treatments (DAT) was recorded in $2x10^8$ conidia ml^{-1} (20.67 per cent ± 2.82 in immature and 27.33per cent ± 2.10 in adult stage) followed by $2x10^7$ conidia ml^{-1} which recorded 18.00 ±2.10 and 20.00 ± 1.66 per cent mortality in immature and adults respectively. At 4 DAT, the highest per cent mortality was observed at 2×10^8 conidia ml^{-1}

(64.00 ± 2.60 in immature and 72.00 ± 2.95 in adults), which was followed by 2×10^7 conidia ml^{-1} (58.67 ± 2.22 inimmature and 64.00 ± 1.02 in adult). The mortality rate of active stages of mite decreased gradually as the concentration of conidia decreased. At 6 DAT significantly higher mortality of both immature and adults was observed at 2×10^8 conidia ml^{-1} (92.00 ± 3.18 % in adults and 89.33 ± 2.60 % in immature), compared to rest of the concentrations (Table 1). The lethal concentration (LC_{50}) value at six days after treatment was (8.15×10^5 conidia ml^{-1} and 1.30×10^5 conidia ml^{-1})

Table 1. Bioefficacy of entomopathogenic fungi against immature and adult stages of *R. indica*

Entomopathogenic fungi	Concentration (Conidia/ml)	Mortality± SE (%)					
		2 DAT		4 DAT		6 DAT	
		Immature stage	Adult	Immature stage	Adult	Immature stage	Adult
Lecanicilliumlecanii	$2x10^8$	20.67± 2.82^c	27.33± 2.10^c	64.00± 2.60^d	72.00± 2.95^d	89.33± 2.60^{cd}	92.00± 3.18^d
	$2x10^7$	18.00± 2.10^{bc}	20.00± 1.66b^c	58.67± 2.22^c	64.00± 1.02^{bc}	68.00± 1.98^c	80.67± 2.66^c
	$2x10^6$	16.00± 1.62^b	14.00± 1.24^b	40.00± 1.58^{bc}	58.67± 1.41^c	47.33± 1.66^b	65.33± 1.90^c
	$2x10^5$	11.33± 1.89^b	10.00± 1.45a	26.00± 1.83^b	36.00± 1.62^b	36.00± 1.91^b	43.33± 2.24^b
	$2x10^4$	6.67± 1.10^a	8.67± 0.90^a	12.67± 1.42^a	24.00± 1.35^a	16.00± 1.04^a	32.67± 1.22^a
Metarhiziumanisopliae	$1.4x10^8$	20.00± 2.05^c	26.00± 1.90^c	60.00± 2.11^d	68.00± 1.60^d	82.67± 3.33^d	90.00± 3.10^d
	$1.4x10^7$	17.33± 1.30^c	22.00± 1.22^c	52.67± 1.95^d	59.33± 1.54^c	64.00± 2.90^d	77.33± 2.42^c
	$1.4x10^6$	13.33± 1.20^{bc}	13.33± 0.90^{ab}	36.00± 1.41^c	48.00± 1.22^{bc}	40.67± 1.66^{bc}	58.00± 1.60^b
	$1.4x10^5$	9.30± 1.48^b	10.00± 1.23^a	24.00± 1.62^b	32.67± 1.60^b	28.00± 1.84^b	39.33± 1.85^{ab}
	$1.4x10^4$	6.00± 0.69^a	8.00± 0.65^a	9.33± 1.10^a	22.67± 0.98^a	14.00± 1.20^a	28.00± 1.24^a
Beauveriabassiana	$2.6x10^8$	20.00± 1.92^d	24.00± 1.66^c	58.00± 1.39^d	66.67± 1.54^d	76.67± 2.22^d	86.67± 2.10^d
	$2.6x10^7$	16.00± 1.83^c	20.00± 1.90^b	49.33± 1.20^{cd}	54.67± 0.93^d	63.33± 1.90^c	73.33± 1.60^d
	$2.6x10^6$	17.33± 1.20^b	12.67± 1.27^{ab}	32.67± 0.88^c	43.33± 0.66^c	36.00± 0.90^{bc}	54.00± 0.98^c
	$2.6x10^5$	8.00± 1.68^a	9.33± 1.57^a	20.00± 1.10^b	28.67± 1.24^b	26.67± 1.22^b	32.00± 0.14^b
	$2.6x10^4$	7.33± 0.59^a	6.67± 0.44^a	9.33± 0.49^a	18.00± 0.40^a	14.67± 0.38^a	26.67± 0.24^a
Untreated Control	0.00	0.00	0.00	5.00± 0.82^e	3.87± 1.23^e	8.24± 0.44^e	5.33± 0.89^e

Table 2.Probit analysis of concentration-mortality responses of immaturesof *R. indica* to entomopathogenic fungi (6 DAT)

Entomopathogenic fungi	Regression Equation (Y= a + bx)	LC_{50} (Conidia/ml) (x 10^5)	LC_{90} (Conidia/ml) (x 10^5)	Fiducial limit (Conidia/ml) (x 10^5) at 95 % CI	Chi^2(÷2)
Lecanicilliumlecanii	Y= - 3.12+0.53 x	8.15	2421.22	5.23-12.66	4.59
Metarhiziumanisopliae	Y= - 3.11+0.50 x	18.05	6727.66	11.46-28.91	1.74
Beauveriabassiana	Y= - 2.91+0.39 x	27.13	17579.00	16.50-46.00	3.27

