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The Journal covered all aspects of basic and applied entomology, viz., agricultural entomology, aquatic entomology, medical entomology, veterinary parasitology, forensic entomology, forest entomology; acarology and arachnology. The publication carries original articles in morphology, systematics, physiology, eco-biology, biological control, biotechnology, pest outbreaks, integrated pest management, toxicology, botanical insecticides, pollinators, vectors, apiculture, sericulture and reports of new species.

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National Academy of Agricultural Sciences (NAAS) score for ENTOMON is 4.69.

University Grants Commission, New Delhi has placed the journal on the official list of UGC Consortium for Academic and Research (CARE) i. e. the UGC Care List of approved journals 2023-24 (see - All UGC-CARE Journals in Science – Group 1/ UGC-CARE List of Journals - Science – 2023). ENTOMON is included in the Web of Science Master Journal List (Clarivate).

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Digitalisation of ENTOMON volumes 1 – 37 (1976 - 2012) is in progress; there are 37 volumes with 146 issues. ENTOMON volumes 38 to 47 (from 2013 to 2022) are already in digital form, in the web site. On completion of the digitalisation of ENTOMON volumes 1 – 37, all issues of the journal since its inception to date will be posted in the archives of our web site.

ENTOMON is grateful to Professor K. Madhavan Nair, the AAE web site Manager for his committed and selfless service. ENTOMON owes allegiance to the late Professor M. Koshy, M/s SB Press, Thiruvananthapuram and the present management for the support. ENTOMON profoundly acknowledges the peer reviewers for their precious inputs, critical evaluation of the manuscripts. Publication of all issues of ENTOMON is as per schedule.

We place ENTOMON volume 48, issue 1 (March, 2023) before you.

Wishing you all enjoyable professional moments!

Regards

Dr M.S. Palaniswami
Chief Editor

ENTOMON

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Short Commuinication

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Article No. ent. 48101

A new subspecies of *Caltoris* Swinhoe, 1893 (Lepidoptera, Hesperiidae) from the Malabar Coast, Kerala, India

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ABSTRACT: The Indo-Australian genus *Caltoris* has over 15 species distributed from India through south China and SE Asia into New Guinea and Solomon Islands. Based on wing colouration, characters of male genitalia, and early larval stages on the host plant *Phragmites karka*, a new subspecies of *Caltoris bromus* (Leech, 1894), *C. b. sadasiva* **ssp. nov.**, is described from the coastal lakes and mangrove associated swamps of Kerala, southern India on the western slopes of the Western Ghats. This is the first record of *C. bromus* from Western Ghats and Peninsular India. This extends the distribution range of the species from NE India to south-western India, adding it to the butterfly fauna of the Western Ghats.

KEYWORDS: Taxonomic descriptions, Baorini, new taxa, life cycle, mangrove, distribution range

INTRODUCTION

The genus *Caltoris* Swinhoe, 1893 (Lepidoptera, Hesperiidae, Hesperiinae, Baorini), with over 15 species, ranges widely over the Indo-Australian Region, from Sri Lanka, India and China to Solomon Islands (Evans, 1949; Vane-Wright and de Jong, 2003). It is represented in India by the following taxa as per Evans (1949): *C. aurociliata* (Elwes and Edwards, 1897) in E. Himalaya and NE India, *C. bromus bromus* (Leech, 1894) in NE India, *C. brunnea caere* (de Nicéville, 1891) in NE India, *C. cahira cahira* (Moore, 1877) in Andaman and Nicobar Islands; *C. cahira austeni* (Moore, [1884]) in E. Himalaya and NE India; *C. canaraica* (Moore, [1884]) in Western Ghats and adjoining areas; *C. confusa* (Evans, 1932) in E. Himalaya and NE India; *C. cormasa* (Hewitson, 1876) in NE India; *C. kumara kumara* (Moore, 1878) in Western Ghats, *C.* (*kumara*) *moorei* (Evans, 1926) in E. Himalaya and NE India; *C. plebeia* (de Nicéville, 1887) in E. Himalaya and NE India; *C. sirius sirius* (Evans, 1926) in NE India, *C. tulsi tulsi* (de Nicéville, [1884]) in E. Himalaya and NE India, and *C. philippina philippina* (Herrich-Schäffer, 1869) in Western Ghats, NE India.

Rearing of a series of reed-feeding hesperiid larvae from 2005 to 2015 from southern Kerala yielded a

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taxon that differed from all other known *Caltoris* from the region. Further comparison of museum specimens revealed that the taxon is similar to *C. bromus* in external morphology as well as the male genitalia but slightly and consistently differentiated from other known subspecies of *C. bromus*, which is described as a new subspecies. This extends the distribution range of the species from NE India to south-western India, adding it to the butterfly fauna of the Western Ghats.

MATERIALS AND METHODS

Early stages of *C. bromus* reared in the lab, with leaves of the larval host plant *Phragmites karka* (Retz.) Trin. and maintaining them in plastic boxes. Some of the newly eclosed butterflies were preserved as vouchers, as a combination of specimens pinned as dry for taxonomic work, and stored wet in 100 per cent molecular grade ethanol for phylogenetic work. These specimens are preserved in the Biodiversity Lab Research Collections at the National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research, Bengaluru (http://biodiversitycollections.in). Male genitalia of paratypes were dissected after dissolving extraneous abdominal tissue with 10 per cent KOH overnight and photographed using a Leica digital camera MC 120 HD mounted onto a Leica S8APO stereomicroscope (Leica Microsystems, Germany). Multiple images were taken and stacked to improve the depth of field with CombineZM. The dissections were preserved in 0.5 ml vials containing anhydrous glycerol at room temperature in an air-conditioned room (22–26°C). These specimens were compared with the historical collections, including type specimens, at the Natural History Museum, London (NHMUK). At both the research collections, museum specimens were photographed using Canon EOS 7D and 1200D DSLR cameras, Canon 50 mm macro, 60 mm macro, and 100 mm macro lenses, and Canon 420EX flashes (Canon Inc., Japan) fitted with photographic umbrellas. The specimens (adults and early stages) in the field were photographed using a Canon EOS 70D DSLR camera body with a 100 mm f 2.8 macro lens.

The terminology used for wing patterns, the numerical system of wing venation, and the male genitalia, in the description below, follows Evans (1949) and Kunte *et al.* (2019). Wing-length measurements follow methods by Van Hook *et al.* (2012). The following abbreviations are used in the text: OD: original description, ssp.: subspecies, FW: forewing, HW: hindwing, UpF: dorsal side of the forewing, UpH: dorsal side of the hindwing, UnF: ventral side of the forewing, UnH: ventral side of hindwing, NHMUK: Natural History Museum, London.

RESULTS AND DISCUSSION

Taxonomic description

***Caltoris bromus sadasiva* Sadasivan & Kunte ssp. nov. (Figs. 1–6)**

Holotype: Voucher code IBC-AD633. Male. Aakulam Lake (8°31’13.79" N; 76°54’21.64" E) in suburbs of Thiruvananthapuram City, Thiruvananthapuram District, Kerala, India. Ex larva, collected by Krushnamegh Kunte, 14 March 2015. Preserved dry, pinned, and deposited in the Biodiversity Lab Research Collections at the National Centre for Biological Sciences, Bengaluru, India (Fig. 1A).

Paratypes: IBC-PX683 (male), IBC-AD639 (male), IBC-AD631 (female, allotype), IBC-PX690 (female), and, IBC-PZ148 (female). Collection data same as the holotype. Preserved dry, pinned, and deposited in the Biodiversity Lab Research Collections (Figs. 1 B–F).

Description of the Holotype (Fig. 1A)

Forewing length: 19 mm. Antenna: 10 mm, including the apiculus.

FW Dorsal side: Dark brown with pale semi-transparent spots. Two spots in the cell, the upper one is triangular, and the lower one is more rounded. Spots in spaces 2, 3, and 4, the spot in 2 is the largest and quadrate, spots in 3 and 4 decreasing in size. A spot in 1b present. Three subapical spots in 6–8, that in 6 slightly shifted out. Ventral side: Paler rusty-brown compared with the dorsal surface. Spotting pattern same as described for the dorsal surface, but the spot in 1b is almost twice as large.

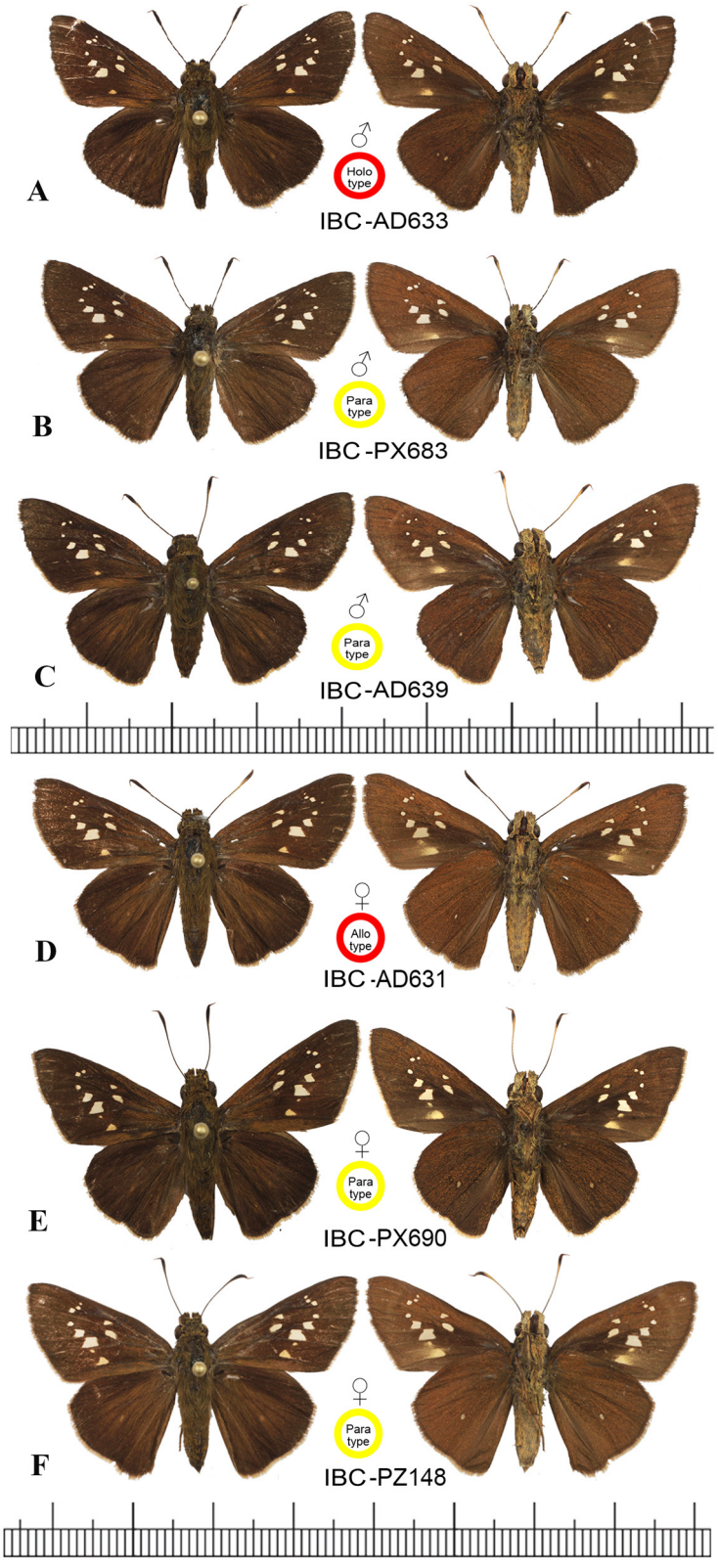

Fig. 1 Type specimens of *Caltoris bromus sadasiva* **ssp. nov.** © Krushnamegh Kunte

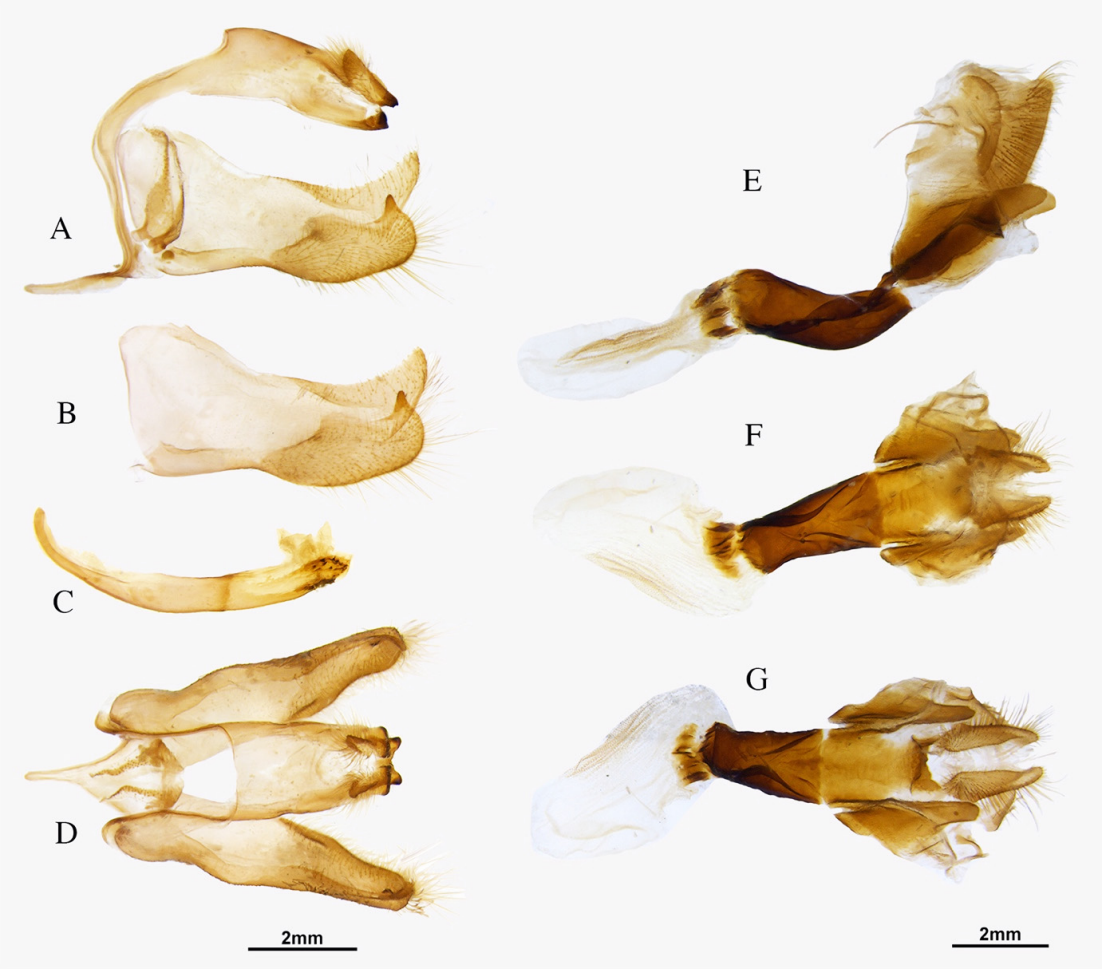

Fig. 2 *Caltoris bromus sadasiva* **ssp. nov.** A–D: Male genitalia and E–G: Female genitalia. © Dipendra Nath Basu

HW Dorsal side: Dark brown with long ochreous-brown scales, especially in space 1 and cell. A small semi-transparent dot in 2. No other spots. Ventral side: Pale rusty-brown with two yellowish-white spots in spaces 2 and 3, the one in 2 almost twice as large as that in 3. Cilia is pale brown.

Variation in paratypes (Figs. 1A–C): Forewing length of male: 43±1 mm; forewing length of female: 44±2mm. Antennal length: 10 mm, including apiculus. A large series of individuals (19 males, 20 females) raised from caterpillars, including the voucher specimens described here as paratypes, show little variation in size, wing coloration, and spotting pattern. Forewing lower cell spots may be reduced or absent. UpH usually has no spots. UnH usually has a small spot in 2, and sometimes in both 2 and 3, but in some individuals these spots are absent. Females are similar to males in wing coloration, but they have an extra-large spot in 1b on the forewing and in some specimens a small spot in 5 UnH. There was no variation between the Aakulam-Veli and Vembanad populations.

Genitalia (Figs. 2A–D, 6C, F): The male genitalia of ***C. b. sadasiva* ssp. nov.** are similar to that of the other subspecies, *C. b. yanuca* (Fruhstorfer, 1911) (Hsu and Wang 2004) and *C. b. bromus* (Huang 2011), but valva broader and more rounded (Figs. 2A–D). The cuiller (dorsal process) is longer than valva, ventral portion of the gnathos develops into two triangular processes, the lateral angle of

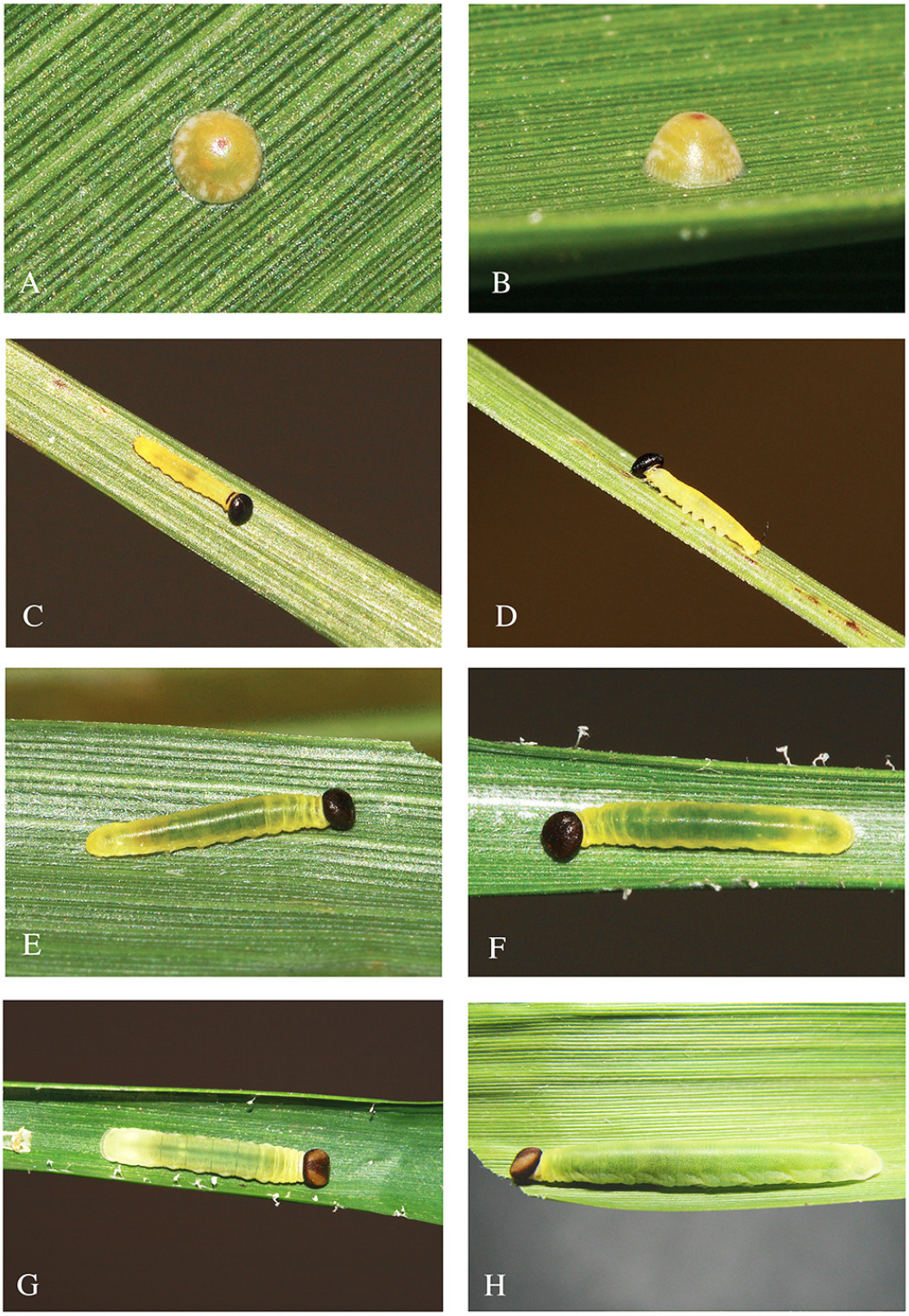

Fig. 3 *Caltoris bromus sadasiva* **ssp. nov.** Early stages. A–B: Egg, C–D: Egg Larva, E: 1st instar, F: 2nd Instar, G: 3rd Instar, H: 4th Instar. © Kalesh Sadasivan

