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Taxonomic studies on the genus *Glyphodes* Guenee (Lepidoptera: Crambidae: Spilomelinae) from Karnataka, India

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ABSTRACT: The specimens collected and reared to an adult stage on their respective hosts utilized to characterize the species of the genus *Glyphodes* based on morphological and genital characters of adults, revealed three species of the genus *Glyphodes* and were documented from Karnataka *viz., Glyphodes caesalis* Walker, *Glyphodes pulverulentalis* Hampson and *Glyphodes vertumnalis* Guenee on jack-fruit, mulberry and jasmine, respectively. These three species differ morphologically in having entire body green colour in *G. vertumnalis,* abdomen with oblique lateral stripes in *G. pulverulentalis* wherein, *G. caesalis* having sub-marginal black edged patch on costa with four spots. In genitalia, uncus greatly curved and beak shaped in *G. vertumnalis,* uncus slim and slightly curved in *G. caesalis,* whereas in *G. pulverulentalis* uncus long, narrow and slightly curved with short setae at apex.

KEY WORDS: Taxonomy, three species, *Glyphodes,*Genitalia

INTRODUCTION

The genus *Glyphodes* was established by Guenee in 1854. This genus is more varied, omnipresent and comprises of 187 species throughout the world. It is one of the most economically important genera comprising fruit borers, shoot borers, leaf webbers, leaf rollers etc. Twenty-five species have been recorded in the Southeast Asia and seventeen species in Australia (Robinson *et al.,* 1994; Shaffer *et al.,* 1996). In India, so far 22 species of the genus *Glyphodes* have been reported (Nuss *et al*., 2003-2019). In Tamil Nadu, three species namely *G. bivitralis*, *G. caesalis* and *G. canthusalis* were recorded by Fletcher (1914) and Nair (1970). Recently, Rathikannu and Chitra (2017) reported 6 species of *Glyphodes viz., G. bivitralis*, *G. caesalis*, *G. canthusalis, G. onychinalis, G. pulverulentalis* and *G. stolalis* from Tamil Nadu by relying on light trap collection. They have provided taxonomic description of genitalia with line diagram and a key. Above studies indicate that in India, the taxonomic studies on the genus *Glyphodes* were carried out by the researchers predominantly based on light trap collections and none of them made any efforts to associate *Glyphodes* species with their host plants. Hence, the description of a species reared from actual hosts

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is the need of the hour for accurate identification and authentication of its host. In this context, in the present investigation, an attempt was made to study the host-based taxonomy of the genus *Glyphodes,* which were collected and reared on their actual hosts. In the current paper, synonyms and taxonomic descriptions for the species of *Glyphodes* are provided with photographic illustrations of genitalia, wing venation and adult habitus. Further, an illustrated key is provided for easy identification of the species.

MATERIALS AND METHODS

To study the adult morphological and genital characters, the specimens already collected (Karnataka) and reared from their host plants at the Department of Agricultural Entomology, College of Agriculture, Bheemarayanagudi, University of Agricultural Sciences, Raichur 584-104, Karnataka, India were utilized. The morphological as well as genital characters of the adult Spilomelinae were studied following Hampson (1896), Clark (1941), Robinson (1976), Thomas (2007) and Nagaraj (2014) with the necessary modifications. Before dissection of genitalia, adult specimens were photographed. Adult structures such as forewing and hindwing, palpi and genitalia were photographed using Trinocular microscope with auto-montage (Leica M 205C).

RESULTS

Genus *Glyphodes* Guenee, 1854; type species: *Glyphodes stolalis* Guenee, 1854

= *Caloptychia* Hubner, 1825; type species: ***Phalaena chrysialis*** Stoll, 1790

= *Calliptychia* Agassiz, 1847; type species: ***Phalaena luciferalis*** Snellen, 1780

= *Morocosma* Lederer, 1863; type species: ***Phalaena margaritaria*** Clerck, 1764

Diagnosis: Labial palpi inverted, the 2nd joint broadly scaled in front, the 3rd porrect and lying along the hair on the 2nd joint; maxillary palpi triangularly scaled; frons rounded; tibiae with inner spur twice the length of the outer spur; tuft of hairs in forewing of the male; costa much arched towards apex.

Wing venation similar in almost all the *Glyphodes* species, but the external markings on wings of each species differs. So, wing venation of each species not discussed here, instead general venation for all the species is given below.

Wing venation: Fore wing with vein R_5 marginally approaching to R_{3+4}; M_1 arises close towards vein R_5; M_3, M_2 arising from angle of cell; Cu_{1a} from below the angle of cell, Cu_{1b} before angle of cell; hind wing with vein Rs stalked with $Sc+R_1$; M_2 and M_3 closely approximated for short distance; Cu_{1b} before angle of cell.

***Glyphodes caesalis* walker, 1859;** type locality: Sri Lanka (Fig. 1 A-H)

= *Glyphodes assimilis* Rothschild, 1915; type locality: Indonesia, Papua

Description: Head brown; labial palpi white; abdomen dark brown with white at side; forewing yellowish brown; an oblique ante medial fuscous line; a large fuscous edged iridescent white patch in and below end of cell; fuscous disco-cellular edged iridescent scales below lower angle of cell; hindwing ground colour, iridescent white with a broad marginal band with fuscous line on its inner edge; fringe yellowish mixed with brown.

Male genitalia: Uncus long, slim, curved, dentate and hooked at apex with hairs; valva broad with hairs; costa sclerotized; harpe thorn-shaped and straight; sacculus half the length of valva, inner surface granulated and thick; saccus triangular and slender, pointed at apex; aedeagus short and stout with cornuti inside vesica at apex.

Female genitalia: Ovipositor slit cut open; anal papillae thick; both the apophyses short, posterior apophysis half the length of anterior apophysis; sub-genital plate small; ductus bursae fairly long and stout; corpus bursae spherical with a scar like signum on the anterior apex.

Materials examined: India: Karnataka: Bellary, 1♂, 07.iii.2017, reared on jackfruit, S. Murthy;

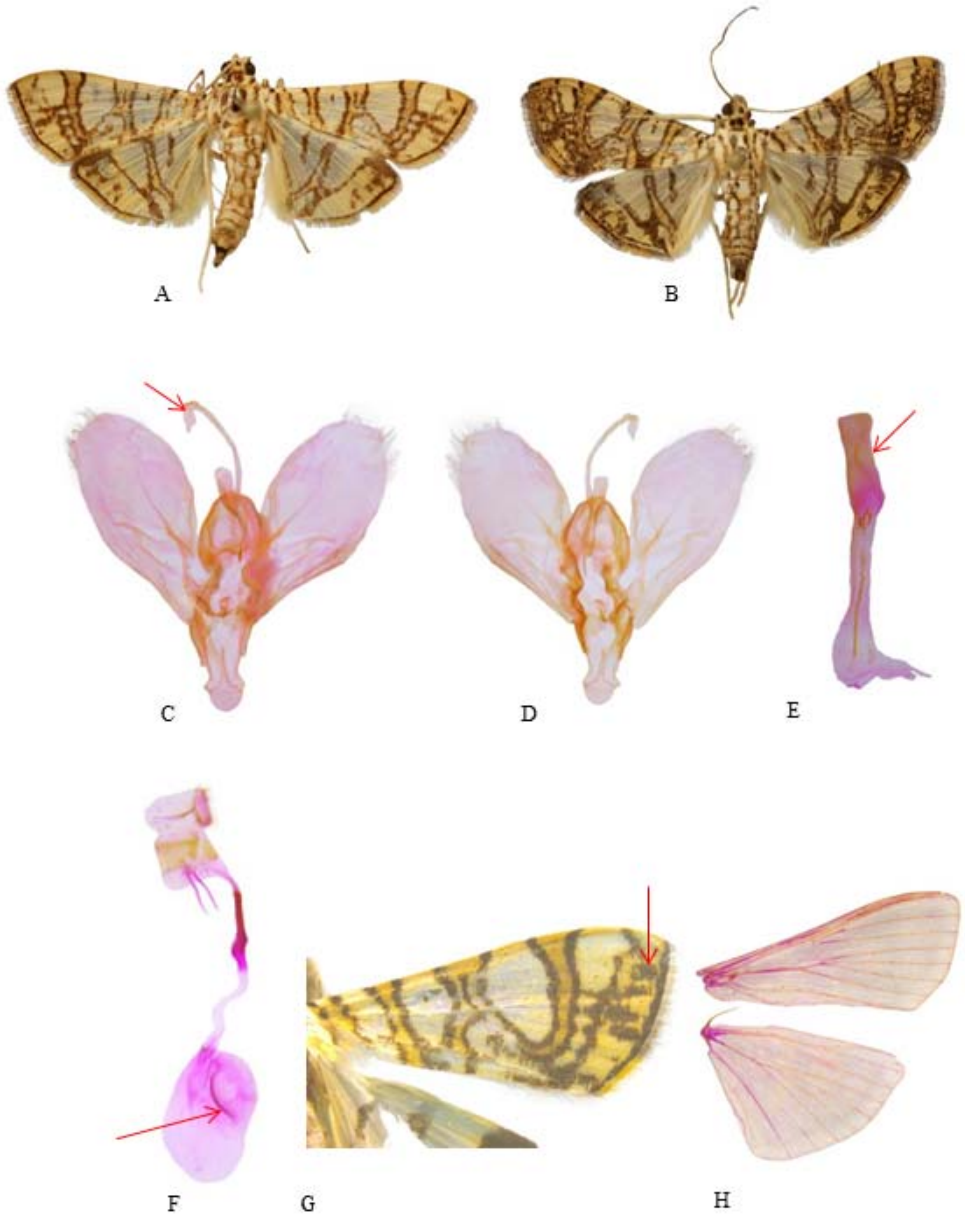

Figure 1. Genital and morphological characters of adult *Glyphodes caesalis* Walker
(A. male; B. female; male genitalia, C. ventral view; D. dorsal view; E. aedeagus; F. female genitalia; G. sub-marginal black edged patch on outer margin with four spots; H. wing venation)

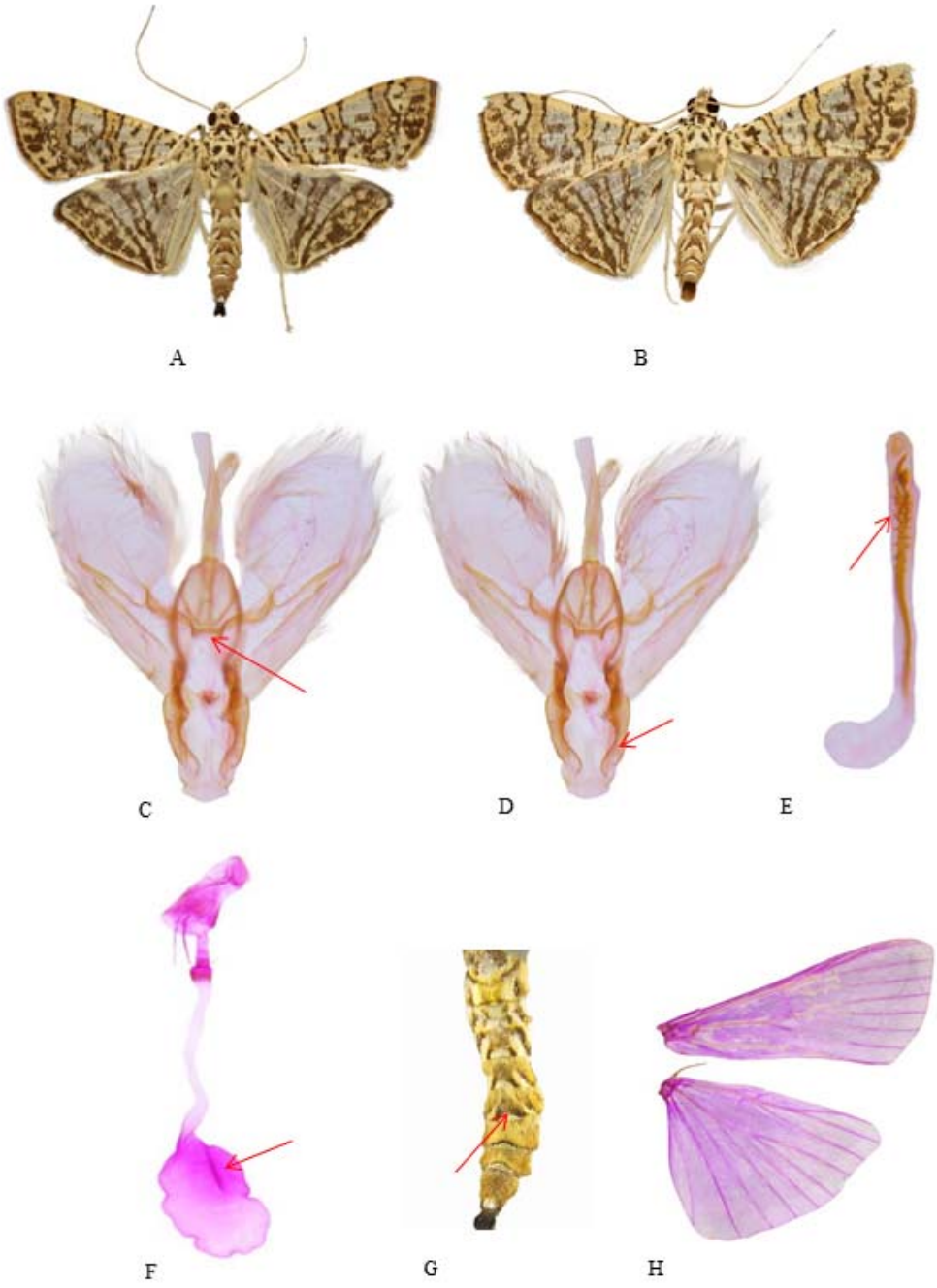

Figure 2. Genital and morphological characters of adult *Glyphodes pulverulentalis* Hampson
(A. male; B. female; male genitalia, C. ventral view; D. dorsal view; E. aedeagus; F. female genitalia; G. abdomen with oblique lateral stripes; H. wing venation)

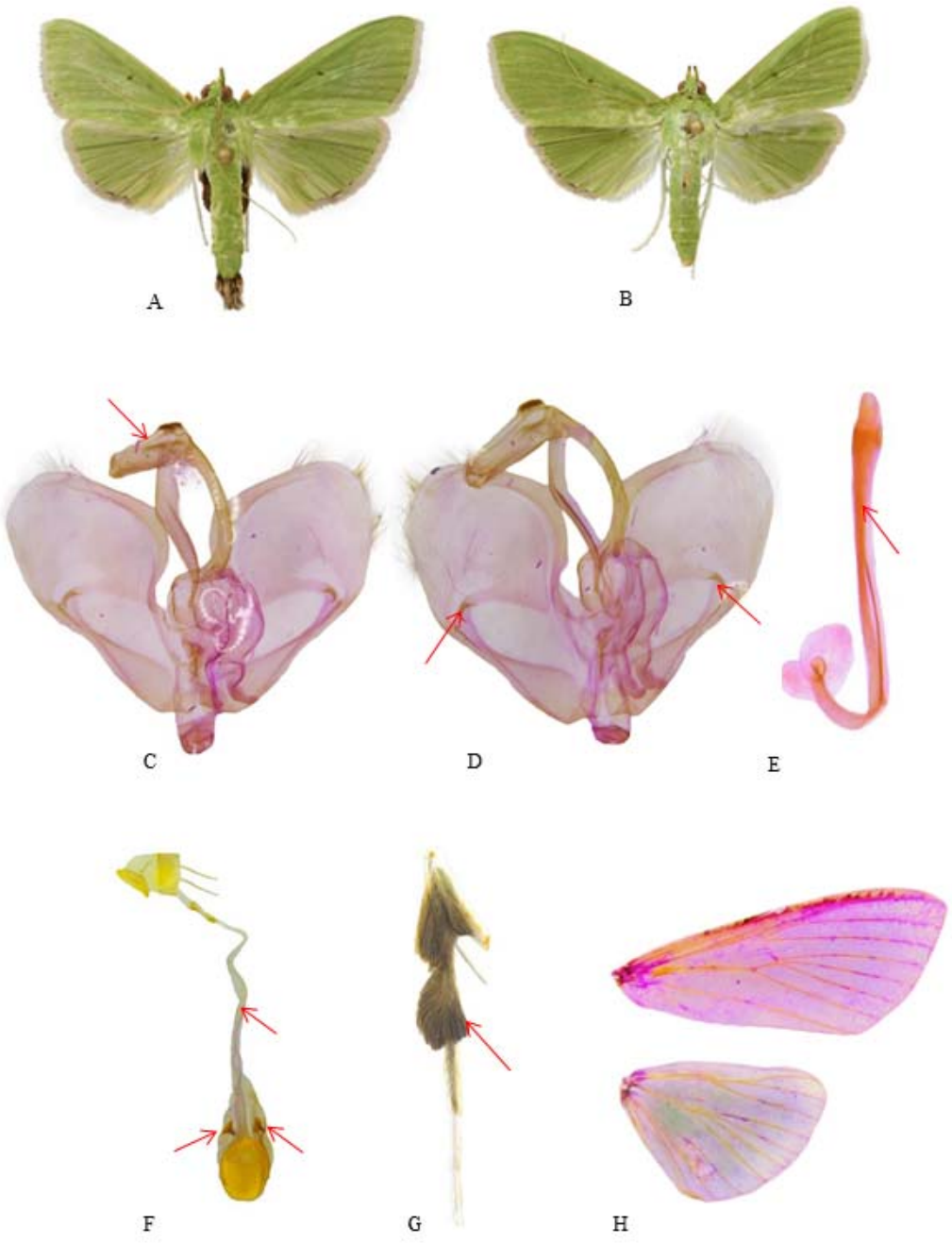

Figure 3. Genital and morphological characters of adult *Glyphodes vertumnalis* Guenee
(A. male; B. female; male genitalia, C. ventral view; D. dorsal view; E. aedeagus; F. female genitalia; G. male hind tibia with tuft of hairs on outer extreme region; H. wing venation)

Bellary, 1♂, 1♀, 06.iii.2017, reared on jackfruit, S. Murthy; Bellary, 1 ♂, 2♂, 15. Vi. 2012, reared on jack fruit, S. Murthy; Bellary, 2♂, 1♀, 17.vi. 2012, reared on jack fruit, S. Murthy.

Remarks: It feeds on jack fruit as a fruit borer. Externally, this can be easily discriminated from other species by the presence of a large sub-triangular medial black edged patch with black below it on inner margin, a post medial band formed of two irregular black edged patches with their inner and outer edged indented and sub-marginal black edged patch on costa with four spots on black suffusion extending from it to inner margin.

***Glyphodes pulverulentalis* Hampson 1896;** type locality: India (Fig. 2 A-H)

Description: General body thickly irrorated, striated with black; abdomen with oblique lateral stripes; anal tuft black with brown middle; forewing with all the markings obscured by the spots and striae, the antemedial, medial and post-medial bands broader and less irregular, the 2nd vein without disco-cellular spot on it, the 3rd with series of pale specks on its outer edge from vein 4 to inner margin; hindwing thickly striated, oblique black edged brown post-medial and sub-marginal bands almost meeting at a point near anal angle; cilia of both wings fuscous, with fulvous and brown lines at base.

Male genitalia: Uncus long and narrow, anterior tip enlarged and pointed, beak-shaped dorsally with short setae; tegumen longer than wide, sclerotized and arched; vinculum long, sclerotized; saccus long, U-shaped; valva long, membranous, apex broadly rounded; costa weakly sclerotized, dorsally fringed with long hairs; juxta narrow, sclerotized, arrow-like; phallus almost straight, vesica with long sclerotized bar with lateral spine-like projection; curved sclerotized hook-like cornutus.

Female genitalia: Ovipositor slit swollen; anal papillae thick; both the apophyses short, thin and tapering, posterior apophysis half the extent of anterior apophysis; ductus bursae fairly short and thick; corpus bursae spherical with a scar like signum.

Materials examined: India: Karnataka: Bellary, 2♂, 10.x.2017, reared on mulberry, Manjunath; Bellary, 1♂, 1♀, 06.x.2016, reared on mulberry, Manjunath; Bellary, 1♂, 01.x.2017, reared on mulberry, Manjunath; Bellary, 1♂, 2.x.2017, reared on mulberry; Manjunath.

Remarks: This is one of the leaf-webber species which sustains on mulberry. Morphologically, this can be easily differentiated from other species of *Glyphodes* by the presence of oblique lateral stripes on the abdomen.

***Glyphodes vertumnalis* Guenee, 1854;** type locality: India (Fig. 3 A-H)

= *Enchocnemi diafuscitibia* Warren, 1896; type locality: Indonesia, Maluku, Tanimbar Islands

= *Margaronia herbidalis* Walker, 1866; type locality: Indonesia, Maluku, Seram

= *Margaronia melanuralis* Walker, 1866; type locality: Indonesia, Flores

= *Margaronia morvusalis* Walker, 1859; type locality: Malaysia, Borneo, Sarawak

= *Margaronia phryneusalis* Walker, 1859; type locality: North India

= *Margaronia proximalis* Walker, 1866; type locality: Indonesia, Maluku, Makian; Sulawesi

= *Pachyarches tibialis* Moore, 1877; type locality: India, South Andamans

Description: Body green, neither of the wings fulvous; marginal specks often obsolescent; cilia fulvous; abdomen small and profuse; male having black anal tuft of hairs; female devoid of anal tuft of hairs on hind tibiae; male with black tuft of hairs on hind tibiae at outer margin and extremity; hindwing of male with the inward area compactly clothed below with clumps of yellowish hair.

Male genitalia: Uncus broad and greatly curved, bending forward giving a beak like appearance; gnathos equal to uncus and broad; base of the gnathos and uncus darken laterally; vinculum wide and V-shaped; coremata with long thick as well as

finehair; valvae small, broad, fan-like having chitinous hook-like clasper in the costal base; phallus equally long,

Female Genitalia: Ovipositor slit swollen, wide dorsally and tapered ventrally; anal papillae thick; apophyses short; anterior apophysis twice the length of posterior apophysis; sub-genital plate small, ductus bursae fairly long and thick; corpus bursae spherical with two triangular signa one on each side near the apex.

Materials examined: India: Karnataka: Gulbarga, Raddewadigi, 1♀, 05.ii.2015, reared on jasmine, Nagaharish; Gulbarga, Hattekuni, 1♀, 12.viii.2015, reared on jasmine, Nagaharish; Yadgir, Bgudi, 4♂, 20.iv.2013, at light, S. Murthy; Gulbarga, Raddewadigi, 1♂, 15.x.2015, reared on jasmne, Nagaharish; Yadgir, B gudi, 5♂, 1♀, 03.xii.2012, jasmine, S. Murthy; Yadgir, B gudi, 1♂, 4.ix.2012, at light, S. Murthy; Yadgir, B gudi, 1♂, 16.vii.2012, jasmine, S. Murthy; Yadgir, B gudi, 1♂, 18.vii.2012, jasmine, S. Murthy.

Remarks: *Glyphodes vertumnalis* Guenee intently looks like *G. marginata* Hampson. Both are recognized by wing character. In *G. marginata* Hampson, wings are fulvous; however, in *G. vertumnalis* Guenee wings are not fulvous.