Table 3. Probit analysis of concentration-mortality responses of adults of *R. indica* to entomopathogenic fungi (6 DAT)

Entomopathogenic fungi	Regression Equation (Y= a + bx)	LC_{50} (Conidia/ml) (x 10^5)	LC_{90} (Conidia/ml) (x 10^5)	Fiducial limit (Conidia/ml) (x 10^5) at 95 % CI	Chi^2(÷2)
Lecancilliumlecanii	Y = - 1.96 + 0.33 x	1.30	751	0.70-2.30	1.83
Metarhiziumanisopliae	Y = - 1.96 + 0.33 x	2.70	1436	1.50-4.30	1.81
Beauveriabassiana	Y = - 2.59 + 0.46 x	4.80	3225	2.92-7.98	4.30

against immature and adults of *R. indica* respectively (Table 2 and 3).

Pathogenicity of Metarhizium anisopliae:

At 2 DAT *M. anisopliae* at 1.4 × 10^8 conidia/ ml caused highest mortality in immatures (20.00% ± 2.05) and adults (26.00 % ± 1.90). The concentrations 1.4 × 10^8 and 1.4 × 10^7conidia/ ml performed on par with each other and significantly superior to other concentrations of *M. anisopliae* (Table 1). *M. anisopliae* at 1.4 × 10^8 conidia/ml caused 60.00 ± 2.11 per cent mortality of immatures and 68.00 ± 1.60 per cent of adult mortality at 4 DAT followed by 2 × 10^7 conidia/ ml which recorded 52.67±1.95 and 59.33 ± 1.54 per cent mortality in immatures and adult mites respectively. The mean mortality of mite increased steadily as conidial concentration increased. At 6 DAT among the different concentration of *M. anisopliae*maximum per cent mortality was recorded at 2 × 10^8 conidia ml^{-1} both in immatures (82.67 ± 3.33) and adult (90.00 ± 3.10) mites, followed by 2 × 10^7 conidia/ ml (64.00 ± 2.90 in immatures and 77.33 ± 2.42 in adults) and were on par each other (Table 1). At 6 DAT and the lowest calculated LC_{50} value was 18.05 x 10^5 conidia/ml with fiducial limit ranging from 11.46 × 10^5 to 28.91 × 10^5 conidia/ml against immature and 2.70 × 10^5 conidia/ml with fiducial limit ranging from 1.50 × 10^5 to 4.50 × 10^5 conidia ml^{-1} against adults of *R. indica* (Table 2 and 3).

Pathogenicity of Beauveria bassiana:

At 2 days after treatment maximum mortality per cent of 20.00 ± 1.92 was recorded in immature and 24.00 ± 1.66 per cent in adults at 2x10^8 conidia/ ml, followed by 2 × 10^7 conidia ml^{-1} , where mortality rate of 16.00 ±1.83 per cent was recorded in immature and 20.00 ± 1.90 per cent in adult mites (Table 1). Different conidial concentration of *B. bassiana* was evaluated against active stages of mites and at four days after treatment, the highest mortality percentage was observed at 2x10^8 conidia/ ml concentration with 58.00 ± 1.39 in immature stages and 66.67 ± 1.54 per cent in adults, followed by 2 × 10^7 conidia/ ml which recorded 49.33 ± 1.20 and 54.67 ± 0.93 per cent mortality of adult and

immatures respectively. However, the mortality decreased gradually with the decrease in concentration. At six days after treatment *B. bassiana*at 2×10^8 conidia/ ml recorded significantly higher per cent mortality in adult stage (86.67 ± 2.10) and 76.67 ± 2.22 in immature stages compared to rest of the concentrations (Table 1). At 6 DAT the calculated lowest LC_{50} values for *B. bassiana* was 27.13×10^5 conidia ml^{-1} and 4.80×10^5 conidia/ml against immatures and adults of *R. indica* respectively (Table 2 and 3).

Overall, the efficacy of entomopathogenic fungi indicated that higher conidial concentration was more effective compared to rest of the treatments. All the five dosages of fungi proved their supremacy to uninoculated treatments. The least mortality of mites was observed at lower conidial concentration, this may be due to hyphal and conidial characters vary between the concentrations. These results indicate variability among the concentration which needs to be realized when being used for the development of effective bioinoculant and mass production. In the present study, it was noticed that at four and six days after infestation maximum mortality was observed in all the three entomopathogenic fungi *viz.,* of *L. lecanii, M. anisopliae* and *B. bassiana.* The different motile stages of *R. indica* varied in their susceptibility to these entomopathogenic fungi. It was observed that immature stages were generally less susceptible to fungal infection than adults. This might be due to integument being penetrated by the fungus and ecdysis. Moulting has been reported to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals (Sewify and Mabrouk, 1991).obtained are close to those reported by Sewify and Mabrouk (1991) who found that adult stages of the citrus brown mite were susceptible to the entomopathogenic fungus, *V. lecanii* Similarly, El Hady (2004) reported that adult stage *Eutetranychus orientalis*, was highly susceptible to *V. lecanii* compared to other motile stages.

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Report of *Celosterna scabrator* (Fabricius, 1781) (Coleoptera: Cerambycidae: Lamiinae) from Goa, India

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ABSTRACT: *Celosterna scabrator* (Cerambycidae, Lamiinae) is reported from Goa for the first time. The diagnostic characteristics, colour images and geographical distribution of *C. scabrator* are given.