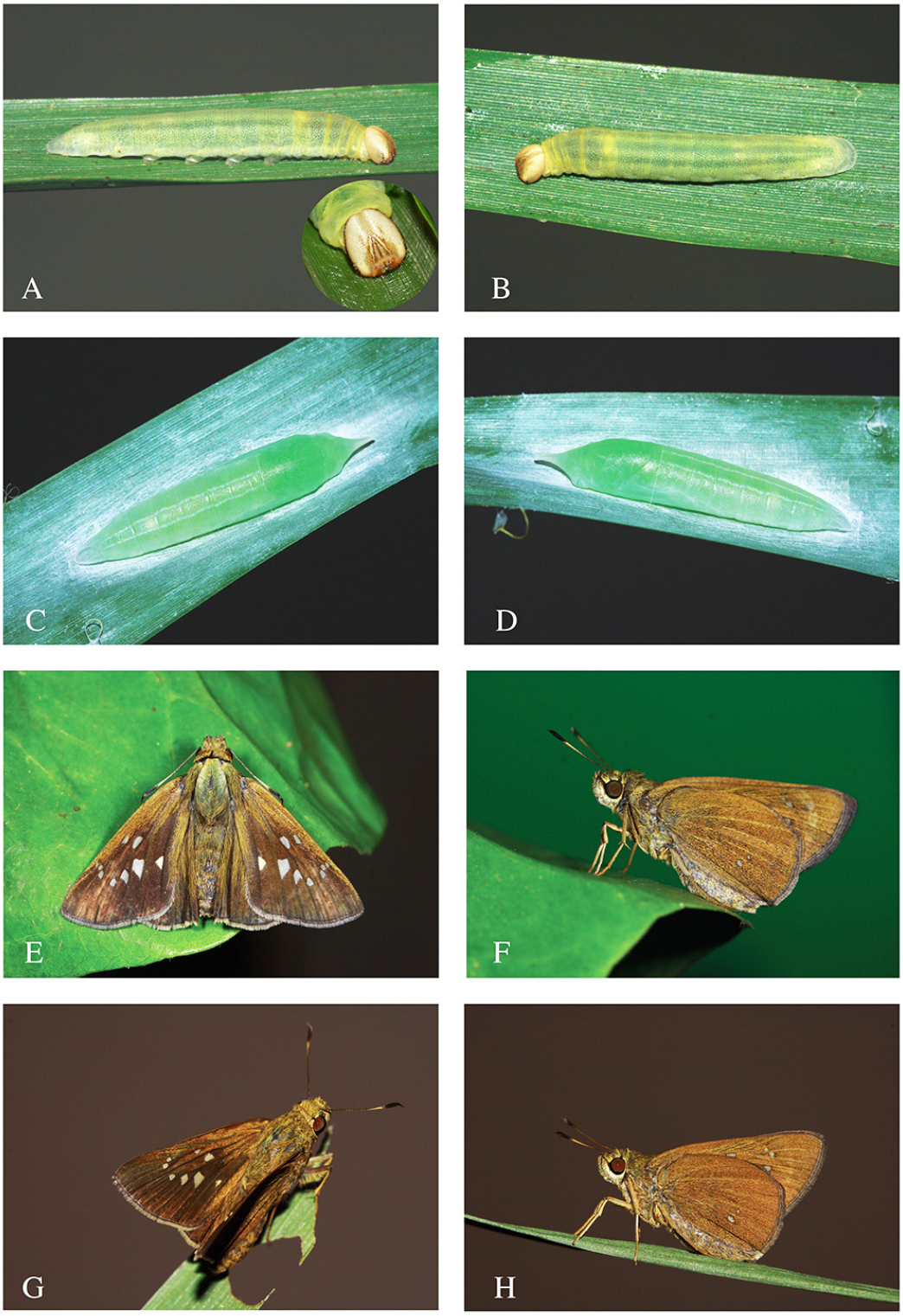

Fig. 4 *Caltoris bromus sadasiva* **ssp. nov**., Early stages, and emerged adults. A–B: Final Instar, C–D Pupa, E–F: Male, G–H: Female. © Kalesh Sadasivan

which extends beyond the uncus. The cuiller of ***C. b. sadasiva* ssp. nov.** is broader and shorter than that of *C. b. yanuca* and *C. b. bromus*. The lower edge of the valva is angular in *C. b. yanuca* and *C. b. bromus* while it is an even curve in ***C. b. sadasiva* ssp. nov.** The aedeagus of ***C. b. sadasiva* ssp. nov.** differs from *C. b. bromus* in that the proximal narrow end is dorsally much more curved and stout, and the middle portion is nearly parallel while in the other two subspecies it expands distally. The tegumen is more slender and evenly curved in comparison with the other subspecies. The female genitalia of ***C. b. sadasiva* ssp. nov.** are also overall similar to other subspecies of *C. bromus* (Figs. 2E–G). The tip of the shoulder of the lamella post-vaginalis is rounded and variable. These are minor but seemingly constant variations, which support the treatment of ***C. b. sadasiva*** as a distinct subspecies.

Diagnosis: ***Caltoris b. sadasiva* ssp. nov.** may be differentiated from the closely related subspecies based on several external characters. Variation in spotting pattern of *C. b. yanuca* (Fruhstorfer, 1911) is considerable, ranging from spotless to being prominently spotted on both surfaces (Evans, 1949; Hsu and Wang, 2004). On the other hand, the spotting patterns of ***C. b. sadasiva* ssp. nov.** is largely constant, showing little seasonal, sexual, and individual variation. In *C. b. bromus*, UpF 1b in females always has a spot, whereas this spot is usually absent in the male (Evans, 1949; Corbet *et al.*. 1992). In ***C. b. sadasiva* ssp. nov.** both sexes usually have a spot in 1b, with females having an additional spot. Moreover, the small but constant differences between the male genitalia described above also distinguish the new subspecies from the closely similar subspecies in NE India, S. China, and Indo-China. The male and female genitalia of the three subspecies are compared (Fig. 6).

Etymology: The species is named after the first author's father who was an inspiration for the author's works in natural history.

Distribution: As far as known, ***C. b. sadasiva* ssp. nov.** is restricted to *Phragmites* reed patches growing in coastal lake systems of Aakulam-Veli in Thiruvananthapuram District and Vembanad in Kottayam District in the state of Kerala, southern India.

Status, habits and habitat: ***C. b. sadasiva* ssp. nov.** is multivoltine, flying throughout the year, although it is more common during the monsoon, from June–November. The adults may be seen flying among *Phragmites* clumps during the day, often settling low on bushes or reed leaves, and flying into thick reed clumps when disturbed. They retire to shaded undergrowth when sunlight is intense.

Reproductive behaviour and early stages: Oviposition was observed during morning hours, in overcast conditions, at dusk, and sometimes in the afternoons. Females lay a single egg at a time on the upper side of reed leaves, preferring fresh leaves of smaller plants over older leaves of robust plants. The larvae are monophagous as far as known, feeding on the reed, *Phragmites karka*. This is a gregarious reed found in coastal lakes, mangrove associated swamps and wetlands of Kerala (Fig. 5C). Two other *Caltoris*, *C. kumara* (Moore, 1878) and *C. philippina* (Herrich-Schäffer, 1869) occur on the adjoining dry lands. Their larvae typically feed on *Bambusa*, not on *Phragmites*.

The egg is dome-shaped. It appears smooth and shiny to the naked eye but has inconspicuous vertical ridges that are especially apparent at the base (Fig. 3A, B). The color of a freshly laid egg is yellow with an orange tinge, the micropyle region is red, and the basal third has suffused white patches. The egg later turns greenish, the micropyle area becoming more broadly red. It measures approx. 1.75 mm in diameter, and 0.75 mm in height.

The head capsule of the egg-larva is black and vertically ovoid. The first thoracic segment has a dark brown or black collar limited to the dorsal half of the body. The anal plate is semi-circular with long anteriorly curved hairy structures. The body is translucent yellow. It measures approx. 5 mm (Figs. 3C, D). The egg-larva moves to the tip of the leaf where it makes its first evenly sutured cell by folding it longitudinally. It eats from the terminal portions of the cell (larval shelter). The first moult usually occurs 24–48 hours after hatching. In

Fig. 5 *Caltoris bromus sadasiva* **ssp. nov.** A: Larval cell, B: Egg parasitoid of the family Scelionidae (Platygastridae), C: Larval host-plant *Phragmites karka* (Retz.) Trin., from Aakulam Lake, Thiruvananthapuram. © Kalesh Sadasivan

subsequent instars the body turns progressively darker green, developing longitudinal alternative pale and dark lines in the last instar (Figs. 3, 4A, B) In successive instars the head turns paler brown, developing broad paler brown, vertical bands in the third and fourth instars, and pale yellow-white lines and bands along with tiny dark brown spots in the final instar (Fig. 4A). Testes of the male larva appear as yellow spots on the back in fourth and fifth instars. The first instar measures approx. 0.5 to 0.7 cm, second instar approx. 0.7–1 cm, third instar approx. 1.0–1.5 cm, fourth instar approx. 1.5–2.5 cm, and the final instar up to 4.5 cm. The cell of later instars is typical of *Caltoris* (Fig. 5A), it is open at both ends, made at the distal part of the leaf by folding it longitudinally, and the walls are left uneaten and intact. These instars continue to feed on leaves from the distal part of the cell. The larvae are reluctant to move from the cell even when disturbed slightly. They are intolerant to heat and direct sunlight, becoming restless when exposed. They usually feed in low light or the dark, resting in the cell motionless during the daytime.

Pupation takes place in a cell similar to the cell of the final instar larva. The floor of the pupation cell is covered in a thick mat of silk, the pupa held in place by the cremaster and a thoracic silk girdle (Figs. 4C, D). The pupa is similar in shape to that of other *Caltoris*; long, tapering at both ends, and with a characteristic down-turned process in front of the head (Figs. 4C, D). From the ventral side, the proboscis extends to the proximal part of the fourth segment distal to wing cases, almost 7 mm long. The pupa is pale grass-green with two dorsal white irregular and discontinuous thin lines that extend from the anterior end of the thorax to the second segment proximal to the tail process. Wing cases are pale translucent green, as is the rest of the ventrum. The tail and head processes are whitish. The pupa measures approx. 3.3–3.55 cm in length, and approx. 0.7–0.8 mm in width. The pupal duration is 10 days. There were no significant

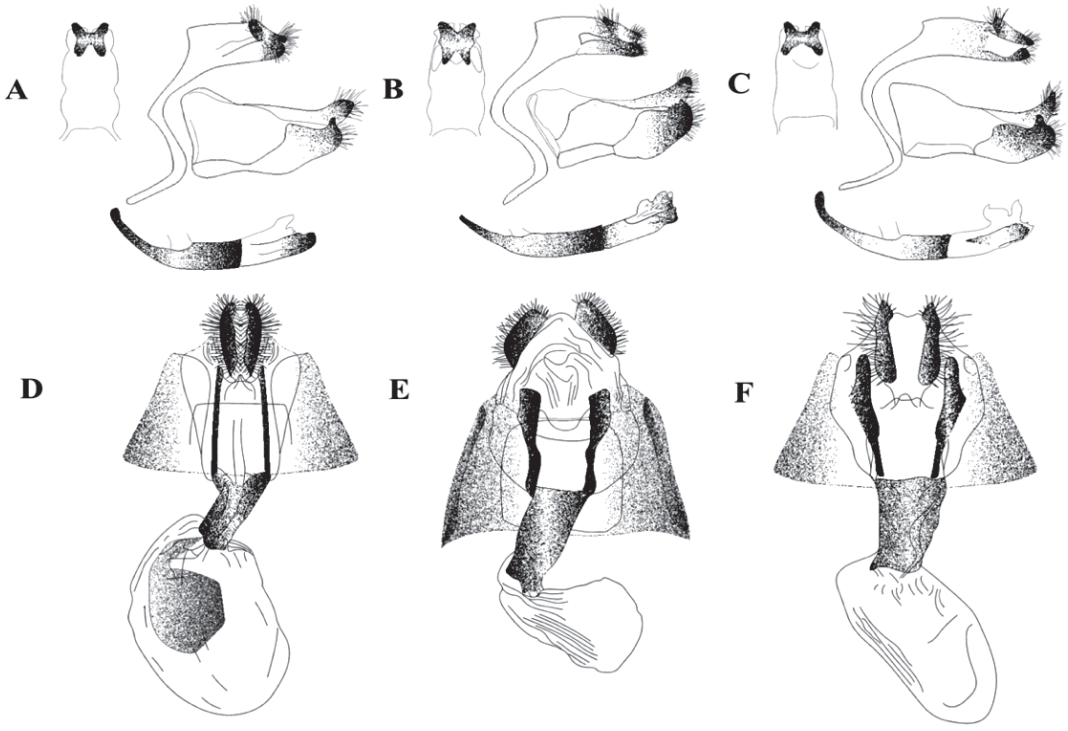

Fig. 6 *Caltoris bromus*. A–C: Male genitalia and D–F: Female genitalia. A: C. b. bromus (Leech, 1894) based on Evans (1949), B: C. b. yanuca Fruhstorfer 1911 based on Shirôzu (1960), C: C. b. sadasiva **ssp. nov,** D: C. b. bromus (Leech, 1894) based on Feng et al. (2015), E: C. b. yanuca Fruhstorfer 1911 based on Hsu and Wang (2004), F: C. b. sadasiva **ssp. nov.** Illustrations not according to scale. © Dipendra Nath Basu

morphological differences in the early stages with that of *C. bromus bromus* on comparison with images in HKLS (2011).

Brood Parasitism: Many instances of egg parasitism by a wasp in the family Scelionidae (Platygastridae) were observed from September to November (Fig.4B). No larval parasitism was observed but a single instance of pupal parasitism was observed during the study period.

Key to subspecies of *Caltoris bromus* (Leech, 1894)

1) Male UpF typically unmarked. Female usually with reduced markings. Male genitalia with inferior border of the valva angular; tegumen thick; aedeagus with proximal narrowed end straighter, middle and distal parts expands caudally (Fig. 6 B) (Formosa, Okinawa)……..…….

Caltoris bromus yanuca Fruhstorfer 1911

– Male and female with well-developed spots UpF in discal spaces 2,3,4 and apical spots in spaces 6,7 & 8……..…....…..2

2) Spot in space 1b may be absent. Female UpH with only single spot in 1b, UnH sometimes with spots in spaces 2 and 3. Male genitalia with inferior border of the valva angular; tegumen thick aedeagus with proximal narrowed end straighter, middle and distal parts expands caudally (Fig. 6A) (Malaya, Borneo, China, Indo-China, North-east India)……. *C. bromus bromus* (Leech, 1894)

– Spot in space 1b always present. Female UpH always with two spots in 1b, UnH with spots in spaces 2 and 3. Male genitalia with inferior border of the valva rounded;

tegumen thinner; aedeagus with proximal narrowed end prominently curved dorsally, middle and distal portion parallel (Fig.6C) (Southwestern India)……..... *C. bromus sadasiva* **ssp.nov**

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Genetic diversity of cotton *Pectinophora gossypiella* (Saunders) (Lepidoptera, Gelechiidae) population inferred from mitochondrial *COI* sequences

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ABSTRACT: The objective was to identify different haplotypes network of pink bollworm and their distribution in south India using the partial mitochondrial *COI* sequence of pink bollworm populations. Genetic diversity analysis exhibited the presence of 5 haplotypes in *COI gene* sequences, and among them, Hap_1 was the most common and present in 17 populations. Distributions of pairwise differences obtained with partial *COI* data from the overall populations were unimodal, suggesting population expansion. A significant neutrality test based on Tajima's D, Fu and Li's D and F test presented a haplotype network with multiple haplotypes. The acceptance of the neutrality test with significant positive values validated the theory of demographic expansion in cotton pink bollworm populations with respect to *COI*. The pink bollworm has a population genetic diversity among the investigated populations collected from south India. © 2023 Association for Advancement of Entomology

KEYWORDS: Haplotype, unimodal, neutrality test, demographic expansion

INTRODUCTION

Cotton is the most important cash crop in India and is used for industrial purposes all over the world (Keerthivarman *et al.,* 2022). The cotton crop is attacked by many species of insects at different stages of crop growth. The pink bollworm, *Pectinophora gossypiella* (Saunders) has become a significant production constraint on cotton production in India. The pest has developed multi-fold resistance to *Cry* toxins in many Indian populations and most of them have developed multifold resistance to *Cry 1 Ac* and *Cry 1Ac* + *Cry 2 Ab* toxins. Year-round cultivation of long-duration cotton hybrids on a large scale has a pronounced impact on the pink bollworm incidence (Prasada Rao, 2022). Mitochondrial DNA is widely used in taxonomy and systematics to explore the phylogenetic relationships of insects (Simon *et al.,* 1994; Boykin *et al.,* 2006) owing to their maternally inherited and well conserved genomic segments.

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As it evolves in a nearly neutral fashion, it reflects the divergence times, which can be used as a robust marker for determining genetic relationships and geographical studies (Bermingham and Lessios, 1993; Armstrong and Ball, 2005; Galtier *et al.*, 2009; Prabhakar *et al.*, 2013). Further, the genetic constitution of a pest is vital in determining the capacity to tolerate adverse climatic conditions and adoption to new conditions (Hayden *et al.*, 2011).