An illustrated key to *Glyphodes* species

1. Moth green coloured, male hind tibia enclosed with tuft of hairs on outer extreme margin, male genitalia with hook like clasper at base of costa, bursa copulatrix with two triangular signum near the apex…….....…….***Glyphodes vertumnalis*** **Guenee (Fig. 3. A, B, C, D, E, F & G)**

- Moth straw-yellow coloured, male hind tibia without tuft of hairs on the outer extreme margin, male genitalia without clasper at base of costa, bursa copulatrix without two triangular signum near the apex…….....**2**

2. Abdomen marked with lateral oblique stripes; both apophysis small, phallus slim and long; ductus bursae thin and narrow…….. .....***Glyphodes pulverulentalis*** **Hampson (Fig. 2. C, D, E, F & G)**

- Abdomen marked without lateral oblique stripes; anterior apophysis larger than posterior apophysis, phallus stout and short, ductus bursae thick and wide …***Glyphodes caesalis*** **walker (Fig. 1. C, E, F & G)**

In the present investigation, three species of the genus *Glyphodes* were identified with *G. caesalis* on jack fruit, *G. pulverulentalis* on mulberry and *G. vertumnalis* on jasmine. The taxonomic descriptions for these species were provided with photographic illustrations of genitalia, wing venation and adult habitus. An illustrated key was also provided for the same for easy identification of the species. Further, current taxonomic status of each species was given.

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Influence of spinosad on the reproductive potential of *Tribolium castaneum* (Herbst), (Coleoptera: Tenebrionidae) infesting wheat

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ABSTRACT: Spinodad of different concentrations were screened against *Tribolium castaneum* (Herbst) reared on four local wheat varieties to observed the effects on reproductive potentials for two successive generations.The lowest number of eggs laid was 119 (23.80±0.97) observed in Shatabdi-21 (S-21) in 45 d in F_1 and 15 (3.00±0.71) in 15 d in F_2 generation when treated at 0.12µl/g of spinosad. Spinosad at all concentration totally inhibited egg laying oroviposition rate on day 45 in all wheat varieties except P-24 variety in F_1 generation.The lowest fertility was found in Shatabdi-21 variety as 12.61percent in F_1 and 6.67 percent in F_2 generation at 0.12µl/g.The latent effect of spinosad on number of eggs, larva, pupa and adult obtained in Shatabdi-21 as 2.00±0.32,0.80±0.37, 0.40±0.40 and 0.20±0.20 at 0.12µl/g of spinosad in F_2 generation.There was no significant difference between the number of males and females in F_2 generation wheat varieties. Spinosad ultimately reduced the fecundity, fertility and decreased the egg to adult survivability in four wheat varieties compared to control and F_1 and F_2 generations. Results of the research revealed that comparatively higher concentrations of spinosad that used in this study would potentially control development and reproductive potentiality of *T. castaneum* in wheat varieties.

INTRODUCTION

In a recent post-harvest compendium *T. castaneum* is reported as most common secondary pest of all plant commodities in store throughout the world (Babarinde and Adeyemo, 2010; Stejskal *et al*., 2014). This external feeder pest makes serious damage on flour and over whelms cereal particularly at larval and adult stages (Baldwin and Fasulo, 2004) making it unfit for human intake.This insect causes substantial loss in storage because of its high reproductive potential (Campbell and Runnion, 2003). Diet type and initial population density may also have an immediate or indirect impact on the reproduction, development rate, number of progeny and body weight of *T. castaneum* (Longstaff, 1995; Assie *et al*., 2008). Shukla and Upadhyay (2011) noticed that the progeny production rate of *T. castaneum* was very excessive and the fourth instar larvae were enormously energetic in rainy season causing very excessive infestation where in adults could live for 2 years or greater in the course of duration the female producing nearly 1000 eggs.

Spinosad is an insecticide product from Dow Agro Sciences (Indianapolis, Indiana, USA) based on

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chemical compounds of a soil bacterium *Saccharopolyspora* was discovered in 1985 (Mertz and Yao, 1990). Hertlein *et al*. (2011) stated that spinosad has an aggregation of high potency, minimum mammalian toxicity, and secure environmental profile which is unique among existing products recently used for stored-grain protection.The mode of action of spinosad is characterized by involving the disruption of nicotinic acetylcholine receptors and γ-amino butiric acid (GABA)-gated ion channels of insect nervous systems (Salgado and Sparks, 2005; Kirst, 2010; Sparks *et al*., 2012). Spinosad must be sprayed directly into the eggs, but larvae and adults can be efficaciously dosed through contact butit is most effective when ingested (Cleveland *et al*., 2001). Spinosad is more toxic through ingestion than via contact (Athanassiou and Kavallieratos, 2014).

Wheat is the major and second staple food/ worldwide and Bangladesh respectively. Different wheat varieties were developed in different countries, among which the factors like pest resistant and insecticide susceptible varieties gaining importance from the point of view of reducing infection stress during post-harvest storage. In this research four local varieties of wheat were used to screen out the variety that is susceptible to spinosad treatment without leaving any hazardous effect on human health. The objective of this experiment was to evaluate the effect of different concentrations of spinosad on the reproductive potentials of *T. castaneum* for two successive generations as a result of contact action to eggs and contact and gustatory effects on the adults of *T. castaneum* in four wheat varieties local of Bangladesh.

MATERIALS AND METHODS

Culturing of *T. castaneum*: *T. castaneum* beetles were obtained from the stock culture maintained in the control temperature (CT) room, at Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh. The standard food medium, mixture of whole wheat flour with powdered Brewer's Yeast (19:1) (Park and Frank, 1948; Park, 1962) was used as a food medium throughout the mass culture of *T. castaneum*. Both flour and yeast were previously passed through a 250µm sieve sterilized at 60°C for six hours in an oven, and kept at least for 15 days before use, to equilibrate the moisture content with the environment (Khan, 1981; Mondal, 1984).

Mass cultures were maintained in plastic containers (2.5 liters) in a Control Temperature (CT) room at 30±1°C and 70±5% RH providing 250g of wheat flour /container. About 500 adults of *T. castaneum* were introduced into each container. The cultures were checked at regular intervals and eggs and larvae were separated to avoid cannibalism. A crumpled filter paper was placed inside each container for easy movement of the beetles. Mouth of the container was covered with muslin cloth using a rubber band, to prevent the possible contamination and escape of insects (Mondal and Parween, 1997). A series of stock culture were maintained for the constant supply of these insects to conduct different experiments.

Preparation of spinosad concentrations:

Spinosad is light grey to white in colour with slight odour stale water. About 500ml of spinosad (PRN-MAPP-12054, cafno 20012-019, Lot No-3068404) was obtained from Dow Agro Sciences, UK. Concentration of spinosad was 120g a.i./l.

Spinosad measuring 0.72µl was pippetted in a glass vial and added 6ml distilled water properly by using 2ml syringe. The vial was shaken vigorously for thorough mixing of spinosad and water serving as stock having 0.12µl spinosad/ml of other desired concentrations of spinosad (0.06 and 0.03µl/g) were prepared by serial dilution by taking 1 ml of solution and adding 2 ml distilled water in each step.

Wheat varieties: Infestation free four wheat varieties viz., BARI-26, BARI-28, Prodip-24 and Shatabdi-21 (B-26, B-28, P-24 and S-21) were used for the experiment. The grains were washed with water and dried at room temperature before adjusting their moisture content to 13.5 by adding tap water, cleaned by sieving through 500 micrometer aperture sieve and sterilized in an oven at 60°C for 8 h. After sterilization wheat grains were

kept in separate plastic containers according to variety.Grains were partially broken down by the hand blender before further use.

Bioassay: The efficacy of spinosad against reproductive potentiality of *T. canstaneum* was investigated by dietary exposure termed Treated Food Method (TEM) (Talukder and Howse, 1994). Newly emerged 50 pupae from culture were sexed by the electric microscopic examination of the exo-genital process. In female the structure of exo-genital lobes are larger than male. The genital papillae of females are pointy but male's genital papillae are stubby, conjoined. Female papillae resemble fingers like 2 combined thumbs (Halstead 1963). After adult emergence, 20 male and females were paired up to 15 days and collected about 350 numbers of freshly eggs with the help of microscope.Two grams of each wheat variety was soaked with different concentrations of spinosad as treated wheat and 2gms of each wheat variety was soaked with distilled water as for control or untreated wheat. About 20 eggs were placed on each petri-dish and provided with 2gms treated wheat and untreated wheat of each variety which was changed after every three days. The larvae were checked regularly until they emerged as pupae. Pupae from each treated concentration were paired by microscopic examination of the exo-genital process. After 48 h emerged adults were paired and 5 pairs of beetles from each concentration were placed in separate petri-dishes and provided with 2gms of treated wheat of each variety of different concentrations for egg collection.

Eggs from each pair were collected after three days up to a 45 day. Total number of eggs laid by each pair up to 45 daywas recorded and daily egg production/female was determined. Fertility of the eggs was assessed by the how many larvae were recovered from the total number of eggs up to 45 days of 5 pairs adults into 100 was recorded. The ultimate effects of the spinosad were assessed by recording the total number of larvae produced, recoveries of total pupae and adults up to 45 d. About fifty pupae were sexed and the males and females were kept separately again.

After emergence, adults were paired again (5 pairs) and kept separately. Similarly, the total number of eggs laid by each pair up to 15 days, fertility of these eggs, the total number of larvae produced, and recoveries of pupae and adults were recorded. The second generation (larvae to adults) was developed on untreated wheat. A similar set of experiment was conducted with same number of beetles in untreated wheat, as the control batch. All the experiments were conducted at CT room (30±1°C) and 75% RH and replicated thrice. Data obtained from the experiments were analyzed using the Analysis of variance (Factorial) using SPSS version 20. Means were compared by Tukey's test, accepting significant differences at $P<0.05$. Sex ratios were determined by χ^2 test ($P>0.05$).

RESULTS AND DISCUSSION

i) Effect on fecundity in two successive generations

The total number of egg laid by *T. castaneum* ranged from 656 to 1125 in control and 119 to 761 in treatments in F_1 and 163 to 384 in control and 15 to 145 in treatments in F_2 generation.The lowest number of eggs laid was 119 (23.80±0.97) observed in S-21 up to 45 days in F_1 and 15 (3.00±0.71) up to 15 days in F_2 generation at 0.12µl/g and the highest number of egg was found in P-24 as 761 (152.20±2.60) in F_1 and 145 (29.00±1.30) in F_2 generation at 0.03µl/g concentration (Tables 1 and 2).

ii) Effect on fertility of the eggs in two successive generations

Spinosad at all concentrations reduced fertility of the laid eggs in a concentration- dependent manner in F_1 and F_2 generations in all wheat varieties (Figure 1). The percentage of fertility of eggs ranged from 74.70 to 89.67 percent in untreated wheat and 12.61 to 64.91 percent in treatments in F_1 generation. The lowest fertility was noted in S-21 variety as 12.61 percent at 0.12µl/g in F_1 generation. In F_2 generation, the percentage of fertility ranged from 69.94 to 85.16 percent in untreated wheat and 6.67 to 45.52 percent in treatments. The same trend was also observed in F_2 generation where the lowest fertility was recorded in S-21 variety as 6.67 percent.

iii) Latent effect of spinosad on the life stages produced in second generations

The latent effect of different concentrations of spinosad at different life stages and sex ratio of *T. castaneum* deviated from 1:1 in F_2 generation; the effects were concentration-dependent and in all wheat varieties (Table 2).The mean number of eggs production ranged from 32.60±1.47 to 76.80±1.39 in control and 3.00±0.71 to 29.00±1.30 in treatments. Out of four wheat varieties, the lowest number of eggs was produced in S-21 as 3.00±0.71 at 0.12µl/g of spinosad compared with control in F_2 generation (Table 2).

Larval mortality was found to be very high especially in spinosad at 0.12µl/g concentration. Few larvae became black and shriveled, failed to shed the old cuticle and died while hatching after 2-3 days. Larval survival ranged from 22.80±1.02 to 65.40±0.86 nos in control and 0.20±0.20 to 13.20±0.86 nos in treatments. The lowest number of larva was recorded in S-21 as 0.20±0.20 at 0.12µl/g compared with the control and rest of other concentrations (Table 2). The mean number of pupal survival ranged from 20.80±1.02 to 50.20±1.80 nos in control and 0.00±0.00 to 9.00±0.711 nos in treatments. The lowest number of pupa was recorded in S-21 as 0.00±0.00 at 0.12µl/g compared with the control and other concentrations (Table 2).

The mean number of adult survival ranged from 17.80±0.86 to 41.60±1.50 nos in control and 0.00±0.00 to 5.60±0.40 nos in treatments. The lowest number of adult was observed in S-21 as 0.00±0.00 at 0.12µl/g of spinosad compared with the control and other concentrations. Few adults became black and shriveled (Table 2). The sex ratios for the treatment variables did not differ significantly from each other in any generation as judged by χ^2 test ($P>0.05$) (Table 3).

Results of the present experiments have demonstrated that spinosad affected egg to adult survivability and reproductive potentiality of *T. castaneum* in F_1 (45 d) and F_2 (15 d) generations. There was significant decrease in egg number of *T. castaneum* laid on different wheat varieties. The lowest number of eggs laid (119) in 45 d was observed in S-21 in F_1 generation; and that was 15 laid in 15 d in F_2 generation; at highest concentration 0.12µl/g. Egg laying was decreased in all wheat varieties and in F_1 generation females laid small number of eggs after 33 d; and at highest concentration was totally failed in the later part of oviposition period.

Spinosad at 0.12µl/g concentration totally inhibited egg laying on day 45 in F_1 and day 15 in F_2 generation in all wheat varieties. Absence of adequate published literature on the spinosad toxicity on the fecundity and oviposition rate of stored product insects, make the present result quite difficult to compare. Similarly fertility rate lowest in S-21 variety as 12.61 percent in F_1 and 6.67 percent in F_2 generation at 0.12µl/g concentration. Accordingly, lowest number of larva, pupa and adult was recorded in S-21 at 0.12µl/g of spinosad in F_2 generation.

Bajracharya *et al.* (2013) reported that spinosad caused high mortality and complete progeny suppression of *Rhizopertha dominica* at five storage periods (2, 84, 168, 252, and 336 d) which was in agreement with Bonjour *et al*. (2006) who demonstrated that stored wheat treated with 1 ppm of spinosad completely controlled *R. dominica* adults and progeny production for all the post treatment storage periods (28, 84, 182, 252, 336, and 672 d). Subramanyam *et al*. (2012) reported that complete suppression in *R. dominica* (0.0±0.0) and near complete suppression of adult progeny in *T. castaneum* (1.2±1.0 and 0.6±0.4) achieved on spinosad-treated wheat (spinosad I and spinosad II) after 42 days.The author additionally reported that no *Plodia interpunctella* adults have been found on liquid spinosad treated wheat after 42 days. These findings are consistent with Huang *et al.* (2004) who stated that spinosad was highly toxic against *P. interpunctella* inhibiting larval survivability and adult emergence on wheat and maize. Adult mortality of *Cryptolestes ferrugineus*, *R. dominica*, *Oryzaephilus surinamensis*, *S. oryzae*, and *S. zeamais* was found as >98% at 1 and 2 mg of spinosad/kg on corn after 12 days. Spinosad absolutely suppressed egg-to-larval

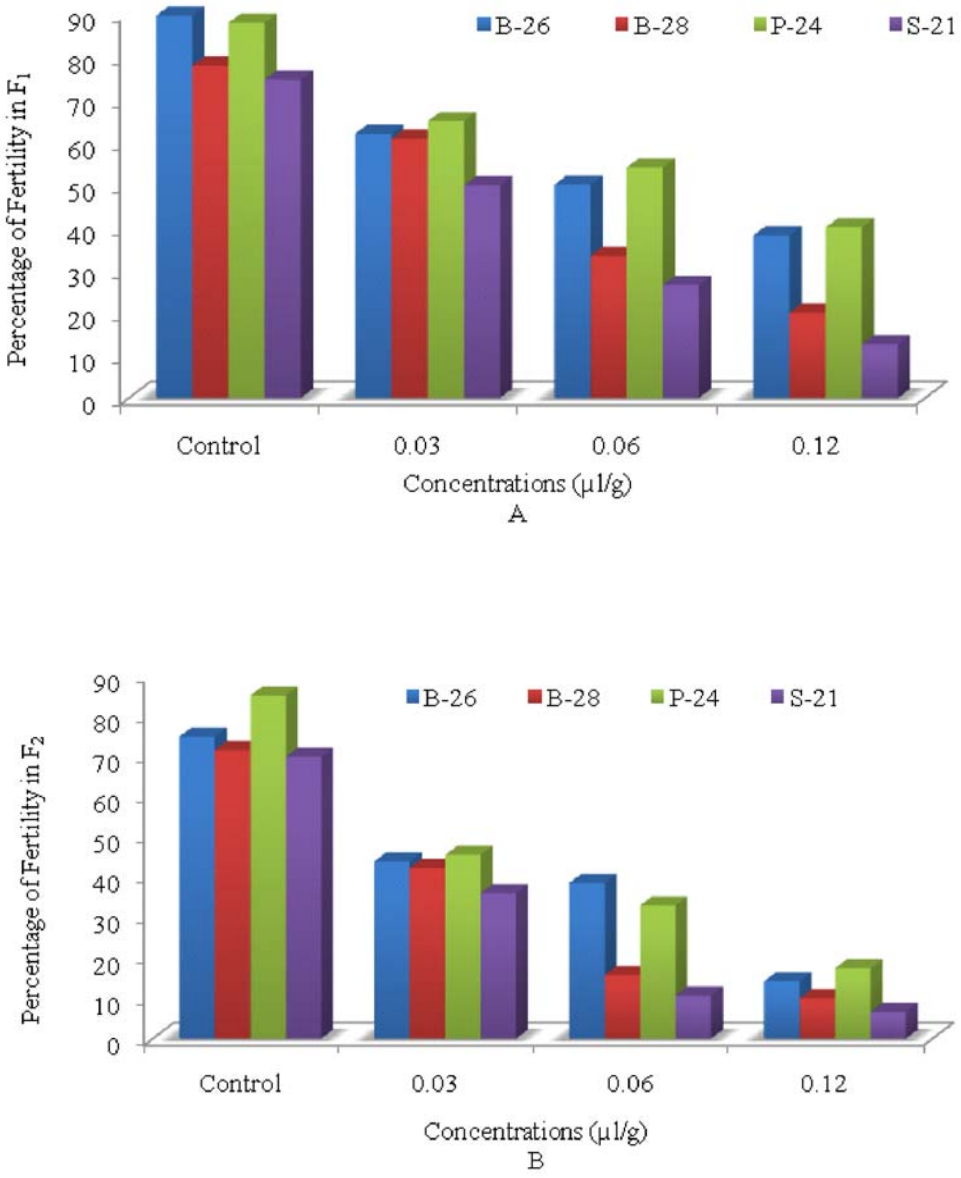

Fig..1 Effect of spinosad on the fertility in F_1 (A) and F_2 (B) generations of *T. castaneum* in wheat varieties.

survival at ≥0.5 mg/kg after 21 days and egg-to-adult emergence of *P. interpunctella* after 49 days, whereas 16 percent adults of *T. castaneum* survived at 1 mg of spinosad/kg of flour after 12 days. Spinosad at 1 or 2 mg/kg provided complete or close to entire suppression of progeny production and kernel damage of all species after 49 days (Huang and Subramanyam, 2007).The present findings are more or less consistent with above results. There was no significant deviation was found in sex ratios at any concentrations of spinosad, wheat varieties and F generations.

Spinosad (Tracer®) showed powerful toxicity against the adults of *R. dominica* and *S. oryzae* and maximum mortality occurred at 250 and 80 ppm

Table 1. Latent effects of spinosad on the life stages of F_1 generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Concentrations (µl/g)	Total no. and Mean ± SE			
		Eggs	Larvae	Pupae	Adults
B-26	Control	1075 215.00±1.30a	964 192.80±1.93a	839 167.80±3.32a	655 131.00±3.32a
	0.03	579 115.80±1.69b	359 71.80±2.31b	314 62.80±2.24b	205 41.00±1.18b
	0.06	352 70.40±1.17c	176 35.20±2.31c	111 22.20±1.83c	65 13.00±1.10c
	0.12	265 53.00±1.22d	101 20.20±2.15d	62 12.40±1.44d	43 8.60±1.17d
B-28	Control	761 152.20±2.01a	593 118.60±1.81a	424 84.80±3.57a	328 65.60±2.38a
	0.03	380 76.00±1.41b	23 146.20±2.35b	14 929.80±2.84b	76 15.20±1.83b
	0.06	174 34.80±2.18c	58 11.60±1.33c	39 7.80±1.16c	17 3.40±0.68c
	0.12	130 26.00±0.89d	26 5.20±0.58d	14 2.80±0.37d	3 0.60±0.24d
P-24	Control	1125 225.00±2.21a	990 198.00±1.76a	735 147.00±3.74a	553 110.60±3.59a
	0.03	761 152.20±2.60b	494 98.80±4.67b	337 67.40±6.19b	220 44.00±6.96b
	0.06	405 81.00±0.45c	219 43.80±1.39c	141 28.20±2.11c	80 16.00±1.87c
	0.12	300 60.00±1.58d	120 24.00±1.30d	69 13.80±0.73d	52 10.40±0.81d
S-21	Control	656 131.20±3.92a	490 98.00±1.30a	368 73.60±3.39a	271 54.20±3.22a
	0.03	331 66.20±2.15b	165 33.00±1.70b	98 19.60±1.72b	44 8.80±1.07b
	0.06	185 37.00±2.10c	49 9.80±0.37c	25 5.00±0.55c	12 2.40±0.60c
	0.12	119 23.80±0.97d	15 3.00±0.32d	10 2.00±0.32d	2 0.40±0.24d

Source	DF	F	Sig.*	F	Sig.*	F	Sig.*	F	Sig.*
Varieties	3	164.58***	0.00	187.22***	0.00	146.68***	0.00	65.72***	0.00
Concentrations	3	1278.25***	0.00	1790.61***	0.00	2120.40***	0.00	1117.38***	0.00
Varieties * Concentrations	9	50.95***	0.00	96.15***	0.00	72.95***	0.00	39.84***	0.00

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test).
Note: *Significant value, *** 0.001 = P < 0.001.

Table 2. Latent effects of spinosad on the life stages of F_2 generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Concentrations (µl/g)	Total no. and Mean±SE			
		Eggs	Larvae	Pupae	Adults
B-26	Control	343 68.60±0.93a	257 51.40±1.63a	207 41.40±0.81a	175 35.00±1.00a
	0.03	123 24.60±2.01b	54 10.80±0.80b	35 7.00±0.55b	21 4.20±0.37b
	0.06	70 14.00±1.22c	27 5.40±0.40c	13 2.60±0.24c	6 1.20±0.37c
	0.12	28 5.60±0.51d	4 0.80±0.20d	3 0.60±0.24d	2 0.40±0.24c
B-28	Control	211 42.20±1.77a	151 30.20±1.43a	138 27.60±1.17a	109 21.80±2.44a
	0.03	123 24.60±2.24b	52 10.40±1.17b	30 6.00±0.55b	18 3.60±0.51b
	0.06	38 7.60±0.74c	6 1.20±0.20c	5 1.00±0.32c	1 0.20±0.20c
	0.12	20 4.00±0.83d	2 0.40±1.75d	0 0.00±0.00d	0 0.00±0.00c
P-24	Control	384 76.80±1.39a	327 65.40±0.86a	251 50.20±1.80a	208 41.60±1.50a
	0.03	145 29.00±1.30b	66 13.20±0.86b	45 9.00±0.71b	28 5.60±0.40b
	0.06	88 17.60±0.81c	29 5.80±0.66c	16 3.20±0.37c	8 1.60±0.24c
	0.12	40 8.00±0.84d	7 1.40±0.40d	3 0.60±0.24d	2 0.40±0.24c
S-21	Control	163 32.60±1.47a	114 22.80±1.02a	104 20.80±1.02a	89 17.80±0.86a
	0.03	100 20.00±0.95b	36 7.20±0.86b	18 3.60±0.40b	12 2.40±0.24b
	0.06	285.60±0.40c	30.60±0.24c	10.20±0.45c	00.00±0.00c
	0.12	15 3.00±0.71d	1 0.20±0.20d	0 0.00±0.00d	0 0.00±0.00c

Source	DF	F	Sig.*	F	Sig.*	F	Sig.*	F	Sig.*
Varieties	3	164.58***	0.00	187.22***	0.00	146.68***	0.00	65.72***	0.00
Concentrations	3	1278.25***	0.00	1790.61***	0.00	2120.40***	0.00	1117.38***	0.00
Varieties * Concentrations	9	50.95***	0.00	96.15***	0.00	72.95***	0.00	39.84***	0.00

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test).Note: *Significant value, *** $0.001 = P < 0.001$.