KEY WORDS: Lamiinae, *Celosterna scabrator,* Goa

Insect diversity of Goa state is very poorly studied as compared to adjoining states of Maharashtra and Karnataka, where good amount of information has been generated on species diversity of class Insecta. Perusal of literature revealed that most studies were carried out in orders Odonata, Lepidoptera and Mantodea (Rangnekar *et al*., 2010; Vyjayandi *et al.*, 2010; Gaude and Janarthanam, 2015 and D'Souza and Pai, 2019) and the rest of orders of class Insecta were ignored by researchers especially on the family Cerambycidae of order Coleoptera. According to recent publication, the Goa state represents 2 subfamilies, 2 tribes and 3 genera of family Cerambycidae and its species composition against India is 0.1% during the year 1758 to 2016 (Kariyanna *et al.*, 2017a). Sen *et al*. (2005) have reported two cerambycids from Goa. As compared to Maharashtra and Karnataka state were represents 3.1% and 5.9% species respectively, the species composition against India during the year 1758 to 2016 (Kariyanna *et al.,* 2017a). The genus *Celosterna* composed of only two species in India (Kariyanna *et al*., 2017b) of them no earlier record from Goa. The species *Celosterna scabrator* is widely distributed and is a very common in India and another one *Celosterna fabricii* is very rare and it is only known from Tamil Nadu. The morphological character of *C. scabrator* is presented in this communication and is being reported for the first time from Goa.

Celosterna scabrator (Fabricius, 1781) (Image 1 and 2)

Lamia scabrator Fabricius, 1781: 224; *Zimsen*, 1964: 167 (Type).

Specimens examined: One male, 26.iv.2018, Sal-Goa (latitude 15° 57' 493'' N and longitude 74°10' 028''E), Coll. S. V. More, Collected from arjun tree (*Terminalia arjuna*), identified by Dr. Hemant Ghate

Adult (male): Body length: 24.4mm; width: 5.3mm. Generally, body colour dark brown, yellow to black. Head gray to brown, vertical, covered with yellow brown colour pubescence, front view of head

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Image 1. *Celosterna scabrator* Morphology
A: dorsal view; B: elytra details; C: ventral view; D:head details

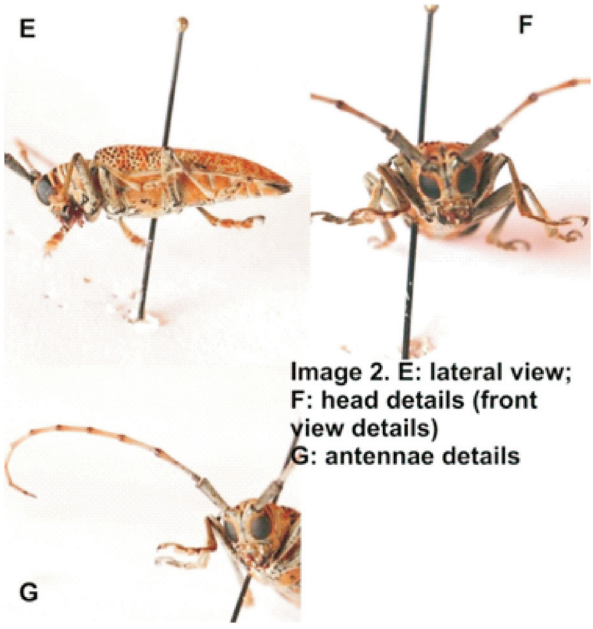

Image 2. E: lateral view; F: head details (front view details)
G: antennae details

brownish to yellow (image 2. F), antennae longer than body, covered with brownish pubescence, scape thick, slender, with slightly gray in colour, remaining antennomeres covered with brownish pubescence and nitid, apical segment short and pointed (image 2. G). Thorax dark gray to brown in colour, with lateral pointed spine, not smooth, with thin punctured, longer than head, vertex brown in colour without punctured, black line extending from vertex and run between upper lobes of eyes and reach below the frons (image 1 and 2. C, D and F). Elytra dark brown, clothed with reddish brown pubescence, thickly punctured on base, large punctured on each humerus and surrounding the scutellum, less punctured at apical area of each elytron, with apical blunt spine, scutellum reddish-brown, 'U' shaped or tongue like (image 1. B). Legs are brownish in colour, claws widely separated with reddish- brown. Abdomen ventrite visible, covered with brownish pubescence, ventrite 1, 2 and 5 occupy large space as compared to ventrite 3 and 4 occupy about equal space (image 1. C). The mesoventrite, metanepisternum and metaventrite without spot and covered with brownish pubescence (image 1. C).

Distribution: Pakistan, Ceylon, Nepal, Vietnam Laos, and India (Tamil Nadu, Maharashtra, Orissa, Chhattigarh, Karnataka, Himachal Pradesh Bengal and Uttar Pradesh).

Remarks: This communication provided here is additional geographical distribution of *C. scabrator* from India.

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Biology of eri silkworm, *Samia ricini* (Donovan) on castor, *Ricinus communis* L.

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ABSTRACT: Studies on biology of eri silkworm, *Samia ricini* (Donovan) on castor, *Ricinus communis* L. under laboratory condition revealed that the eggs were laid in clusters on *kharika*. A female laid average 360.10±23.88 eggs with 97.17±2.09 per cent hatching and 8.83±0.91 days of incubation period. The duration of first, second, third, fourth and fifth instar larva were 3.77±0.43, 3.23±0.43, 3.70±0.47, 4.60±0.50 and 7.67±0.55 days, respectively with total larval duration of 22.97±0.85 days. The pre-pupal and pupal periods were2.63±0.49 and 15.73±0.74 days, respectively. The pre-oviposition, oviposition and post-oviposition period were 11.61±0.37, 70.47±1.78 and 122.73±4.81hrs, respectively. The female and male longevity were 204.82±5.24and155.99±7.99hrs, respectively with sex ratio of 1:2.01 (Male: Female).