Population genetic structure and genetic diversity define the level of adaptation of a population to environmental change and susceptibility to selection pressure (Mopper, 1996; Pauls *et al.*, 2013). Gene flow through dispersion and migration, which is responsible for determining genetic variation leads to the evolution of local populations (Kremer *et al.*, 2012). In some lepidopteran species, the genetic diversity and structure were reported to be related to the migration capacity (Chen and Dorn, 2010; Men *et al.*, 2013). Studies on population structure and genetic diversity of pink bollworm (PBW) have been explored more in Asiatic countries such as India, Pakistan and China owing to the development of resistance to Bt cotton by PBW populations (Liu *et al.*, 2010; Sridhar *et al.*, 2017). Liu *et al.* (2009) studied the population genetic structure of Chinese PBW using mitochondrial *COI* and *Nad4* and found extremely low genetic variability among all populations examined. However, sequence variation in the *Nad4* region differentiated the Chinese populations from the Pakistani and American populations. Haplotypes and differentiation in PBW populations of China were identified using piggy Bac-like elements (Wang *et al.*, 2010). Sridhar *et al.* (2017), based on the analysis of the pink bollworm population from 19 districts, found that the pink bollworm population in India exhibited a low level of genetic diversity, and based on haplotype diversity results, they also opined that the populations might be experiencing population expansion; However, they could not provide the evidence through neutrality tests owing to the small population size. Thus the present investigation was designed to analyze the genetic diversity and distribution of *P. gossypiella* from selected populations using the *mitochondrial cytochrome oxidase I (mt COI)* gene.

MATERIALS AND METHODS

Pink bollworm samples were collected from the major cotton-growing tracts of Tamil Nadu *viz.* Coimbatore, Srivilliputhur, Veppathanttai and Salem were used. The collected larvae were stored in Eppendorf tubes with 70 per cent ethanol @ -20°C before DNA extraction. The genomic DNA was extracted from pink bollworm larvae using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle, 1991) with necessary modifications. Homogenization of larvae with 600 ìl of CTAB buffer (CTAB buffer: 1 mM Tris HCl; 0.5 mM EDTA; 5 mM NaCl; 2 % beta-mercaptoethanol, 2 % CTAB and pH 8.0) and made up to 1 ml with CTAB buffer. The mixture was incubated for 30 - 60 minutes at 65°C. Then treated with 500 ìl phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged at 12000 rpm for 10 minutes. The supernatant was transferred to a fresh Eppendorf tube containing 300 ìl chloroform-isoamyl alcohols (24:1) and the mixture was centrifuged at 12000 rpm for 10 min. Again, the supernatant was transferred to the fresh Eppendorf tube containing 250 to 500 ìl of isopropanol, for precipitation. The pellets were recovered by centrifugation @ 12000 rpm for 10 minutes and washed with 70 per cent ethanol and re-suspended in 30 - 50 ìl DEPC treated water. The quality and quantity of DNA were assessed using a NanoDrop spectrophotometer (Thermos Scientific, USA) and 0.8 per cent agarose gel.

PCR amplification and sequencing: The mitochondrial genes are inherited maternally and show sufficient diversity among the insect species. Hence, the primers (Table 1) which amplify the mitochondrial genes *viz*., *cytochrome c oxidase subunit I* was employed to generate PCR products. PCR analyses were performed in 25 ìl total reaction volume using 1 ìl of each forward (LCO1490) and reverse (HCO2198) primer. Polymerase Chain Reaction (PCR) was carried out in a thermal cycler with the following cycles: 94°C for 4 minutes as initial denaturation followed by 35 cycles of 94°C for 30 seconds, 48°C for 45 seconds, 72°C for 45 seconds and 72°C for 20 minutes as a final extension.

Table 1. Details of the COI primers used

No	Primer	Sequence (5'-3')	Tm*	Reference
		***COI* Primers**		
1	LCO1490	GCTCAACAAATCATAAGATATTGG	61.2°C	Folmer *et al.,* 1994
2	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	66.9°C	Folmer *et al.*, 1994

The amplified products were resolved in 1.5 per cent agarose gel, stained with ethidium bromide (10 ìg ml^{-1}) and visualized in a gel documentation system (Asokan *et al.,* 2011). The amplicons were commercially sequenced bidirectionally using the SANGER sequencing method (@ Biokart India Pvt. Ltd., Bangalore). The sequences were characterized using bioinformatics tools such as BLAST to check their homology. The sequences including all of the geographic populations were submitted to GenBank repositories and registered with accession numbers (Jiang *et al.,* 2014).

Phylogenetic analysis: The phylogenetic analysis based on the maximum likelihood (ML) method was performed using MEGA X version 7.0.9 (Kumar *et al.*, 2018) for investigating the degree of consistency of mutation patterns in different regions of India.

Data analysis for genetic divergence and haplotype distribution: Mitochondrial *COI gene* sequences were edited using Bio edit (Hall, 1999) and aligned using the ClustalW program (Thompson *et al.*, 1994) using MEGA X (Kumar *et al.*, 2018). Descriptive statistics on the number of haplotypes (H), haplotype diversity (Hd), variance and standard deviation of haplotype diversity were calculated using DnaSP version 5.10.01 (Librado and Rozas, 2009). Population history was inferred based on values of haplotype diversity (Hd) and nucleotide diversity (Pi). Small values of Hd and Pi (Hd < 0.5 and Pi < 0.005) represent population bottelneck or founder event by single or a few mtDNA lineages; high Hd and low Pi (Hd > and Pi < 0.005) represent population bottleneck folllowed by rapid population growth and accumualtion of mutations; low Hd and high Pi (Hd < and Pi > 0.005) represent divergence between geographically subdivided populations; large values of Hd and Pi (Hd > 0.5 and Pi > 0.005) represent large stable population with long evolutionary history or secondary contact between differentiated lineages To depict the evolutionary and geographical relationships among haplotypes, a minimum spanning haplotype network was constructed with PopART version 1.7 (Bandelt *et al.,* 1999).

Neutrality test and genetic differentiation: Fu and Li's D test; Fu and Li's F test and Tajima's D tests of neutrality index and genetic diferentiation are useful for demographic history information, with demographic expansion related to negative values and subdivided populations at equilibrium leading to positive values and hence they were also investigated using DnaSP version 5.10.01 for detecting the range of population expansions (Librado and Rozas, 2009).

RESULTS AND DISCUSSION

Phylogenetic analysis: The homology search of the sequences using NCBI BLAST matched with the *mt COI* sequences of pink bollworm in NCBI database, and the sequence similarity varied from 98 - 100 per cent. The nucleotide sequences were further aligned using MEGA version 7.0.9 and used for further analysis. The trimmed sequences were deposited in NCBI Gene Bank and accession numbers were obtained (Table 2). In these types of analyses, the nucleotide mismatch for each region was selected using the Tamura-Nei model. The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. The evolutionary distances were computed using the Tamura 3 parameter method (Tamura, 1992) and were in the units of the number of base substitutions per site. This analysis involved 28 nucleotide sequences (Fig. 1).

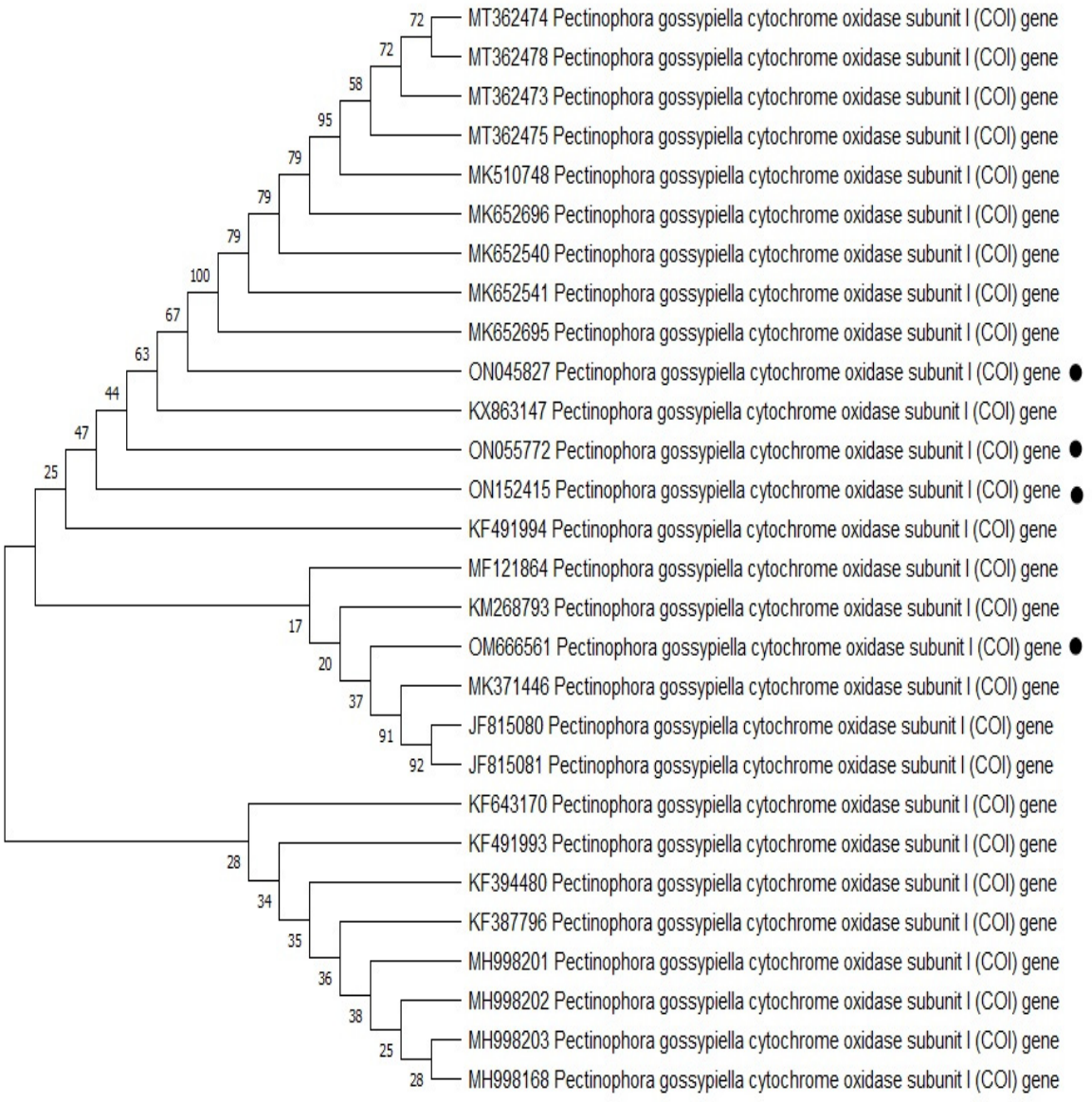

Fig. 1 Phylogenetic tree of the given *mt COI* gene sequences of *Pectinophora gossypiella*

The GenBank accession number with scientific names was included. Phylogenetic tree for the isolates of pink bollworm were constructed using the sequences of *COI* gene. The isolated *COI* genes were labelled as black spot (Table 2). Other sequences for the *COI* gene phylogenetic analysis were also obtained from the GenBank database (Table 3).

Table 2 *Pectinophora gossypiella mt COI* sequences developed and used in the current study

Collection	Location	Accession	*mt COI*
Coimbatore	11.0167N, 76.9350E	OM666561	638bp
Srivilliputhur	9.5045N, 77.6440E	ON055772	624bp
Veppanthattai	11.3516N, 78.8043E	ON045827	551bp
Salem	11.6560N, 78.4690E	ON152415	624bp

Table 3. *P. gossypiella mt COI* sequences from GenBank used in the current study

Location	Accession	*mt COI*
New Delhi, India	MH998201	651bp
New Delhi, India	MH998202	651bp
New Delhi, India	MH998203	651bp
Pakistan	KX863147	570pb
Pakistan	MK371446	687bp
USA	JF815080	688bp
USA	JF815081	688bp
Australia	KF387796	658bp
Australia	KF394480	658bp
USA	KF491994	642bp
USA	KF491993	658bp
Kenya	KF643170	658bp
Kenya	MF121864	658bp
Junagarh, India	MH998168	651bp
Bangalore, India	KM268793	658bp
Parbhani, India	MK652696	686bp
Parbhani, India	MK652695	686bp
Bangalore, India	MT362473	753bp
Guntur, India	MT362474	753bp
Raichur, India	MT362475	753bp
Warangal, India	MT362478	753bp
Akola, India	MK510748	711bp
Akola, India	MK652541	686bp
Akola, India	MK652540	686bp

Minimum spanning network among haplotypes: A minimum spanning network of haplotypes (Bandelt *et al.,* 1999) was generated using PopART version 1.7 (Fig. 2). The results confirmed five haplotypes *viz.* Hap_1, Hap_2, Hap_3, Hap_4 and Hap_5 for the selected populations of the pink bollworm. The network suggests that the most common haplotype of *COI gene* is Hap_1 followed by Hap_5, which was linked by Hap_3. These could be proposed as ancestral/ original haplotypes. Haplotypes were generally low in frequency with few populations formed and connected to nearby major haplotypes through several steps. The phylogenetic relationship studies revealed that most haplotypes were closely related to each other with the *COI* of the pink bollworm populations. The populations used in the study which were collected from Tamil Nadu falls under Hap_1 and Hap_2, which confirmed its association between the populations (Table 4).

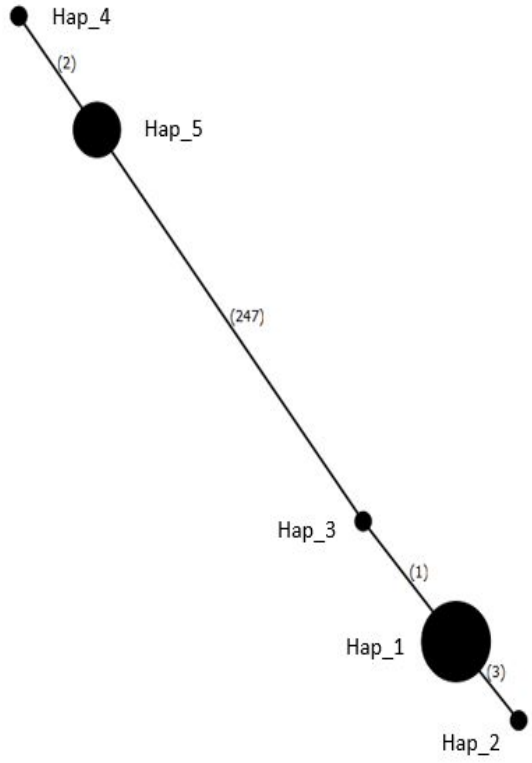

Fig. 2 *Haplotype network of Pectinophora gossypiella generated by DnaSP version 5.10.01 based on cytochrome c oxidase I sequences. A minimum spanning network was constructed using PopART version 1.7. Sequences with unverified species assignment and potential contaminants were not considered in the analyses. The circle size is proportional to the frequency of haplotypes. The numbers on the lines represent the number of nucleotide substitutions between linked haplotypes.*

Table 4. Comparison of the outputs of the presented identification tool and result of genetic analysis- Gene *COI*

Sequences	Haplotype - sequence analysis
Hap_1	OM666561_*Pectinophora gossypiella*
	ON152415_*Pectinophora gossypiella*
	ON055772_*Pectinophora gossypiella*
	MH998201_*Pectinophora gossypiella*
	MH998202_*Pectinophora gossypiella*
	KX863147_*Pectinophora gossypiella*
	MK371446_*Pectinophora gossypiella*
	JF815080_*Pectinophora gossypiella*
	JF815081_*Pectinophora gossypiella*
	KF387796_*Pectinophora gossypiella*
	KF394480_*Pectinophora gossypiella*
	KF491994_*Pectinophora gossypiella*
	KF491993_*Pectinophora gossypiella*
	KF643170_*Pectinophora gossypiella*
	MF121864_*Pectinophora gossypiella*
	MH998168_*Pectinophora gossypiella*
	KM268793_*Pectinophora gossypiella*
Hap_2	ON045827_*Pectinophora gossypiella*
Hap_3	MH998203_*Pectinophora gossypiella*
Hap_4	MK652696_*Pectinophora gossypiella*
Hap_5	MK652695_*Pectinophora gossypiella*
	MT362473_*Pectinophora gossypiella*
	MT362474_*Pectinophora gossypiella*
	MT362475_*Pectinophora gossypiella*
	MT362478_*Pectinophora gossypiella*
	MK510748_*Pectinophora gossypiella*
	MK652541_*Pectinophora gossypiella*
	MK652540_*Pectinophora gossypiella*

Population level diversity: Genetic diversity parameters including the number of haplotypes (5), haplotype diversity (0.566 Hd), nucleotide diversity (Pi 0.21625), variance of haplotype diversity (0.00631), and standard deviation of haplotype diversity (ì 0.079) that were determined with DnaSP version 5.10.01 suggested that they represented the large stable population with long evolutionary history or secondary contact between differentiated lineages.

Demographic history analysis: Tajima's D test (2.88869, P < 0.01**), Fu and Li's D test (1.82374, P < 0.02**), and Fu and Li's F test (2.57145, P < 0.02**), for *COI gene* in populations of cotton pink bollworm revealed high significance. Neutrality tests were accepted for all populations with significant positive values which confer to the hypothesis of past population expansion events. Three neutrality tests were performed and values were positive for populations of *COI,* indicating there was no excess of mutations which favours population expansion or growth. Thus, the results obtained in this study accept the hypothesis of neutral evolution for the investigated cotton PBW population and showed the demographic history among them.

A haplotype is a set of DNA variations (polymorphisms such as SNPs and InDels) adjacent to one another at the same locus that tend to be inherited together. This set of alleles is often referred to as linked polymorphisms (Durbin and Lathrop, 2010; Li *et al.,* 2010). The studies revealed that total of 5 unique haplotypes while 17 individuals at Hap_1 and 8 individuals at Hap_5 were inferred from mitochondrial *COI* sequences. It has been supported by Sridhar *et al.* (2017) who found that 12 (15.18 %) haplotypes in 79 individuals were distributed in 19 populations in Indian subcontinent. The first most predominant haplotype was found in 143 individuals and the second most predominant haplotype was shared with 32 individuals as confirmed with the haplotype network shared by populations across different zones.

This study, reported low mitochondrial DNA variations (0.566 Hd) in taxa that might have undergone severe bottlenecks or founder effects. These results have supported by Sridhar *et al.* (2017) who found 0.3028 Hd in overall populations of pink during 2011 - 2012 populations before they broke the resistance to both *Cry1Ac* and *Cry2Ab* in India. Similarly extremely low level of population genetic variation was also observed in the two mitochondrial regions (*COII and Nad4*) among the nine Chinese Pink Bollworm populations (Liu *et al.,* 2010) and such variations were attributed to invasion bottlenecks, which had subsequently strengthened by its non-migratory biology and the mosaic pattern of agricultural activities (Liu *et al.,* 2010). These

haplotype information can be categorized by its applications in the identification of pink bollworm as well as its adaptation to diverse ecological niches and mechanisms of insecticide resistance. Proper assimilation and understanding of the genetic diversity of an insect pest have been found essential to mitigate and improve its monitoring that further facilitating the implementation of need based management strategies (Naik *et al.,* 2020).