Table 3. Effect of spinosad on the sex ratio of the 2nd generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Concentrations (µl/g)	Male No. (%)	Female No. (%)	Sex ratio (Male: Female)	÷² value
B-26	Control	91(43.96)	116(56.04)	1:1.27	1.81^{NS}
	0.03	14(40.00)	21(60.00)	1:1.50	1.76^{NS}
	0.06	9(69.23)	4(30.77)	1:0.44	3.61^{NS}
	0.12	3(100.00)	0(0.0)	0:0.00	0.00^{NS}
B-28	Control	79(57.25)	59(42.75)	1:0.75	2.42^{NS}
	0.03	14(46.67)	16(53.33)	1:1.14	2.11^{NS}
	0.06	4(80.00)	1(20.00)	1:0.25	0.00^{NS}
	0.12	0(0.0)	0(0.0)	0:0.00	0.00^{NS}
P-24	Control	116(46.22)	135(53.78)	1:1.16	3.72^{NS}
	0.03	25(55.56)	20(44.44)	1:0.80	1.80^{NS}
	0.06	10(62.50)	6(37.50)	1:0.60	2.84^{NS}
	0.12	2(66.67)	1(33.33)	1:0.50	0.00^{NS}
S-21	Control	53(50.96)	51(49.04)	1:0.96	1.45^{NS}
	0.03	9(50.00)	9 (50.00)	1:1.00	2.20^{NS}
	0.06	1(100.00)	0(0.0)	1:0.00	0.00^{NS}
	0.12	0(0.0)	0(0.0)	0:0.00	0.00^{NS}

Note: NS= Not significant at 5% level.

spinosad after 20 days exposure for *R. dominica* and *S. oryzae*. These results inspired the usage of Tracer® as an safe agent for insect pests' management (Sadeghi and Ebadollahi, 2015). Huang and Subramanyam (2007) reported that progeny production was 28.0±4.2 in control treatment after 49 days and decreased by 91 percent at 0.5 mg/kg and >96 percent at 1 and 2 mg/kg for *T. castaneum*. These consequences certify our results that spinosad is powerful in suppressing progeny production and there is an inverse relationship between progeny production and spinosad concentrations.

The present result revealed that higher concentrations of spinosad were highly effective against progeny production of *T. castaneum* in all wheat varieties in both generations. Among the wheat varieties used S-21 was found to give best result. So, it is revealed that spinosad inhibits the potentiality of *T. castaneum* at 0.12µl/g, and the latent effect transmitted to the next generation. It ultimately reduced the fecundity, fertility and decreased the egg to adult survivability in four wheat varieties in F_2 generations. So, it can be said that effect of spinosad at higher concentrations could effectively control development and reproductive potentiality of *T. castaneum* in stored wheat.

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Relative abundance and foraging activity of hymenopteran pollinators in cucurbitaceous vegetables

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ABSTRACT: A study was conducted to investigate the diurnal activity patterns of hymenopteran pollinators in culinary melon and the dynamics of hymenopteran pollinators of five selected cucurbitaceous vegetables *viz.,*culinary melon, bitter gourd, pumpkin and ridge gourd in 34 locations of Kerala from 06:00 h to 18:00 h with a cone type hand net. The study revealed that *Apis cerana indica* was dominant in culinary melon, pumpkin and ridgegourd and *Tetragonula travancorica* was dominant in bitter gourd and ash gourd, *A. cerana indica, T. travancorica* and *Halictus* sp. recorded highest foraging speed during 10:00 h to 11:00 h; *Ceratina hieroglyphica* and *Lasioglossum* sp. recorded highest foraging speed during 09:00-10:00 h; *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. recorded maximum foraging rate during 10:00 h to 11:00 h; *A. cerana indica* and *Halictus* sp. recorded highest foraging rate during 11:00-12:00 h and 09:00-10:00 h.

KEY WORDS: Pollinators, composition, relative abundance, diurnal activity, cucurbitaceous, foraging speed, foraging rate

INTRODUCTION

Insect pollinators play an important role in effecting optimum pollination of several crops and contribute to the raise of their productivity and quality. Their essentiality is more significant in crops like cucurbitaceous vegetables. Among the vegetable crops, cucurbits are cultivated extensively in India. The cucurbitaceous family comprises of cucumber, pumpkin, chow-chow, bitter gourd, bottle gourd, ridge gourd, ash gourd, watermelon, muskmelon, etc. Globally, the family cucurbitaceae comprises of 118 genera and 825 species. At present in India, cucurbits are cultivated in an area of 555,000 ha with a productivity of 9,912,000 MT and in Kerala cucurbits are cultivated in an area of 2,970 ha with a productivity of 41,610 MT (NHB, 2018). FAO estimates show that, in India about 6% of the total vegetables produced are from eight species of cucurbitaceous vegetables. In India, studies have been conducted on some of the important cucurbit crops to record the insect visitors and to understand the pollinators diurnal activity.

Cucurbits being monoecious, bearing male and female flowers separately on the same plant, depends mainly on insects for pollination and also, their pollen grains being large and sticky, cannot be blown away by the wind. Hence, pollination by insects is essential to bear improved quality of fruits

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and seeds (Free, 1970; Mc Gregor, 1976). In cucurbitaceous vegetables, among all the pollinating insects, the honeybees are known to be the most efficient. For maximizing the yield of cross pollinated crops, utilization of pollinators especially honeybees are considered as one of the cheapest and eco-friendly approach (Free, 1970).

Grewal and Sidhu (1978) recorded that in Punjab, the most frequent visitors of bittergourd flowers were *Apis florea* F. and various species of Anthophoridae and Halictidae with 28, 10 and 5.2 per-cent, respectively. In Vellayani, Kerala, on sponge gourd the most abundant pollinator was *Tetragonula iridipennis* Smith (Mohan, 2000). Pateel and Sattagi (2007) observed that in Karnataka, *A. florea, Apis cerana* F. and *Apis dorsata* F. were the most abundant insect pollinators visiting cucumber in Rabi season.

In Kannur, Kerala on ash gourd flowers, *T. iridipennis* was the most frequent pollinator followed by *Halictus timidus* Smith, *A. cerana* F., *Ceratina hieroglyphica* Smith, and *Halictus taprobanae* Cameron (Leena and Nasser, 2015). Lalita and Yogesh (2015) observed that in Hisar (Haryana), on pumpkin flowers, *A. dorsata* was the most efficient pollinator followed by *Apis mellifera* F., *A. cerana* and *A. florea*.

Hence, keeping in view of the pollination requirements of cucurbits, bee conservation requires rapid and effective tools for identification and delineation of species. For this purpose, composition, relative abundance and diurnal activity of hymenopteran pollinators is to be studied.

MATERIALS AND METHODS

The study was conducted during the year 2018 - 2019 on five selected cucurbitaceous vegetables *viz.,* culinary melon (*Cucumis melo var. acidulus*), bitter gourd (*Momordica charantia* L.), ash gourd (*Benincasa hispida* Thunb. and Cogn.), pumpkin (*Cucurbita moschata* L.) and ridge gourd (*Luffa acutangula* (Roxb.) L.). surveyed once in 34 locations in Thiruvananthapuram and four other districts of Kerala *viz.,* Kollam, Pathanamthitta, Alappuzha and Kasaragod in the months of September, October, November and December during the year 2018 and in January, February and March during the year 2019 from 06:00 h to 18:00 h of the day with a cone type hand net during the blooming period. The four southern districts were selected based on the mandate of the RARS whereas the Kasaragod district was included to represent north Kerala. The selected number of locations covered in each district is mentioned in the table 1.

Table 1. Localities of sample collection

District	Localities covered	Number of locations
Thiruvananthapuram	Vellayani, Karamana, Kulathoor, Karyavattom, Karode, Venkulam, Balaramapuram,Vellarada, Pangode, Parassala, Idinjar, Pallichal, Mukkola, Oorutukaala, Azhicode, Melvettoor, Kalliyoor, Muttakkad, Venganoor, Vizhinjam, Athiyanoor, Pothencode, Puliyancode, Vattiyoorkavu, Perumkadavila	25
Kollam	Edamon, Kottarakkara, Karunagappally	3
Kasaragod	Padannakkad, Nileshwar	2
Alappuzha	Moncompu, Kavalam	2
Pathanamthitta	Padam, Thiruvalla	2
	Total	34

The composition and relative abundance of the different hymenopteran pollinators visiting flowers of five selected cucurbitaceous vegetables from the randomly marked one square meter area were recorded from 06:00 h to 18:00 h of the day at an hourly interval for 5 minutes during flowering period and expressed as mean number of pollinators / m2/ 5 min. Diurnal activity observations were recorded from the flowers of one selected cucurbit viz., culinary melon in College of Agriculture, Vellayani during October to December. Foraging speed (time spent by the bee per flower) from landing till takeoff was recorded by using a stop watch and was expressed in seconds per flower, foraging rate (number of flowers visited by bee per minute) of bees were recorded. These observations were recorded from 06:00 h to 18:00 h at an hourly interval for 5 minutes during flowering period.

RESULTS AND DISCUSSION

A total of twenty-nine species of hymenopteran pollinators were recorded from five cucurbitaceous vegetables (Table 2).

The results on relative abundance (RA) and diurnal activity are presented in tables 3 to 7 and figures 1 to 4. The study on composition and relative abundance of hymenopteran pollinators from Thiruvananthapuram and four other districts of Kerala revealed that, *A. cerana indica* was the dominant pollinator in culinary melon (42.51%), pumpkin (38.76%) and ridgegourd (35.16%) whereas, *T. travancorica* was the dominant pollinator in bittergourd (31.86%) and ashgourd (33.50%). During two seasons, the foraging speed of *A. cerana indica, T. travancorica* and *Halictus* sp. was found to be highest during 10:00-11:00 h. The foraging speed of *Ceratina hieroglyphica* and *Lasioglossum* sp. was found to be highest during 09:00-10:00 h. The foraging rate of *T. travancorica*, *C. hieroglyphica* and *Lasioglossum* sp. was found to be highest during 10:00-11:00 h. The foraging rate of *A. cerana indica* and *Halictus* sp. was found to be highest during 11:00-12:00 h and 09:00-10:00 h respectively.

Study of hymenopteran pollinators composition and relative abundance in cucurbits

A. cerana indica was the most frequent pollinator followed by *T. travancorica.* Jangaiah (2007) reported that in Kerala, on culinary melon flowers, *A. cerana indica* was the most dominant and frequent floral visitor. *T. travancorica* (31.86 per cent) was recorded as the dominant pollinator on bitter gourd flowers followed by *A. cerana indica* (29.90 per cent) and *Ceratina* sp. (11.76 per cent). Subhakar *et al*. (2011) reported that, in Tirupathi on bitter gourd flowers, *T. iridipennis* (86.31 per cent) was the most frequent visitor. The abundance of bees depends on so many factors such as anthesis, weather parameters, competing flora, nectar concentration and its volume (Free, 1970). At peak flowering, the availability of flowers is more than commencement and cessation of flowering, and maximum number of insects would visit the crop during this period to increase the pollination process. Therefore, the flower number clearly influences the pollinator abundance, and in turn, the level of pollination.

A. cerana indica (38.76 per cent) was the frequent floral visitor followed by *T. travancorica* (24.03 per cent). Hemanthkumar (2006) and Mohapatra and Sontakke (2012) observed that on pumpkin flowers *A. cerana* was the most frequent and dominant pollinator followed by *A. dorsata. A. cerana indica* (35.16 per cent) was observed as the most frequent floral visitor on ridge gourd followed by *Xylocopa verticals* (18.68 per cent). Kuberappa *et al*. (2008) and Lakshmi (2013) also reported that on ridge gourd flowers *A. cerana* was the most frequent and dominant pollinator. *T. travancorica* (33.50 per cent) was the most dominant pollinator on ash gourd followed by *A. cerana indica* (26.73 per cent). Leena and Nasser (2015) reported that, on ash gourd flowers, *T. iridipennis* was the most frequent pollinator followed by *H. timidus*, *A. cerana*, *C. hieroglyphica* and *H. taprobanae* in Kannur (Kerala).

Table 2. List of hymenopteran pollinators in cucurbitaceous vegetables

Common name	Scientific name	Family	Vegetable
Indian bee	*Apis cerana indica* F.	Apidae	Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
Rock bee	*Apis dorsata* F.		Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
Little bee	*Apis florea* F.		Culinary melon
Stingless bee	*Tetragonula travancorica* Shanas and Faseeh		Culinary melon, Bitter gourd, Pumpkin, Ash gourd
	Tetragonula sp. nov.1		Pumpkin
Small carpenter bee	*Ceratina hieroglyphica* Smith, *Ceratina simillima* Smith, *Ceratina binghami* Cockerell, *Ceratina unimaculata javanica* van der Vecht		Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
Blue- banded bee	*Amegilla zonata* L.		Culinary melon, Ridge gourd
Carpenter bee	*Xylocopa verticals* Smith		Culinary melon, Ridge gourd
Sweat bee	*Lasioglossum* sp.	Halictidae	Culinary melon, Bitter gourd, Pumpkin, Ash gourd
	Halictus sp. 1, *Halictus* sp. 2, *Halictus* sp. 3		Culinary melon, Bitter gourd, Pumpkin, Ash gourd, Ridge gourd
Alkali bee	*Nomia eliotti* Smith, *Nomia westwoodi* Gribodo *Nomia curvipes* F., *Nomia* sp.		Culinary melon, Ashgourd
Leaf- cutter bee	*Megachile lanata* F., *Megachile disjuncta* F.	Megachilidae	Bitter gourd, Ash gourd
Paper wasp	*Ropalidia brevita* Das &Gupta	Vespidae	Culinary melon, Ridge gourd
Potter wasp	*Eumenes* sp.		Ridge gourd
	Anterhynchium abdominale abdominale Illiger		Culinary melon
Mud dauber	*Sceliphron madraspatanum* F.	Sphecidae	Culinary melon
Blue mud dauber	*Chalybion bengalense* Dahlbom		
Scoliid wasp	*Phalerimeris phalerata phalerata* de Saussure	Scoliidae	Ash gourd
	Campsomeriella annulata annulata F.		
Mole cricket hunters	*Larra maura* F.	Crabronidae	Culinary melon

Table 3. Composition and relative abundance of different hymenopteran pollinators in culinary melon

Pollinator	Trivandrum	Kollam	Pathanam-thitta	Alappu-zha	Kasara-god	Total	% RA
Apis cerana indica	167	21	13	4	8	213	42.51
Tetragonula travancorica	77	5	6	-	5	93	18.56
Ceratina Sp.	46	6	5	-	-	57	11.38
Apis dorsata	44	2	4	-	1	51	10.18
Lasioglossum sp.	10	4	5	-	4	23	4.59
Nomia sp.	12	-	-	5	2	19	3.79
Halictus sp.	5	3	3	3	-	14	2.79
Apis florea	7	1	2	-	-	10	1.96
Xylocopa verticals	6	-	-	-	3	9	1.80
Amegilla zonata	5	-	-	2	-	7	1.40
Wasps	3	-	1	1	-	5	1.00
Total	382	42	39	15	23	501	42.51

No. of locations – 29
Total no. of pollinators collected - 501
% RA - mean number of pollinators/m^2/5 min

Table 4. Composition and relative abundance of different hymenopteran pollinators in bittergourd

Pollinator	Trivandrum	Kollam	Pathanam-thitta	Alappu-zha	Kasara-god	Total	% RA
Tetragonula travancorica	34	7	8	6	10	65	31.86
Apis cerana indica	39	6	7	4	5	61	29.90
Ceratina sp.	19	-	2	3	-	24	11.76
Megachile sp.	5	4	4	-	2	15	7.35
Lasioglossum sp.	8	2	-	1	3	14	6.86
Apis dorsata	7	2	-	3	1	13	6.37
Halictus sp.	6	3	1	2	-	12	5.88
Total	118	24	22	19	21	204	

No. of locations – 27
Total no. of pollinators collected - 204
% RA - mean number of pollinators/m^2/5 min

Foraging Behavior of Bees

On culinary melon flowers, *A. cerana indica, T. travancorica* and *Halictus* sp. recorded maximum foraging speed during 10:00 h to 11:00 h. *C. hieroglyphica* and *Lasioglossum* sp. recorded highest foraging speed during 09:00 to 10:00 h. *T. travancorica*, *C. hieroglyphica* and *Lasioglossum*

Table 5. Composition and relative abundance of different hymenopteran pollinators in Pumpkin

Pollinator	Trivandrum	Kollam	Pathanam-thitta	Alappu-zha	Kasara-god	Total	% RA
Apis cerana indica	28	7	6	5	4	50	38.76
Tetragonula travancorica	15	4	4	3	5	31	24.03
Ceratina sp.	8	3	-	-	3	14	10.85
Apis dorsata	7	-	1	3	2	13	10.08
Lasiog lossum sp.	2	5	2	1	1	11	8.53
Halictus sp.	5	1	2	2	-	10	7.75
Total	65	20	15	14	15	129	

No. of locations – 19
Total no. of pollinators collected – 129
% RA - mean number of pollinators/m^2/5 min

Table 6. Composition and relative abundance of different hymenopteran pollinators in ash gourd

Pollinator	Trivandrum	Kollam	Pathanam-thitta	Alappu-zha	Kasara-god	Total	% RA
Tetragonula travancorica	43	6	7	4	6	66	33.50
Apis cerana indica	39	4	5	2	4	54	27.41
Ceratina sp.	10	-	4	-	3	17	8.62
Halictus sp.	8	3	-	5	-	16	8.12
Nomia sp.	7	2	2	-	2	13	6.59
Wasps	-	5	2	4	-	11	5.58
Lasioglossum sp.	6	-	2	-	1	9	4.56
Apis dorsata	6	1	-	-	-	7	3.55
Megachile sp.	4	-	-	-	-	4	2.03
Total	123	21	22	15	16	197	

No. of locations – 24
Total no. of pollinators collected – 197
% RA - mean number of pollinators/m^2/5 min

sp. recorded the highest foraging rate during 10:00 h to 11:00 h. While *A. cerana indica* and *Halictus* sp. were observed to have maximum foraging rate during 11:00 to 12:00 h and 09:00 to 10:00 h. Rapp (1981) reported that, on cucumber flowers, honey bees started foraging at 06:00 h and their activity was maximum from 09:00 to 12:00 h and was found decreasing in the afternoon hours. The peak foraging activity during morning hours can be correlated with the abundant availability of pollen and nectar during this period.

In the present study the maximum time was spent by *T. travancorica* (11.23 sec and 11.46 sec) for pollen collection, followed by *Lasioglossum* sp. (11.06 sec and 11.30 sec), *A. cerana indica* (10.61

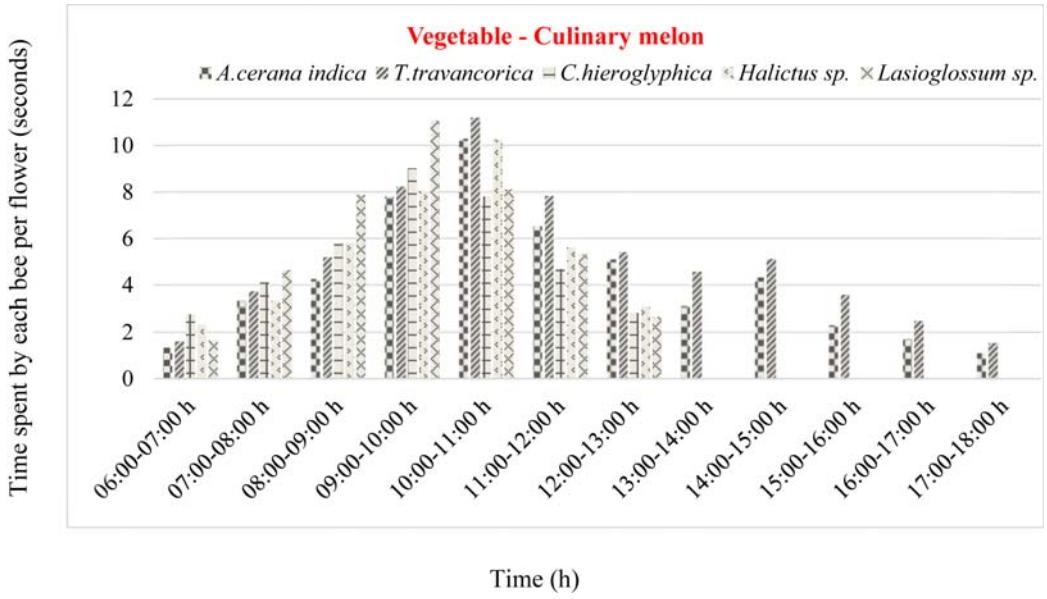

Fig. 1. Foraging speed (seconds) of hymenopteran pollinators during season I (October to November)

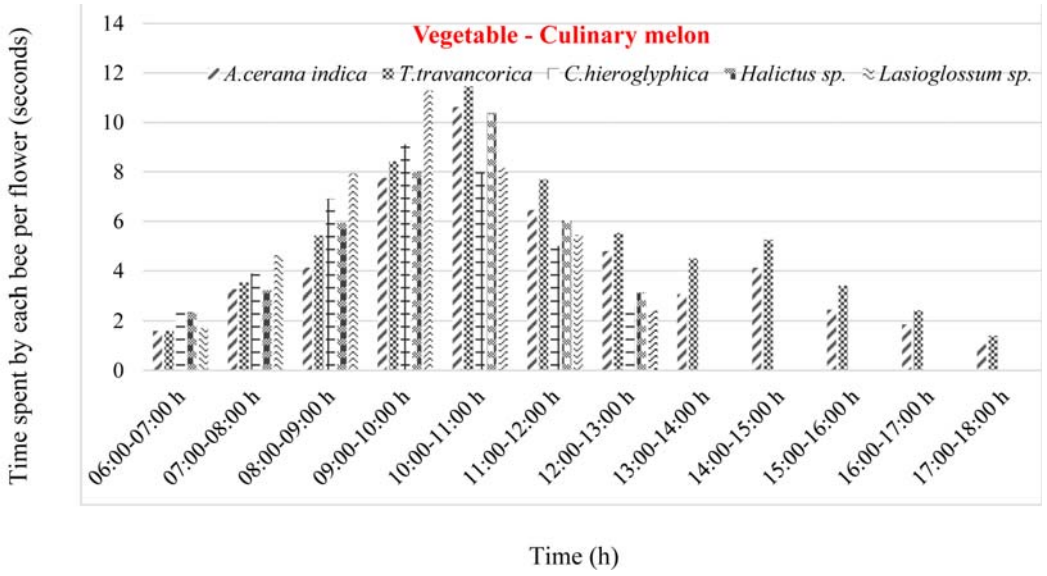

Fig. 2. Foraging speed (seconds) of hymenopteran pollinators during season II (November to December)

sec and 10.63 sec), *Halictus* sp. (10.26 sec and 10.40 sec) and *C. hieroglyphica* (9.02 sec and 9.11 sec) during two seasons. Prakash (2002) reported that in cucumber, among the honey bees, maximum time was spent for pollen collection, by *A. florea* (13.49 sec), followed by *T. iridipennis* (11.44 sec), *A. cerana* (9.65 sec), *A. mellifera* (8.74 sec) and the least in *A. dorsata* (7.22 sec). The difference in the foraging speed of bee species, may be due to different climatic conditions, type of