KEYWORDS: Bionomics, morphometrics, silkworm**,** *Samia ricini*

Sericulture is broadly classified into two distinct sectors *viz.,* mulberry and non-mulberry. Mulberry sericulture is concerned with mulberry silk production. Whereas, non-mulberry sericulture includes eri, tasar, and muga culture. India holds a unique distinction in producing all three kinds of non-mulberry silks. Among the non-mulberry silkworm species only eri silkworm is completely domesticated and reared indoors. It is a multivoltine insect completing at least six to seven generations in a year. The word "Eri" is derived from the Sanskrit term "Erranda", which refers to the Castor plant. *Ricinus communis* L., which is the primary host plant.

Ericulture is relatively a less remunerative occupation as compared to the production of other silks, but has its own advantages. Eri silkworms require comparatively minimum care as they are neither as wild as muga or tasar worms nor so much domesticated as mulberry silkworms. Eri silk has always been identified as 'Ahimsa silk' because there is no need to kill the pupae for getting silk. The rearer can easily preserve the cocoons till a reasonable price is offered. It is advantageous to producers. Eri silk is widely used for preparing warm clothing like chadars, quilts and scarves, which are used by the poor rural folk and the silk is referred as poor man's silk. The present investigation has been designed with the objective of measuring the potential of sustainable utilization of the non-mulberry silk moth for rearing through understanding basic biology and various stages of eri silkworm.

The rearing of eri silkworm was carried with the use of castor (GCH-7) leaves from well grown

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castor plot near Sericulture laboratory, Department of Entomology, N. M. College of Agriculture, N.A.U., Navsari during 2018-19. The studies on biology of eri silkworm on castor conducted under laboratory conditions. In order to study the biology of various stages of eri silkworm 20 freshly emerged male and female adult moths were kept in tray for mating and paired moths were covered with cloth. After six hours, unpaired female moths were allowed to lay eggs on cloth (*kharika*). The *kharika* with eggs were collected on next day morning. The eggs were detached from *kharika* and used for further studies. The study on biology started with at least 30 neonate larvae of the same age and reared individually. The size of eggs and initial instar larvae measured with stereo trinocular microscope (Make: Olympus-SZ 61) fitted with Brand Catcam-130 camera having software power Scopephoto (Version 3.1) while, later instar larvae were measured with scale.

The freshly laid eggs by female were sticky, oblong in shape and yellowish in colour, after few second it turned milky-white in colour. Eggs were usually laid on the *kharika* (to which the moth kept) sticking in cluster one above the other in an orderly manner due to their secretion of gummy substances. The eggs were initially creamy white and later became darker to violet in colour at the time of hatching (Plate 1). At the time of hatching, the chorion cracked at one end of egg and larva wriggled out from the eggs. Renuka and Shamitha (2014) and Brahma *et al.* (2015) described same results on eggs of eri silkworm.

The data on morphometrics of the eggs are presented in Table 1 indicated that the length and breadth of eggs were 1.29±0.05 and 1.19±0.05mm, respectively. The results are more or less similar with Brahma *et al.* (2015) who reported the length and breadth of eggs were 1.7±0.02 and 1.1±0.06mm, respectively on castor. The data (Table 2) revealed that the incubation period of *S. ricini* was 8.83±0.91days with 97.17±2.09 per cent hatching. The results are more or less agreement with Patil (2004) who recorded 8.00 days of incubation period of *S. cynthia ricini* on castor leaves. Moreover, Naik and Murthy (2014) found 95.18 per cent hatching in *S. ricini.*

Table 1. Morphometrics of various stages of eri silkworm, *S. ricini* (n = 25)

Sr. No.	Stage	Length			Breadth		
		Min.	Max.	Av.±SD	Min.	Max.	Av.±SD
1.	Eggs (mm)	1.18	1.40	1.29±0.05	1.06	1.26	1.19±0.05
2.	Larva						
	1st instar (mm)	2.28	2.62	2.49±0.07	0.55	0.67	0.59±0.04
	2nd instar (mm)	8.23	9.70	8.75±0.35	1.83	2.23	2.07±0.11
	3rd instar (mm)	22.50	30.10	26.00±1.57	3.40	7.10	4.54±0.81
	4th instar (mm)	35.40	39.10	37.10±1.18	8.10	9.42	8.71±0.42
	5th instar (mm)	55.23	60.20	58.45±1.19	11.10	12.40	11.93±0.36
3.	Pupa (mm)	21.35	25.20	24.08±1.06	10.23	12.57	11.51±0.73
4.	Cocoon (mm)	40.12	52.32	49.43±2.46	20.10	22.36	21.27±0.71
5.	Adult wing span						
	Female (cm)	12.10	13.00	12.52±0.27	-	-	-
	Male (cm)	10.30	11.70	10.90±0.42	-	-	-
6.	Adult size						
	Female (cm)	3.40	4.00	3.70±0.21	0.70	0.90	0.79±0.08
	Male (cm)	2.80	3.10	2.94±0.11	0.70	1.00	0.81±0.09