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Dissipation kinetics, effect of household processing, and dietary risk assessment of the insecticide chlorantraniliprole residue in bitter gourd and soil

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ABSTRACT: A single quadrupole liquid chromatography-mass spectrometry (LC-MS) method was validated to determine the insecticide chlorantraniliprole residues in bitter gourd fruit, juice, and soil, according to the SANTE guidelines. A linear curve was obtained ($R^2 > 0.99$) with LOD and LOQ at 0.003 mg kg^{-1} and 0.01 mg kg^{-1}. The accuracy (87–102%) and precision (RSD <5%) of the method was found to be satisfactory. The dissipation pattern of chlorantraniliprole 18.5% SC was studied by spraying twice at ten days intervals, at the recommended dose (X) (25 g a.i. ha^{-1}), and double the recommended dose (2X) (50 g a.i. ha^{-1}). The initial deposit on bitter gourd was 0.72 and 1.41 mg kg^{-1} and residues persisted up to 15 and 20 days with a half-life of 2.44 and 2.79 days at X and 2X doses, respectively. Simple decontamination techniques were found to reduce residues to the extent of 30 to 80 per cent. The reduction of chlorantraniliprole residues in bitter gourd juice was 40-50 per cent by different washing techniques. The estimated level of Risk quotient indicated (<1) chlorantraniliprole residues pose no dietary risk to consumers at the level detected.

KEY WORDS: LC-MS, validation, residue, persistence, dissipation pattern, decontamination, fruit, juice

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is a most common and preferred vegetable among the Cucurbitaceae family, grown in India (Singh and Sagar, 2013). The fruit is a rich source of vitamins (B1, B2, B3, B9 and C (88 mg100 g^{-1})), minerals (magnesium, zinc, manganese and phosphorus) and dietary fibre (Krishnendu and Nandini, 2016). The immature fruit contain anticancer and antiviral characteristics, and is useful in treating diabetic diseases (Tan *et al*., 2016). During the year 2020-21 the area and production of bitter gourd were 1.07 lakh hectares and 12.96 lakh metric tonnes (MT) in India respectively, whereas it was 0.24 lakh hectares and 0.44 lakh MT in Tamil Nadu respectively (NHB, 2021). As that of any cultivated vegetables, in bitter gourd also yield is threatened by different insect pests *viz.* aphids, melon fruit fly, hadda beetle, pumpkin caterpillar, and leaf miner. Fruit flies are the major pest of cucurbitaceous

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crops, causing output loss of 30 to 100 per cent depending on crop growth stages and seasons (Dhillon *et al*., 2005), thereby, warranting the application of insecticides for crop protection.

Approximately 13-14 per cent of total pesticide usage (0.678 a.i. kg/ha), including 4 per cent of insecticides is sprayed on vegetables and yields of most vegetables would fall by 50-90 per cent without insecticide (Subash and Kulvir, 2018). Notably, every dollar ($1) spent on pesticide for crops might save up to four dollars ($4) (Zhang, 2018). Inspite of economic protection, frequent application and indiscriminate pesticide usage particularly during the fruiting stage and unsafe waiting periods, may result in an accumulation of pesticide residues in vegetables (Lozowicka *et al*., 2014). The pesticide residues, left in the harvested vegetables may be hazardous to human health and affect trade due to pesticide residues exceeding Maximum Residue Limits (MRL) (Pimentel and Burgess, 2014). The investigation on overall pesticide usage profile in gourds ecosystem showed Chlorantraniliprole 18.50 per cent SC (76.67%) was commonly used insecticide for the management of insect pests (Mawtham *et al*., 2022). Keeping this background, a study was carried out to determine the dissipation and decontamination of chlorantraniliprole in bitter gourd.

Chlorantraniliprole, anthranilic diamide systemic insecticide is effective against Coleopteran, Lepidopteran and few Dipteran pests infesting crops such as bitter gourd, okra, chilli, brinjal and tomato (fruit borers), cabbage (diamondback moth), legumes (pod borers) (CIBRC, 2022). This compound has a unique mode of action and act on ryanodine receptor channels leading to inhibited regulation of muscle contraction due to internal Ca^{2+} store exhaustion (Bentley *et al*., 2010). The greater structural differences at ryanodine receptors between insects and mammals makes the insecticide highly selective and safe (Lahm *et al*., 2007). The physiochemical properties of chlorantraniliprole are water solubility (0.880 mg L^{-1} at 20^0C), vapor pressure (6.3 x 10^{-12} Pa at 20°C, 2.1 x 10^{-11} Pa @ 25°C), octanol/water partition coefficient (P) (pH 7 – 7.24 X 10^2 K_{OW} @ 20^0C) and dissociation constant (10.88 ± 0.71 pKa) (PPDB, 2022). It is an alternative for synthetic pyrethroids to vegetables because it is mentioned as a "low risk" insecticide (USEPA, 2008).

Instrumental analytical procedures for the quantitative analysis of chlorantraniliprole residues in crops like, capsicum, cauliflower, berseem, tomatoes, corn and soil using gas chromatography mass spectrometry, liquid chromatography mass spectrometry, high performance liquid chromatography and liquid chromatography with Orbitrap Mass spectrometry are available (Pathipati *et al*., 2017; Ahlawat *et al*., 2019; Malhat *et al*., 2012; Kar *et al*., 2013; Mandal *et al*., 2014; Dong *et al*., 2011). As there are no available published literature on chlorantraniliprole in bitter gourd, this research on developing and validating an analytical approach for detection and confirmation of chlorantraniliprole residues in bitter gourd fruit, juice and soil using LC-MS (liquid chromatography coupled with mass spectrometry) was undertaken. Furthermore, washing, peeling, cooking, blanching and other household techniques were reported to reduce the residual levels in food (Byrne and Pinkerton, 2004). Therefore, a study was also undertaken to evaluate whether or not simple culinary processes such as washing and cooking can minimise the pesticide residues in bitter gourd fruit and juice.

MATERIALS AND METHODS

Chemical and reagents: Certified reference material (CRM) of chlorantraniliprole (purity, 98.3%) was procured from Sigma-Aldrich Pvt. Ltd. (Bangalore, India). The commercial formulation (chlorantraniliprole 18.5% SC) was obtained from a local pesticide shop in Coimbatore, Tamil Nadu, India. Acetonitrile, formic acid and ammonium formate of LC-MS grade (Sigma Aldrich), LC-MS grade methanol (MeOH) (Fisher chemical, USA), Sodium chloride (NaCl) (>99% purity), anhydrous magnesium sulphate ($MgSO_4$) (>99.5% purity) and sodium sulphate (Na_2SO_4) (>99%) (Merck, Mumbai, India), Sorbents like graphitized carbon black (GCB) and primary secondary amine (PSA, 40 ìm) (Agilent Technologies, USA) were purchased from commercial suppliers as indicated.

Before usage, the magnesium sulphate was baked in a muffle furnace at 400 °C for 4 hours and maintained in an airtight desiccator in order to prevent the moisture absorption. Throughout the analysis, purified Millipore water (18.2 MÙ) from a lab-scale (Q3 Merck) Millipore unit was used.

Preparation of standard solutions: The chlorantraniliprole stock solution (400 mg L^{-1}) was prepared in methanol (LC-MS grade) by accurately weighing 10.17 mg of analytical standard into a calibrated (Class A) 25 ml volumetric flask. The intermediate standard (40 mg L^{-1}) was prepared by transferring 2.5 ml from the stock solution (400 mg L^{-1}) into a 25 ml volumetric flask and the volume was made with methanol. Serial dilution from the intermediate standard solution was made in the range of 0.0025 – 0.5 mg L^{-1} and matrix match standard solutions were prepared at 0.01, 0.025, 0.05, 0.075 and 0.1 mg L^{-1}. All standard solutions were kept in a -20 ^{0}C freezer until further use.

Field experiment: A supervised field trial was carried out in farmer's field at Annur block, Coimbatore district, Tamil Nadu, India (11.22^0 N latitude and 77.10^0 E longitude) from November 2021-March 2022, to study the dissipation pattern and decontamination of chlorantraniliprole in bitter gourd fruit. Bitter gourd (Eastwest F1 hybrid) was raised in 250 m^2 plot/treatment with three treatments following good agronomic practices. Chlorantraniliprole was applied at recommended dose @25 g a.i ha^{-1}(X) and double the recommended dose @50 g a.i ha^{-1} (2X) as per CIB&RC (2022) recommendations. The first spraying was done after 45 days of sowing followed by second spray at 10 days interval. An untreated plot (water spray) was maintained throughout the study period. Two consecutive sprays were done using a 500 L ha^{-1} spray fluid, high-volume knapsack compression sprayer during morning hours. During the field experiment, average maximum (29.0 ^{0}C) and minimum temperature (18.2 ^{0}C) and relative humidity (77.2 %) were recorded and there was no rainfall.

Sample collection and preparation: Two kilograms of bitter gourd fruit samples were randomly collected at each sampling intervals 0 (within 2 hr), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days from treated and control plot after last insecticide application for dissipation study. The soil samples (15 days after spraying) collected at 0-15 cm depth from each plot were mixed, air-dried, homogenised, crushed and sieved (2 mm pore size). The collected samples were labelled separately and transported to the laboratory to carry out residue analysis. A high-volume blade homogeniser (Robot Coupe, Blixer 6 VVA, France) was used to homogenise the samples. Bitter gourd juice was extracted from homogenised extract of treated and untreated samples diluted 200 ml with water (100 ml) and filtered through a strainer. All the samples were stored at -20 ^{0}C until residue analysis.

Extraction and clean-up: The chlorantraniliprole residues were extracted and cleaned up from bitter gourd fruit, juice, and soil matrices by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Anastassiades *et al*., 2003). Representative samples (3 replicates each) of 10 g were weighed in a 50 ml polypropylene centrifuge tube, 20 ml of acetonitrile was added and vortexed for one minute. Then four gram of anhydrous magnesium sulphate and one gram of sodium chloride were added, mixed thoroughly using vortex and centrifuged for 10 minutes at 6000 rpm. After centrifugation, the upper acetonitrile layer (10 ml) was passed through anhydrous sodium sulphate (4 g) to remove moisture traces. A six-millilitre supernatant was transferred into a 15 ml centrifuge tube containing PSA (150 mg), GCB (25 mg) and $MgSO_4$ (900 mg), vortexed for one minute and centrifuged at 3000 rpm for 10 min. A four-millilitre supernatant acetonitrile phase was carefully pipetted out to a clean glass tube and evaporated to near dryness at 35°C using turbovap LV with a gentle stream of nitrogen. The residues were then redissolved in one millilitre of methanol, filtered through a 0.2-micron PTFE syringe filter (Millipore, USA) and transferred to 1.5 ml autosampler glass vials for LC-MS analysis.

Chlorantraniliprole residue was estimated using Shimadzu 2020 series LC-MS containing reverse phase C18 (Eclipse plus- Agilent) column (250 mm length x 4.6 mm id, 5 µm particle size) at a column oven temperature of 40°C. Solvents A (ultra-pure

water with two mM ammonium formate, 0.05 per cent formic acid) and B (Methanol with two mM ammonium formate, 0.05 per cent formic acid) were used as mobile phase (30:70 V/V). Before use, mobile phases were degassed for 20 min in a sonicator. Then, the mobile phase was discharged at a constant isocratic flow rate of 0.5 ml/min using the LC-MS pump in binary mode at a pressure of 48 kg/cm^2. Shimadzu lab solutions software version 5.6 was used to operate the instruments and analyse the chromatograms. The chromatograms and sample were ionized by positive electrospray ionization (ESI+) in selected ion monitoring (SIM) at m/z 483 and interface mode with 0.1 ìA^o of interface current. The optimised instrument parameters were capillary voltage 3.5 kV; heat block temperature 200 ^{0}C; desolvation line temperature 250 ^{0}C; nebulizer gas (N_2-99.99%) flow (1.5 lmin^{-1}), drying gas (15 lmin^{-1}), injection volume was 10 µl and 15000 sec scan speed. The residue detection method was developed and validated for the parameters such as linearity, sensitivity, accuracy, and precision and matrix effect (SANTE, 2021).

Linearity: Chlorantraniliprole linearity curves were obtained for solvent and matrix match calibration standards by injecting seven concentrations ranging from 0.0025 to 0.5 mg kg^{-1} in LC-ESI-MS with six replications. The linear relationship between the concentration and signal area were calculated.

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ) were calculated by injecting the matrix match standards of chlorantraniliprole starting from lowest concentration level (0.01 mg kg^{-1}). Based on the calibration curve, LOD and LOQ were calculated.

LOD = 3 x (Standard Deviation/Slope)
LOQ = 10 x (Standard Deviation/Slope)

Accuracy (recovery): Experiments were carried out by spiking five different concentrations of chlorantraniliprole (0.01, 0.025, 0.05, 0.075 and 0.1 mg kg^{-1}) in bitter gourd fruit, juice and soil samples with six replications. Recovery (%) was calculated by comparing the peak area of the known quantity of analytes in the spiked sample (prior extraction) and matrix match standard.

Precision (repeatability): The precision of the method was evaluated through relative standard deviation (RSD) for each spiking level (0.01 to 0.1 mg kg^{-1}) of bitter gourd fruit, juice and soil matrix.

Matrix effect (ME): The peak area of the matrix standard was compared with the peak area of the solvent standard to measure matrix effects (Mariappan and Kaithamalai, 2020). The ME was calculated using the following equation:

$$ME\ (\%) = \frac{\text{(Peak area of matrix standard - Peak area of solvent standard)}}{\text{Peak area of matrix standard}} \times 100$$

Data Analysis: The concentration of chlorantraniliprole residue was calculated using—

$$\text{Residue (mg kg}^{-1}) = \frac{A_1 \times C \times I_1 \times F}{A_2 \times W \times I_2}$$

Where A_1= Peak area of chlorantraniliprole in the sample solution,

A_2 = Peak area of chlorantraniliprole in the standard solution,

C = Concentration of standard solution (mg kg^{-1}),

I_1 = Injected volume of standard (ìl), I_2 = Injected volume of sample (ìl), W = mass of the sample (g) and F = Final volume of the sample (ml).

The dissipation of chlorantraniliprole residue followed first-order rate of kinetics equation $Ct = Coe^{-kt}$, where, Ct is the insecticide concentration (mg kg^{-1}) at time t, k is the dissipation rate constant and Co is the apparent initial concentration (mg kg^{-1}) (Mariappan and Kaithamalai, 2020). $T_{1/2} = \ln (2)/k$ was used to compute the half-life of chlorantraniliprole and pre-harvest interval (PHI) was calculated using the formula PHI = [ln Co - ln MRL]/k (Hoskins, 1961; Handa *et al*., 1999). According to the Codex Alimentarius and the European pesticide (EU) database, the maximum residue limit (MRL) for chlorantraniliprole in bitter gourd fruit is 0.3 mg kg^{-1}.

Decontamination studies: The effect of simple culinary practices in removing chlorantraniliprole residues from bitter gourd fruit was assessed through laboratory experiment. After second spraying, samples were collected at 0, 1, 2, 3, 5, 7 and 10 days. For each treatment, a sample of one kilogramme of bitter gourd fruit was taken. The fruits were then subjected to decontamination methods *viz*., washing with tap water (pH 7.0), 2% salt solution, lukewarm water (40 ^{0}C), 2% tamarind solution, 2% lemon solution, and also subjected to cooking. Washing was done for one min which cooking was done for 10 min under open cook method. The treatments were also combined and evaluated as methods like, tap water washing + 2% salt solution + 10 min cooking, tap water washing + 2% tamarind solution + 10 min cooking, tap water washing + 2% lemon solution + 10 min cooking. Decontamination solutions (2%) were made by mixing 20 g of each common salt, tamarind and lemon juice in one litre of water and the fruits were gently rubbed in the solution for about 1 min. In lukewarm (40 ^{0}C) and tap water (1L) treatment, fruit were immersed for 1 min and then gently rubbed with hands. In cooking treatment, part of fruit sample was cut into small pieces and cooked in boiling water (1 L for each 500 g sample) for 10 min. Washed and cooked samples were dried using blotting paper and then homogenised. Chlorantraniliprole residues were estimated by following the above standardized methodology.

Processing factor: The Processing Factor (PF) is a method to determine the risk of insecticide residue intake in processed foods. The PF less than one suggests a decrease in residue in the processed food, while a PF more than one indicates concentration of residue (Scholz *et al.*, 2017).

$$PF = \frac{\text{Residue of processed product (mg kg}^{-1})}{\text{Residue of raw agricultural commodity (mg kg}^{-1})}$$

Dietary risk assessment: The maximum residue obtained from the field trial was multiplied by the average food consumption rate (60 g day^{-1}) (NIN, 2020) divided by the average adult male (65 kg) and female weight (55 kg) to compute the estimated daily intake (EDI) of chlorantraniliprole residue (Dong *et al*., 2018). The risk quotient (RQ) was derived by dividing the EDI by acceptable daily intake (ADI) of the insecticide and expressed in mg kg^{-1} body weight (BW)/day. The ADI for chlorantraniliprole is 1.58 mg kg^{-1} BW day^{-1} (EFSA, 2012). The risk of long-term human dietary consumption of chlorantraniliprole residues in food is acceptable when RQ is less than one and unacceptable if RQ is more than one.

RESULTS AND DISCUSSION

Method validation

The results of method optimization were satisfactory for all validation parameters studied following the SANTE guidelines (SANTE, 2021). Linear response was assessed for different solvent concentrations (0.0025-0.5 mg kg^{-1}) and matrix match standard concentration (0.01-0.1 mg kg^{-1}) (Fig. 1). Good linear curve and correlation coefficient (R^2) values of chlorantraniliprole in solvent (0.999), fruit matrix match standards (0.997), fruit juice (0.998) and soil (0.998) (Fig. 2) were obtained. LOD was 0.003 mg kg^{-1} and LOQ was 0.01 mg kg^{-1}. In bitter gourd fruit, juice, and soil, the recovery was within 87.45-101.08 per cent and RSD was 1.02-4.22 per cent (Table 1 and Fig. 3 (a,b,c)). The LOQ estimated using above method was less than the MRL value (0.3 mg kg^{-1}). The matrix effect was within 0.35-9.86 per cent of the spiked chlorantraniliprole standards in the bitter gourd fruit and soil. Matrix effect was not found in fruit juice. Matrix effect values were less than 20 per cent in both matrices, indicating that the samples had no apparent matrix effect and that the purification effect was acceptable.