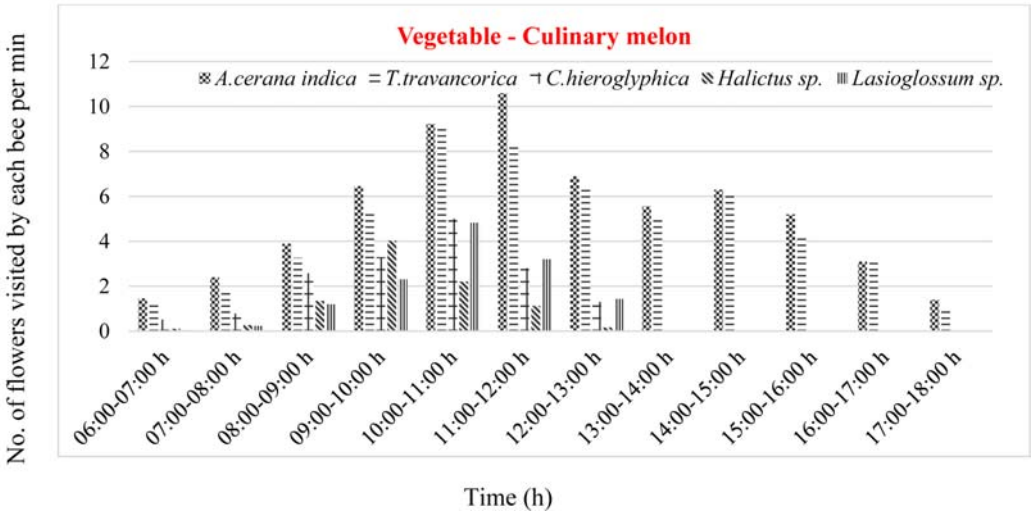

Fig. 3. Foraging rate of hymenopteran pollinators during season I (October to November)

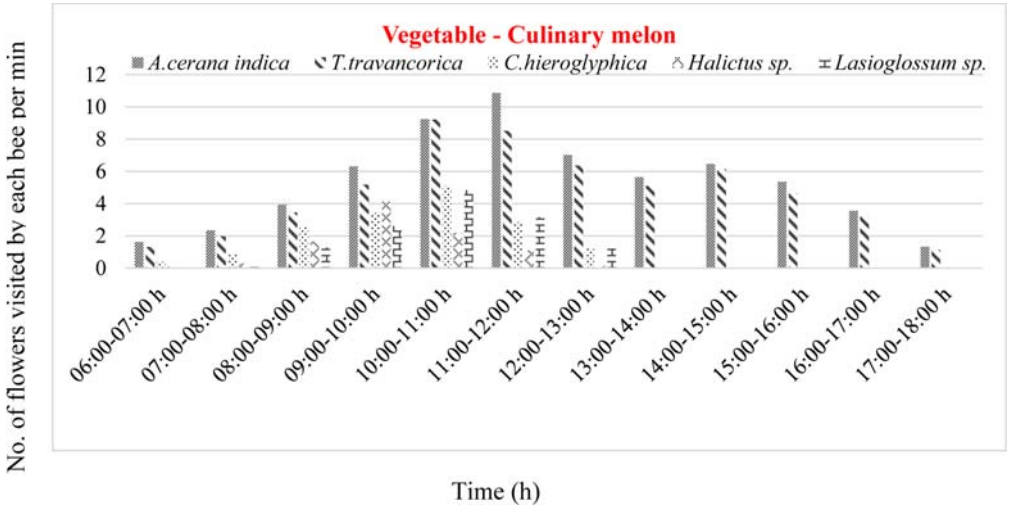

Fig. 4. Foraging rate of hymenopteran pollinators during season II (November to December)

crop, geographic location and species specific differences and variation in the availability of foraging source.

In culinary melon flowers, the mean foraging rate was found highest in *A. cerana indica* (10.60 & 10.88 flowers/min) followed by *T. travancorica* (9.16 and 9.23 flowers/min), *C. hieroglyphica* (5.01 and 5.10 flowers/min), *Lasioglossum* sp. (4.83 and 4.85 flowers/min) and *Halictus* sp. (4.03 and 4.13 flowers/min) during the two seasons. In Hisar, the data on the foraging activity of insect visitors in

Table 7. Composition and relative abundance of different hymenopteran pollinators in ridge gourd

Pollinator	Trivandrum	Kollam	Pathanam-thitta	Alappu-zha	Kasara-god	Total	% RA
Apis cerana indica	11	6	5	4	6	32	35.16
Xylocopa verticals	5	5	-	6	1	17	18.68
Wasps	5	-	4	3	2	14	15.38
Apis dorsata	3	4	1	-	4	12	13.19
Lasioglossum sp.	3	-	-	2	4	9	9.89
Amegilla zonata	1	3	3	-	-	7	7.69
Total	28	18	13	15	17	91	

No. of locations – 13
Total no. of pollinators collected – 91
% RA - mean number of pollinators/m^2/5 min

cucumber hybrids, *viz.*, Evergreen, NBH-Manu, Damini and Rani showed that the mean foraging rate irrespective of different day hours was highest in *A. dorsata* (8.63 flowers/min.) followed by *C. sexmaculata* (5.03 flowers/min.), and it was lowest in *Halictus* sp. (4.38 flowers/min.) (Hanh *et al*., 2014). Fluctuation in visits of insect pollinators on culinary melon flowers reveals that the visits were low at the time of commencement and cessation of flowering but these remained high during mid flowering period. This difference might be due to variation in the floral density during the span of blooming and changes in climatic conditions.

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On a new species of *Neoceratobaeus* Rajmohana (Hymenoptera: Scelionidae) from India

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ABSTRACT: *Neoceratobaeus* Rajmohana (Hymenoptera: Scelionidae) was described from India as a monotypic genus. The present paper describes and illustrates the second species *N. dwitiyus* sp. nov. from West Bengal, India. Morphological affinities with the known species are discussed.

KEYWORDS: *Neoceratobaeus*, new species, Baeini, egg parasitoid

INTRODUCTION

Neoceratobaeus Rajmohana 2014, (Hymenoptera: Scelionidae) with type species *Neoceratobaeus gibbus* Rajmohana, 2014, has been a monotypic genus, described from India. With 7 segmented antenna ending in a large unsegmented clava in females, forewing rather spoon shaped distally and T1 having a large, backward directed and laterally compressed horn, the genus is easily diagnosed. It belongs to subfamily Scelioninae and tribe Baeini, Members of the tribe Baeini are unique among Scelonidae since they attack spider eggs (Austin *et al,* 2004; Carey *et al.* 2006). Majority of the Baeini species fall under 4 genera with 433 species known globally. *Ceratobaeus* Ashmead (165 species), *Idris* Förster (160 species), *Odontacolus* Kieffer (55 species), and *Baeus* Haliday (53 species). In India Baeini comprises 47 species under five genera - *Baeus* Haliday (1 species), *Ceratobaeus* Ashmead (12 species), *Idris* Förster (31species), *Neoceratobaeus* Rajmohana (1species) and *Odontacolus* Kieffer (2 species) (Johnson 1992, Johnson *et al.* 2018). In this paper *Neoceratobaeus dwitiyus* sp. nov. collected from West Bengal, India, the second species of the genus is described and illustrated. Morphological affinities with *N. gibbus* are discussed.

MATERIALS AND METHODS

Specimens were collected using yellow pan traps, set in a vegetable garden. 30 traps were kept for 10 hours. The collected specimens were preserved in 70% ethanol and later mounted on point-card tips. Descriptions and images were prepared using Leica M205-A stereomicroscope, with 1X objective and Leica DFC-500 digital camera and processed with LAS version 3.6 extended focus software. Morphological terminology follows Masner (1976), Austin and Field (1997) and Mikó *et al.* (2007, 2010).

Abbreviations used in the description are as follows: F1 to F4- Funicular Segments; L-Length; W-Width; H-Height; IOS- Inter Occipital Space; HW-Head Width, HH- Head Height; stgv- Stigmal vein; mv-Marginal vein; pmv- Post marginal vein; T1-T7-Metasomal tergites 1 to 7.

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Both the holotype and paratype of *N. dwitiyus* sp. nov. are deposited in the National Zoological Collection, Zoological Survey of India, Kolkata.

***Neoceratobaeus dwitiyus* sp. nov. Holotype Female (Figs. 1-6)**

urn:lsid:zoobank.org:act:C43ADC42-B34D-42C4-B2CB-D2FBF2E86286

DESCRIPTION

Holotype Female. Length = 1.02 mm. Head and mesosoma black, metasoma yellow, contrasting in colour with rest of body, horn on T1 concolorus with metasoma, blackish brown only towards tip and; legs pale; eyes silvery; mandibles as well as base of antennal clava yellowish brown; wings clear and hyaline; veins brown (Figs 1, 2).

Head : (L: W): 35: 6 transverse dorsally, slightly wider than mesosoma; in anterior view subtriangular; vertex straight, upper frons, frons, vertex and occiput with coarse granulations; with fine and dense setigerous punctate; frons entirely, face and gena entirely sculptured except for a large smooth and glabrous speculum(Fig 4) , on antennal scrobe, extending upto three fourth eye level on frons medially; cheeks with fine radiating striae, lacking granulations in between striae, and gradually merging with granulations near lower margin of eyes, pilosity scanty; IOS : HW to HH =22 : 35 : 14 (in front view); eyes densely pubescent; mandibles with three pointed and equal teeth; central keel distinct, complete and expending till anterior ocelli; lateral ocelli contiguous with orbital margin; hyper-occipital carina complete; occipital carina distinct, striate-scrobiculate; temples coarsely granulate, without any striae; antennae with 7 segments, 4 funicular segments; clava large (Figs. 3, 6), segmentation indistinct; length and width of F1 a little less than that of pedicel, F1 longest, > 2x length of F2; F3 and F4 much transverse; clava large, 4 segmented, segmentation indistinct. L: W ratio of antennal segments being 17: 3; 7: 2; 1: 2; 1:2; 1:2; 1:2; 1:1; 14:8.

Mesosoma : (L : W = 31 : 33); narrower than head dorsally (Fig. 1); surface with granulations as on vertex; mesoscutum with dense pubescence; notauli distinct as a short strip, but visible only till 0.2 of mesoscutum, trans scutellar sulcus not crenulated; mesoscutellum moderately convex (Fig. 1), surface with granulations as on mesoscutum, pilosity scattered; posterior margin of mesoscutellum feebly excavate, a row stiff setae present on posterior border; metascutellum with an arched row of horizontal foveolae; propodeum feebly hairy, drawn into pointed spines at lower corners; propodeal lamellae and flanges on either sides of metasomal horn developed into spines laterally; pronotum laterally striate longitudinally and with rough rugosity, mesopleural carina distinct (Figs. 5), bordered, though not continuous, with longitudinal striae; acetabular carina, flanked by irregular punctate and irregular longitudinal elements; lower, stem of forewing emarginated inwards, fitting to curvature of metasomal horn when at rest; L : W =61 : 17; pmv longer than stgv; (mv : stgv : pmv = 2: 7 :8), pmv with 5-6 large setae; marginal fringe short; hindwing wider than usual, width of hindwing: forewing = 14 : 23; basal vein absent (Fig. 2).

Metasoma : (L : W = 58 : 34); T3 is widest among all the tergites ; all tergites transverse, T2 onwards with fine pubescence laterally; metasomal horn in dorsal view extending backwards, upto anterior half of T2, horn dorsally with closely placed concentric striae; median suture between T1 and T2 distended downwards; T1 longest among tergites; proportions of width to length of T1 to T7 medially being 23: 14; 31: 8; 34: 11; 27: 6; 22: 2; 14: 1; 8: 8 T1 and T2 with similar sculpture, weak and wavy longitudinal striae, T3 onwards with dense and fine irregular reticulate sculpture; anterior margin of T3 with a narrow smooth band; posterior margins of T3-T6 with a narrow smooth band; T7 entirely sculptured, with sparse long hairs (Fig. 1); ovipositor extended.

Male: Unknown.

Etymology: The species is named '**dwitiyus**', this being the second species under the genus ('Dwitiya' in Sanskrit = second)

Material examined: Holotype. Female (20678/ H3). India: West Bengal: Sagar Island, Phulbari (N21° 51' 43.94", E088° 07' 46.32"); Coll: Sunita; 21.iii.2018; yellow pan trap.

Fig. 1

Fig. 2

Fig. 3

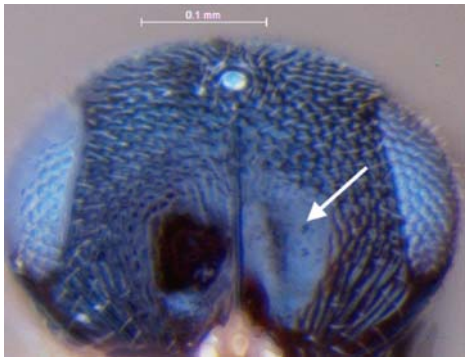

Fig. 4

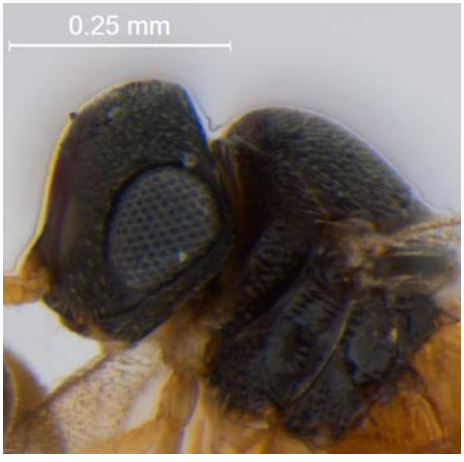

Fig. 5

Fig. 6

Figs. 1-6. *Neoceratobaeus dwitiyus* sp. nov. Holotype, female
1) Dorsal view; 2) Lateral view; 3) Wings; 4) Head front view (Speculum indicated); 5) Head with Mesopleuron; 6) Head with antenna

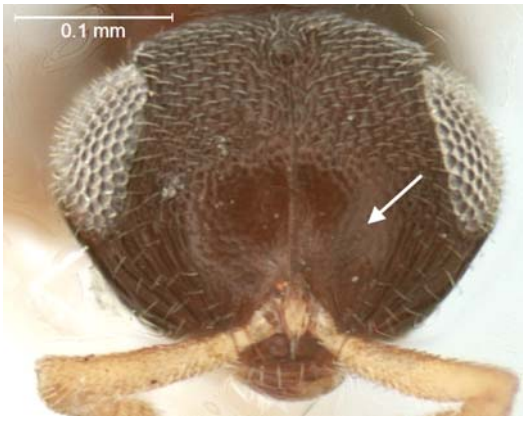

Fig. 7

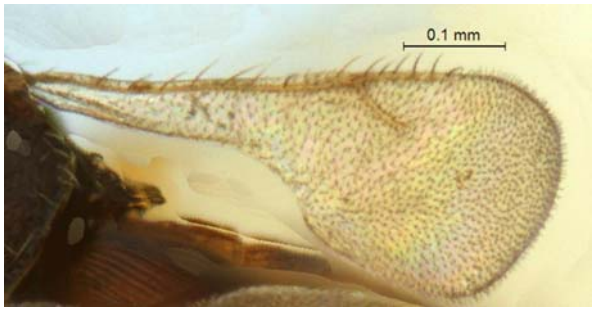

Fig. 8

Figs. 7-8. Female *Neoceratobaeus gibbus* Rajmohana
7) Head frontal view (Speculum indicated); 8) Forewing

Paratype: 1 female (20679/H3). India: West Bengal: Sagar Island: Gangasagar Island, Bharat Seva Ashram Campus (N21° 38' 22.05", E088° 04' 48.32"); Coll: Sunita; 23.iii.2018; yellow pan trap.

Diagnosis:

Neoceratobaeus dwitiyus sp. nov. is very similar to *N. gibbus*, but the former can be identified by the smooth, shiny and glabrous speculum on antennal scrobe, which is very conspicuous. *In N. gibbus*, speculum is not smooth (Fig. 7), instead with impressions or traces of coriaceous sculpture. In *N. dwitiyus*, forewing is comparatively longer, 3.5X as long as wide, with distal margin slantingly convex. In *N. gibbus*, forewing is wider, only 2.5X as long as wide, with a more rounded distal margin (Fig. 8). Both the species differ largely in the shape and size of the forewing (Figs. 3, 8).

Comments:

Genus *Neoceratobaeus* has been known by females only. The metasomal horn on T1, serve as a recess for the internally retracted ovipositor and the horn length is correlated to ovipositor length (Austin *et al,* 2004). The prominent metasomal horn of *N. dwitiyus* gives a clue of the length of the ovipositor and also that the eggs of the host spider would have a thick sac. Since only one specimen each was trapped, in both the collecting events, this indicates the rarity of the species.

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Occurrence of multiple nest entrance in the stingless bee *Tetragonula travancorica* (Hymenoptera: Meliponini)

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ABSTRACT: Multiple nest entrance occurs in the stingless bee *Tetragonula travancorica* Shanas & Faseeh at a low frequency (2.3%). Different types of multiple nest entrances are described and its significance discussed.

KEY WORDS: stingless bee, nest architecture, Kerala, India.

INTRODUCTION

The stingless bees (Hymenoptera, Apidae, Meliponini) are the most diverse eusocial bees in the tropical regions of the world. They are 60 times more speciose than the *Apis* bees. Nesting habits and nest architecture vary greatly not only among genera but also among species within this genus. The nesting habitats of stingless bees vary widely from cracks and crevices, walls, tree hollows, the base of trees, bird nests, underground structures, limestones etc. According to Roubik (2006) the nest entrance is normally associated with physio-chemical regulation, maintenance of microclimate inside the nest, foraging activity and defence of a colony and it is the check point at which the individual stingless bees entering the nest are recognized. He also mentioned the possibility of occurrence of innovations in the nest architecture within a taxon.

Attributes of nest structure and nesting habits can be used as a tool for ecological, phylogenetic and taxonomical studies (Rasmussen and Camerago, 2008; Lima *et al.,* 2013). Kelly *et al.* (2014) observed that the nest entrance of stingless bees differs with genus. Most of the studies were concentrated on ecology, biology, morphology and pollination of these bees (Vijayakumar 2014). They use the waxy secretion from their dorsal body along with resins collected from plants for construction of nests (Virkar, 2014). Only limited information is available on the nesting behaviour of stingless bees in India (Roopa *et al*., 2015; Patel and Pastagia, 2016).

Shanas and Faseeh (2019) described three new species of stingless bees from south India and provided keys to the species of *Tetragonula* of the Indian subcontinent. They also established that, *T. iridipennis* (Smith, 1854) does not occur in India and *Tetragonula iridipennis*, mentioned in publications from south India most probably refers to *T. travancorica* Shanas & Faseeh. Multiple nest entrance in *Tetragonula travancorica* from Kerala is described and its significance discussed here.

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MATERIALS AND METHODS

The study was conducted during 2017-2018 in Kerala, India. Stingless bees were collected from different geographical regions in the state. The colonies were located at Manakkadavu, Kannur District (12^{0}13'12.21"N 75^{0}30'07.29"E, altitude 223 m), Peechi, Thrissur District (10^{0}30'56.06"N 76^{0}21'31''.35"E, altitude 115 m), Vellayani, Thiruvananthapuram District (8^{0}25'40.85"N 76^{0}59'05.68"E, altitude 29 m), Kadannamanna, Malappuram District (11^{0}01'53.58"N 76^{0}10'07.90" E, altitude 55 m), Ambanad, Kollam District (9^{0}00'50.99"N 77^{0}05'21.88"E, altitude 362 m), Thenmala, Kollam District (8^{0}57'45.58"N 77^{0}03'53.38"E, altitude 153 m). A total of 207 colonies were located during the study. The colonies with more than one entrance were recorded with the number of guard bees, shape of entrance mouth, length and width of entrance mouth, number of entrance tubes, length of each entrance tube, number of active entrances, height from the ground level and nest habitat. Each nest entrance was photographed with a 55mm macro lens mounted on a Nikon D80 camera. Latitude, longitude, and altitude of each colony were noted with the help of Google earth pro app.

Transparent bottles of 15-20 cm height were kept covering the mouth of the entrance and gently tapped around the nearby areas of nest which resulted in bees getting trapped inside the bottle. An average of 20 stingless bees was collected from each colony and no colonies were harmed during the entire collection. Collected bees were killed using ethyl acetate and preserved in 70% ethyl alcohol, few of which were pinned, dried and stored in insect boxes for further studies.

RESULTS AND DISCUSSION

The samples were collected from various locations in Kerala (comprising all geographical regions such as coastal areas, planes, and hills). Out of the 207 colonies, six colonies had multiple nest entrance openings, of which four were from the hill tracts and two were from the plains. The shape of entrance tubes identified were either round, oval or slit. Three of the nest entrances were oval and

Table 1. Nest entrance characters of colonies with more than one entrance

Character	Location*					
	M	P	V	T	A	K
No of entrance tubes	2	2	2	2	2	5
No of active entrance tubes	2	1	1	2	2	5
Shape of entrance	Round	Oval	Oval	Round	Slit	Round
No of guard bees	5+5	5	6	4+4	7+7	4+1+1+1+1
Length of entrance mouth (cm)	1.2	1.2	2.1	1	2.5	
Length of each entrance tube (cm) 1		2.1	7.6	5.4	7	04.3
2						3.8
3						2.3
4						5.4
5						5.4
Height from ground level (cm)	34	67	28.7	25.4	138	7.8
Nest habitat	Stone wall	Stone wall	Stone wall	Stone wall	Building wall	Stone wall

*(M-Manakkadavu, P-Peechi, V-Vellayani, T-Thenmala, A-Ambanad, K-Kadannamanna)

Fig. 1 Vellayani

Fig. 2 Thenmala

Fig. 3 Manakadavu

Plate 1. Multiple entrances recorded in *Tetragonula travancorica*

Fig. 4 Kadannamanna

Fig. 5 Peechi

Fig. 6 Ambanad

Plate 1. Multiple entrances recorded in *Tetragonula travancorica*

Fig.7 Arrows indicate the openings in the entrance tube at Thachampara, Palakkad (Host: Fig tree)

two of them were round and one was slit in shape. It was also observed that the shape of entrance mouth was similar for all entrance tubes of the same colony.

The number of guard bees varied between colonies, however, the number of guard bees were same in each active entrance tube of the same colony, except in the case of a single colony where in, out of five active entrance tubes, one entrance tube had 4 guard bees and the remaining entrance tubes were guarded by a single bee (Fig. 4).

The number of entrance tubes was two for colonies identified from Vellayani (Fig. 1), Thenmala (Fig. 2), Manakkadavu (Fig. 3), Peechi (Fig. 5), and Ambanad (Fig. 6) whereas five entrance tubes were found in a single colony from Kadannamanna (Fig. 4). The number of active entrances differed in each colony. Colonies from Thenmala, Ambanad and Manakkadavu had two active entrances, whereas colonies from Vellayani and Peechi were with only one entrance tube which was active and the other one was closed entirely or joined with the active entrance tube mouth (Vellayani). A colony from Kadannamanna had five entrance tubes (Fig.4) and all of them were active.

The length and width of entrance mouth were almost same for different entrance tubes in a single colony. Length of entrance mouth varied from 1.0to 2.5cm and width varied from 0.2 to 1.2 cm in different locations. The height of colony from ground level varied in different locations. All colonies were located at a height of less than 50cm, except the ones in Ambanad (138 cm) and Peechi (67 cm). Five colonies were found associated with foundations of buildings made of stones, and one colony was observed inside the wall of a building.

All the five colonies were having the entrance tubes one above the other presenting an overall look of vertical arrangement of entrance tubes. In one colony, the five entrances were arranged in a different manner: two pairs of entrance tubes arranged vertically (one below to the other) and the fifth, in line with the upper entrance tubes (three tubes were in one line and two tubes were in another line) (Fig.4).