Plate 1. Different stages of eri silkworm, *S. ricini*

During present investigation, it was observed that the larva of eri silkworm moulted four times thus, there were five larval instars. The present findings are similar to findings of Kavane (2014) and Yaligar (2014). The newly hatched neonate larvae were dark yellow in colour with black linings and hairs. The head capsule was dark brown in colour with black hairs. Small black spots observed in between two rows of larger black spots. The larva had black thoracic prolegs. On the second day, it changed its colour to creamy yellowish colour with two dorsal black spots on prothorax. However, the other thoracic and abdominal segments had two parallel brownish round spots with a row of small brown spot till the last abdominal segment, which has a darker brown spot. The head covered with black hairs while the body had hairs on the lateral sides of larva on each segment. Second instar larva has

Table 2. Biology of eri silkworm, *S. ricini* on castor, *R. communis*

Sr. No.	Particulars	No. Observed	Periods		
			Min.	Max.	Av.±SD
1	Incubation period (Days)	30	8.00	11.00	8.83±0.91
2	Hatching (%)	3212	93.13	99.39	97.17±2.09
3	Larval period (Days)				
	1st instar	30	3.00	4.00	3.77±0.43
	2nd instar	30	3.00	4.00	3.23±0.43
	3rd instar	30	3.00	4.00	3.70±0.47
	4th instar	30	4.00	5.00	4.60±0.50
	5th instar	30	7.00	9.00	7.67±0.85
	Total larval period (Days)	30	22.00	25.00	22.97±0.85
4	Pre-pupal (Days)	30	2.00	3.00	2.63±0.49
5	Pupal- period (Days)	30	15.00	17.00	15.73±0.74
6	Pre-oviposition period (hrs)	25	11.00	12.10	11.61±0.37
7	Oviposition period (hrs)	25	68.00	72.50	70.47±1.78
8	Post-oviposition period (hrs)	25	118.00	135.00	122.73±4.81
9	Adult emergence (%)	159	86.67	100.00	92.30±5.16
10	Sex ratio (Male: Female)	147	1:1.25	1:3.33	1:2.01
11	Adult longevity (hrs)				
	Female (F)	25	198.50	218.10	204.82±5.24
	Male (M)	25	144.00	168.10	155.99±7.99
12	Total life cycle (Days)				
	Male	30	52	55	52.85±0.93
	Female	30	55	58	56.50±0.95
13	Fecundity (No. of eggs per female)	10	322.00	409.00	360.10±23.88
14	Temperature (°C)	-	22.30	25.40	23.71±0.64
15	Relative humidity (%)	-	43.00	63.10	51.63±6.06

a dark brown head with yellowish white fleshy body. A pair of black colour spots presented on the thoracic region. The larvae had black tubercles with whitish hair and pairs of black spots observed longitudinally on the body. Third instar larva did not exhibit any variation except size however, the small black coloured spots observed on body with short white tubercles. Longitudinal black spots observed on the body, pairs on dorsally and uneven manner on dorso-ventrally with yellow colour legs, anal flap and claspers. Fourth instar larva has a yellow colour head, creamy white body colour with short white tubercles and whole body covered with white powder like substance. The fifth instar larva was similar in general appearance to fourth instar larva, excluding large in size and the larva was white in colour (Plate 1). Kavane (2014) reported the same descriptions of larvae of eri silkworm. The length and breadth of first, second, third, fourth and fifth instar larva was 2.49±0.07 and 0.59±0.04mm, 8.75±0.35 and 2.07±0.11mm, 26.00±1.57 and 4.54±0.81mm, 37.10±1.18 and 8.71±0.42mm, 58.45±1.19 and 11.93±0.36mm, respectively (Table 1).The average larval period of first, second,

third, fourth and fifth instar larva were3.77±0.43, 3.23±0.43, 3.70±0.47, 4.60±0.50 and 7.67±0.55 days, respectively with the total larval period of 22.97±0.85 days (Table 2). The results are more or less in agreement with Yaligar (2014).

The full grown larvae were voracious feeder, consuming a large quantity of food and finally stopped the feeding once they accomplished their larval stage and enter maturity. At the beginning of this stage, the larva retracted its body and remained still until emptying the last excreta both in solid and semi-solid form. The mature larva dropped in their weight, became soft and yellowish transparent in colour. Matured larva avoided feeding, started to move away in search of a proper place for cocooning in the early morning to till noon and by spinning the silk around formed cocoons on both leaves and rearing tray if it was not properly placed on mountage. A sound of hollowness is produced while, picking up the matured worms and rubbing in between fingers. Such worms were then collected and kept for cocooning on plastic mountage. Pupa was obtect type, the pro-legs were shrivelled up and secretly curved, the thoracic legs, as well as wing pads, were developed. Newly formed pupa became soft and yellow to initiate the final moult inside the cocoon. The pupa became robust and changed its appearance into dark brown to reddish brown colour. The cocoons were shining white in colour elongated, spindle shape with an opening at one end and could be easily distinguished from those of other silkworm cocoons. The cocoons were compact and hard without peduncle (Plate 1). The description of pupa and cocoon are similar to Renuka and Shamitha (2014). The duration of the pre-pupal and pupal period were 2.63±0.49 and 15.73±0.74 days, respectively (Table 2). The results are similar with Naik *et al.* (2010), Yaligar (2014) and Deori and Khanikor (2015). The data on morphometrics of pupa and cocoon presented in Table 1. The results revealed that the length and breadth of pupa were 24.08±1.06 and 11.51±0.73mm, respectively. Whereas, the length and breadth of cocoon were 49.43±2.46 and 21.27±0.71mm, respectively. The results are more or less similar to Brahma *et al.* (2015).