Dissipation of chlorantraniliprole in bitter gourd and soil

Bitter gourd and soil samples were collected at periodical interval from 0 to 30 days after the last application for residue analysis. The average residues of chlorantraniliprole in bitter gourd fruits were 0.72 and 1.41 mg kg^{-1} as initial deposit at single and double the doses (Table 2 and Fig. 3d). After third day, more than 50 per cent of the residues

Table 1 Recovery percentage of chlorantraniliprole in different matrices of bitter gourd

Spiked (mg kg-1)	Recovered conc	Recovery* (mg kg-1)	Repeatability (RSD %)
		Fruit	
0.01	0.01	101.08 ± 1.03	1.02
0.025	0.03	100.03 ± 1.66	1.66
0.05	0.05	96.16 ± 3.48	3.62
0.075	0.07	99.21 ± 2.56	2.58
0.10	0.10	96.97 ± 2.70	2.78
		Juice	
0.01	0.01	100.86 ± 3.90	3.87
0.025	0.02	96.72 ± 4.08	4.22
0.05	0.04	93.97 ± 3.47	3.69
0.075	0.06	92.57 ± 4.18	4.51
0.10	0.09	90.94 ± 3.04	3.34
		Soil	
0.01	0.01	92.62 ± 1.50	1.62
0.025	0.02	92.82 ± 1.96	2.12
0.05	0.04	87.45 ± 1.26	1.44
0.075	0.07	94.50 ± 2.68	2.83
0.10	0.09	92.49 ± 2.63	2.85

*Mean of six replications, RSD- Relative Standard Deviation

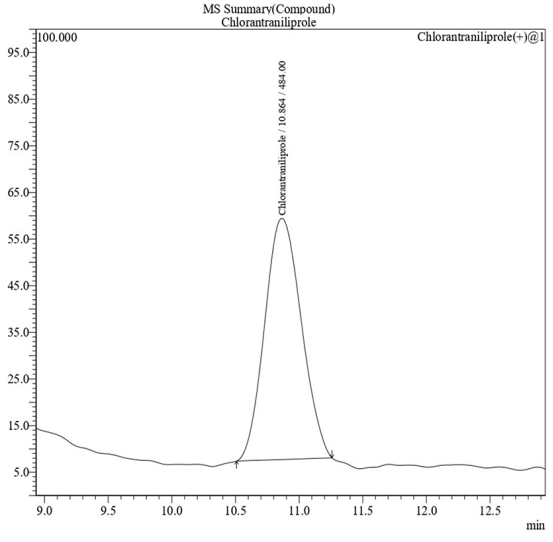

Fig. 1. LC-MS chromatogram of chlorantraniliprole standard (0.01 mg kg^{-1})

dissipated from bitter gourd and reached BLQ (0.01 mg kg^{-1}) on 15 and 20 days after application in single and double the doses, respectively (Fig. 4). The chlorantraniliprole residue was not detected in soil samples collected at harvest (15 days after second spray). The residual deposition and persistence of pesticides are affected by several factors: type of pesticide, its formulation, active ingredient, carrier material, meteorological parameters, plant growth, and type of plant (Lavtizar *et al*., 2014). Initial concentration and persistence of chlorantraniliprole residues varied with crops. In capsicum, chlorantraniliprole residue levels were 3.16 and 4.18 mg kg^{-1} on 0 day after treatment at 30-60 g a.i. ha^{-1} (Ahlawat *et al*., 2019). In tomato fruit the initial residue concentration of chlorantraniliprole was 2.31 mg kg^{-1} and reached BLQ (0.01 mg kg^{-1}) after 21 days of application at 30 g a.i. ha^{-1} (Malhat *et al*., 2012). Chlorantraniliprole initial residues were 0.18 and 0.29 mg kg^{-1} and reached BLQ after 5 and 7 days from last application on cauliflower (Kar *et al*., 2013). In okra, chlorantraniliprole residue in soil was BLQ after 15 days of last application and was attributed to microbial and chemical degradation (Singla *et al*., 2020).

Half-life and pre-harvest interval (PHI)

The half-life (DT_{50}) values of chlorantraniliprole were 2.44 and 2.79 days at 25 and 50 g a.i. ha^{-1}, respectively (Table 2). The pre-harvest interval (PHI) was calculated using the kinetic equation obtained from a graph of residues vs time. The PHI was found to be 3.09 and 6.24 days for 25 and 50 g a.i. ha^{-1} of chlorantraniliprole, respectively (Table 2). This shows that consumption of bitter gourd is safe at 3.09 days if recommended dose is followed and at higher doses of chlorantraniliprole 6.24 days is recommended for safe harvest of bitter gourd fruit. The chlorantraniliprole residues dissipated with the half-life of 2.70 days in grapes and 10.0 and 15.2 days in cabbage (Malhat, 2012; Lee *et al*., 2019). Treatment of chlorantraniliprole at 30 and 60 g a.i. $ha^{"1}$ in brinjal required a PHI of 0.69 and 2.38 days (Vijayasree *et al*., 2015).

Decontamination of chlorantraniliprole residue in bitter gourd fruits and juices

The effect of different household techniques on the reduction of chlorantraniliprole residues was studied. All decontamination procedures were effective in terms of reducing chlorantraniliprole residues from the bitter gourd fruits (Table 3,4). Among the various decontamination methods, tap water washing + salt solution+ cooking, tap water washing + lemon solution + cooking and tap water washing + tamarind solution + cooking were all very effective in removing chlorantraniliprole residues to the extent of 75.10 - 80.70 per cent in both doses of treatments. In cabbage and cauliflower (17-40%), reduction of chlorantraniliprole residues was observed by washing with tap water and above 90 per cent by boiling (Kar *et al*., 2012). Chlorantraniliprole is a systemic insecticide with moderate solubility (pH 7- 0.880 mg/L) in water. Solubility of pesticide compound in polar and non-polar solvent determines the octanol/water partition coefficient (Kow) ratio. Higher the Kow value higher will be the absorption and retention (Finizio *et al*., 1997). The Kow value of chlorantraniliprole is moderate (7.24×10^2) and hence less removal by washing was expected (<48%). Bitter gourd treated with, phorate, chlorpyriphos (4.70×10^2), parathion (2.5×10^4), permethrin (6.1×10^1) and captafol showed 17.0 to 78.89 per cent loss of residues by washing (Joshi *et al*., 2015). Among the household techniques, cooking process caused the maximum reduction (62.42-71.21%) of chlorantraniliprole residues. The rate of degradation or break down of the pesticide residue is highly dependent on the physico-chemical properties of chemical. The vapour pressure of chlorantraniliprole is moderate (6.3×10^{-12} Pa at 20°C) and hence, the cooking method has resulted in moderate reduction of pesticide residues in the sample (Kwon *et al*., 2015).

Among the washing treatments, 2 per cent salt solution was the most effective, by eliminating 46.40 to 48.68 per cent of residues in bitter gourd fruit. Washing with 2 per cent lemon solution or 2 per cent tamarind solution reduced chlorantraniliprole residue in bitter gourd fruit by 38.49 to 46.65 per cent, whereas washing with lukewarm water reduced chlorantraniliprole by 43 per cent and washing with tap water reduced 33-37 per cent of chlorantraniliprole residues. In bitter gourd juice, sodium chloride washing effectively influenced the loss of residues to an extent of 54 per cent followed by tamarind solution (51%), lemon solution (50%), lukewarm water (42%) and tap water (40%) (Table 5&6). The Processing Factor (PF) calculated was in the range of 0.42-0.67 for bitter gourd juice (Table 5&6). PF was less than one in juice extracted from all washing treatments indicating insignificant transfer of residues from raw fruit into juice. After three days of insecticide application, the washing treatments decreased the residues below the limit of quantification (0.01 mg kg^{-1}) in both 25 and 50 g a.i. ha^{-1}. In capsicum, chlorantraniliprole residues were eliminated up to 68 per cent by NaCl (5%) followed by hot water and plain tap water treatment which were in the range of 55-58 per cent (Ahlawat *et al*., 2019). In vegetable cowpea, lime and vinegar were more effective in removing chlorantraniliprole residues (87.47-91.70%) (Vijayasree *et al*., 2013). In comparison to other salts, sodium chloride has a high reduction potency and its high solubility in water might have resulted in a higher removal of pesticide residues. Citric acid in lemon is a chelating agent and effectively eliminates pesticide residues from bitter gourd fruit (Chandra *et al*., 2015). The tamarind solution has an acidic pH (1.8 to 3.7) with higher rate of furan derivatives and carboxylic acids (44.4 and 38.2 %). These volatile constituents along with acidic nature of tamarind, might have contributed for the removal of chlorantraniliprole residues (Nowowi *et al*., 2016).

In bitter gourd, a combined treatment of tap water + salt solution + cooking, one followed by another, eliminated 86 per cent of chlorantraniliprole residues on 0 day, more than 70 per cent on second and seventh days after application. The other treatments, tap water + lemon and tamarind solution + cooking caused maximum reduction on 0 day (79.94 to 84.86%), followed by second (69.83-77.50%) and seven (68.46-77.01%) days after second application. On 10^{th} day in both single and double the doses, chlorantraniliprole residue reached BLQ (0.01 mg kg^{-1}) in the combined

Table 2 Persistence and dissipation of chlorantraniliprole 18.5% SC residues in/on bitter gourd

Chlorantraniliprole @ 25 g a.i ha^{-1} (X dose)				Chlorantraniliprole @ 50 g a.i ha^{-1} (2X dose)		
Days after treatment	Mean residues (mg kg^{-1}) ± SD*	RSD (%)	Dissipation (%)	Mean residues (mg rkg^{-1}) ± SD*	RSD (%)	Dissipation (%)
0 (2hrs)	0.72 ± 0.02	3.16	-	1.41 ± 0.04	3.13	-
1	0.48 ± 0.01	1.62	33.83	0.96 ± 0.01	0.86	32.09
3	0.30 ± 0.01	2.33	58.03	0.62 ± 0.03	4.37	55.85
5	0.19 ± 0.01	3.37	73.25	0.45 ± 0.01	1.64	68.07
7	0.10 ± 0.01	7.98	86.48	0.27 ± 0.01	2.93	80.75
10	0.04 ± 0.01	13.62	94.71	0.11 ± 0.01	6.03	92.54
15	0.01 ± 0.00	6.83	99.19	0.03 ± 0.00	6.64	98.01
20	BLQ	-	-	0.01 ± 0.00	6.64	99.57
25	BLQ	-	-	BLQ	-	-
Kinetic equation	$y = 0.703e^{-0.283x}$			$y = 1.3723e^{-0.248x}$		
R^2 value	0.992			0.990		
Half-life	2.44 days			2.79 days		
PHI	3.09 days			6.24 days		

*Mean of three replications, SD- Standard Deviation, RSD- Relative Standard Deviation, ND- Not Detected, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), PHI- Pre-Harvest Interval

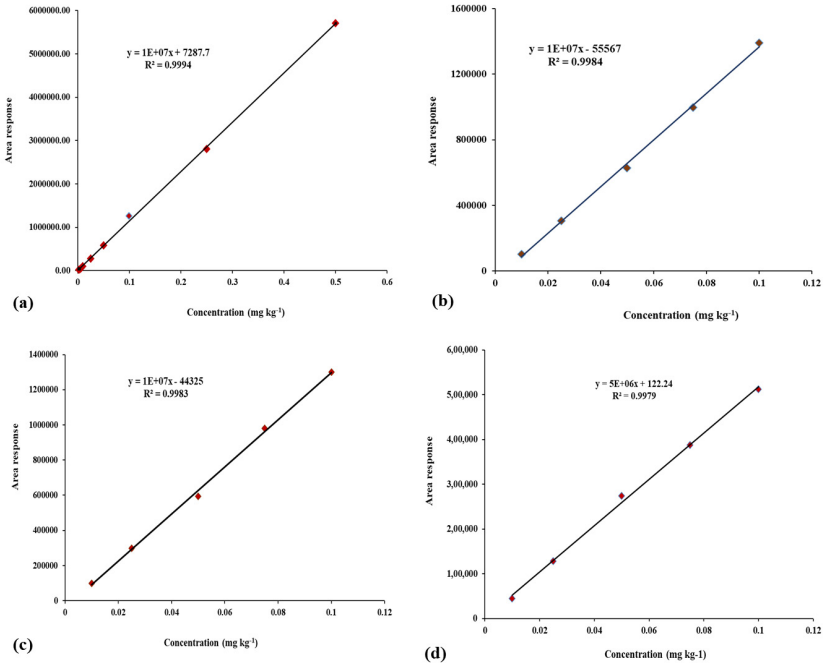

Fig. 2 Calibration curve for the chlorantraniliprole in methanol solvent (a), bitter gourd fruit matrix (b), soil matrix (c) and bitter gourd juice matrix (d)

Table 3 Effect of different decontamination techniques on residues of chlorantraniliprole @ 25 g a.i ha^{-1} on bitter gourd fruit

Treatments	Residues in mg/kg and reduction (%)*							Mean Per cent reduction
	0 day (2 hr)	1 day	2 day	3 day	5 day	7 day	10 day	
Washing in tap water	0.39 (42.83)	0.28 (39.25)	0.24 (37.96)	0.22 (31.62)	0.12 (34.58)	0.07 (33.87)	0.03 (34.82)	36.99
Washing in lukewarm water	0.33 (50.84)	0.24 (46.83)	0.22 (43.44)	0.20 (39.90)	0.11 (40.27)	0.07 (40.46)	0.02 (39.47)	43.23
Washing in (2%) salt solution	0.32 (52.41)	0.23 (49.36)	0.21 (46.41)	0.18 (46.09)	0.11 (43.35)	0.06 (44.19)	0.02 (44.78)	46.40
Washing in tamarind solution (2%)	0.37 (45.36)	0.27 (40.73)	0.24 (37.60)	0.20 (39.39)	0.12 (36.26)	0.07 (36.77)	0.03 (36.06)	38.49
Washing in lemon juice (2%)	0.33 (51.63)	0.25 (46.46)	0.21 (45.45)	0.18 (44.91)	0.11 (42.14)	0.06 (43.72)	0.02 (42.15)	45.02
Cooking	0.19 (71.21)	0.13 (71.23)	0.14 (63.56)	0.11 (65.44)	0.07 (64.32)	0.04 (64.23)	BLQ	67.47
Tap water washing + salt solution (2%) + cooking	0.09 (86.43)	0.08 (82.23)	0.08 (77.26)	0.05 (78.85)	0.04 (78.10)	0.02 (78.33)	BLQ	80.70
Tap water washing + tamarind solution (2%) + cooking	0.12 (82.88)	0.10 (77.19)	0.10 (73.96)	0.07 (72.31)	0.05 (72.40)	0.03 (72.70)	BLQ	75.78
Tap water washing + lemon juice (2%) + cooking	0.10 (84.86)	0.09 (80.23)	0.09 (76.74)	0.06 (77.83)	0.04 (76.39)	0.03 (77.01)	BLQ	79.33
Untreated (control)	0.68	0.46	0.38	0.32	0.19	0.11	0.04	

* Mean of three replications, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

treatments. Chlorantraniliprole residues were found to be less than the MRL (0.3 mg kg^{-1}) after 1 and 3 days of individual washing treatments and after 0 and 1 day when treatments were combined in single and double doses. Hence concluded that safe consumption of raw fruit after 0 and 1 day subjecting to combined treatments at chlorantraniliprole 25 and 50 g a.i. ha^{-1}. Bitter gourd juice consumption after 0 day poses no risk to the consumer at chlorantraniliprole 25 and 50 g a.i. ha^{-1} when washing treatments were followed.

Risk assessment

The PHI for chlorantraniliprole applied at 25 and 50 g a.i. ha^{-1} was estimated at 3.09 and 6.24 days. In bitter gourd, the MRL for chlorantraniliprole is 0.3 mg kg^{-1} (Codex). Though the residues were exceeding MRL up to 3 days in X dose and up to 5 days in 2X dose, RQ calculated taking into the quantity consumed (60 g/day), showed no risk. The

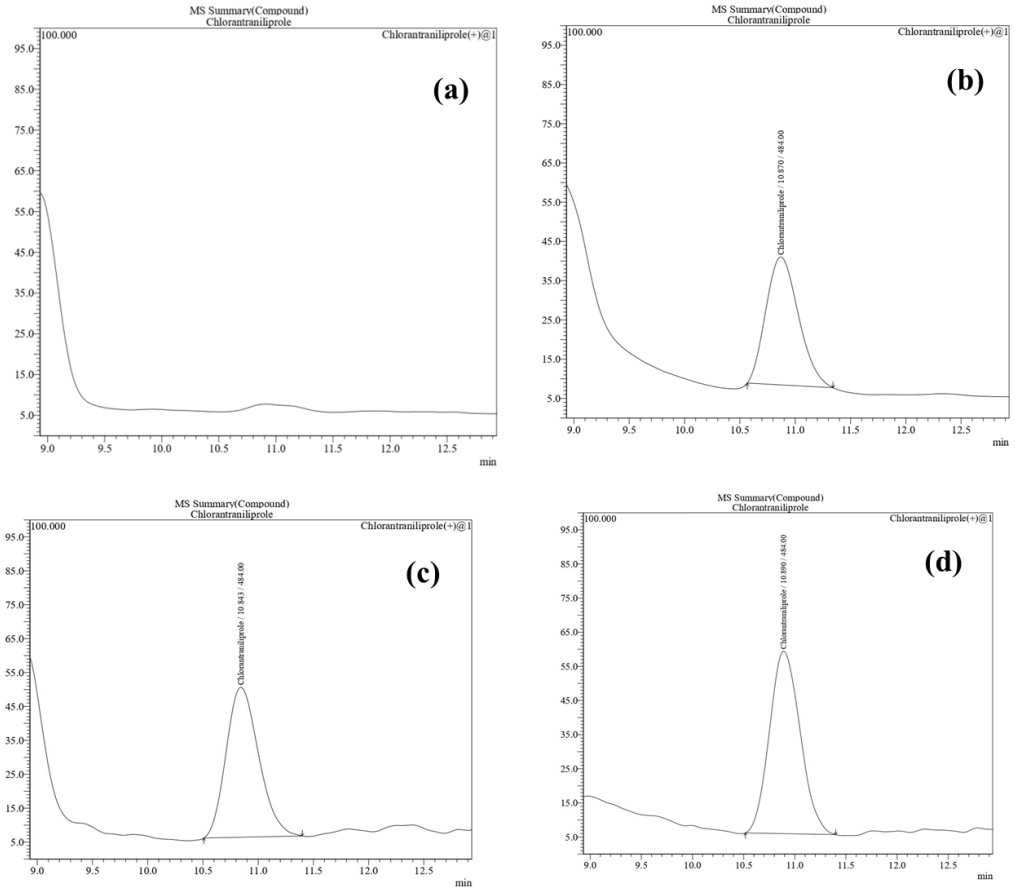

Fig. 3 LC-MS chromatogram of chlorantraniliprole bitter gourd control (a), fruit matrix match (b), fruit recovery (c) and treated field sample (d)

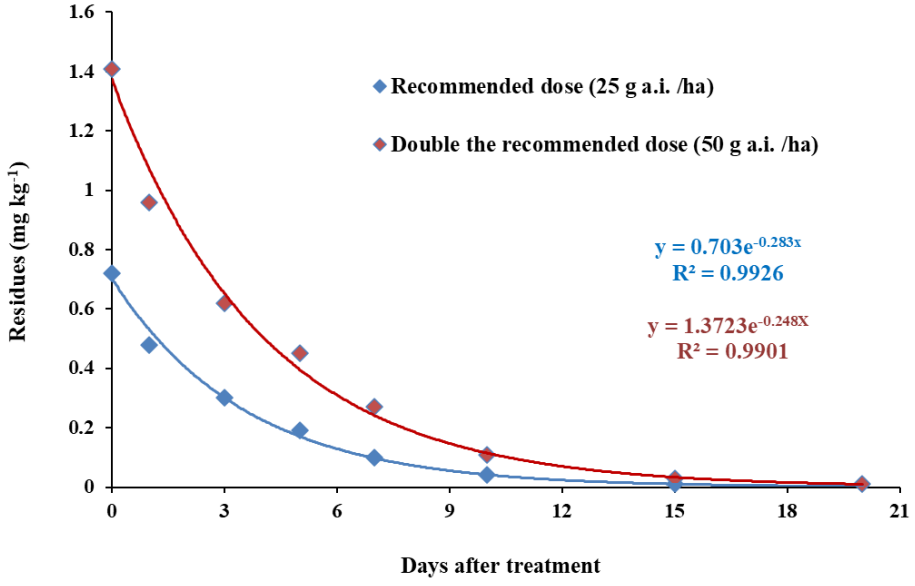

Fig. 4 Dissipation kinetics of chlorantraniliprole in bitter gourd fruit

Table 5 Effect of washing on the reduction of chlorantraniliprole residue in bitter gourd juice at 25 g a.i ha^{-1}