In addition to these six colonies with multiple entrances, one more colony was identified from Thachampara (Palakkad dist) with multiple nest entrance openings. Three to four small openings were observed in the entrance tube, in addition to

opening on the apex. Except for the apex, all openings were non-functional and without any guard bees. The main entrance was guarded by 4-5 bees and the colony was located 186cm above the ground level on a fig tree (Fig.7).

Wille and Michener (1973) reported two species of stingless bees *Tetragonisca angustula* and *Trigona fulviventris,* those rarely form nests with two or three entrances. Roubik (2006) stated the existence of multiple entrances in some genera such as *Lepidotrigona, Scaptotrigona, Plebia, Tetragona*, and *Hypotrigona* and these multiple entrances cannot be considered as a consistent character in these genera. Benziger and Benziger (2010) observed two exceptional nests of minute stingless bees, *Lisotrigona cacciae* and *L. furva* having two nest entrances for a single colony. The nest entrances of *Pariotrigona klossi* obtained from calcareous rocks were having several tubelets (highest reported were with 300 tublets) arranged on interconnected clumps (Benziger, 2011). Such clumps were observed only in one of the five colonies (colony which had five entrance tubes originating from a clump) in the current study. The nests with two entrances had no clumps, however, each tube was very closely constructed or merged partially at the base. This may enhance the stability of the nest entrances as the number of entrance tubes increases. Divya *et al*. (2016) observed two nests of *Tetragonula iridipennis,* each with two entrances, from Poojappura and Tholicode in Kerala. Jose (2015) observed two separate entrance funnels in a *T. iridipennis* nest.

This behaviour of constructing multiple entrances can be considered as a defensive strategy of the stingless bees or to avoid the huge foraging traffic in the strong colonies. The building of a large, single entrance may result in variation in the microclimate of the bee nest, which may adversely affect the colony. This could be a plausible for the construction of multiple entrance holes instead of one big entrance. It is also difficult to manage the intruders as the size of the nest entrance increases and at the same time, an efficient distribution of guard bees can be done by dividing the nest entrance, and thus fortify the defence. All the nests mentioned in the current study were obtained inside stone walls of the foundations of buildings and all of them were free from harsh weather and other disturbances. Benziger *et al.* (2011) stated, in order to construct complex nest entrances they need to be protected well and the nests of *Pariotrigona klossi* obtained from crevices of limestones were protected by overhanging rocks.

Multiple exit holes and platforms were observed in *Tetragona clavipes* and the species also varied with broad and lamellate nest entrances (Roubik, 1979). Roubik (2006) reported construction of tubercles or hollow tubes around the nest entrance of *Lestrimelitta* and occurrence of dual entrances in colonies of *Meliponula ferrugenia* and *Lepidotrigona ventralis*. Jose (2015) reported two openings in a single entrance tube of an artificial hive in *T. iridipennis,* in which both the openings were guarded by bees. In the current study, we observed single entrance tube with multiple openings in a natural habitat (in feral colonies) however, none of them were functional except the opening at the apex of the tube (Fig.7).

The shape of nest entrance of *T. iridipennis* varied from slit-like, circular, oval, funnel-shaped and feral colonies are dominant with slit and circular nest entrance apex (Pavithra *et al*., 2013; Jose, 2015). The shape of entrance apex was similar with in a colony while it varied between colonies such as slit-like, oval or circular. The reported nest entrance length in *T. iridipennis varies* from 6 to 18 mm (Roopa *et al*., 2015), 10.6 to 102.6 mm (Jose, 2015) and 5 to 90mm (Ramya *et al*., 2015). *Tetragonula* colonies without an entrance tube were also reported from Kerala (Jose, 2015). The length of the entrance tube varied from 2.1 cm to 7.6 cm and among them, one colony was without a nest entrance tube and was with a slit-like opening. In addition to environmental cues, the pheromone-regulated nest mate interaction also acts as a stimulus for nest building, when these stimuli become more complex and vigorous, it changes the nest building behaviour and results in the formation of novel building action (Pavithra *et al.*, 2013).

The behaviour of *Tetragonula travancorica* in constructing nests with multiple entrance is

prevalent in different regions of Kerala, however, its frequency is low (2.3%). The factors behind this behaviour or instinct are not known. The knowledge on nesting and nesting site can be a useful tool for designing stingless beehives and their conservation for honey as well as pollination purpose.

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Influence of wind direction on parasitization behaviour of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) in brinjal ecosystem

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ABSTRACT: Investigations were carried out to assess the impact of wind direction on parasitization behaviour of *Trichogramma chilonis* Ishii on brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee indicated maximum number of parasitization in windward direction. Findings indicate importance of air stream in the parasitization behaviour of *T. chilonis* in field condition.

KEY WORDS: Biocontrol, *Leucinodes orbonalis, Trichogramma* parasitoid, wind

Trichogrammatids are the most widely used egg parasitoids against lepidopterous pests on a wide range of agricultural and forest crops across the world for more than 70 years (Li, 1994) as inundative releases. More than 25 species reported in India, *Trichogramma chilonis* Ishii occurs widely all over the country throughout the year. Farmers in Tamil Nadu are known to use *Trichogramma* egg parasitoid for shoot and fruit borer *Leucinodes orbonalis* management. It is well known that movement activity of *Trichogramma* species may depend on environmental conditions, primarily temperature (Fournier and Boivin, 2000), host density, release rates (Singh and Jalali, 1992), host plant density, wing size (Kolliker- Ott *et al*., 2004) and sex (Canto-Silva *et al*., 2006). This study was conducted to understand the orientation behaviour of *T. chilonis* influenced by wind direction to decide on parasitoid release points in an inundative release during wind influence situation for the management of brinjal shoot and fruit borer (BSFB) in brinjal ecosystem.

Orientation behaviour of *T. chilonis* in brinjal field

1. Sentinel Egg Card Technique

A field trial conducted *kharif* 2017 in Agricultural College and Research Institute, Killikulam in Thoothukudi district using brinjal variety KKM 1. The experiment was conducted in supervisory trial. The experiment was organized on brinjal crop in vegetative stage 25 DAT having shoot damage

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caused by *L. orbonalis* to the extent of more than 10 per cent. *T. chilonis* orientation behaviour study conducted adopting sentinel egg card technique (Niranjana, 2015). *Corcyra* egg card having approximately 0.0625 cc parasitized eggs fixed in centre surrounded by each 0.0125 cc unparasitized *Corcyra* egg placed in 60 cm and 120 cm distance in all four directions constituted an experimental unit. Five such units were arranged randomly in half acre of the cropped area. A minimum of more than five meter distance was maintained between the experimental units. After three days of parasitoid release, the baited cards were brought to the laboratory and the parasitized eggs (blackened eggs) were counted. Data was compared based on the number of parasitized eggs in each bait cards. Weather data *viz.,* wind direction and wind speed corresponding to the experimental period recorded in Meteorological unit of Agricultural College and Research Institute, Killikulam was used for further interpretation. Experiment was repeated eight times and the data were compared observation week wise with wind direction that previlled.

2. On field host *L. orbonalis* egg in brinjal crop

To understand the parasitization behaviour of *T. chilonis* on the field host *L. orbonalis* eggs *insitu* in field condition, the parasitoid was released at the rate of 1cc per hectare in brinjal crop in the vegetative stage having a shoot damage caused by *L. orbonalis.* The extent of damage observed was more than 20 per cent. The *L. orbonalis* eggs (10 eggs) were located by using hand lens and tagged with bright colour tags before the parasitoid release for easy identification. *Corcyra* egg card having approximately 0.0625 cc parasitized eggs were placed in the prefixed parasitoid release spots. After three days of parasitoid release, the parasitized eggs were counted and brought to the laboratory for further observation. Data was compared based on the number of parasitized eggs in each direction and the parasitoid emergence, the species identity of *T. chilonis* was established based on the taxonomic features (Niranjana, 2015). Experiment was repeated six times. Weather data *viz.* wind direction and wind speed corresponding to the experimental period recorded in Meteorological unit of Agricultural College and Research Institute, Killikulam was used for further interpretation. Experiment was replicated five and repeated seven times and data along with direction prevailed during the parasitoid release period was tabulated observation wise.

Orientation behaviour of *T. chilonis* under field condition (Sentinel Egg Card Technique)

The result of the field experiment conducted using sentinel egg card technique is furnished in Table 1. During first observation conducted during 39th std. week, the mean level of parasitization ranged from 20 per card in South direction and 47 in North direction, during this period wind ward direction was in South. During 45th std. week (Observation II), a maximum parasitization was recorded in North direction (20.20 eggs/ card) and a minimum parasitization was observed in East direction (9.20 eggs/ card) and during this period wind ward direction was in South. During observation III (46th std. week) the mean level of parasitization ranged from 7.80 per card in South direction and 35.80 eggs per card in West direction and during this period the wind ward direction was in East. In the observation conducted during 48th std. week, the wind ward direction was remained same, a maximum level of parasitization was recorded in West direction (33.00 eggs/ card) and a minimum level of parasitization was observed in South direction (15.80 eggs/ card). During the subsequent observation V (6th std. week), the mean level of parasitization ranged from 6.80 eggs per card in North direction and 18.80 eggs per card in South direction. During this period the wind ward direction was in North. During 6th observation (8th std. week), a maximum parasitization was recorded in East direction (19.60 eggs/ card) and a minimum parasitization was observed in South direction (9.60 egg/ card) and during this period the wind ward direction was in West. During observation VII (10th std. week), the peak level of parasitization was recorded in East direction (15.40 eggs/ card) and minimum level of parasitization was observed in South direction (8.00 eggs/ card). In the final observation repeated during 13th std. week, a maximum level of parasitization was recorded in

North direction (10.80 eggs/ card) and a minimum level of parasitization was recorded in West direction (8.00 eggs/ card), during this period the wind ward direction was in South.

Orientation behaviour of *T. chilonis* on *L. orbonalis* eggs in-suit under field condition

Based on the above result of the preliminary studies conducted on the orientation behaviour of *T. chilonis,* involving laboratory host egg (*Corcyra* egg) a subsequent experiment was conducted to assess the orientation behaviour of *T. chilonis* on the *L. orbonalis* in field condition. Result is presented in Table 2.

During the first observation (18th std. week), the mean level of parasitization recorded ranged from 1.60 eggs in South direction and 3.60 eggs in East direction, during this period wind ward direction was West. During observation II made in 19th std. week, maximum level of parasitization was recorded in West direction (2.20 eggs) and a minimum level of parasitization was observed West direction (0.60

Table 1. Extend of parasitization of *T. chilonis* in brinjal field influence by wind direction (Sentinel Egg Card Technique)

Direction	Number of parasitized eggs/ card							
	Observation I	Observation II	Observation III	Observation IV	Observation V	Observation VI	Observation VII	Observation VIII
	(39th std. week)	(45th std. week)	(46th std. week)	(48th std. week)	(6th std. week)	(8th std. week)	(10th std. week)	(13th std. week)
East	22.00	09.20	15.40	19.80	12.20	19.60	15.40	07.80
West	23.00	16.60	35.80	33.00	10.20	17.20	10.60	08.00
North	47.00	20.20	16.00	20.00	06.80	15.60	10.00	10.80
South	20.00	10.40	07.80	15.80	18.80	09.60	08.00	08.40
Wind direction	North	North	West	West	South	East	East	North

Table 2. Extend of parasitization by *T. chilonis* on *L. orbonalis* egg in brinjal field influence by wind direction

Direction	Number of parasitized eggs					
	Observation I	Observation II	Observation III	Observation IV	Observation V	Observation VI
	(18th std. week)	(19th std. week)	(20th std. week)	(21th std. week)	(23th std. week)	(24th std. week)
East	3.60	2.20	1.40	2.60	1.80	0.40
West	2.00	0.60	3.20	3.00	0.80	1.40
North	3.00	1.80	5.40	4.20	5.60	2.20
South	1.60	2.00	4.00	3.00	4.60	2.20
Direction	East	East	North	North	North	North

egg). During this period wind ward direction was west. During 3rd observation period *i.e.* on 20th std. week ththth, the mean number of parasitization recorded ranged from 1.40 eggs in East direction and 5.40 eggs in North direction, during this period the wind ward direction was South. During the subsequent week (21th std. week), the wind direction remained same and maximum parasitization was recorded in North direction (5.60 eggs) and a minimum parasitization was observed in East direction (2.60 eggs). During subsequent observations the wind direction remained the same at North to South direction, a maximum level of parasitization (5.60 eggs) was observed in North direction in observation V and 2.20 eggs observed in observation VI. In East direction during this period of observation a minimum parasitized egg were recorded.

In the observation made on the orientation behaviour of *Trichogramma* under field condition, it is interesting to note that the wind direction has an influence on orientation behaviour of *T. chilonis*. The change in wind direction has resulted in shifting the parasitoid orientation on the host egg towards the wind stream. The consistency of observation indicates existence of positive correlation between parasitization and wind stream direction under field condition was recorded both in the sentinel egg card technique and *L. orbonalis* eggs. The kairomone fumes carried by the air current may attribute such variations in the orientation behaviour. This is the first such research report for the dispersal behaviour of *T. chilonis* under field condition on both the *C. cephalonica* and *L. orbonalis* eggs under field condition.

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Evaluation of pongamia oil soap against leaf hopper, *Amrasca biguttula biguttula* (Ishida) infesting okra

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ABSTRACT: Field studies on evaluation of pongamia and neem oil soap at various concentrations against okra leaf hopper, *Amrasca biguttula biguttula* (Ishida) was carried out during the year 2018-19. Treatments i.e. T_1: Pongamia oil soap 0.6%; T_2: Pongamia oil soap 1%; T_3: Pongamia oil soap 2%; T_4: Neem oil soap 0.6%; T_5: Soap solution 0.5%; T_6: Quinalphos 25 EC @ 0.05%; T_7: Standard check applied once at vegetative stage and twice during reproductive stage. Quinalphos 25 EC @ 0.05 % was effective followed by pongamia oil soap 2 per cent, pongamia oil soap 1 per cent, neem oil soap 0.6 per cent and pongamia oil soap 0.6 per cent. The effectiveness of the soap reduced after seven days of treatment.

KEY WORDS: Pongamia. neem oil soap, leaf hopper

Okra, *Abelmoschus esculentus* (L). Moench also known as lady's finger native to West Africa is a warm season vegetable crop cultivated for its tender and delicious fruits which remains productive even in the long summers of South East. India stands first in okra production with 62 per cent share of world production. With a production of 6094.94 MT during 2017-18, okra is cultivated in 509.02 ha of area with a productivity of 11.97MT/ha (Anon., 2018). One of the major constraints for okra production is heavy infestations of several insect pests which exert both quantitative and qualitative loss. Insect pests caused 48.97 per cent loss to the tune of 77.78 q/ha (Kanwar and Ameta, 2007). Early stages of crop is infested by sucking pests like leafhoppers, aphids and whiteflies that cause huge economic loss due to sucking of the cell sap and making the plant weak. Krishnaiah (1980) reported that leafhoppers alone can cause a yield loss of 54.04 per cent in okra.

Okra being harvested at frequent intervals, application of synthetic insecticides may lead to toxic residues in fruits causing health hazards. Non judicious use of synthetic pesticides over the last four to five decades have resulted in many negative consequences like resurgence and resistance of pests and pesticide residues in farm products (Kabir *et al.,* 1994; Mahapatro, 1999). Hence, to control these pests and to reduce such risks, alternative environmentally safe methods like bio pesticides, botanicals etc., are to be adopted (Khade *et al*., 2014).

Pongamia pinnata (L.) is a multipurpose tree species of pea family Fabaceae which is widely distributed in India, China, Bangladesh and Australia. It is commonly called as Indian Beech Tree or Karanj. Karanj oil is thick yellowish red/brown non edible oil, extracted from seed. It is used for the treatment of rheumatism and skin diseases, in soap

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industry, as a fuel, lubricant and pesticide. The secondary metabolites like flavanoids, chalcones, steroids and terpenoids in pongamia oil serve as natural pest repellents (Pavela, 2009). It has insecticidal (antifeedent) properties similar to neem oil and act against a number of insect pests. Tripathi *et al.* (2012) stated that pongamia oil is safe to humans and other mammals. Hence an experiment was carried out to evaluate the efficacy of botanical product "pongamia oil soap" at various concentrations against leaf hopper infesting okra..

Pongamia oil soap was prepared according to the technology used for the preparation of Ready To Use neem oil garlic soap, the first botanical of KAU, approved by Kerala Agricultural University (Varma, 2018).

A field study was carried out in the Instructional farm, College of Agriculture, Padannakkad during 2018 – 19. Seeds of okra (Variety: Arka Anamika) were sown at a spacing of 60 x 45 cm^2 during October to January under randomized block design (RBD). There were seven treatments (Table 1). Spraying of pongamia oil soap solution was done at 30 days after sowing (DAS) during vegetative phase and during the reproductive stage at 55 and 80 DAS using 16 L Knapsack sprayer. Spraying was carried out during evening hours and precautions were taken to avoid drift. Four plants out of eight were randomly selected and tagged in each plot for recording the observations. Population density of leaf hopper was recorded by counting the number of nymphs and adults from five leaves (one top, two middle and two lower) of selected four plants on one day prior to and 1, 3, 5, 7 and 14 days after spraying of various treatments. Data on the population density of sucking pests were analyzed after square root transformation. The data were analysed using analysis of variance (ANOVA). Web Agri Stat Package (WASP) was used to compare the significance of each treatment.

The results revealed that a day after the first application, the plot treated with quinalphos 25 EC at 0.05 per cent (standard check) recorded the least count of 1.75 leaf hoppers/5 leaves, followed by pongamia oil soap 2 per cent (2.81 leaf hoppers/5 leaves) and pongamia oil soap 1 per cent (3.75 leaf hoppers/5 leaves) which were on par with the standard check. All the treatments stood significantly superior over the control (5.38 leaf hoppers/5 leaves) whereas soap solution 0.5 per cent (5.19 leaf hoppers/5 leaves) was on par with control. It was further observed that after three days of application, among the botanicals, pongamia oil soap 2 per cent showed lowest population of 1.19 leaf hoppers/5 leaves which was at par with standard check (1.00 leaf hoppers/5 leaves). It was followed by pongamia oil soap 1 per cent, neem oil soap 0.6 per cent and pongamia oil soap 0.6 per cent (2.81, 3.00 and 3.69 leaf hoppers/ 5 leaves) respectively which was at par with each other. Soap solution 0.5 per cent and control showed highest population count of 5.31 and 5.56 leaf hoppers/5 leaves which was at par with each other. Five days after first application lowest hopper population was observed in standard check (0.31 leaf hoppers/ 5 leaves) which was immediately followed by pongamia oil soap 2 per cent (0.81 leaf hoppers/5 leaves). A gradual decrease in the population was observed in all the treatments except control and soap solution 0.5 per cent on the seventh day after first application with minimum population recorded in standard check (0.13 leaf hoppers/ 5 leaves) which was on par with pongamia oil soap 2 per cent (0.44 leaf hoppers/ 5 leaves). Pongamia oil soap 1 per cent was at par with neem oil soap 0.6 per cent with a population count of 1.00 and 1.25 leaf hoppers/5 leaves respectively. A gradual increase in leaf hopper population in all the treatments on fourteenth day after first application and among the botanicals pongamia oil soap 2 per cent recorded the lowest leaf hopper population of 0.75 leaf hoppers/5 leaves which was at par with standard check 0.31 leaf hoppers/5 leaves. Soap solution 0.5 per cent and control showed the highest population count of 7.44 and 7.50 leaf hoppers/5 leaves which was at par with each other (Table 1).

Precount of leaf hopper population prior to second application was at a range of 5.37 - 8 leaf hoppers/ 5 leaves /plant. A day after the second application, a reduction in the leaf hopper population was observed in all the treatments except in soap solution 0.5 per cent (8.06 leaf hoppers/ 5 leaves) and control (8.1 leaf hoppers/ 5 leaves) which were on

par with each other. Quinalphos 0.05 per cent (standard check) recorded the least count of 3.13 leaf hoppers/ 5 leaves, followed by pongamia oil soap 2 per cent (4.63 leaf hoppers/5 leaves). Pongamia oil soap 1 per cent (5.81 leaf hoppers/ 5 leaves), neem oil soap 0.6 per cent (6.06 leaf hoppers/ 5 leaves) and pongamia oil soap 0.6 per cent (6.19 leaf hoppers/ 5 leaves) were found on par with each other.

A similar trend was observed at three days after second application with lowest population recorded in standard check (1.00 leaf hoppers/ 5 leaves) followed by pongamia oil soap 2 per cent (2.50 leaf hoppers/ 5 leaves). Pongamia oil soap 1 per cent, neem oil soap 0.6 per cent and pongamia oil soap 0.6 per cent (3.88, 4.25 and 4.50 leaf hoppers/ 5 leaves) respectively were found on par with each other. Standard check showed the lowest hopper population of 0.19 leaf hoppers/5 leaves followed by pongamia oil soap 2 per cent (0.94 leaf hoppers/ 5 leaves) at five days after second application while Soap solution 0.5 per cent and control showed the maximum leaf hopper population with 9.00 and 9.31 leaf hoppers/5 leaves. Observations at seventh day after second application during rabi season revealed standard check as the best treatment with 0.00 leaf hopper population as significantly superior treatment followed by pongamia oil soap 2 per cent (0.56 leaf hoppers/ 5 leaves) and pongamia oil soap 1 per cent (1.69 leaf hoppers/ 5 leaves). However all the treatments were significantly superior to control and soap solution 0.5 per cent. A gradual increase in leaf hopper population was observed in all the treatments at 14 days after application with standard check showing the lowest population count of 0.56 leaf hoppers/ 5 leaves, while among the botanicals pongamia oil soap 2 per cent recorded the lowest leaf hopper population of 1.06 leaf hoppers/5 leaves. Neem oil soap 0.6 per cent (3.94 leaf hoppers/5 leaves) and pongamia oil soap 0.6 per cent (4.06 leaf hoppers/5 leaves) were on par with each other.

Precount of leaf hopper population prior to third application was at a range of 7.93 – 11.25 leaf hoppers/5 leaves /plant. A day after the third application pongamia oil soap 2 per cent showed least population of 5.50 leaf hoppers/5 leaves among the botanicals which was on par with standard check (11.94 leaf hoppers/5 leaves). Pongamia oil soap 1 per cent (7.00 leaf hoppers/5 leaves) was the next best treatment followed by neem oil soap 0.6 per cent (7.75 leaf hoppers/5 leaves) which was on par with pongamia oil soap 0.6 per cent (8.31 leaf hoppers/5 leaves). Soap solution 0.5 per cent (11.19 leaf hoppers/5 leaves) was at par with control. Third day count of leaf hopper population recorded the lowest population of 2.00 leaf hoppers/ 5 leaves in standard check followed by pongamia oil soap 2 per cent, pongamia oil soap 1 per cent, neem oil soap 0.6 per cent and pongamia oil soap 0.6 per cent with 3.38, 4.44, 5.00 and 5.44 leaf hoppers/ 5 leaves respectively. A similar trend was observed at five days and seven days after third application were standard check showed the lowest hopper population of 1.19 and 0.81 leaf hoppers/ 5 leaves followed by pongamia oil soap 2 per cent (2.38 and 2.13 leaf hoppers/ 5 leaves). While the population was 12.50 and 13.00 leaf hoppers/5 leaves at seven days after third application in soap solution 0.5 per cent and control respectively which were on par with each other. A gradual increase in leaf hopper population in all the treatments at 14 days after application was observed while among the botanicals pongamia oil soap 2 per cent recorded the lowest leaf hopper population of 2.81 leaf hoppers/ 5 leaves after the standard check 1.25 leaf hoppers/ 5 leaves which was followed by pongamia oil soap 1 per cent, neem oil soap 0.6 per cent which was on par with pongamia oil soap 0.6 per cent with 4.19, 4.75 and 5.06 leaf hoppers/ 5 leaves respectively. Soap solution 0.5 per cent and control showed the highest count of 13.19 and 14.06 leaf hoppers/ 5 leaves respectively (Table 1).