The adult moths were stout, brownish or blackish in colour and covered with white scales (Plate 1). The male moth was smaller and had a larger and longer bipectinate antenna while, the female moth was having a larger abdomen with a thinner and smaller bipectinate antenna. The wings were broad, buff coloured with white coloured strips in the marginal portion. Wings covered with scales of different colour and shape. The prominent veins visible from both sides. Forewings observed longer and narrower than hind wings. The forewings of both sexes were more or less similar in structure and colour pattern. The characteristics anti median line observed bright chocolate in colour with a white border on each sides and almost run through the centre. The post median line was black in colour with a single dull grey border on each sides. A conspicuous black spot and pterostigma with a whitish tinge was present at the top of the wing apex. Furthermore, the wing had a few white oblique lines. The ocellus in both the sexes was crescent shaped. The hyaline area almost unseen and located in the anterior region of the ocellus. The space between the ocellus and post median line was darker. The remaining colouration of both fore and hind wings identical apart from the yellow strips of ocellus which was broader and prominent in hind wings. The data presented in Table 1 revealed that the average wing span of female and male were 12.52±0.27 and10.90±0.42cm, respectively. The results are more or less similar with Naik *et al.* (2010) and Subramanian *et al.* (2012) and Brahma *et al.* (2015). The observation on measurements of body length and breadth of female and male moths are presented in Table 1. The data revealed that the body length and breadth of female were 3.70±0.21and 0.79±0.08cm, respectively. Whereas in male, the length and breadth 2.94±0.11 and0.81±0.09cm, respectively. More or less similar measurements of adults had been recorded by Subramanian *et al.* (2012). The average adult emergence was 92.30±5.16 per cent. Naik *et al.* (2010), Naik and Murthy (2014) and Yaligar (2014) observed more or less similar results in eri silkworm. The sex ratio of eri silkworm (male to female) varied from 1:1.5 to 1:3.3 with an average of 1:2.01. The pre-oviposition, oviposition

and post-oviposition period were 11.61±0.37, 70.47±1.78 and 122.73±4.81hrs, respectively (Table 2).

The longevity of male and female was 155.99±7.99 and 204.82±5.24hrs, respectively (Table 2). The results are more or less similar with Gomma (1973) and Reddy *et al.* (1989). The egg laying capacity of female moth varied from 322 to 405 eggs per female with an average of 360.10±23.88 eggs per female. The present findings are more or less in agreement with Naik *et al.* (2010) who reported that the fecundity was 339.50 eggs per female. The total life cycle of female and male were 56.50±0.95 and 52.85±0.93 days, respectively (Table 2). The results are more or less similar with Naik and Murthy (2014) who reported the total life cycle of eri silkworm completed in 47.50 days.

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Gender associated morphological differences in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)

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ABSTRACT: The sexual dimorphism in pupal and adult stages of rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) was studied. Distinct slit on eighth abdominal segment is present in female pupa, while it absent in male pupa. Female moths have longer and snout- like palpi and male moth has shorter and blunt labial palpi.

KEYWORDS: *Corcyra cephalonica*, Pyralidae, labial palpi, morphology

Corcyra cephalonica (Stainton) (Lepidoptera: Pyralidae) is an economically important stored grain insect pest. The biological aspects such as incubation period, larval instars, larval and pupal developmental period, adult activities, and number of generation (Ayyar, 1934; Pruthi and Singh, 1950; Atwal, 1976; Cox *et al*., 1981; Nathan *et al*., 2006; Bhubaneshwari *et al.*, 2013, and Dulera *et al*., 2015) and other aspects like food and rearing environment (Hugar and Jairao, 1991; Kumar and Kumar, 2002; Jagadish *et al.,* 2009; Nasrin *et al*., 2016) has been extensively studied. However, very few observations have been reported on the gender associated differences in *Corcyra cephalonica* (Ayyar, 1934). Hence, this study was undertaken on the sexual dimorphism in *C. cephalonica*.

Corcyra cephalonica eggs were obtained from National Bureau of Agricultural Important Insects (NBAIR), Bengaluru. Eggs were inoculated on sterilized sorghum grains in plastic box covered with muslin cloth for aeration. Experiment was conducted in department of Agricultural Entomology, UAS, Bengaluru at room temperature 27±1.6 ^{0}C and 46± 6 % of RH. On hatching, larvae fed on sorghum grains and constructed webs. After the attainment of full growth, larvae pupated within the web. The pupae were removed carefully from webs and examined them under binocular compound microscope (Nikon SMZ645) to look at sexual dimorphism of pupae. Further, the sexed pupae were kept in separate petri plates inside the cage (45×45×45 cm) for adult emergence. On emergence, adults were observed for sexual dimorphic characters under the binocular compound microscope (Nikon SMZ645).

On examination of the pupae, it was observed that the female pupa has slit on eighth abdominal segment and abdominal slit is absent in male pupa (Fig. 1). Adult female moth has long and snout - like labial palpi, whereas, adult male moth possessed shorter and blunt labial palpi (Fig. 2). The difference in the labial palpi in male and female moths was earlier reported by Ayyar (1934).