Treatments	Residues in mg kg^{-1} and reduction (%)*								
	0 day (2 hr)		1 day		2 day		3 day	5 day	Reduction (%)
	Residues	PF	Residues	PF	Residues	PF			
Washing in tap water	0.10 (42.39)	0.59	0.06 (41.58)	0.6	0.04 (36.58)	0.57	BLQ	BLQ	40.19
Washing in lukewarm water	0.09 (44.27)	0.53	0.06 (43.28)	0.6	0.04 (39.99)	0.57	BLQ	BLQ	42.52
Washing in salt solution (2%)	0.08 (54.76)	0.47	0.05 (56.47)	0.5	0.03 (51.49)	0.43	BLQ	BLQ	54.24
Washing in tamarind solution (2%)	0.08 (52.55)	0.46	0.05 (52.85)	0.5	0.03 (48.43)	0.43	BLQ	BLQ	51.28
Washing in lemon juice (2%)	0.08 (50.93)	0.47	0.05 (51.52)	0.5	0.03 (49.31)	0.43	BLQ	BLQ	50.59
Untreated (control)	0.17	-	0.10	-	0.07	-	0.01	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

Table 6 Effect of washing on the reduction of chlorantraniliprole residue in bitter gourd juice at 50 g a.i ha^{-1}

Treatments	Residues in mg kg^{-1} and reduction (%)*									
	0 day (2 hr)		1 day		2 day		3 day		5 day	Reduction (%)
	Residues	PF	Residues	PF	Residues	PF	Residues	PF		
Washing in tap water	0.22 (41.26)	0.58	0.13 (40.89)	0.56	0.09 (39.22)	0.6	0.04 (36.09)	0.67	BLQ	39.37
Washing in lukewarm water	0.20 (47.13)	0.53	0.13 (44.62)	0.56	0.09 (41.60)	0.6	0.04 (37.57)	0.67	BLQ	42.73
Washing in salt solution (2%)	0.16 (57.90)	0.42	0.10 (56.50)	0.43	0.07 (53.51)	0.47	0.03 (51.88)	0.50	BLQ	54.95
Washing in tamarind solution (2%)	0.18 (53.34)	0.47	0.11 (52.55)	0.47	0.07 (50.33)	0.47	0.03 (46.14)	0.50	BLQ	50.60
Washing in lemon juice (2%)	0.17 (53.71)	0.44	0.11 (51.95)	0.48	0.08 (49.1)	0.53	0.03 (47.72)	0.50	BLQ	50.63
Untreated (control)	0.38	-	0.23	-	0.15	-	0.06	-	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

Table 7 Dietary risk assessment of chlorantraniliprole in bitter gourd at 25 g a.i ha^{-1} (X) and 50 g a.i ha^{-1} (2X)

Days after treatment	X dose				2X dose			
	Dietary risk assessment (Male-65kg)		Dietary risk assessment (Female-55kg)		Dietary risk assessment (Male-65kg)		Dietary risk assessment (Female-55kg)	
	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)
0 (2hrs)	0.000665	0.000421	0.000785	0.000497	0.001301	0.000823	0.001538	0.000974
1	0.000443	0.000280	0.000524	0.000331	0.000886	0.000561	0.001047	0.000663
3	0.000277	0.000175	0.000327	0.000207	0.000572	0.000362	0.000676	0.000428
5	0.000175	0.000111	0.000207	0.000131	0.000415	0.000263	0.000491	0.000311
7	0.000092	0.000058	0.000109	0.000069	0.000249	0.000157	0.000295	0.000186
10	0.000037	0.000023	0.000044	0.000027	0.000102	0.000064	0.000120	0.000076
15	0.000009	0.000006	0.000011	0.000007	0.000027	0.000018	0.000033	0.000021
20	-	-	-	-	0.000009	0.000006	0.000011	0.000007
25	-	-	-	-	-	-	-	-

EDI-Estimated Daily Intake, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1})

risk quotient (RQ) was calculated by dividing the EDI by ADI in mg kg^{-1} body weight (BW)/day. Even on the 0 (within 2 hr) day of spraying, the RQ value was found to be less than one in both single and double the doses, indicates that chlorantraniliprole is safe for consumption and the risk is acceptable (Table 7).

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Evaluation of the residual activity of newly developed aqueous formulation of novel biopesticide *Bacillus cereus* VCRC-641 through simulated field trial

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ABSTRACT: In the study a new aqueous formulation with the new mosquitocidal isolate of *Bacillus cereus* VCRC 641 was developed, and the residual activity was assessed from simulated field trials conducted in the natural environment. From laboratory bioassays, it was observed that the efficacy of aqueous formulation of *Bacillus cereus* was on par with lyophilized cell mass, as such. The LC_{50} values of formulated *B. cereus* against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* were 0.002, 0.009 and 0.008 mg L^{-1} respectively. Correspondingly, the LC_{50} values of lyophilized cell mass of *B. cereus* against these three mosquito species were 0.0019, 0.005 and 0.004 mg L^{-1} respectively. Finally, simulated field trial was carried out using formulated *B. cereus* and the residual efficacy against all three mosquito larval species revealed 100 per cent larval mortality up to seven days and 69 to 78 per cent mortality were up to 15 days. Thereafter, the mortality was declining gradually up to 21 days. It is concluded that the formulation of *B. cereus* may be used for mosquito control program. © 2023 Association for Advancement of Entomology

KEY WORDS: Bioassays, LC_{50}, *Culex quinquefasciatus, Anopheles stephensi. Aedes aegypti*

INTRODUCTION

Mosquito-borne diseases are a serious global public health concern. Dengue fever, filariasis, chikungunya, malaria, and other mosquito-borne diseases are producing high level of illnesses and death in many areas throughout the world (WHO, 2016). Many vector control programmes in many countries are focusing on adult mosquito control and larval management to combat these diseases. For nearly four decades, widespread use of chemical insecticides against vector mosquitoes for the management of malaria and other mosquito-borne diseases has resulted in the development of pesticide resistance in vector mosquitoes and environmental dangers (Mittal, 2003; Etang and Fondjo., 2006; Mohan and Ramaswamy, 2007; Becker *et al*., 2010; Raghavendra *et al*., 2011). Despite the widespread and long-term use of chemical pesticides, many diseases continue to be prevalent and can produce outbreaks (Mittal, 2003).

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As a result, alternative mosquito control strategies must be investigated urgently in order to reduce dependency on chemical insecticides. Chemical larvicides, which are famous to be eco-friendly, particular to target species, and ideally suited for community use, could be replaced by bacterial-derived biolarvicide (Mittal *et al.,* 1999). Some registered formulations of *Bti* and *B. sphaericus* have been established and experienced to kill mosquito larvae.

The effectiveness of *Bti* and *B. sphaericus* against the larvae of *Anophelines* and *Culicines* have been meticulously studied (Lacey, 2007). If *Bti* is free of biologically active substances other than insecticidal crystal proteins and non-*Bt* microorganisms, it is unlikely to be toxic to humans, other vertebrates, or non-target invertebrates (WHO, 2006). According to recent studies, the sequential evolution of the taxonomic organization and taxa richness of non-target populations of aquatic invertebrates are not significantly impacted by the long-standing application of *Bti* in coastal wetlands (Lagadic *et al*., 2013). In the present study, an aqueous formulation from *Bacillus cereus* VCRC 641 was developed and examined the toxic efficacy against the mosquito larvae of *Aedes aegypti* (Linnaeus), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say in the simulated field conditions.

MATERIALS AND METHODS

Isolation of Bacteria: The gut content of fresh water fishes have been collected and about 1gram of sample was mixed with 9 ml of sterile water and processed by standard serial dilution method and then kept in a water bath (Technico serological water bath, Made in India) at 80ºC for 30 minutes in order to kill the non-spore formers (Radhika *et al*., 2011). After cooling, 50µl of the sample was streaked on LB media agar plates and incubated at 37^0C overnight. Single pure colony from the culture plate was taken and inoculated into 10 ml of Luria Bertani broth and incubated overnight in an Orbitek Incubator shaker (Scigenics Biotech LT4676, Made in India) at 250 rpm. 10µl of this culture was inoculated into 100ml of LB broth in a 500ml Erlenmeyer flask and incubated for 72 hours at 250 rpm in an orbital shaker. Further, the culture was harvested and centrifuged at 10,000 rpm for 15 minutes in a Hitachi high speed refrigerated centrifuge (CR22III, Made in Japan). The bacterial cell mass was collected and kept in deep freezer (-80°C) overnight and freeze-dried in a lyophilizer Lark Innovative (Penguin classic, Made in India). The lyophilized bacterial powder was preserved in a refrigerator until further use.

Preparation of aqueous formulation: Aqueous formulation of *B. cereus* VCRC 641 was prepared (5%) by combination of Congo red (0.025%), sodium benzoate (0.5%), calcium chloride (4%) and sodium alginate (1%). Composition is - *B. cereus* VCRC 641 lyophilized cell powder 5g, Congo red 0.025g, sodium benzoate 0.5g, calcium chloride 4g, sodium alginate 1g and water 100ml. The formulated product was stored at room temperature (4ºC) until further use.

Laboratory bioassay: Toxicity bioassays were conducted against three mosquito species of laboratory reared late third instars (*Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti*). The toxicity test was performed in disposable wax-coated paper cups using a homogenous stock solution of the liquid formulation. Serial dilutions were made by dissolving appropriate known volume of formulation (7 different doses) in 100 ml of chlorine-free tap water and 25 late third instar larvae of each mosquito species were added for bioassay (WHO, 1985).

Simulated field trials: *Bacillus cereus* VCRC 641, 5 per cent aqueous formulation was developed and potency of the formulation evaluated for perceiving the residual activity of the strain. Laboratory reared (Unit of Mosquito Rearing and colonization, Vector Control Research Centre, Puducherry) late third instar larvae were used for the simulated field trial. Cement tanks with a capacity of 75 litres (*i.e.,* Diameter of outer surface 60cm, height 47.5cm) were used to study the *B. cereus* VCRC 641 aqueous formulation and test the residual efficacy. The tanks were filled with 50L of water and covered with a mosquito net. To

avoid direct exposure to rain and sunshine, the cement tanks used in the simulated field trial were placed under shaded roof shelter.

The primary goal of simulated field investigation was to test and determine the residual efficacy of the *B. cereus* VCRC 641 formulation in the field condition. 50 numbers of late third instar larvae of each species were introduced in each experimental and control cement tanks. *B. cereus* was tested at 3 dosages from the stock solution of 5mg $10ml^{-1}$ ($625\mu l\ 50L^{-1}$, $625\mu l\ 50L^{-1}$ and $1.25ml\ 50L^{-1}$) against *Ae. aegypti, Cu. quinquefasciatus* and *An. stephensi* respectively. Dosage of formulation for simulated field trial was calculated based on the LC_{90} values of the isolate. Three replicates were kept for each dosage in each experiment for all the three species. The experiments were repeated three times at different time intervals. Larvae were counted and removed from the cement tank every day after treatment; mortality of larvae was recorded for the assessment of residual activity. After recording the mortality values, fresh alive larvae of each species were introduced in alternative days.

RESULTS AND DISCUSSION

Formulation of *Bacillus cereus:* In the present study, a new aqueous formulation was standardized from the lyophilized cell mass and toxicity assay was tested against laboratory reared mosquito larval species (*An. stephensi, Cx. quinquefasciatus,* and *Ae. aegypti*). It was observed that there was no significant variation in the toxicity levels (at LC_{50} and LC_{90}) of cell mass and the formulation of *B. cereus* (Table 1).

Simulated field trials: The residual activity of formulated *Bacillus cereus* VCRC 641 against three major mosquito larval species using cement tanks. The percentage mortality was recorded till 20^{th} day of each experiment. From the findings of residual activity of *B. cereus* against *Cu. quinquefasciatus, An. stephensi* and *Ae. aegypti* revealed that end of first week (7 days) all species showed 100 per cent mortality, on the second week (14 days) it was shown 75, 78 and 69 percent respectively. Subsequently, the mortality was declining gradually up to 21 days (Fig. 1). Indicating that the new formulation of *B. cereus* was efficient in mosquito control.

Due to its proven safety for both the environment and human health, *B. thuringiensis* (Bt) has been used in bio pesticide formulations on a large scale for the past 40 years. Increased manufacturing and formulation costs generally impede the widespread use of *Bt*. Complementary local media have progressively replaced expensive synthetic media, but the actual barrier is the cost of the formulation. Since formulation affects cost, shelf life, ease of use, and field efficacy, it serves as a crucial bridge between manufacturing and use. UV light, rain, pH, temperature, and foliage physiology are all environmental factors that reduce the efficacy of Bt formulations. To overcome the negative environmental consequences, various formulations – solid and liquid – have been developed based on application target and practicality (Brar *et al*., 2006).

The new formulated *B. cereus* toxicity assay in laboratory (bioassays) revealed that the LC_{50} and LC_{90} values for *Cu. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were 0.002 and 0.007 mg L^{-1}, 0.008 and 0.0180 mg L^{-1} and 0.009 and 0.019 mg L^{-1} respectively. The results from simulated field trials revealed that the aqueous formulation was on par with lyophilized cell mass toxicity assay and pronounced effect on mosquito larvae. Gunasekaran *et al.* (2004) reported Teknar HP-D, an enhanced biolarvicide formulation of *Bti,* effectiveness in the field against *Cx. quinquefasciatus*. The formulation's toxicity was tested in the laboratory on larvivorous fish, water bugs. Teknar HP-D was evaluated in the ground at three suggested doses, 1, 1.5, and 2 L ha^{-1}, in five different habitats. In the lab, *Ae. aegypti* was the most susceptible to the *Bti* toxin. Up to day 6 post-treatment in drains, all three doses resulted in a >80 per cent reduction, implying that a weekly application at the lowermost level would be required for long-term management. The formulation's residual activity lasted longer in unused wells, resulting in a >80 per cent drop in pupal recruitment

Table 1. LC50 and LC90 values of aqueous formulation of *B. cereus* VCRC 641 cell pellet

Larval species	LC_{50} (mg L^{-1}) (UCL-LCL)	LC_{90} (mg L^{-1}) (UCL-LCL)	χ^2	p-value
Aedes aegypti	**0.0089** (0.011-0.007)	0.018 (0.02-0.014)	80.55	<0.05
Anopheles stephensi	**0.009** (0.01-0.006)	0.019 (0.035-0.013)	94.14	< 0.05
Culex quinquefasciatus	**0.002** (0.003-0.002)	0.007 (0.01-0.003)	89.65	<0.05

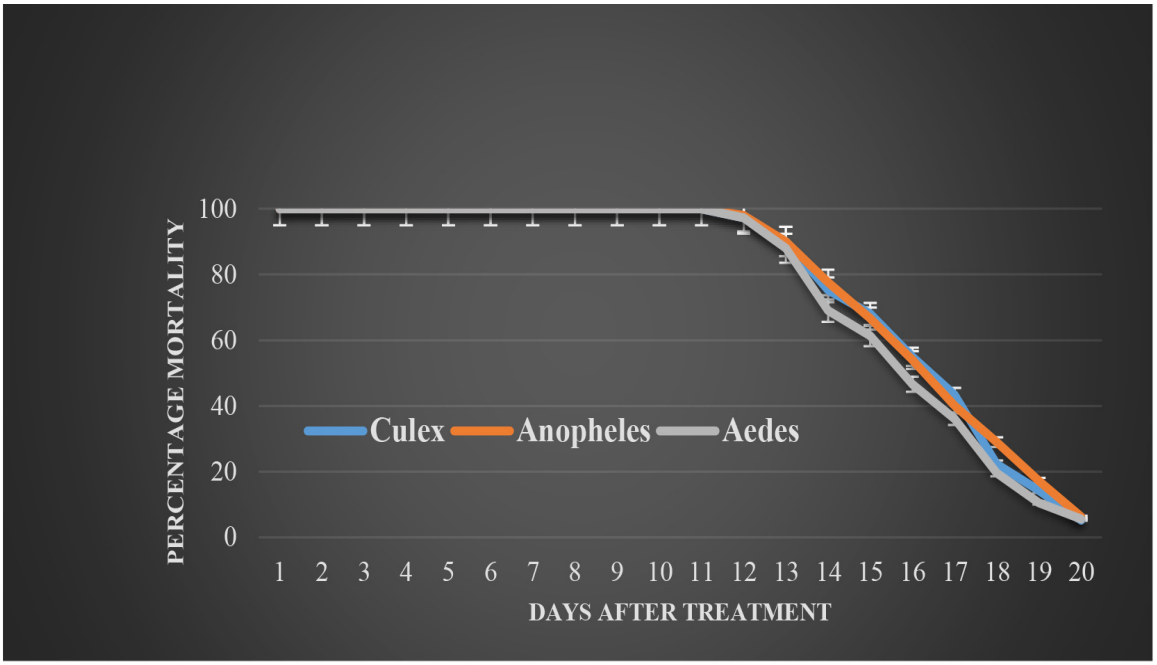

Fig. 1 Average value of simulated field trial using *B. cereus* VCRC 641formulation against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*

for 17 days after treatment (Gunasekaran *et al*., 2004).

For evaluating the efficacy and residual activity of *B. cereus* aqueous formulation simulated field experiment revealed that, *B. cereus* efficacy was long lasting upto 20 days. The residual activity of *B. cereus* against *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* revealed that, end of the first week all species showed 100 per cent mortality, on the second week its showing 75, 78 and 69 per cent respectively. Residual efficacy of *B. cereus* against all three larval species showed 100 per cent mortality up to 11thday. Field application of *B. cereus*, after 24 hours of exposure to *B. cereus* VCRC B540 in the laboratory, doses of 0.0047 and 0.037 mg l^{-1} resulted in 50 and 95 percent mortalities, respectively, and in the field, the necessary concentration was 0.047 grams per square meter. The target mosquito larvae (*Cx. quinquefasciatus*) studied were particularly sensitive to the *B. cereus* VCRC B540 liquid formulation (various places in Puducherry, India), with the early instars being the most sensitive. This study confirms 17 days of residual activity (100 to 80% reduction) of *B. cereus* VCRC B540 like *B. sphaericus*, which is better than residual activity of *Bti* demonstrated earlier (Margalit and Dean, 1985; Mani *et al*., 2018). Uragayala *et al*. (2018)

described, in natural habitats in Bengaluru, India, the effectiveness and residual activity of a novel formulation of Bti over larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. In Phase III tests, Bactivec SC at 1 ml/50 litre dosage produced 10-17 days' effectiveness (>80% reduction in pupae) in fresh water environments examined, whereas 0.5 ml/50 litre dosage produced residual activity from 7 to 14 days against *Ae. aegypti* and *An. stephensi*. In Phase III, efficacy against *Cx. quinquefasciatus* could be measured for 4-7 days in polluted water environments. It is concluded that the formulation of *B. cereus* may be used for mosquito control program.