From the data observed during the rabi season it is evident that all the treatments except soap solution 0.5 per cent was effective in reducing the leaf hopper population significantly as compared to that of control. In general the efficacy of pongamia oil soap at 0.6, 1 and 2 per cent and neem oil soap 0.6 per cent were significantly superior over control, however the standard check (Quinalphos 25 EC @ 0.05 per cent) was superior to pongamia and

Table 1. Average population density of leaf hoppers during rabi season from October 2018 to January 2019

Treatments	Number of leaf hoppers/5 leaves																	
	First application						Second application						Third application					
	1 DBA	1 DAA	3 DAA	5 DAA	7 DAA	14 DAA	1 DBA	1 DAA	3 DAA	5 DAA	7 DAA	14 DAA	1 DBA	1 DAA	3 DAA	5 DAA	7 DAA	14 DAA
Pongamia oil soap 0.6%	4.94 (2.21)	4.31 (2.07)^{ab}	3.69 (2.02)^{ab}	2.50 (1.56)^{b}	2.38 (1.68)^{b}	3.31 (1.94)^{b}	7.75 (2.87)^{a}	6.19 (2.48)^{b}	4.50 (2.23)^{b}	3.06 (1.88)^{b}	3.38 (1.96)^{b}	4.06 (2.01)^{b}	10.37 (3.21)^{ab}	8.31 (2.88)^{b}	5.44 (2.33)^{b}	4.94 (2.22)^{b}	4.44 (2.10)^{b}	5.06 (2.24)^{b}
Pongamia oil soap 1%	4.44 (2.07)	3.75 (1.90)^{abc}	2.81 (1.81)^{b}	1.63 (1.27)^{c}	1.00 (1.22)^{c}	1.56 (1.42)^{c}	7.06 (2.73)^{ab}	5.81 (2.40)^{b}	3.88 (2.08)^{b}	1.88 (1.53)^{c}	1.69 (1.46)^{c}	2.06 (1.43)^{c}	10 (3.16)^{b}	7.00 (2.63)^{b}	4.44 (2.09)^{bc}	3.63 (1.90)^{c}	3.19 (1.78)^{c}	4.19 (2.04)^{c}
Pongamia oil soap 2%	5.06 (2.22)	2.81 (1.58)^{bc}	1.19 (1.25)^{c}	0.81 (0.89)^{d}	0.44 (0.96)^{d}	0.75 (1.11)^{d}	6.31 (2.60)^{bc}	4.63 (2.15)^{c}	2.50 (1.72)^{c}	0.94 (1.18)^{d}	0.56 (1.02)^{d}	1.06 (1.02)^{d}	8.75 (2.95)^{c}	5.50 (2.34)^{c}	3.38 (1.82)^{c}	2.38 (1.52)^{d}	2.13 (1.45)^{d}	2.81 (1.67)^{d}
Neem oil soap 0.6%	4.13 (2.01)	4.06 (2.00)^{ab}	3.00 (1.86)^{b}	2.06 (1.43)^{bc}	1.25 (1.31)^{c}	1.69 (1.46)^{c}	7.18 (2.77)^{ab}	6.06 (2.45)^{b}	4.25 (2.17)^{b}	2.94 (1.85)^{b}	2.81 (1.81)^{b}	3.94 (1.97)^{b}	10.25 (3.20)^{ab}	7.75 (2.78)^{b}	5.0 (2.23)^{b}	4.44 (2.10)^{bc}	4.00 (1.99)^{b}	4.75 (2.17)^{b}
Soap solution 0.5%	4.25 (1.97)	5.19 (2.25)^{a}	5.31 (2.40)^{a}	6.00 (2.43)^{a}	6.63 (2.65)^{a}	7.44 (2.81)^{a}	7.87 (2.89)^{a}	8.06 (2.83)^{a}	8.44 (2.98)^{a}	9.00 (3.08)^{a}	9.63 (3.18)^{a}	10.25 (3.19)^{a}	11 (3.31)^{a}	11.19 (3.34)^{a}	11.63 (3.40)^{a}	12.06 (3.47)^{a}	12.50 (3.53)^{a}	13.19 (3.63)^{a}
Quinalphos 25 EC 0.05%	4.00 (1.97)	1.75 (1.27)^{c}	1.00 (1.17)^{c}	0.31 (0.55)^{e}	0.13 (0.78)^{d}	0.31 (0.88)^{d}	5.37 (2.41)^{c}	3.13 (1.76)^{d}	1.00 (1.19)^{d}	0.19 (0.82)^{e}	0.0 (0.70)^{e}	0.56 (0.72)^{e}	7.93 (2.81)^{c}	4.69 (2.14)^{c}	2.0 (1.38)^{d}	1.19 (1.08)^{e}	0.81 (0.89)^{e}	1.25 (1.11)^{e}
Control	4.69 (2.10)	5.38 (2.25)^{a}	5.56 (2.41)^{a}	6.25 (2.49)^{a}	6.81 (2.70)^{a}	7.50 (2.82)^{a}	8 (2.91)^{a}	8.1 (2.92)^{a}	8.38 (2.97)^{a}	9.31 (3.13)^{a}	10.0 (3.23)^{a}	11.0 (3.31)^{a}	11.25 (3.35)^{a}	11.94 (3.45)^{a}	12.19 (3.48)^{a}	12.75 (3.56)^{a}	13.0 (3.60)^{a}	14.06 (3.74)^{a}
C.D (0.05)	NS	0.62	0.49	0.28	0.25	0.25	0.24	0.17	0.24	0.19	0.23	0.22	0.15	0.25	0.29	0.20	0.14	0.13

Figure in parenthesis denotes square root transformed value. Means followed by similar letters are not significantly different; DBA- Day before application; DAA- Days after application: NS- Non significant.

neem oil soap. Similar findings were reported by Kumar (2013), where he stated that imidacloprid followed by triazophos, quinalphos and neem based insecticides were effective in reducing jassid population as compared to that of control.

After three sprays, pongamia oil soap 2 per cent was effective in reducing the leaf hopper population followed by pongamia oil soap 1 per cent, neem oil soap 0.6 per cent and pongamia oil soap 0.6 per cent. Efficacy of pongamia oil soap was reduced with the reduction in concentration. Even though ponamia oil soap 0.6 per cent and neem oil soap 0.6 per cent showed similar results, better efficacy was showed by neem oil soap 0.6 per cent in reducing leaf hopper population. Similar results were observed by Anitha (2007) who reported that among the botanicals and myco pathogens, neem oil 2 per cent recorded least leafhopper population (2.90 leafhoppers/3 leaves) followed by pongamia oil 2 per cent (3.44 leafhoppers/3 leaves) on okra. Superiority of neem based insecticides have been reported by Mandal *et al.* (2006) and Sinha and Sharma (2007). Higher efficacy of pongamia oil soap 2 per cent against leaf hopper as observed in this study is in line with Sardana and Krishnakumar (1989), who stated that maximum reduction in hopper population to the extent of 17.51 leafhoppers per plant was recorded in case of karanj oil (2 per cent) as compared to neem oil (0.5 per cent) and garlic oil (0.5 to 1%).

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Effect of carbofuran on quantitative and qualitative alterations in haemolymph of larva of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)

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ABSTRACT: Investigation to evaluate the toxicity of carbofuran pesticides on haematological parameters of third instar larvae of *Oryctes rhinoceros* L. Indicated alterations in total haemocyte count and differential haemocyte count for toxicity assessment. Various doses of carbofuran (0.05g, 0.010g and 0.015 g) applied on insect through oral route and its impact after 24 hours of its application revealed that various doses of carbofuran exert specific alterations in both total and differential haemocytes of insect haemolymph.

KEYWORDS: carbofuran,changes, *Oryctes rhinoceros*, toxicity, haemocytes

Haemocytes and immune responses are considered to be potential indicators of toxicity in insects. Hence the study of changes in either the total or part of insect haemolymph is a proper system for detecting effects of toxic substances. Toxic substances induce an irreversible cytopoiesis of the host's haemocytes (Vladimir *et al.*, 1991).There are very little study on the haemograms of insects exposed to toxic substances and the role of haemocytes and direct detoxification of pesticides. Cytopoiesis has been proven in insects exposed to lethal doses of arsenates, nicotine dichloro-diethyl ether, carbon tetrachloride and DDT (Vladimir *et al.*, 1991).

Carbofuran is a very toxic pesticide widely used by farmers and registered for more than 25 crops in India. As a result of widespread use, air, water and food are polluted with carbofuran and its metabolites (Bushway *et al.,* 1992; Kross *et al.,* 1992; Waite *et al.,* 1992). Carbofuran has high toxicity to human through the oral and inhalation routes of exposure affecting the nervous system It is highly toxic to birds, bees, fish and non- target species due to its high acute toxicity. The residues of these chemical have been reported in plant, soil and water there for its use has been restricted or banned in many countries (Goulart *et al.,* 2015). Present investigation is focused to evaluate the toxicity of carbofuran pesticides on haematological parameters of third instar larvae of *Oryctes rhinoceros* L. to understand the impacts on circulatory system of insects.

Third instar larvae of coconut beetle, *O. rhinoceros* one of the important economic pests of coconut palm having long life span, voracious feeding habit, sensitivity to insecticides or any control agent and ease in rearing and handling the larvae of *Oryctes rhinoceros* being excellent experimental animal was used as test animal. Various stages of larvae

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were collected from the local manure pits and reared in the laboratory on cow dung which formed the stock. In the present study the third instar larvae, those have long lifespan, peculiar voracious feeding habit and ease of rearing were considered for the isolation from the stock and each larva kept separately in plastic containers with cow dung as feed.

Carbofuran is broad spectrum commercial grade carbamate insecticide used for control of insects, mites and nematodes and being also used against soil and foliar pests of field, fruits, vegetables and forest crops. Carbofuran is highly toxic by inhalation or ingestion and moderately toxic by dermal absorption.

The chemical name of carbofuran is 2, 3- dihydro 2, 2- dimethyl-7- benzofuranyl methyl carbamate. Formulations of carbofuran include flowable or granular formGranular form like Furadan 3G used in the present study is usually prepared by mixing technical grade materials with silica based particles in required proportions. Pure form is a white crystalline solid with slight phenolic odour. It has a melting point of 153-154^{0}C. It is slightly soluble in water. It is highly soluble in N- methyl-2-pyrrolindone, dimethyl formaldehyde, dimethyl sulfoxide, acetone, aectonitrite, methylene chloride, cyclohexanone, benzene and xylene. It is stable under alkaline conditions. Degradation of carbofuran in soil takes place by microbial action. In water, direct photolysis and photo irradiation via hydroxyl radical, 2-hydroxy furadan and furadan phenol are the major pathways of degradation. In the air, degradation occurs by photolysis. Half-life in water is 5.1 weeks at pH 7.0 and 1.2 hours at pH 10 and in the soil several days to over three months (HSDB, 1998)

Three doses of the of the toxicity *viz*., 0.05, 0.010 and 0.015g doses of carbofuran mixed with 30g cow dung each was kept in containers for a day. Then actively feeding larvae after 30-35 days of moulting with an average weight of 9.6 $\pm$ 0.01 g were introduced into each experimental container with four replications along with a control without furadan. Pesticide dose was chosen based on result of preliminary continuous bioassays and probit analysis, (Finney, 1971). Behavioural changes and toxic sighs were recorded daily.

Haemolymph was collected from both treated and control larvae. A puncture was made on the body wall so as to draw the exuded haemolymph using a capillary tube and kept in eppendorf tubes containing a few crystals of phenylthiourea (Wyatt and Pan, 1978) to prevent melanisation. The collected samples were used immediately, for analysis.

Haemolymph smear was prepared according to the method of Arnold and Hinks (1979). The smear of haemolymph was prepared by placing a drop of freshly extracted haemolymph on a clean glass slide and a thin uniform smear was drawn by using a rectangular cover slip at 45 degrees. After air drying for few minutes, the smear fixed in methanol and stained with Giemsa's stain for 5 min., washed in double distilled water and mounted in DPX.

Total haemocyte count (THC) was done by the method of Gosh and Roy (1984). A Newbaur Haemocytometer was used for this purpose (Witting, 1966). The haemolymph was initially collected on micro slides. This haemolymph was taken into a clear WBC pipette filling up to the mark 0.5. Care was taken to avoid air bubbles. The blood sticking to the tip of the pipette was wiped out. Dilution medium (Turk's dilution fluid) was pipette up to the mark 1.1. The haemolymph was allowed to mix thoroughly with the dilution medium. The fluid from the lower end of the pipette was discarded. The counting chamber of the haemocytometer was charged and the preparation was kept aside for the haemocytes to settle. The haemocytes were counted from the four corners squares with the aid of a microscope. Total number of haemocytes was calculated by multiplying the average number of cells in one chamber with the volume of one square of haemocytometer and dilution factor, *i.e.,* average number of cells in one chamber X10 X 20. Following formula of Jones (1962) was adapted for calculations:

$$\frac{\text{Haemocytes in 1mm Squares X dilution X depths at the Chamber}}{\text{number of 1mm square counted}}$$

Haemolymph smears prepared from each experimental larva were examined under a light

microscope, and all cells were counted. The total cells were counted and the percentage of each kind of haemocytes Prohaemocytes (PRC), Plasmatocytes (PLC), Granulocytes (GRC), Adipocytes (ADC), Spherulocytes (SPC) and Oenocytes (OEC) were calculated to arrive the differential haemocytes. All the data were analysed statistically at $p < 0.005$. The significance was calculated by using ANOVA.

Differential Haemocyte Count (DHC) of control and exposed to carbofuran for 24 hours

Differential Haemocyte Count in experiment and control larvae is given in Table 1. Proportion of the PRC in the control was 46.00 ± 3.79per cent while 18.33 ± 1.45 PLC, 20.0 ± 0.58 GRC , 6.67 ± 0.88 ADC, 5.67 ± 1.45 SPC and 3.33 ± 0.88 OEC indicating the population size of PRC was the highest followed by GRC and PLC. The least population was observed in OEC followed by SPC and ADC. When the larvae exposed to 0.005g carbofuran for 24 h the PRC, PLC and GRC were 17.67 ±1.45, 9.00 ± 1.15 and 60.0 ± 2.31 per cent respectively expressing a steep elevation of GRC count over control as against PLC showing a significant reduction in their population. Proportion of the other cell types were 7.00 ± 0.58, 4.66 ± 0.33 and 1.67 ± 0.33 per cent respectively for ADC, SPC and OEC. A slight decrement was noted in the count of SPC and OEC from control value. Exposing to the higher dose of to 0.01g carbofuran, the mean population of PRC was 13.33 ± 0.88 per cent while PLC- 8.00 ± 1.53, GRC- 68.33 ± 0.88 , ADC - 4.67 ± 0.88, SPC - 3.33 ± 0.67 and OEC- 2.33 ± 0.88 per cent wherein, GRC showed steep significant elevation as against the decrement of ADC, SPC and OEC though statistically insignificant.

However 0.015 g carbofuran treated larvae haemolymph exhibited that the mean counts of haemocytes were PRC- 11.00±1.15 per cent of PRC, 6.33±0.88 PLC, 72.00±1.73 GRC, - 5.33± 0.33 ADC, 4.00 ± 0.58 SPC and 1.33 ± 0.33 per cent OEC respectively indicating significant increase in case of PRC, PLC and GRC, however, insignificant in the case of ADC, SPC and OEC count. Overall assessment expressed a significant sharp increase of granulocytes due to exposure larvae corresponding with increase in dose of carbofuran as against other haemocytes got decreased over control.

Cytological study of haemolymph of *O. rhinoceros* larvae-control and exposed to carbofuran

Haemocytes of control larvae comprised of PRCs, PLCs, GRCs, ADCs, SPRs and OECs (Fig. 1). PRCs found in numerous groups were small round cells with dense homogenous cytoplasm and large nucleus. PLCs were spindle shaped cells with a centrally placed round nucleus and surrounding of abundant cytoplasm. GRCs were observed as large spherical or oval cells having more granular cytoplasm and centrally located round or elongated nucleus. ADCs were small round or slightly elongated and centrally or eccentrically located nucleus. The cytoplasm contained characteristic small to large refringent fat droplets and other non-lipid granules in addition to vacuoles. SPCs were round with small central or eccentric nucleus. A number of spherules are found in the cytoplasm. These cells are larger than granulocytes. OECs were small to large oval or spherical cells with a granular, thick, homogenous cytoplasm and centrally located small round nucleus.

High total haemocytes counts with moderate increase in granulocytes were found in the larvae exposed to 0.005g carbofuran for 24h (Fig. 2). Degeneration and nuclear pyknosis was observed in granulocytes. Reactive changes were observed in PLCs whose cytoplasm was darkly stained. Smears of the larvae exposed to 0.010g carbofuran for 24 h showed mild decrease in total haemocytes with increase in number of GRCs which were degenerated with distorted shape having irregular nucleus. Some of them showed enlargement. (Fig. 3).

Blood smear of larvae exposed to 0.015g exhibited mild decrease in total haemocytes with increase in GRCs which were more basidophilic with thickened granules. Clustering of cells with abnormal staining

was observed with ruptured cell membrane and distorted cell shape (Fig. 4). Carbofuran exposed larvae showed a significant decrease of total haemocytes and various cytopathological changes, particularly an increase in GRC sand increased cellular damage compared to control.

The haemocytes of insects constitute a complex system of cells circulate in the haemolymph which play an essential role in immunity against invading substances through coagulation, phagocytosis, encapsulation and detoxification process. Haemocytes are several types and their primary functions also include storage and distribution of nutritive materials. Six types of haemocytes were identified in the haemolymph of *O. rhinoceros* larvae such as PRC, GRC, PLC, SPC, OEC and ADC (Annie, 1995). In the present study their response to different doses of carbofuran at various time periods were investigated. A drastic change in total haemocytes (THC) with various histopathological changes in haemocyte morphology was observed due to carbofuran intoxication. This is because haemocytes are known to respond to various intrinsic or extrinsic factors. Under adverse conditions and at the time of experimental stress, numbers of haemocytes were reported to get increased (Shapiro, 1979, Gupta, 1985). However, the present investigation indicated a decrease in the total haemocytes due to carbofuran treatment which could be the result of degeneration of pathological cells caused by toxicity of carbofuran.

Number of haemocytes is a key factor in compaction of the encapsulation of invading foreign bodies (Sendi and Salehi, 2010). Moreover, PLC and GRC have been found to be most sensitive and the main phagocytic haemocytes in most of the insect studied (Crossley 1964, Arnold, 1970; Neuvarth, 1974; Akain and Sato, 1978). In this sense, differential heamocyte count (DHC) is more meaningful, because, present investigation found sharp increase of GRC in the haemolymph of treated larvae and other haemocytes showed numerical decline over control. Such an increase in the population size of GRC might be connected with the growing demand for cellular immunity (Gupta, 1985, 1986, 1991). This is because granular

Table 1.Total and differential haemocytes in the haemolymph of control *Oryctes rhinoceros* larvae exposed to carbofuran for 24 h.

Doses of carbofuran (g/larvae)	Total haemocyte count (THC) (cu/mm))	Differential haemocyte count (DHC) (%)					
		Prohae-mocyte (PRC)	Plasma-tocyte (PLC)	Granulocyte (GRC)	Adipocyte (ADC)	Sperulocyte (SPC)	Oenocyte (OEC)
Control	7973.33± 1065.85a	46.00± 3.79a	18.33± 1.45a	20.0± 0.58a	6.67± 0.88a	5.67± 1.45a	3.33± 0.88a
0.005	13766.6± 523.87b**	17.67± 1.45 b**	9.00± 1.15 b**	60.0± 2.31b**	7.00± 0.58a	4.66± 0.33 a	1.67± 0.33a
0.010	6563.33± 545.60a	13.33± 0.88 c **	8.00± 1.53c**	68.33± 0.88c**	4.67± 0.88 a	3.33± 0.67 a	2.33± 0.88 a
0.015	3933.33± 348.01d*	11.00± 1.15 d **	6.33± 0.88d**	72.00± 1.73d**	5.33± 0.33 a	4.00± 0.58 a	1.33± 0.33 a
ANOVA	F=37.78 P=.000	F=56.83 P=.000	F=17.73 P=.001	F=242.37 P=.000	F=2.43 P=0.141	F=1.32 P=0.333	F=1.75 P = 0.234

Values are the mean of five observations ±SE
Common alphabets denote insignificant difference between control and doses
Different alphabets denote significant difference between control and doses
(*) significant at 5% level (**) significant at 1% level

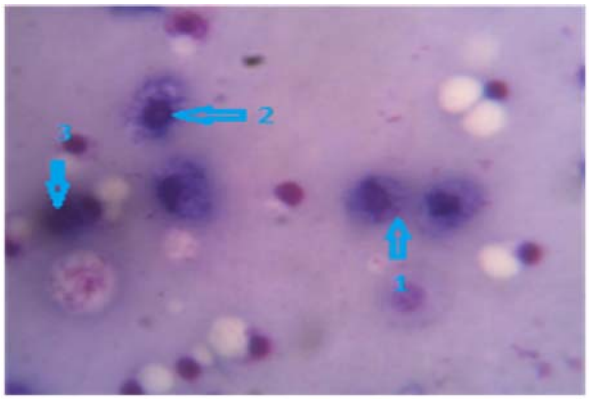

Fig. 1. Blood smear shows heamocytes of control larvae X40x

1. Proheamocyte
2. Plasmatocyte
3. Adipocyte
4. Sperulocyte

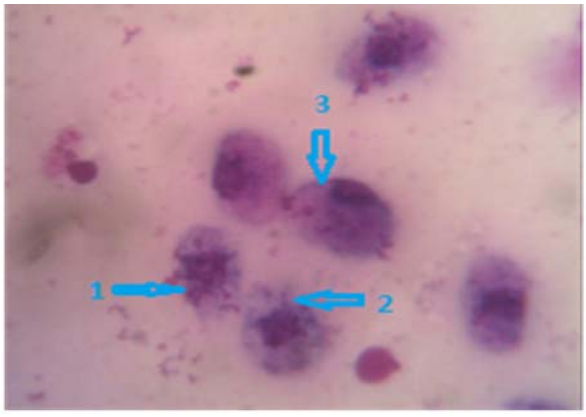

Fig. 2. Blood smear shows heamocytes of 0.005g Carbofuron/ 24 hours

1. GRC shows degeneration
2. GRC shows nuclear pykinosis
3. PLC shows darkly stained cytoplasam

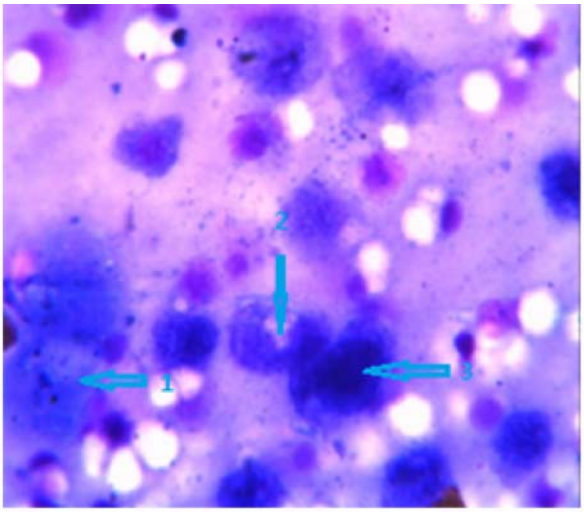

Fig. 3. Blood smear shows heamocytes of 0.010g Carbofuran /24 hours

1. Distortion in cell shape
2. Degeneratative changes with irregular nucleus
3. GRC shows cellular enlargement

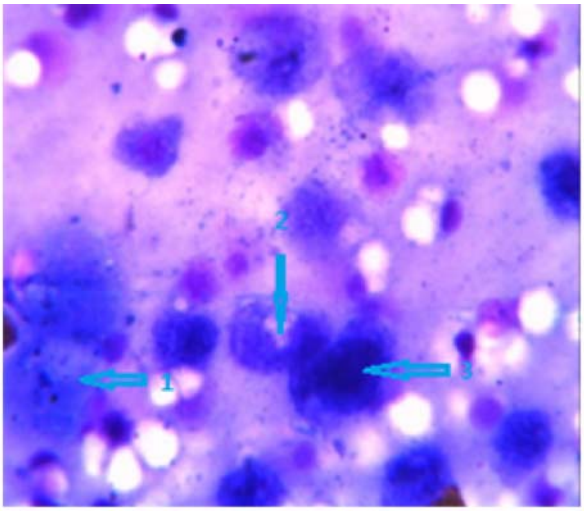

Fig. 4. Blood smear shows heamocytes of 0.015g Carbofuron/ 24 hours

1. GRC shows clustering with ruptured cell membrane
2. Ruptured cell membrane and distorted cell shape
3. Basidophillic with thickened granules

haemocytes are primarily involved in body defence activities (Ambrose and George, 1996). A study of *Atemisia annua* extract in *Eurygaster integriceps* revealed an alteration in the number of hemocytes and their phagocytic activity (Zibaee and Bandani, 2010).