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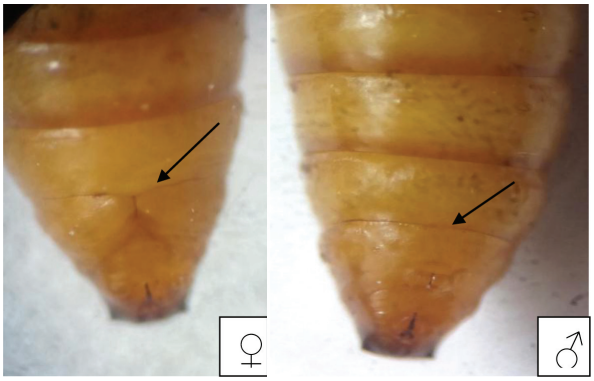

Fig. 1. Pupa of female (slit present) and male (no slit) *C. cephalonica*

Fig. 2. Labial palpi in adults

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Identification of a species of deer fly attacking human and live stocks in Assam, India

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ABSTRACT: During April 2018, a sudden appearance of some unknown flies was observed in some villages under Sibsagar district of Assam, Northeast India. The flies attacked in groups and had more attraction towards human than livestock. The present article describes the entomological and molecular identification of the fly at species level in Sibsagar district of Assam, northeast India. Preliminary examination revealed the fly to be an insect of order Diptera, Family *Tabanidae* and Genus *Chrysops* as they posses short proboscis, ocelli, third antennal joint with five divisions and wings demarcated with dark median cross-band. This was supported by molecular data where the partial nucleotide sequences of Mitochondrial COI gene revealed maximum identity (90.6%-92.3%) with genus *Chrysops*. The mitochondrial COI sequence data of *Chrysops flavocinctus* has been made available in NCBI Gen Bank. Gen Bank Accession No. MH998226.

KEY WORDS: *Chrysops flavocinctus*, mitochondrial COI gene,

During April 2018, a sudden appearance of some unknown flies was observed in some villages under Sibsagar district of Assam, Northeast India which was reported in local media and national newspapers (Karmakar, 2018). The flies created panic among the residents as they were attacking human and live stocks in large numbers. Moreover people of the locality never experienced or heard about such type of incidence in their life time. As the fly was seen for the first time, it was necessary to identify the fly so as to implement control measures in the population. The present article describes the entomological and molecular identification of the fly at species level in Sibsagar district of Assam.

A team of scientists from ICMR-RMRC NE Dibrugarh investigated the affected area after getting the information from local health authorities of Sibsagar district, Assam and collected several specimens of flies. The flies were brought to ICMR-RMRC Dibrugarh for morphological identification and molecular characterization. DNA barcoding of the fly specimen was done commercially (Eurofins, Genomics India) using amplification followed by sequencing of a part of mitochondrial cytochrome oxidase I gene. Molecular Phylogenetic analysis and rate of pairwise nucleotide divergence was calculated in MEGA software 7.0.

The flies were seen to attack in groups mostly on the exposed part of the human body usually in legs and hands. Bites resulted in allergy like reaction including severe itching, redness, and swelling in the affected site for a day or two. In some cases

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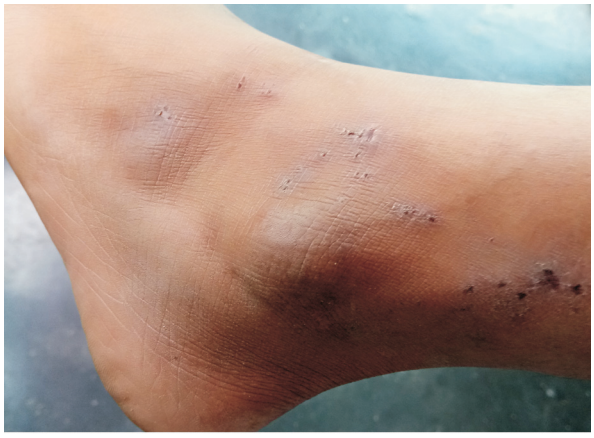

Fig. 1. Skin infection caused due to biting of fly species.

Fig. 2. DeerFly (*Chrysops flavocinctus*).

secondary skin infection were also observed due to severe itching (Fig. 1). Field observations revealed that at least ten villages situated in both sides of a stretch of a dead river were mainly affected. Among these ten villages, five villages were affected more due to their close proximity to the water body.

Preliminary examination revealed this fly to be an insect of order Diptera, Suborder Brachycera, Infraorder Tabanomorpha, Family *Tabanidae* and Superfamily Tabanoidea (Maity *et al*., 2016) Species belonging to this group are commonly called as horse flies (*Tabanus*), deer flies (*Chrysops*) or clegs (*Haematopota*) depending upon the genus (Maity *et al*., 2016). The present species belonging to the family *Tabanidae* was further keyed to Subfamily Chrysopsinae and Genus *Chrysops* as they posses short proboscis, ocelli, third antennal joint with five divisions, and wings demarcated with dark median cross-band (Chandra *et al*., 2015) (Fig. 2). This was supported by molecular data where the NCBI BLAST search using partial Sequences of Mitochondrial COI gene revealed maximum identity with genus *Chrysops* (Gen Bank Accession no. MH998226). Nucleotide sequences corresponding to mitochondrial COI gene showing maximum homology with fly specimen identified in Assam were downloaded from NCBI genBank. Few other genera of related fly's sequences were also included in the phylogenetic tree. The sequences were aligned using CLUSTAL W in BioEdit Software v 7.0 and phylogenetic tree constructed in MEGA v 7.0 using the Maximum Composite