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Antixenosis of Thodan (AAB), a resistant *Musa* cultivar to *Odoiporus longicollis* (Olivier) and characterization of larvicide molecules

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ABSTRACT: Banana pseudostem borer (BPB) *Odoiporus longicollis* (Olivier) (Coleoptera, Curculionidae) is a serious pest of *Musa* cultivars. Experimental maintenance of larvae in the live pseudostem of cultivar Thodan, a resistant AAB Musa cultivar has resulted antixenosis followed by death of larvae within a week. Antixenosis was characterized by significant decrease of total hemocytes and sharp changes on the proportionate distribution of different types of hemocytes in larvae. As the number of plasmatocytes, prohemocytes, splenocytes and adipohemocytes decreased the number of granulocytes and oenocytes increased. Antixenosis also caused accumulation of 20-hydroxyecdysone (20E) and significant inhibition on the activities of trypsin like serine protease (TlSP) and phenoloxidase (PO). Phytochemical analysis of Thodan resulted characterization of three larvicides such as Betulinic acid (BA), Stigmasterol-3-O-glucoside (SOG) and Sulfoquinovosyl diacyl glycerol (SQDG), and the content in the pseudostem ranged 0.0027 to 0.007 per cent. All the three larvicides were highly toxic to the larvae with LD_{50} of 0.38 ppm for SOG, 0.41 ppm for SQDG and 0.83 ppm for BA. Simultaneous action of three larvicides in the live pseudostem resulted resistance in Thodan against infestation by BPB. Susceptible Nendran showed negligibly low content of SOG (0.0011%) and SQDG (0.0013%) but no detectable quantity of BA. Intoxication by all the three larvicides caused significant changes on the proportionate distribution of hemocytes, accumulation of 20-hydroxy ecdysone (20E) and inhibition of TlSP and PO, the enzymes involved in larval metamorphosis and cuticle sclerotisation. This study demonstrated that resistance of Thodan against *O. longicollis* is due to adverse effect of these larvicides on endocrine system, cuticle development and cytotoxicity of hemocytes. As these larvicide molecules are stable compounds, there is scope for them to be used as substitutes in place of deleterious insecticides for the management BPB.

KEY WORDS: Betulinic acid, Stigmasterol–3-O-glucoside, Sulfoquinvosyl diacylglycerol, endocrine system, cytotoxicity, metamorphosis

INTRODUCTION

Banana and plantains are important fruit crops of tropical and subtropical regions of the world and India is one of the major Centres of diversity of these plants (Bhat and Jarret, 1995; Reshma *et al.,*

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2016). *Odoiporus longicollis* (Olivier) (Coleoptera, Curculionidae), the banana pseudostem borer (BPB) is a major problem in the commercial cultivation of banana and plantains (Padmanaban *et al*., 2001; Kavitha *et al*., 2015a, b; Ajitha *et al*., 2017). Larvae are the destructive stages in the lifecycle and they are feeding on the pseudostem. If infestation is in the early growth phase, the plant will not set fruit and if it is in the late vegetative phase the plant topples down before the maturity. In the absence of proper control measures yield loss will be 80 per cent or more (Visalakshi *et al*., 1978; Padmanaban *et al*., 2001). Since the pest is an internal feeder, topical application of insecticides is futile and farmers are adopting pseudostem injection of insecticides (Aishwarya, 2019; Kavitha, 2019). Field study conducted in various agroecosystems of Kerala, India proved *O. longicollis* is extremely host specific (Kavitha *et al*., 2015a, b; Ajitha *et al*., 2017). India has rich diversity of more than 512 *Musa* cultivars and Kerala, a southern state of India has 217 *Musa* cultivars (Uma, 2010; Reshma *et al.,* 2016; Ajitha *et al*., 2018a). Among these diverse cultivars only few are commercially viable (CV) and are abundant in agroecosystem (Kavitha *et al*., 2015a, b). All the CV *Musa* cultivars are highly susceptible to BPB. Activity of supporting enzymes such as phenyl alanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (PO) are extremely low in CV cultivars (Ajitha *et al*., 2018a, b; Kavitha *et al*., 2020a). Most of the commercially nonviable (CNV) *Musa* cultivars are seen in villages and crushed juice extracted from the fresh pseudostem of some of these *Musa* cultivars are folk lore remedy for urolithiasis, hyperacidity and diabetes. CNV *Musa* cultivars are rare in agroecosystems and interestingly they face sporadic or no infestation by BPB (Kavitha *et al*., 2015a, b; Ajitha *et al*., 2018a, b). Experimental maintenance of *O. longicollis* larvae in the live pseudostem of some of the CNV *Musa* cultivars resulted 100 per cent mortality of larvae within a week (Kavitha *et al.,* 2020a, b, 2021). Those *Musa* cultivars which caused death of the larvae within a week of experimental maintenance are termed as resistant to BPB and larvae taken out on the fourth day of were weak, flaccid and hemocytes of the larvae showed cytopathological changes (Kavitha *et al.,* 2016). Kavitha *et al.* (2020b, 2021) and Ajitha *et al.* (2023) reported antixenosis on the pest, is characterized by sharp decrease of total hemocyte count, quantitative and qualitative changes on protein, sharp decrease of free amino acids and imbalance on the activities of enzymes such as transaminases, leucine aminopeptidase and cathepsin, and disruption of carbohydrate metabolism.

All the BPB resistant *Musa* cultivars possessed extremely high content of phenols, flavonoids and elevated activities of PAL, PPO and PO, compared to that of susceptible *Musa* cultivars. Phytochemical analysis of the pseudostem of resistant *Musa* cultivar Thenkaali (an indigenous triploid AAB cultivar) resulted in the identification of Stigmasterol-3-O-glucoside (SOG) and Pisanglilin (an exotic diploid AA cultivar) resulted characterization SOG and Sulfoquinovosyl diacylglycerol (SQDG) as larvicidal agents. Experimental rearing of *O. longicollis* larvae for three days in the excised pseudostem pieces of susceptible *Musa* cultivars, which were impregnated with either SOG or SQDG also resulted antixenosis and death (Kavitha *et al.,* 2020b, 2021). Thodan, a giant CNV plantain (an indigenous triploid AAB cultivar) was not infested by BPB under field condition and experimental maintenance indicating strong antixenosis (Ajitha *et al.,* 2018a, 2023). Bioassay guided isolation, identification and mode of action of larvicides responsible for the antixenosis on *O. longicollis* larvae were identified and reported in this paper.

MATERIALS AND METHODS

Actively feeding larvae of *O. longicollis* (fourth instar) collected from susceptible *Musa* cultivar banana (Nendran) were used for bioassay. Younger instars are small and highly sensitive to handle and fifth instar larvae are not in active feeding state and hence they were not used for bioassay (Kavitha *et al*., 2020b). Thodan, a BPB resistant *Musa* cultivar which was used for bioassay guided extraction and isolation of active larvicidal molecules. The cultivar, indigenous to Vettikavala Grama Panchayath of Kottarakara Taluk in

Kollam district of Kerala, India was identified as resistant to *O. longicollis* through field observation and subsequent laboratory observations (Ajitha *et al*., 2017, 2018a). Nendran *Musa* cultivar, which is highly susceptible to BPB was used as control.

Experimental maintenance of larvae in the live pseudostem: Four month old Thodan and Nendran cultivars, maintained in the College campus were used for the study. Crown of the cultivars with trunk circumference of 25 to 30 cm was cut down in such a way that the live stump of 1.0 m remained in viable state. Fourth instar larvae of *O. longicollis* (six numbers) were carefully placed on the free cut end of the pseudostem and allowed them to bore themselves into it. Cut end was covered with a piece of mosquito net and kept undisturbed for four or seven days. Either on the fourth day or on the seventh day, pseudostem bearing larvae was cut 25 cm below the free cut end and observed. Active wriggling movements of exposed larvae, in search of pseudostem to bore into it and creamy white colour indicate health of the larvae but flaccid body with bluish white colour indicates death of the larvae. As antixenosis by the resistant Thodan caused 100 per cent mortality of them within seven days of experimental period (Ajitha *et al.,* 2018a) larvae were removed on the fourth day of experimental period from the live pseudostem of Thodan and used for collecting hemolymph for further analysis.

Bioassay guided extraction and isolation of larvicide molecules: Fresh pseudostem of healthy Thodan cultivar weighing 50 kg was chopped into small pieces and kept for drying under shade for one week at room temperature of 26 to 31^{0}C. Dry pieces were powdered in electric motor with mesh size of 0.5 mm. Pseudostem powder (1kg) was sequentially extracted with hexane, chloroform and methanol in soxhlet and 2L of solvent was used for each extraction under controlled condition of 60^{0}C. Extracts were evaporated to dryness in rotary vacuum evaporator, limiting the temperature to 50^{0}C.

Fractionation of active extract: Chloroform extract was fractionated by column chromatography (silica gel 60-120 mesh) with solvent mixture at different proportion *viz.,* 1. hexane-chloroform (50:50), 2. hexane-chloroform (25:75), 3. hexane-chloroform (0:100), 4. Chloroform-methanol (90:10), 5. chloroform-methanol (80:20) and 6. chloroform-methanol (0:100). Volume of each elution was limited to one litre.

Sub fractionation of active fraction: Fraction 5 (active larvicidal fraction) was sub fractionated with following solvent mixture with silica gel (60-120 mesh) as stationary phase. 1. hexane-ethyl acetate (45:55), 2. hexane-ethyl acetate (35:65), 3. hexane-ethyl acetate (25:75), 4. hexane-ethyl acetate (0:100), 5. ethyl acetate-methanol (99.5:0.5), 6. ethyl acetate-methanol (85:15) and 7. ethyl acetate-methanol (80:20). Volume of each solvent mixture was limited to 500 ml.

Isolation of active compounds from 5th sub fraction: Sub Fraction 5 (active larvicidal sub fraction) was again fractionated with ethyl acetate-methanol (99.5:0.5), which resulted isolation of three compounds. Phytochemical isolation was done in the Phytochemistry and Phytopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden Research Institute, Palode, Thiruvananthapuram, Kerala.

Spectral analysis and structural elucidation: Compounds were subjected to ^{1}H NMR, ^{13}C NMR and HRMS. One of the three compounds possessed attached fatty acids, which were identified by GC-MS. All spectral analysis was performed in the sophisticated Instrumentation Centre, University of Kerala, Kariyavattom, Thiruvananthapuram, Kerala. The structural elucidation of compounds was done by using the *chemdraw* software with the expertise of Phytochemistry and Phytopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden Research Institute, Palode, Thiruvananthapuram.

Experimental maintenance of larvae in pieces of excised pseudostem: Each extract/ fraction/ active compound was dissolved in 0.5 per cent Tween80 in such a way to get 1 to 20 mg extract/ mL water. Aqueous suspension of extracts (1.0 mL) was injected evenly at 10 sites into 100g fresh pseudostem pieces of susceptible Nendran cultivar.

A small hole was made on the pseudostem piece and a single larva was allowed to bore into the pseudostem. Pseudostem injected with 0.5 per cent Tween (1.0 mL) was used as control. Every day each piece of pseudostem containing a single larva inside was kept very close to the ear of the investigator to listen the feeding sound of the larva, which is the indication of the health of the larva inside. Each observation lasted for five minutes and each piece with larva was observed six times a day. Fresh pseudostem impregnated with test material was provided every two days by gently taking the larvae out of the old pseudostem and allowing them to bore into the new one. Ability of the larvae to bore themselves into the pseudostem is also an index on the health of the larvae (Kavitha *et al*., 2020b, 2021). On the seventh day of experiment, control and treated larvae were taken out with much care and percentage of mortality was tested as described in detail (Kavitha *et al*., 2020b). All the larvae of the treatment group were either became immobile or dead within one week.

Determination of LD_{50}: Toxicity study and percentage mortality were calculated as described below (Kavitha *et al*., 2020b, 2021) and LD_{50} values determined by Probit analysis.

$$\frac{\text{Percentage of test mortality} - \text{Percentage of control mortality}}{100 - \text{Percentage of control mortality}} \times 100$$

Mode of action studies on larvicide molecules: Larvicide molecules at the dose of their LD_{20} concentration were used for mode of action study on the fourth day of toxicity. Betulinic acid (0.4 ppm), Stigmasterol-3-O-glucoside and Sulfoquinovosyl diacylglycerol (0.2 ppm) are the doses fixed. Larvae were carefully taken out from the pieces of pseudostem, washed in distilled water, blotted in tissue paper and hemolymph was collected. A small slit was made on the ventral portion of neck, without puncturing the gut and hemolymph was collected in chilled Eppendorf tubes and stored in deep freezer at -20^{0}C. The samples were centrifuged in refrigerated centrifuge at 1000 g and used for biochemical estimations. Hemolymph samples without centrifugation were used for the study of haemocytes.

Estimation of 20-hydroxyecdysone (20E) : Cell free hemolymph 50µl was diluted to 0.5 ml using methanol, mixed well and centrifuged at 1000 g for ten minutes. Supernatant was collected and allowed to evaporate gradually in dust free condition at room temperature. The dry sample was dissolved in Enzyme Immunoassay Buffer provided in the assay kit. 20E of hemolymph was estimated by Enzyme immunoassay (Porcheron *et al*., 1989) using an assay kit (A05120, Cayman Chemicals, France), provided with 96 well micro plate and absorbance measured at 405 nm by Micro plate reader.

Assay of Trypsin like Serine Protease: Activity of this enzyme was done as described in standard protocol (Erlanger *et al*., 1961). Cell free hemolymph 100µl was diluted to 1.0 mL with ice cold, Tris HCl buffer (0.05 M, pH 8.2) and used as enzyme for the assay. The enzyme 100µl was made up to 1.0 ml with buffer and was incubated at 37^{0}C for 90 minutes. 1.0 ml of BApNA (40 mg of N-benzoyl-dl- arginine-paranitroanilide dissolved in 0.5 ml dimethyl sulfoxide and then made up to 100 ml with Tris HCl buffer) was added to the above solution and incubated at 37^{0}C for 60 minutes. The reaction was stopped by the addition of 30 per cent acetic acid. The solution was centrifuged at 12,000 g for 4 minutes and supernatant was read at 410 nm. Trypsin standards were run alongside.

Assay of Phenol oxidase: Phenoloxidase (tyrosinase) was measured colorimetrically using dopa as substrate (Lerch, 1987). Cell free hemolymph 100µl was diluted to 1.0 ml of phosphate buffer (0.1 M, pH 6) under ice cold condition and kept in the refrigerator until use. Diluted hemolymph 100 µl was used enzyme, which is applied into 2.9 ml of substrate solution (L-3,4 dihydroxyphenylalanine, 0.8 mg ml^{-1} in buffer), followed by rapid mixing. Enzyme reaction took place at 30^{0}C and read at 475 nm. One unit of enzyme activity corresponds to absorption change of 0.6/minute. A small aliquot of enzyme was subjected to protein estimation. Activity of enzyme is expressed as Units/minute/mg protein.

Estimation of Protein: The quantification of protein in the cell free hemolymph was estimated using Folin-Ciocalteu reagent (Lowry *et al*., 1951).

Cell free hemolymph 100 µl was made up to 1.0 mL with distilled water and 1.0 ml of 10 per cent aqueous trichloro acetic acid was applied. The mixture was kept in ice bath for complete precipitation of protein, followed by centrifugation at 1000 g for ten minutes. Supernatant was decanted and precipitate was dissolved in 1.0 ml of 0.1 N NaOH and used for estimation. The dissolved sample of protein was treated with 3.5 ml of alkaline $CuSO_4$ solution, followed by 0.5 ml of Folin's reagent. The colour developed was estimated spectrophotmetrically at 620 nm.

Hemocytes count: Total hemocytes count of the collected hemolymph was carried out by adopting the protocol of Jones (1963) using Neubauer Hemocytometer, after diluting five times the hemolymph, with phosphate buffered saline (PBS, pH 7.2) possessing 0.1 per cent Giemsa stain.

Differential hemocytes count: A small drop of hemolymph was placed on a clean dry glass slide and a thin film of it was prepared with the help of another glass slide by drawing the second slide across the first one at an angle of 45^0. The smear was air dried for five minutes and stained using Giemsa stain for 15 minutes. Excess stain was washed in running water and slides were observed under microscope at 45x. Different types of hemocytes were identified by their distinguishing features (Wigglesworth, 1972).

Statistical analysis: SPSS 24.0 software (IBM, USA) was used. Data obtained was expressed as mean ± standard error of six numbers of observations. One way Analysis of variance (ANOVA) was applied on the data to assess the effect of individual larvicides and antixenosis exhibited by Thodan with respect to susceptible Nendran cultivar (control).

RESULTS

Antixenosis of resistant Thodan on *O. longicollis*

Thodan (AAB) is a tall, giant *Musa* cultivar which is resistant to infestation by *O. longicollis*. The name Thodan (meaning of *thodu* in local language is covering) was originated because of the exceedingly thick rind of the fruits. Ripe fruits are not as palatable as fruits of CV cultivars and hence they are used as vegetable. Nendran *(*AAB) the most common CV cultivar of Kerala (India) is highly susceptible to infestation by BPB. Experimental maintenance of *O. longicollis* larvae in the live pseudostem of resistant *Musa* cultivar Thodan with three days existence caused strong antixenosis characterized by weak, flaccid and unable to feed the tender pseudostem and their hemolymph exhibited significantly high content of 20-hydroxy ecdysone (20E) compared to that of the larvae in the susceptible, control pseudostem. Elevation of 20E was more than two-fold with that of control. Activity of trypsin like serine protease (TlSP) and phenol oxidase/tyrosinase (PO) showed significant inhibition in larvae maintained in cv Thodan, compared to susceptible cv Nendran (Table 1). Total hemocytes count of larvae maintained in the resistant pseudostem showed significant decrease (70%) in number than control. Proportionate distribution of different types of hemocytes also showed significant difference between the test and control larvae. In healthy larvae, plasmatocytes occupy major proportion and is almost 40 per cent of total hemocytes. After three days of existence larvae in resistant Thodan, plasmatocytes decreased to 18.75 per cent. The second major population of hemocytes is prohaemocytes which accounts to 35 per cent of total cells, decreased to 26 per cent. In healthy normal larvae, proportion of spherulocytes and adipohaemocytes occupy 6 and 8 per cent respectively was decreased to 3.5 per cent. Interestingly two types of cells such as granulocytes and oenocytes showed significant increase during antixenosis. In normal healthy state, proportion of granulocytes is only 8 per cent, which increased to 38 per cent (Table 2). Among the hemocytes, the oenocytes accounts to 2 per cent in normal healthy larvae, which exhibited fourfold increase in larvae under antixenosis.