Pathological study of haemolymph indicated dose dependent cellular degeneration due to carbofuran treatment. Numerous changes in cytoplasm and nucleus observed in haemolymph of larvae treated with the highest dose. Vacuolization of cytoplasm and cellular clumbing were the feature of high dose of carbofuran (0.015g) treated larvae as result of aggregation of several cells due to loss of their cells boundaries. Similar pathological systems were reported by using some of the insecticides (Yeager and Manson, 1942; Gupta and Sutherland, 1968; Zaidi and Khan, 1977; Azam and Ilyas, 1986; Younes, *et al.*, 1999; Haq, *et al.,* 2005; Sendi and

Salehi, 2010). Phytochemicals like plumbagin and neem induced similar changes (Sharma *et al.,* 2003). *O. rhinoceros* larvae exhibited a high cytological response to carbofuran indicating that carbofuran exerted peculiar changes in the circulatory system of insect.

Several reports were published on the impact of insecticides in altering the number and morphology of insect heamolymph. Electron microscopic studies of *Spodopteralitura* Fabricious larvae treated with neem gold and *Artemisia calamus* oil found cytoplasmic projections andrapid regeneration in granulocytes. Vacuolization in the cytoplasm and degeneration in organelles in PLCs and GRCs leading to degenerative transformation and degranulation were observed within a period of 48 hours of exposure resulting in the disintegration of immunity-building mechanism (Sharma *et al.,* 2003, 2008). In the present study similar observation could be observed in *O. rhinoceros* treated with different doses of carbofuran.

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Bioefficacy of horticultural mineral oil against spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) on okra

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ABSTRACT: A field study was conducted to evaluate the efficacy of horticultural mineral oil (HMO), combination of HMO + neem oil and neem oil 2 per cent along with a synthetic acaricide, Spiromesifen 240SC and an untreated control against *Tetranychus truncatus* on okra during March, 2018. The plots treated with HMO at 2.5 (92.60%) and 3.0 per cent (93.90%) as well as combination treatments HMO 2.5 per cent + neem oil 2.0 per cent (94.14%) and HMO 3.0 per cent + neem oil 2.0 per cent (96.79%) recorded significant reduction in mite population and were superior to plots treated with either spiromesifen (91.08%) or neem oil alone at 2.0 per cent (90.42%). The high efficacy of HMO against the spider mite *T. truncatus* brought out in the study suggests that HMO can be an effective tool for mite management in vegetable crops.

KEYWORDS: Horticultural mineral oil (HMO), neem oil, spiromesifen, *Tetranychus truncatus*

Okra is cultivated in an area of 5,11,000 hectares in India with a production of 58,49,000 metric tonnes (GOI, 2017). Among several factors responsible for the low productivity of okra, the damage inflicted by insect and mite pests has been considered important (Varadaraju, 2010). More than hundred species of insects have been reported as pests of okra (Santhoshkumar *et al*., 2013). However, among them, only to a few insects such as leaf hopper, aphid, whitefly and shoot and fruit borer are considered as economically important. Among the mite pests, spider mites belonging to the genus *Tetranychus* have emerged as a major pest of okra causing considerable yield loss (Ghosh *et al*., 1996; Srinivasa and Sugeetha, 1999; Kumaran *et al*., 2007). Recent studies at Kerala Agricultural University identified *Tetranychus truncatus* Ehara as the predominant species of mite infesting vegetable crops in Kerala, including okra (Bennur *et al*., 2015).

The spider mites colonise undersurface of the leaves and cause significant damage by feeding on sap. This results in yellowing and speckling of leaves, webbing, premature leaf fall, stunted growth, reduction in photosynthetic activity and ultimately death of the whole plant (Damirel and Cabuk, 2008). Apart from its polyphagous nature, high reproductive potential and short life cycle, factors such as change in climatic conditions and over-use of plant protection chemicals also help to compound the mite problem.Though conventional pesticides offer good control, they have high residue levels and cause resurgence and resistance (Khajehali *et*

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al., 2011; Sharma and Bhullar, 2018). Moreover they cannot be recommended during the later stages of the crop, when mite damage typically intensifies. Consequently, biocontrol agents, botanicals and mineral oils are increasingly being evaluated against the mites.

Mineral oils have been used for centuries to control insect and mite pests on several crops (Egho and Emosairue, 2010). With recent advances in technology, refinement of petroleum oil to summer spray oils commonly called as horticultural mineral oils (HMOs) or agricultural mineral oils (AMOs) has made it possible to use them all the year round, without any risk of phytotoxicity (Davidson *et al*., 1991; Agnello, 2002). Oils have several advantages over conventional pesticides, such as low mammalian toxicity, low residual toxicity, minimal risk of resistance development and limited effects on beneficial organism (Beattie *et al*., 2002). Laboratory bioassay studies conducted at Kerala Agricultural University revealed that HMO at 2.0 and 2.5 per cent possess appreciable efficacy against both egg and active stages of *T. truncatus* and is relatively safer to the predatory mite, *Neoseiulus longispinosus* (Yadav and Bhaskar, 2018).

A field experiment was carried out to test the efficacy of HMO against *T. truncatus* on okra (variety Arka Anamika) at College of Horticulture, KAU, Vellanikkara during March, 2018. Two concentrations of HMO namely 2.5 and 3.0 per cent and its combinations with neem oil *viz.,* HMO 2.5 per cent + neem oil 2 per cent and HMO 3.0 per cent + neem oil 2.0 per cent were evaluated along with neem oil 2.0 per cent alone, an acaricide spiromesifen 240 SC @ 0.02 per cent and a control treatment with water spray. The crop was raised as per the Package of Practices Recommendations (KAU, 2016) at a spacing of 60×30cm in plots of 2×2m size. The experiment was laid out in Randomized Block Design with seven treatments and four replications. Mites were released on 45days old okra plant at the rate of 25 active mites/ leaf by stapling mite infested mulberry leaf bit of 3cm^2 size each on top, middle and bottom leaf of okra plant. Treatments were imposed two weeks after the release of mites using a hand sprayer.

The number of mites from three windows of 1cm^2 each from top, middle and bottom leaves of randomly selected five plants per replication were recorded. The mite count was recorded *in situ* by using a hand lens of 10X magnification one day before spraying and 1, 3, 7, 10 and 14 days after spraying. Data on mean population of mites were transformed using square root transformation. Population difference on one, three, seven, ten and fourteen days after treatment were tested by one way ANOVA. The mean per cent reduction in population of mites over pre count was also worked on seven and fourteen days after treatment application.

The results of the field experiment to evaluate the efficacy of different treatments against *T. truncatus* are presented in Table 1. The mean population of *T. truncatus* before the application of treatments ranged from 17.73 to 18.59 per cm^2 leaf area. One day after spraying, all the treatments significantly reduced the population of mite as compared to untreated control. The mean mite population ranged from 4.85 to 18.51 per cm^2 leaf area. The lowest mean mite count of 4.85/cm^2 leaf area was recorded by HMO 3.0 per cent + neem oil 2.0 per cent followed by HMO 2.5 per cent + neem oil 2.0 per cent (5.15/cm^2 leaf area) which were on par with each other. HMO 2.5 and 3.0 per cent recorded 5.46 and 5.33 mites/cm^2 leaf area respectively and were on par with each other and also with HMO 2.5 + neem oil 2.0 per cent, HMO 3.0 + neem oil 2.0 per cent and neem oil 2 per cent (5.43 mites/ cm^2 leaf area). The acaricide spiromesifen 240 SC recorded mite count of 5.88 mites/ cm^2 leaf area which was on par with HMO 2.5 per cent, HMO 3.0 per cent and neem oil 2.0 per cent.

Three days after spraying, the plants treated with combination of HMO + neem oil harboured significantly lower mite popultion compared to other treatments. HMO 3.0 per cent + neem oil 2.0 per cent and HMO 2.5 per cent + neem oil 2.0 per cent recorded 1.59 and 1.43 mean mite per cm^2

leaf area respectively. This was followed by HMO 2.5 per cent (2.49/cm^2 leaf area) and HMO 3.0 per cent (2.31 mites/cm^2 leaf area) which were on par with each other and the above treatments. The acaricide, spiromesifen 240 SC recorded 2.87 mites/ cm^2 leaf area and was on par with neem oil 2 per cent (3.10 mites/cm^2 leaf area) and with HMO 2.5 and 3.0 per cent.

At seven days after treatment, all the treatments significantly reduced the mite population as compared to untreated control (15.53 mites/cm^2 leaf area). The lowest mite population of 1.04 per cm^2 leaf area was recorded by HMO 2.5 per cent + neem oil 2.0 per cent followed by HMO 3.0 per cent (1.08/cm^2 leaf area) , HMO 3.0 per cent + neem oil 2.0 per cent (1.11/cm^2 leaf area) and HMO 2.5 per cent (1.33/cm^2 leaf area). However, these treatments did not differ significantly. Spiromesifen 240 SC (1.82/cm^2 leaf area) and neem oil 2.0 per cent (2.32 mites /cm^2 leaf area) were on par with each other and differed significantly from treatments of HMO and combination of HMO + neem oil.

Per cent reduction in mite count after seven days of treatment aplication was worked out. By seventh day of treatment, HMO 2.5 per cent + neem oil 2.0 per cent recorded 96.21 per cent reduction in the mite count closely followed by HMO 3.0 per cent + neem oil 2.0 per cent (93.95%), HMO 3.0 per cent (93.90%) and HMO 2.5 per cent (92.60%). This was followed by treatments *viz.,* spiromesifen 240 SC (89.99 %) and neem oil 2.0 per cent (87.26 %).

Similar trend was observed on ten days after spraying where treatments of HMO and combination of HMO + neem oil continued to record lower mean mite counts. The treatments, HMO 2.5 per cent (0.70/cm^2 leaf area), HMO 3.0 per cent (0.73/cm^2 leaf area), HMO 2.5 per cent + neem oil 2.0 per cent (0.79/cm^2 leaf area) and HMO 3.0 per cent + neem oil 2.0 per cent (0.87/cm^2 leaf area) recorded mean mite count on par with each other which was significantly lower. Spiromesifen 240 SC and neem oil 2.0 per cent recorded mean mite counts of 1.57 and 2.04 per cm^2 leaf area respectively which were on par with each other, but significantly differed from treatments of HMO and combination of HMO + neem oil.

At fourteen days of spraying, all the treatments recorded significant reduction in mite population as compared to untreated control (12.86 / cm^2 leaf area). HMO 2.5 per cent recorded lowest mean mite count of 0.54 per cm^2 leaf area, followed by HMO 3.0 per cent (0.58/cm^2 leaf area), HMO 3.0 per cent + neem oil 2.0 per cent (0.59 /cm^2 leaf area) and HMO 2.5 per cent + neem oil 2.0 per cent (0.67/cm^2 leaf area) which were all on par with each other and significantly superior over Spiromesifen and neem oil. Mite population count in Spiromesifen 240 SC (1.62/ cm^2 leaf area) and neem oil 2.0 per cent (1.74/ cm^2 leaf area) were on par with each other.

Per cent reduction in mite count after fourteen days of treatment aplication was worked out. By fourteenth day, the highest reduction in mite population over untreated control was observed in HMO 2.5 per cent (96.99%) followed by, HMO 3.0 per cent + neem oil 2.0 per cent (96.79%), HMO 3.0 per cent (96.72%) and HMO 2.5 per cent + neem oil 2.0 per cent (96.21%), Spiromesifen 240 SC (91.08 %) and neem oil 2 per cent (90.42 %).

In the field experiment, HMO alone (2.5 and 3.0%) and in combination with neem oil (2.0%) were significantly superior to both the acaricide Spiromesifen and neem oil. Spiromesifen, an inhibitor of lipid biosynthesis, is highly toxic to eggs and immature stages of spider mites, while it acts more slowly against adult females, causing reduction in fertility and fecundity (Marcic *et al*., 2011). Krishna and Bhaskar (2013) reported a higher reduction in egg count (15.40%) of *T. urticae* due to Spiromesifen, while it recorded very low adult mortality (3.40%). Similarly, Sato *et al*. (2011) studied the toxicity of Spiromesifen to different developmental stages, and found egg stage of *T. urticae* was most sensitive. But laboratory bioassay had revealed that HMO possess very high efficacy against both egg and adult of *T. truncatus* and recorded 100 per cent mortality of eggs at concentrations of 1.5, 2.0, 2,5 and 3.0 per cent

Table 1. Efficacy of Horticultural Mineral Oil (HMO) against *Tetranychus truncatus* on okra

Treatments	Mean no. of mite/cm^2 leaf area				Per cent reduction on 7 DAT	Mean no. of mite/cm^2 leaf area		Per cent reduction on 14 DAT
	Pre count	1 DAT	3 DAT	7 DAT		10 DAT	14 DAT	
T_1 - HMO 2.5%	17.98 (4.24)	5.46 ^{bc} (2.33)	2.49 ^{bc} (1.56)	1.33 ^{c} (1.15)	92.60	0.70 ^{c} (0.83)	0.54 ^{c} (1.01)	96.99
T_2 - HMO 3.0%	17.73 (4.21)	5.33 ^{bc} (2.31)	2.31 ^{bc} (1.49)	1.08 ^{c} (1.04)	93.90	0.73 ^{c} (0.84)	0.58 ^{c} (1.03)	96.72
T_3 - HMO 2.5% + neem oil 2.0%	17.76 (4.21)	5.15 ^{c} (2.27)	1.59 ^{c} (1.22)	1.04 ^{c} (1.02)	94.14	0.79 ^{c} (0.88)	0.67 ^{c} (1.08)	96.21
T_4 - HMO 3.0% + neem oil 2.0%	18.36 (4.28)	4.85 ^{c} (2.20)	1.43 ^{c} (1.16)	1.11 ^{c} (1.05)	93.95	0.87 ^{c} (0.93)	0.59 ^{c} (1.04)	96.79
T_5 - Neem oil 2.0%	18.21 (4.26)	5.43 ^{bc} (2.33)	3.10 ^{b} (1.75)	2.32 ^{b} (1.51)	87.26	2.04 ^{b} (1.43)	1.74 ^{b} (1.49)	90.42
T_6 - Spiromesifen 240SC - 0.02%	18.19 (4.26)	5.88 ^{b} (2.42)	2.87 ^{b} (1.66)	1.82 ^{b} (1.34)	89.99	1.57 ^{b} (1.24)	1.62 ^{b} (1.45)	91.08
T_7 - Control	18.59 (4.31)	18.51 ^{a} (4.29)	17.91 ^{a} (4.23)	15.53 ^{a} (3.94)	16.46	12.35 ^{a} (3.51)	12.86 ^{a} (3.65)	30.83
CD value (p=0.05)	NS	0.15	0.43	0.18	0.34	0.21	0.26	0.65

DAT = Days after treatment. Means followed by same letter in the column do not differ significantly. Figures in the parentheses are square root transformed values.

(Yadav and Bhaskar, 2018). High efficacy of HMO against both egg and active stages of spider mite might have resulted in significant reduction in the mite count on treated okra plants compared to Spiromesifen, which possess relatively lower efficacy against adult mite. However, field evaluation of HMO at 1.0 and 2.0 per cent concentration against *T. urticae* on brinjal had shown HMO to significantly reduce mite infestation on par with Spiromesifen (Kavya *et al*., 2015). In the present study, HMO was evaluated at higher concentrations of 2.0 and 2.5 per cent. This could be another reason for higher efficacy of HMO over the acaricide, Spiromesifen.

In the study, HMO showed greater persistence in field as indicated by progressive reduction in mite count up to 14 days of treatment application. In addition to its high efficacy against spider mites, HMO was also found to be safe to the predominant mite predator, *Neoseiulus longispinosus* (Yadav and Bhaskar, 2018). These attributes auger well for HMO to be a safer alternative to synthetic acaricides for mite pest management in vegetable crops.

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First report of *Aeolesthes holosericea* (Fabricius, 1787) (Cerambycidae: Lamiinae) from Goa, India

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ABSTRACT: *Aeolesthes holosericea* is reported for the first time for Goa with its dorsal, ventral and lateral photographic views and current geographical distribution.

KEY WORDS: *Aeolesthes,* Goa

Aeolesthes holosericea is commonly known as apple stem borer or cherry stem borer (Tara *et al.,* 2008). It was reported as polyphagus pest which damage wide variety of trees and fruit plants (Gupta and Tara, 2013). There are eight host plant of this species reported by (Stebbing, 1914) and it was also reported from thirty seven host plant species by (Beeson, 1941). The genus *Aeolesthes* is composed of 6 species from Indian subcontinent (Tara et al., 2008) of them no previous record of this genus from Goa. There are 1536 current species of longhorn beetles known for India (Kariyanna *et al.,* 2017). On the basis of its external characters, the species *A. holosericea* is confirmed on the original description by (Gahan, 1906) and also it was confirmed by Dr. Hemant Ghate, PG research center, Modern College Pune. The present communication gives additional geographical location of this species in India.

Aeolesthes holosericea (Fabricius, 1787) (image 1)

Ceramryx holosericeus Fabricius, 1787: 135 (m. s.); Fabricius, 1801: 281; Zimsen, 1964: 166 (Syn.).

Specimens examined: One male, 11.iii.2019, Sal-Punarvasan Goa (latitude 15.687381 N and 73.962045 E), Coll. S. V. More, damaged species, collected from light pole, host plant-unknown.

Adult (male): Body length: 31mm; width: about 7 to 8mm. Antennae longer than body (65mm in length), antennomere five to eight with spine at apex, segment first to five partially dark brown and remaining antennomeres brownish, first antennomere dorsally wrinkled and thickened, second antennomere short, segment third smooth, segment first to four gradually thickened at apex, segment four and five about equal length, apical segment much longer than others (16mm in length). Head with a straight, dark brown to reddish brown, covered with brownish fine hairs, front view of head or on the frons region slightly covered with wrinkled, eyes divided into upper and lower lobes, upper eye lobes widely separated. Prothorax dark brownish, rounded at each side, with irregularly wrinkled on dorsal side, central portion smooth, pronotum covered with very fine silky pubescence at lateral side. Scutellum slightly whitish, tongue like, elytra with bands or patches, duller to brighter (19mm in

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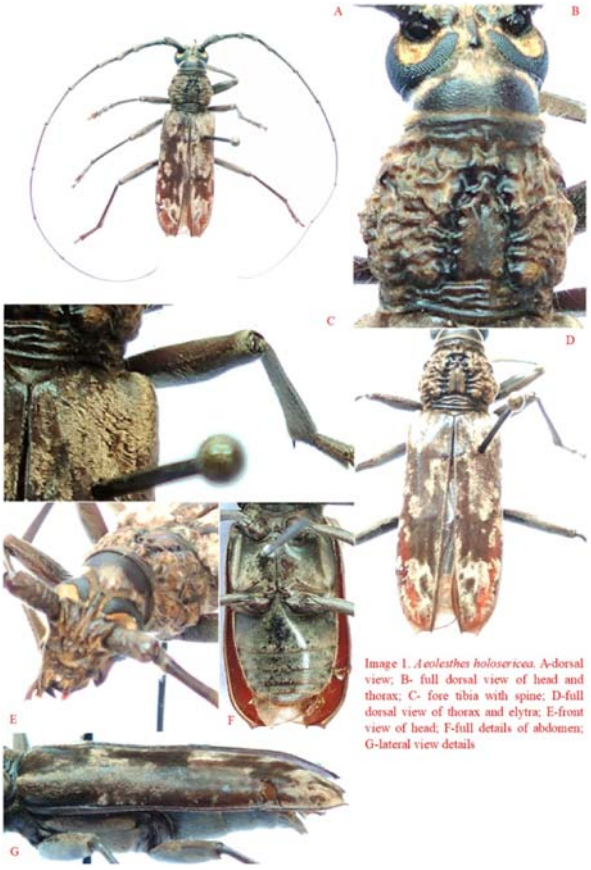

Image 1. *Aeolesthes holosericea*. A-dorsal view; B- full dorsal view of head and thorax; C- fore tibia with spine; D-full dorsal view of thorax and elytra; E-front view of head; F-full details of abdomen; G-lateral view details

length), each elytra with pointed spine at apex of elytra suture other one blunt spine its opposite side. Legs thinner, covered with greyish pubescence, protibia, mesotibia and metatibia with spine at apex, first tarsal segment of mid and hind legs longer than others, claws brownish, widely separated. Gula dark brown in colour, prosternum brownish, the mesoventrite, metanepisternum, metaventrite and discrimen covered with brownish to whittish pubescence, abdomen ventrite one to five covered whitish pubescence, apical abdomen ventrite covered with fine brownish hairs, more on tip, ventrite first occupied large space as compared to others.

Distribution: Pakistan, Indonesia, Vietnam, Laos, Malaysia, Myanmar, China, Srilanka, Thailand and India (Tamil Nadu, Maharashtra, Andhra Pradesh, Arunachal Pradesh, Karnataka, Madhya Pradesh Andaman and Nicobar Island, Assam, Rajasthan, Punjab, Nagaland and Jammu Kashmir).

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First report of incidence of eriophyid mite *Aceria* sp. on *Amaranthus*

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ABSTRACT: Eriophyid mite *Aceria* sp. damaging Amaranthus (*Amaranthus tricolor*) is reported from India for the first time. The mite cause severe malformation of the shoot, making it fibrous and reducing the yield. Foliar application of spiromesifen or fenpyroximate reduced the damage symptoms. DNA data for the mitochondrial COI gene and nuclear gene (ITS 2 region) are being generated for accurate species delineation.