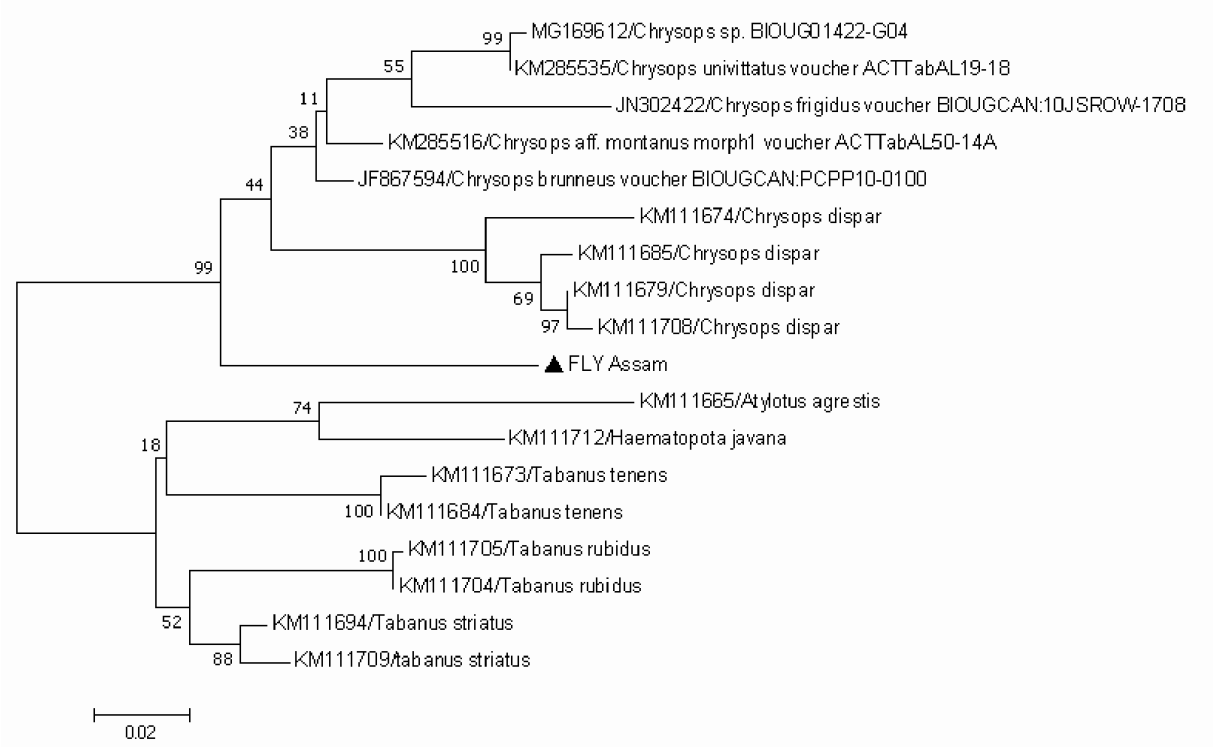

Fig. 3. Molecular phylogenetic analysis of deer fly specimens from Asaam

Likelihood (MCL) approach with Maximum Likelihood method based on the General Time Reversible model based on best fit model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories) (+*G*, parameter = 0.2733) (Fig. 3).

There are about 300 described species in the genus *Chrysops* worldwide (Burger and Chainey, 2000). In the Oriental region, which includes India, contains 34 described species of *Chrysops* and in India 13 species of *Chrysops* recorded (Maity *et al*., 2016). Burger and Chainey (2000) provided the identification key and description of all valid taxa of genus *Chrysops* prevalent in Oriental and Australasian regions (Maity *et al*., 2016). Following the key the present species was identified as *C. flavocinctus* Ricardo, 1902. Summarized diagnostic characters provided by them for this species are slender black-brown species with very large frontal callus, shining black-brown frontoclypeus, undivided cross-band on wing and tergite two with yellow basal band. These characters completely matched with our specimens. *Chrysops dubiens*, a species reported from southern India (Kerela) is closely related with *C. flavocinctus*. However, *C. dubiens* can be separated from *C. flavocinctus* as it possesses characters like distinctly concave outer margin of the cross band in the wing, tergite 2 yellow basally but apical black band with a large triangular (rounded in case of *C. flavocinctus*) marking extending anteriorly, tergites 3, 4, and occasionally 5 with median yellow to light brown spots of variable sizes. *Chrysops flavocinctus* was originally described from specimens collected from Khasi hills of Meghalaya by Ricardo in 1902. In addition to that, this species was also reported from Assam, Arunachal Pradesh, Sikkim, and West Bengal. Occurrence records of this species in countries other than India are Myanmar, China, Laos, Malaysia, Taiwan, Thailand, and Vietnam (Burger and Chainey, 2000).

Scanty information is available regarding the habits and abundance of Oriental *Chrysops* (Maity *et al*., 2016). The available information on the feeding habit of *C. flavocinctus* stated that this species commonly attack human and their bite resulted in

swelling, pain, and intense itching which may persist for several days (Stekhoven Jr., 1926). This observation reported long years back have similarity with the present feeding behavior of this fly attacking human in Assam. Very little information is available on the medical importance of Oriental *Chrysops*. However, some species are reported to transmit *Pasteurella multocida*, *Trypanosoma evansi* (Surra) and anthrax (*Bacillus anthracis*) mechanically in animals (Burger and Chainey, 2000).

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