KEY WORDS: *Aceria* sp, damage symptoms, *Amaranthus*

Amaranth (*Amaranthus tricolor*) is a common leafy vegetable cultivated all over the country and its major growing season in Kasaragod district of Kerala is summer after the cessation of North East monsoon. Extensive cultivation of leaf amaranth is a common practice by farmers in the coastal area of Kanhangad in Kasaragod district. The crop sown during October is continued up to second week of June when the fields are usually submerged due to continuous rains. Shoots of amaranth are harvested every 10 days during this nine-month crop period. The marketability of the produce is good since crop is red in colour and farmers used to get a remunerative price. But from the last three years, growers of Padannakad area close to the Agricultural college campus are experiencing the problem of eriophyid mite infestation in the crop. February onwards mite affected plants show crinkling, deformity and malformation of tender leaves, severe reduction in leaf size and stunting (Plate 1). As the affected shoots become more

Plate 1. Crinkling & malformation of *Amaranthus* shoot

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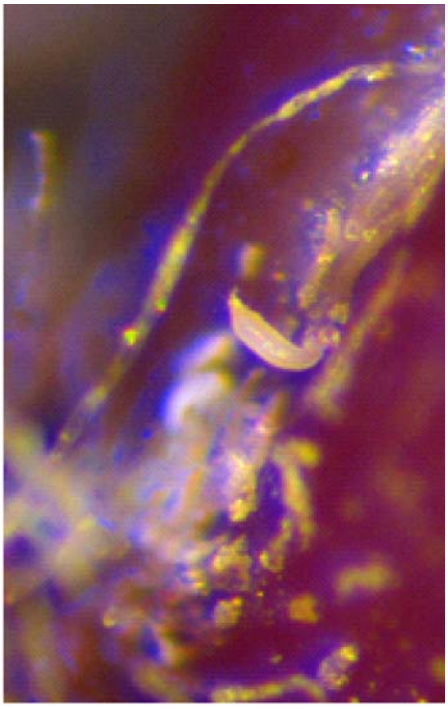

Plate. 2. stereomicroscopic view of *Aceria* sp

fibrous, causes difficulty in harvesting. Such damage symptoms get extended rapidly and by April month most of the plants in the field show the symptoms and the crop appears sickly because of stunted growth. Upon stereozoom microscopic examination of the affected shoot, eriophyid mites were noticed in good numbers. During May 2019, on an average as many as twenty mites were recorded in 5 cm length of tender shoot with the mite number ranging from 8 - 28. Because of severe stunting, the number of periodical tender shoot harvests from April to June was found reduced to almost 50 per cent. Since there was severe crinkling, malformation and fibrous nature of the shoots, marketability was severely affected, fetching the growers a very low price. Altogether there was more than fifty per cent reduction in the revenue as surveyed from 10 to 15 farmers in the affected area. An observational trial to contain the pest with foliar application of spiromesifen (@100g ai/ha) or fenpyroximate (@30g ai/ha) immediately after an harvest, reduced the mite damage symptoms almost completely for a period of at least 15 days when the new shoots would be ready for the next harvest (POP of KAU, 2016).

Eriophyid mites collected from the infested shoots were identified by the AINP (Agricultural Acarology) at UAS, Bengaluru as *Aceria* sp (plate 2). Of course, it is the first report of eriophyid mite on amaranth *Amaranthus tricolor* in India. *Aceria amaranthi* recorded on *Amaranthus* sp. from Tanzania in 1992 has been reported in great numbers (70 to 200 per gall) causing numerous galls on both the surfaces of young and matured leaves with all the developmental stages within the same gall. But the presently recorded *Aceria* sp. do not cause galls on leaves or shoots. Also taxonomically important the pattern of median, admedian and submedian lines on the dorsal shield are distinct for these two species under the common genus *Aceria*. Supportingly, DNA data of *Aceria* sp. for both mitochondrial COI gene and nuclear gene (ITS2 region) are being generated for more accurate species delineation.

It is worth undertaking detail studies on symptom or damage, ecological and economic aspects of this eriophyid mite infestation on the important vegetable crop *Amaranthus*.

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First report of *Rinamba opacicollis* Cameron (Hymenoptera: Braconidae, Doryctinae) in India as parasitoid of coffee stem borer, *Xylotrechus quadripes* (Chevrolat) (Coleoptera: Cerambycidae)

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ABSTRACT: *Rinamba opacicollis* Cameron (Hymenoptera: Braconidae) was collected from Chikkamagaluru, Karnataka, India for the first time from the larvae of white stem borer, *Xylotrechus quadripes* Chevrolat infesting arabica coffee. Its role in the biological or integrated control of *X. quadripes* remains to be evaluated. White stem borer could be the first host record of this parasitoid all over the world.

KEY WORDS: Ectolarval parasitoid, *Rinamba opacicollis, Xylotrechus quadripes*

The coffee white stem borer (CWSB), *Xylotrechus quadripes* (Coleoptera: Cerambycidae) is a major and economically important pest of arabica coffee in India. This pest was first noticed in India during 1838 (Stokes 1838) and its distribution is confined to only Asian countries ((i.e. Burma, China, India, Java, Nepal, Sri Lanka, Thailand and Vietnam) and has not been reported in other coffee growing countries (Le Pelly, 1968). The adult beetles are active in bright sunshine hours and female lays eggs in the cracks and crevices under the loose scaly bark of the main stem and thick primaries. The infestation starts with the feeding of early instar larvae on the outer surface and then gradually entering inside the main stem. Extensive feeding leads to the formation of tunnels inside the stem interrupting the nutrient supply and thereby leading to substantial reduction in the yield (Seetharama *et al*.. 2005). The annual loss due to the CWSB in India is about $17.5-26 million (Venkatesha *et al*., 2012). Severe infestation leads to yellowing of leaves, defoliation and subsequent death of the plant. The Coffee White Stem Borer has two peak flight periods, summer flight during April - May and winter flight during October to December. The winter flight is very crucial, as all the adult beetles tend to emerge during this period (Seetharama *et al*., 2005). The concealed feeding habit of this pest necessitates timely implementation of IPM practices to manage the pest under the economic threshold level. Cultural practices like maintenance of optimum shade, tracing and uprooting of the infested plants before

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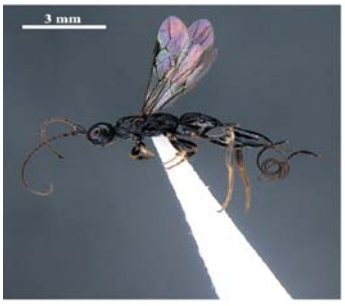
the flight period (March and September) and the judicial use of chemical pesticide, Chlorpyrifos @ 3ml/l (First fortnight of April and October) are the recommended IPM practices for the CWSB.

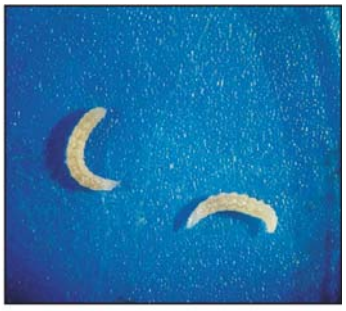
Nevertheless, use of bio-control strategies for management of CWSB has not been very successful. Though, some parasitoids were earlier identified on CWSB, they could not be included in the IPM because of their low potential against CWSB and failure in the establishment in field (Prakasan *et al*., 1986; Shylesha *et al*., 1992; Veeresh, 1993; Venkatesha *et al*., 1997; Seetharama *et al.,* 2008). Hence, efforts have been continued, to identify potential natural enemies against CWSB. In order to achieve potential biological control programs, severely CWSB infested coffee plants were collected before the emergence period (March 2017) from Central Coffee Research Institute (CCRI) farm, Chikkamagaluru Dist, Karnataka, India and stored in isolated closed room and regularly monitored for the emergence of natural enemies, if any. During the routine observations on the stored stems, we found the emergence of parasitoids in large numbers. These insects were collected using aspirator and observed under laboratory for confirmation. Further, the infested plants were split opened and found that some of the CWSB larvae were found parasitized. The parasitized larvae were taken to laboratory and reared till emergence of adults. Both the insects which emerged from cut stems in the storeroom and the parasitized CWSB larvae were found to be similar. The adults of these parasitoids were sent to Insect Ecology and Ethology laboratory, University of Calicut, for taxonomic identification and identified as *Rinamba opacicollis* Cameron (Hymenoptera: Braconidae, Doryctinae) by Ranjith. The same specimens were submitted to the Insect depository of National Bureau of Agriculturally Important Insects (NBAIR), Bengaluru, India to maintain as type specimen (NBAIR/HYM-BRAC/23819). Based on the literature, this is the first report of this parasitoid in India and even from Oriental region. More interestingly, this could be the first host record for the genus. Braconidae is one of the largest families within the Hymenoptera, which act as parasitoids on some immature stages of holo and hemimetabolous insects. Braconids include some important parasitoids that develop endoparasitically in the host body though majority of the species in Braconidae are ectoparasitoids on late instars of host larvae. *Rinamba*- one small genus with less than ten species described. The synonyms for *Rinamba* are *Pseudorhoptrocentrus* Granger, 1949 (synonymized by Belokobylskij in 2004) and *Rhoptrocentroides* Marsh 1993 (synonymized by Belokobylskij in 1995 with *Pseudorhoptrocentrus*). This is distributed in Afrotropical, Neotropical and Oceanic regions. The genus *Rinamba* belongs to the tribe Doryctini including approximately 35 Palaearctic genera (Belokobylskij *et al.,* 2004). This is a moderately large subfamily of the family Braconidae with more than 1000 described species worldwide. Most of the known doryctine species are idiobiont gregarious ectoparasitoids of the larvae of xylophagous or bark-boring Coleoptera, while some species live on Lepidoptera or Hymenoptera-Symphyta (sawfly) larvae (Loni *et al*., 2005).

The adult parasitoids collected from the CWSB infested stored stems were maintained on 10% honey solution in the laboratory. Various methods were tried to standardize the mass rearing techniques for this parasitoid using the CWSB larvae. The adult parasitoids along with the CWSB infested stems were placed inside cages for two days. Adults were found active and hovering around the feeding WSB larvae inside the cut stems exhibiting good searching ability by means of cues. *Rinamba opacicollis* found to be a greagarious ectoparasitoid as female individual deposit eggs on CWSB larvae through its long ovipositor. The spot of oviposition was marked, and the egg laying was confirmed by splitting of the stem. In another method which was originally used for rearing the Braconid, *Parallorhogas pallidiceps* by Balakrishnan and Anishkumar (2008), a microscopic slide was glued on cardboard (5 mm thickness) and the other side of the cardboard was covered with muslin cloth. Arabica stem powder was sparsely sprinkled over the muslin cloth to elicit adults towards the slide-cardboard. A small slit (2.5 cm × 0.5 cm) was made on the cardboard and a late instar larva of CWSB was placed inside the

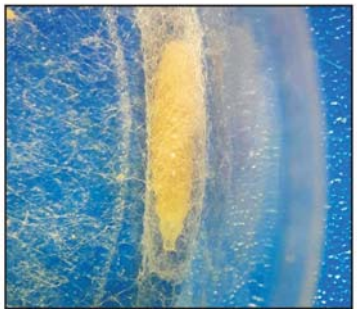


Adult female

Eggs laid on WSB larva



Parasitoid - Larvae



Parasitoid - Pupa

Parasitized larva of WSB

slit and covered with cotton plug. Oviposition was observed in the larva present inside the muslin cloth. Long and slender eggs were laid in groups and hatched larvae were found to feed externally on the WSB larva. The female parasitoid was larger in size (1 to 1.3 cm) compared to male (0.3 to 0.4cm). The life cycle of the parasitoid was studied on WSB larvae using the slide technique. The development period of egg, larvae and pupa were recorded as 6.14 ± 0.89 (5-7), 11.14 ± 1.86 (9-14) and 27.14 ± 3.6 (24-31) days, respectively. The study revealed that the time taken for completion of the life cycle ranged from 38 to 44 days.

Description of *Rinamba opacicollis* Cameron

Body length 6.84 mm, fore wing 4.96 mm, ovipositor 8.84 mm. Antenna short with 32 segments. Face convex in lateral view, rugose-reticulate, setose. Eyes glabrous with crenulated margin. Torular region raised. Clypeus strongly sculptured with distinct ventral carina. Tentorial pit deep. Lateral temple transversely striate. Frons longitudinally striate with a deep pit medio-basally. Ocellar area sculptured. Vertex smooth, sparsely setose. Occipital carina crenulate. Maxillary palp with five segments. Labial palp with three segments. Propleuron transversely striated. Mesosoma moderately dorso-ventrally flattened. Median lobe of mesoscutum with strong medial longitudinal grioove. Mesoscutum smooth, sparsely setose with lateral carina. Notauli crenulate anteriorly, meeting posteriorly. Scutellar sulcus divided by six carinae. Scutellum smooth, sparsely setose. Median area of metanotum with pits anteriorly, smooth medio-posteriorly. Propodeum rugose-reticulate with median longitudinal and lateral carinae anterior half, posteriorly with two anteriorly diverging carinae and with a pair of deep smooth pits sub medially. Mesopleuron smooth medially and anteriorly rest transversely striate. Sternaulus deeply impressed, crenulate, running along almost entire length of mesopleuron. Metapleuron smooth mid anteriorly, sparsely setose, rest transversely rugose striate. Fore wing 3.9× longer than maximum width. Pterostigma 3.9× longer than maximum width. Fore wing vein r arising before mid level of pterostigma. Second submarginal cell narrowing apically. Vein cu-a postfurcal. Fore femur robust. Mid and hind

femora transversely striate dorsally. Metasoma 1.0× longer than mesosoma and head combined. First metasomal tergite rugose reticulate, smooth medio-basally with moderately angulate corners basally and with apically converging pair of strong longitudinal carinae and small smooth medio-posterior area. Ovipositor with dorsal nodus and ventral serrations.

Considering the spontaneous occurrence of *R. opacicollis* in coffee white stem borer infested areas and its efficiency in parasitizing the CWSB larvae inside the stem, systematic studies to assess and exploit the potential of this parasitoid against *X. quadripes* are being pursued.

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Scorpion Hemocyte- Plasmocyte

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ABSTRACT: The plasmocytes in five different scorpions- *Mesobuthus tumulus tamulus, M. tumulus concanesis, Orthochirus bicolor, Heterometrus xanthopus, H. phipsoni* were studied in the present investigation. The hemocytes are polymorphic and their population is about 70 – 80%. The length varied from 6- 12 µm and the width is 6- 12 µm in *M. tumulus tamulus, M. tumulus concanesis and O. bicolor.* In *H. xanthopus and H. phipsoni* the length ranged between 10- 35 µm and width between 3- 11 µm. The cytological characteristics were studied in all five species of scorpions. The nucleus is basophilic and placed at the centre. The cytological detail of plasmocyte in *H. xanthopus* of was studied by TEM. It is polymorphic with rod shaped mitochondria and centrally placed nucleus.

KEY WORDS: *Mesobuthus tumulus tamulus, M. tumulus concanesis, Orthochirus bicolor, Heterometrus xanthopus, H. phipsoni* cytological characteristics, TEM

Arthropoda is the largest phylum on the earth, inhibiting in air, water and on land. The study of hemocytes is the most fascinating area of research. Kollmann (1908) first reported the morphology and types of hemocytes- the blood cells, in arthropoda. Just like vertebrate blood cells, the hemocytes play important role in immune system, coagulation of hemolymph after rupture of cuticle, in wound healing etc (Millar and Ratcliff, 1989). The hemocytes are embryologically derived from intermediate mesoderm (Anderson, 1972). The morphology and nomenclature of hemocytes in different arthropods is confusing and varied. Jones (1962) and Price and Ratcliffe (1974), presented a new nomenclature system applicable for all arthropods. Though there is disagreement about the types of hemocytes, ultra structurally seven types of hemocytes are identified as- Prohemocytes (PRs), Plasmocytes (PLs), Granulocyte (GRs), Spherulocytes (SPs), Adipohemocytes (ADs), Oenocytes (OEs) and Coagulocytes (COs) in almost all arthropods. A perusal of literature indicates that some groups of arthropoda have received detailed attention about study of hemocytes, are- insects, crusaceans and myriopods. Though some work was done on arachnids, scorpions – the living fossil, the oldest group in arthropods remain neglected. Ravindernath (1974) did a pioneer work about hemocytes of *Palamnaeus swammerdami*. In the present investigation the morphology of plasmocytes were studied in five different species of scorpion- *Mesobuthus tumulus tamulus, Mesobuthus tumulus concanesis, Orthochirus bicolor, Heterometrus xanthopus* and *Heterometrus phipsoni.*

During this investigation five different species of scorpions were collected from different localities

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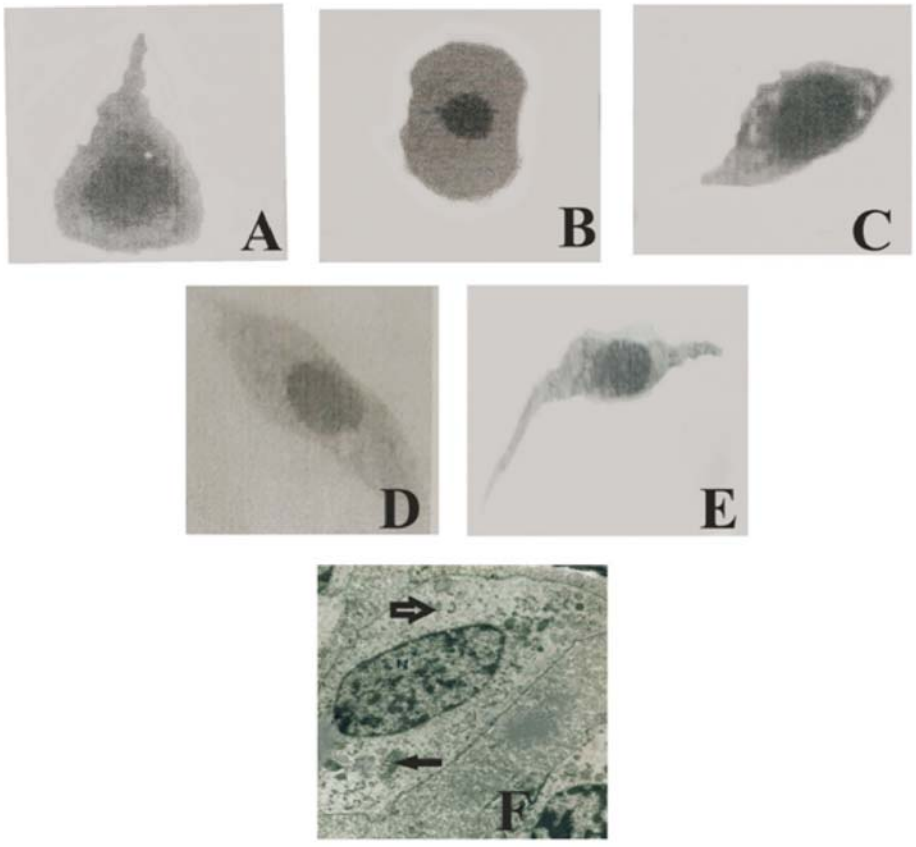
PLATE I



Scorpion Plasmocytes(X940)

A- *Mesobuthus tamulus tamulus* **B-** *Mesobuthus tamulus concanesis*
C-*Orthochirus bicolor* **D-***Heterometrus xanthopus*
E- *Heterometrus phipsoni*
F- TEM of plasmocyte from *Heterometrus xanthopus* (X1200)
Solid arrow- mitochondria
Hallow arrow- ribosomes

from Maharashtra (India). *H. xanthopus, H. phipsoni* were collected from their natural burrows while *M. tumulus tamulus, M. tumulus concanesis, O. bicolor* were collected underneath the stones. The males and females were placed in separate perforated jars with hibiscus leaves and soil from its natural habitat. These were fed with cockroaches (Shah and Patil, 2011).

For collection of hemolymph the method employed as described by Padmanabha (1967) with some modifications. It is collected by aspiration through hypodermic needle inserted through arthroidal membrane of pedipalps. For morphological study Pappenheim'spanchrome, Giemsa stain and Leishman's stains were employed. Janus Green B was used to observe mitochondria while Sudan

Table 1. Plasmocyte Characteristics in Different Species of Scorpions

Sr. No.	Species of scorpion	Shape variation		Size variation (μm)		Nucleus-cytoplsmic ratio	Position of nucleus	Nature of staining reaction	
		cell	nucleus	cell	nucleus			cytoplasmic inclusion	nucleus
1	*Mesobuthus tumulus tamulus,*	P	R	7- 21♂ 4-11♀	3-5	35-45	C	AG	B
2	*Mesobuthus tumulus concanesis*	P	R	6- 18♂ 4-18♀	3-4	25-30	C	AG	B
3	*Orthochirusbicolor*	P	R	9- 20♂ 3-12♀	3-5	35-60	C	SG	B
4	*Heterometrusxanthopus*	P	O	10- 30♂ 3-10♀	4-10	40-50	C	SG	B
5	*Heterometrusphipsoni.*	P	E	11- 32♂ 4-11♀	4-10	45-50	C	SG	B

B- Basophilic C- Centric E- Elliptical O- Oval P- Polymorphic R- Round
AG- Agranular SG- Slightly Granular

Black B and PAS were used for cytochemistry. These techniques include light microscopy, phase contrast microscopy and transmission electronic microscopy (TEM). The quantitative methods include Total Hemocyte Count (THC) and Differential Hemocyte Count (DHC).

The morphological study was done in different five species of scorpion in both male and female. All morphological features of scorpion are similar to that of insect with slight differences. In this investigation Jones system of classification was fallowed.

In all five species of scorpion clearly differentiated seven types of hemocytes were observed- Prohemocytes (PRs), Plasmocytes (PLs), granulocytes (GRs), Spherulocytes (SPs), Adipohemocytes (ADs), Oenocytoids (ODs), and Coagulocytes (COs).

Plasmocyte is the unique type of hemocyte in scorpion due to its polymorphic nature. It is fusiform in *M.tamulus tamulus*, elongated in *M. tumulus concanesis*, irregular in *O. bicolor* and spindle shaped in *H. xanthopusand* and in *H. phipsoni.* However these shapes are not characteristics of the species. During preparation of hemolymph smear PLs adhere the glass slide to produce broad cytoplasmic extensions. Due to this it was very difficult to measure the dimensions of the cell. This was overcome by using 2% versene fixative. In *M. tumulus tamulus, M. tumulus concanesis* and *O. bicolor* the length and width is 6- 12 μm. In *H. xanthopus* and in *H. phipsoni* the length ranged from 10- 35 μm while the width was 3- 11 μm (Table 1). The cytoplasm was agranular in *M. tamulus concanesis* and granular in *H. xanthopus, O. bicolor* and *H. phipsoni.* The granules are Sudan Black B positive indicating lipid material in it. The nucleus is centrally placed and measured about 3-

10 µm in diameter. It is round in *M. tumulus tamulus, M. tamulus concanesis,* and *O. bicolor.* It is oval in *H. xanthopus* and elliptical in *H. phipsoni* (Plate I).

The values PLs was 72% in *M. tumulus tamulus,* 65% in *M. tamulus concanesis, 75%* in *O. bicolor,* 82% in *H. xanthopus* and 79% in *H. phipsoni* as compare to that of other types of hemocytes.

Examination of scorpion hemocytes with TME was particularly useful in distinguishing the PLs from other hemocytes. In *H. xanthopus*, the plasma membrane of PLs clearly showed cytoplasmic projections. TEM photography also showed pinocytic vesicles indicating phagocytic nature of it.

The selection of proper technique becomes important since several methods are available for the study of arthropod hemocytes. During the course of this investigation, it was noticed that any one technique was not suitable for all hemocytes; therefore certain modifications were made with combination of some methods. In all five species the PLs represents bulk of hemocyte population. A common feature of PLs is presence of vacuoles which was not reported in all five species of scorpions. In fixed preparation PLs showed stiff spike like projections (plate I).

As described by Gupta (1985a), PLs are having large nucleo-cytoplasmic ratio, which is considered as the stem cell nature of the hemocytes (Srivastava and Richards, 1965) which gives rise to other types of hemocytes. In the present investigation the PLs of all species having common features to that of GRs. The granules in PLs are small and refractile as compare to PLs as described by Kollaman (1908).

The large number of population and presence of stiff projections might be because of its phagocytic activity. The functional importance of the different hemocytes is still not clearly defined; hence there is ample scope to study scorpion hemocytes.

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