Bioassay guided phytochemical analysis of pseudostem

Extraction of pseudostem powder of Thodan with organic solvents resulted localization of larvicidal activity in chloroform extract. Fractionation of chloroform extract by column chromatography

Table 1. Antixenosis by a resistant *Musa* cultivar and its larvicide molecules on 20-hydroxyecdysone and activities of enzymes related to cuticle sclerotisation in *Odoiporus longicollis*

Host Plant/ Larvicide molecules	20-hydroxy ecdysone	Activity of Trypsin like Serine Protease	Activity of Phenol Oxidase
Susceptible cv.Nendran (Control)	96.38 ± 06.42	0.96 ± 0.05	48.52 ± 3.91
Resistant cv Thodan	202.19 ± 14.38*	0.42 ± 0.03*	33.41 ± 3.01*
Betulinic acid	142.16 ± 10.54*	0.61 ± 0.04*	36.53 ± 3.23*
Stigmasrerol-3-O-glucoside	168.33 ± 12.18*	0.53 ± 0.04*	37.80 ± 3.02*
Sulfoquinovosyl diacyl glycerol	136.47 ± 11.70*	0.51 ± 0.03*	34.71 ± 2.96*

*Values are mean ± SE; *Values are significantly different from control, $p \leq 0.05$, n = 6; Quantity of 20-hydroxyecdysone is expressed as picogram / mL Hemolymph; Activity of Trypsin like serine protease is expressed as Units/ mg of protein; Activity of Phenol oxidase is expressed as Units / mg of protein; (BA, SOG and SQDG were administered into pieces of pseudostem at their LD_{20} concentration)*

Table 2. Effect of antixenosis by resistant host plant and its individual larvicide molecules on the total and differential hemocytes count in *Odoiporus longicollis*

Host Plant/ Larvicide molecules	Total hemocyte count	Differential hemocyte count (%)					
		PLA	PRO	GRA	OEN	SPL	ADH
Nendran (Susceptible)	4540.57 ± 33.45	40.82 ±3.80	34.69 ±3.05	8.15 ±0.63	2.04 ±0.21	6.12 ±0.56	8.17 ±0.71
Thodan (Resistant)	1877.34 ± 16.67*	18.75 ±1.60*	26.57 ±2.34*	38.12 ±3.11*	9.33 ±0.83*	3.42 ±0.27*	3.63 ±0.21*
Betulinic acid	2011.18 ± 18.12*	20.19 ±1.86*	27.83 ±2.41*	36.42 ±3.23*	8.37 ±0.67*	8.37 ±0.65*	3.43 ±0.25*
Stigmasteol-3-O-glucoside	1938.32 ± 17.34*	18.68 ±1.61*	25.24 ±2.30*	39.21 ±3.82*	9.23 ±0.78*	9.23 ±0.79*	4.61 ±0.40*
Sulfoquinovqsyl diacyl glycerol	1630.23 ± 14.56*	14.59 ±1.04*	26.38 ±2.14*	40.16 ±3.90*	12.14 ±0.98*	10.15 ±0.97*	4.33 ±0.38*

*PLA –Plasmatocyte, PRO – Prohaemocyte, GRA – Granulocyte, OEN – Oenocyte. SPL – Splenocyte, ADH – Adipohaemocyte; Values are mean ± SE; n=6, *Values significantly different from control, $p \leq 0.05$; (BA, SOG and SQDG were administere into pieces of pseudostem at their LD20 concentration)*

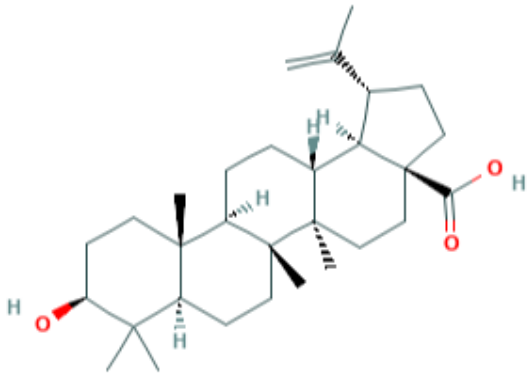

Fig. 1 Structure of Betulinic acid

resulted six fractions and among them 5th fraction showed larvicidal activity. Sub fractionation of 5th fraction gave seven sub fractions, of which 5th sub fraction exhibited larvicidal activity (Table 3). Further chromatographic separation of the 5th sub fraction has resulted isolation of three larvicides in pure form. Spectral analysis of the isolated compounds revealed that they are Betulinic acid (BA), Stigmasterol-3-O-glucoside (SOG) and Sulfoquinovosyl diacylglycerol (SQDG).

Intoxicated larvae with isolated compounds were weak, flaccid and unable to feed depending on the concentration of the molecules. At LD_{20} concentration (0.2 ppm for SOG and SQDG and 0.4 ppm for BA), larvae stopped feeding on the fifth day and were unable enter into the pseudostem provided on sixth day. Control group of larvae developed into pre pupa. Larvae allowed to feed pseudostem impregnated with different concentration of individual larvicide molecules did not develop into pre pupa and died within two weeks.

SOG is the most toxic of all the three larvicide molecules followed by SQDG and BA. SQDG is the most abundant larvicide (0.007%) followed by SOG (0.0036%) and BA (0.0027%). Pseudostem powder of susceptible cv Nendran was also subjected to extraction and fractionation by column chromatography. The cultivar showed the presence of SOG (0.0011%) and SQDG (0.0013%). No detectable quantity of BA was yielded from this cultivar. Fresh pseudostem weighing 50 kg yielded only 1.0 kg of dry powder and hence the presence of larvicides molecules in the live pseudostem ranged between 0.000054 and 0.00014 per cent (Table 3).

Betulinic acid (BA) is chemically 3-b-hydroxyl-lup-20(29)-en-28-oicacid. It is a pentacylic lupine type triterpene (Fig. 1). The compound is present at a very low concentration of 0.0027 per cent on dry weight basis with LD_{50} of 0.832 ppm (Table 3). The spectral characteristics as per ^{13}C NMR, ^{1}HNMR and HRMS of BA is explained below.

_δC(125 MHz): C-1(40.0), C-2 (29.3), C-3 (79.7), C-4 (39.9), C-5 (56.8), C-6 (19.5), C-7 (34.4), C-8(40.8), C-9 (49.2), C-10 (38.1), C-11 (24.4), C-12 (126.7), C-13 (139.8), C-14 (43.3), C-15(27.9), C-16 (25.4), C-17 (47.1), C-18, (54.5), C-19 (40.4), C-20 (40.5), C-21 (31.9), C-22 (38.2), C-23 (28.8), C-24 (16.4), C-25 (16.1), C-26 (17.9), C-27 (21.7), C-28 (182.3), C-29 (17.7), C-30 (24.1), C-1' (145.5), C-2' (123.4), C-3' (150.1), C-4' (127.0), C-5' (128.7), C-6' (143.8), C-7' (170.4).

^{1}H NMR ($CDCl_3$): 0.65, 0.77, 0.98, 1.14 and 1.34 (5s, 15H, all tertiary $-CH_3$), 1.37 (m, 2H, H-21), 1.38 (m, 2H, H-16), 1.45 (m, 2H, H-20), 1.51 (m, 4H, H-18, H-19 and H-15), 2.09 (m, 3H, H-1 and H-9), 2.13 (m, 2H, H-14), 3.17 (t, 2H, J = 7 Hz, H-2), 3.38 (s, 2H, H-7), 4.56 (s, 2H, H-11), 4.59 (s, 2H, H-12).

Table 3. Larvicidal fractions and isolated active compounds from chloroform extract of Thodan against *O. longicollis*

Fractions/Active compound	Fraction / compound (mg)	Yield (%)	LD_{50} (ppm)
Fraction 05	2100	0.21	2.6
Sub Fraction 05	180	0.018	1.21
Betulinic acid	27.3	0.0027	0.832
Stigmasterol-3-O-glucoside	35.8	0.0035	0.380
Sulfoquinovosyl diacylglycerol	72.7	0.0073	0.411

Larvae intoxicated by BA at LD_{50} concentration of 0.8 ppm stopped feeding on the third day of toxicity. They were unable to bore themselves into the pseudostem after 72 hours of toxicity at 0.4

ppm BA. Larvae allowed living for 48 hours in pseudostem with 0.4 ppm BA and later life in control pseudostem could not recover to normal life and die within one week, without undergoing development. During the course of study control larvae successfully moulted into pre pupa.

Stigmasterol-3-O-glucoside is a glycosylated sterol (Structure: Kavitha *et. al*., 2020b) in which glucose is linked with third carbon of the sterol ring with the hydroxyl group of the first carbon of glucose. It is a stable compound with 29 carbons, forming three hexacarbon rings and other carbon atoms are attached with it as a pentameric ring and as free carbon skeleton. The compound is present at a very low concentration (0.0035% on dry weight basis) and possessed LD_{50} of 0.38 ppm and (Table 3) and LD_{20} of 0.2 ppm. The spectral characteristics as per ^{13}C NMR, ^{1}HNMR and HRMS are described below.

_δC(125 MHz): C-1(37.20), C-2 (31.83), C-3 (78.84), C-4 (42.10), C-5 (140.34), C-6 (121.82), C-7 (31.72), C-8(31.83), C-9 (50.19), C-10 (36.60), C-11 (18.96), C-12 (38.50), C-13 (42.10), C-14 (56.72), C-15(24.11), C-16 (28.82), C-17 (55.91), C-18, (11.79), C-19 (18.57), C-20 (39.61), C-21 (20.93), C-22 (138.33), C-23 (129.19), C-24 (51.28), C-25 (31.83), C-26 (19.35), C-27 (18.96), C-28 (24.11), C-29 (11.79), C-1' (101.09), C-2' (73.58), C-3' (76.12), C-4' (70.22), C-5' (77.19), C-6' (61.61)) .

_δH(500 MHz): 1.04(1H, m) H-2, 1.51 (1H, m) H-3, 5.19 (s, 1H) H-6, 0.70 (3H, s) H-18, 1.02 (3H, s) H-19, 1.6 (3H, d, J=4Hz) H-21, 1.18 (2H, m) H-28, 0.84 (3H, t, J=8Hz) H-29, 4.39 (1H, d, J=8Hz) H-1', 3.30 (1H, m) H-2', 3.30 (1H, m) H-3', 3.31 (1H, m) H-4', 3.39 (1H, m) H-5', 2.31 (1H, dd, J=4Hz, 3.6Hz) H-6'. From all the above spectral data, structure of the compound is identified as stigmasterol-3-O- glucoside, which is perfectly matching with the previous reports (Kojima *et al*., 1990) and that of a single larvicide identified in another pest resistant *Musa* cultivar (Kavitha *et al*., 2020b)

Sulfoquinovosyl diacyl glycerol is a sulfur containing compound and is characterized as (2S) -1-2-DL-O palmitoyl-3. O-(6-sulfo-a-D-quinovodiacsyl) glycerol (Structure: Kavitha *et.al*., 2021) It has six fatty acids in which two of them are attached with this compound at a time and they are Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Penta decenoic acid and 9-hexadecnoic acid. The compound is present at 0.007 per cent in pseudostem on dry weight basis and showed LD_{50} of 0.41 ppm (Table 2). The spectral characteristics as per ^{13}C NMR, ^{1}HNMR and HRMS are described below.

It is amorphous white powder; 197-198^{0}C; positive ESI-MS 795 $(M+1)^{+}$; IR (KBr) *v* (cm^{-1}) 3426, 2919, 2851, 1736, 1634, 1465, 1221, 1170, 1110, 1060, 1038, 764, 720.

^{1}H NMR (Methanol-d_4, 400 MHz): ˆ (ppm) 5.76 - 5.72 (m, 1H), 5.48 (dd, J = 15.3, 7.3 Hz, 1H), 5.26 (t, J = 4.5 Hz, 1H), 4.66 (s, 1H), 4.15 (d, J = 7.8 Hz, 1H), 4.00 - 4.04 (m, 2H), 3.86 - 3.89 (m, 2H), 3.73 (d, J = 11.5 Hz, 1H), 3.35 - 3.40 (m, 2H), 3.24 - 3.33 (m, 1H), 2.10 - 2.11 (m, 5H), 2.01 - 2.03 (m, 1H), 1.27 - 1.41 (m, 2H), 0.90 (s, 39H), 0.87 (t, J = 6.5 Hz, 6H).

^{13}C NMR (METHANOL-d_4, 100 MHz): ˆ (ppm) 175.8, 133.5, 130.8, 130.36, 103.0, 77.8, 77.6, 76.32, 73.4, 71.9, 71.7, 68.2, 61.3, 53.2, 34.4, 32.4, 32.3, 32.0, 31.7, 29.6, 29.5, 29.4, 29.0, 27.1, 26.5, 25.9, 25.0, 22.5, 13.6. Also, the ^{13}C NMR showed two distinct set of peak regions corresponding to fatty acids (14.5 - 35.9) and sugar (62.7 - 78.0). Attachment of sulfur to sugar was observed via a peak at 54.6 in ^{13}C NMR spectrum. The two methyl group protons at 0.84 in the ^{1}H NMR spectrum and 14.5 in the ^{13}C NMR spectrum revealed the molecule must be diacylated. The values at 62.7, 69.8 and 71.6 are characteristics of glycerol unit in SQDG (Sulfoquinovosyl diacylglycerol). Based on the above molecular features the compound is identified as Sulfoquinovosyl diacyl glycerol. The spectral characters are identical with that of the larvicide molecule identified in a diploid AA *Musa* cultivar Pisanglilin (Kavitha *et al*., 2021).

Toxicity by individual larvicides

Larvae allowed to exist in the live pseudostem of Thodan for three days were weak and flaccid.

Identical changes were observed in larvae which were maintained in 100 g pieces of pseudostem with individual identified larvicide molecules at LD_{20} (BA at a dose of 0.4 ppm or SOG or SQDG 0.2 ppm). Hemolymph of weak and flaccid larvae, which were either taken out from the live and resistant pseudostem or intoxication by larvicides showed sharp significant decrease on total hemocytes count. Differential hemocytes count of control and intoxicated larvae showed sharp difference on the percentage distribution of hemocytes (Table 2). In the affected larvae granulocytes increased from 8 to 38 per cent. Proportion of oenocytes was also increased from 2 to 8 per cent. Significant decrease on the number of plasmatocytes (50 to 60% decrease) and prohemocytes (20 to 25% decrease) were also observed.

Toxicity by individual larvicides molecule resulted exponential increase on the content of 20E in the hemolymph. Accumulation of 20E was significantly high in larvae existed for three days in live pseudostem than toxicity by individual larvicide molecule. Content of 20E in non- feeding pre pupae was estimated as 136.07±10.32 ng ml^{-1} of hemolymph, which is almost equal to 20E of fourth instar larvae under intoxication by larvicides molecules. Significant inhibition on the activities of trypsin like serine protease (TlSP) and phenoloxidase (PO) were also observed in the hemolymph of the affected larvae. TlSP exhibited 60 per cent inhibition while the larvae in the live and resistant pseudostem for three days (Table 2). Individual larvicide molecules extracted from the resistant plantain at the dose LD_{20} caused similar effect but inhibition of TlSP activity was only 40 per cent. Under identical experimental condition activity of PO exhibited 25 to 30 per cent inhibition.

DISCUSSION

Ajitha *et al*. (2018b) and Kavitha *et al*. (2020b, 2021) reported *Musa* cultivars Yangambi, Thenkaali and Pisanglilin showed resistance to the BPB, as they showed 100 per cent mortality on the reared larvae. Hemolymph of the larvae which spent three days in the live pseudostem of Thenkaali and Pisanglilin showed rupture of hemocytes, qualitative and quantitative changes in the protein profile of hemolymph and imbalance in the activities of enzymes regulating free amino acid pool (Kavitha *et al*., 2020b, 2021). In the present study, larvae spent three days in the live pseudostem of Thodan exhibited severe hemocytopenia with sharp increase on certain hemocytes such as granulocytes and oenocytes. Granulocytes are cells which are actively involved in cellular immune response such as nodulation, encapsulation, phagocytosis of pathogen and removal of necrotic cells (Youngwoo and Sayoull, 2019). Numerical increase of granulocytes in the hemolymph of *O. longicollis* larvae in Thodan may be for the removal of dead cells formed as a result of antixenosis by the resistant host plant.

Recent studies on the mechanism of pest resistance of *Musa* cultivars revealed the presence of a single larvicide molecule (SOG) in the pseudostem of Thenkaali (AAB) (Kavitha *et al*., 2020b) and two larvicide molecules such as SOG and SQDG in a diploid AA *Musa* cultivar Pisanglilin (Kavitha *et al*., 2021). Present study in Thodan revealed that in addition to SOG and SQDG it possessed BA as third larvicide molecule. Besides the larvicide molecules Thodan possessed very high quantities of other secondary metabolites such as phenols and flavonoids in the pseudostem, compared to susceptible *Musa* cultivars. Activities of supporting enzymes such as PAL, PPO and PO were also high in Thodan, compared to susceptible cultivars (Ajitha *et al*., 2018a). In the present study the susceptible Nendran also possessed low content of SOG (40%) to that of its content in resistant Thodan.

Bioassay guided isolation and characterization of phytocompounds responsible for resistance exhibited by *Musa* cultivar Thodan revealed the presence of three larvicides in the pseudostem. Among them, two larvicides isolated from Thodan such as SOG and SQDG are highly toxic to *O. longicollis* and are previously reported from two indigenous *Musa* cultivars of Kerala (Kavitha *et al*., 2020b; Kavitha *et al*., 2021). SOG was isolated first time from the bark of *Prunella vulgaris* as a glycosylated sterol (Kojima *et al*., 1990) and in the present study the spectral

characters obtained in respect of SOG was identical with that of the previous study. SOG has no mammalian toxicity and exhibited antihyperglycaemic (Panda *et al*., 2009), hypocholesterolaemic (Chandler *et al*., 1979) and hepatoprotective activity (Kaur *et al.,* 2011) in humans and animal models.

SQDG exhibited cytotoxicity on the haemocytes and resulted lysis of the midgut epithelium of the intoxicated larvae of *O. longicollis* (Kavitha *et al*., 2021). SQDG was isolated first time as a sulfur containing lipid (Benson, 1959) of the thylacoid membrane (Kaichiro *et al*., 2016). Lysis of midgut cells and hemocytes in *O. longicollus* larvae during toxicity by SQDG may be due to inhibition of DNA polymerase action. Among the three larvicides, SQDG is the most abundant and its toxicity was almost equal to that of SOG (Kavitha *et al.,* 2021). BA the third larvicide molecule identified from Thodan, is a known insect growth regulator seen in many plants which did not exhibit any attack by insects, but the presence of this compound in resistant *Musa* cultivar is new to science. Larvae of *Callasobruchus chinensis,* treated with BA resulted wide spread abnormalities and death (Madhavi *et al*., 2019). Treatment with BA on the larvae of *Spodoptera littoralis* resulted cytotoxicity of hemocytes (Coloma *et al*., 2011) and in larvae of *Papilio demoleus,* BA caused strong anti-feedant action (Srinivasa Rao *et al*., 2014). In the present study, larvae of *O. longicollis* in pseudostem administered with 0.4 or 0.8 ppm BA stopped feeding on the fifth and third day of toxicity respectively. Exposure of larvae to BA at 0.8 ppm for 24 hours in control pseudostem was sufficient for inhibiting their development to 5th instar (pre pupa). Larvae did not die within seven days due to toxicity by BA at a dose of 0.4 ppm (LD_{20} concentration) but they were unable to feed or develop into pre pupa within 14 days.

Intoxication by all the three larvicides or three days existence in resistant host plant resulted accumulation of 20E in the hemolymph of fourth instar larvae of *O. longicollis*, which is almost equal to the quantity of 20E in the non-feeding pre pupa (Kavitha, 2019). Cessation of feeding, observed in larvae during the initial phase of toxicity may be due to accumulation of 20E. In *Helicoverpa armigera*, it was observed that 20E binds to dopamine receptor, a G protein to stop larval feeding and promote pupation (Xin *et al.,* 2019, 2021). Another effect observed in the hemolymph of intoxicated larvae is the inhibition of TlSP and PO. These two enzymes play important role in cuticle sclerotisation and melanisation and also important in defense against parasites (Amado *et al*., 2004). In *Drosophila ananassae* and *Culex quinquefasciatus* TlSP and PO are parallely acting enzymes and are exhibiting very high activity in larvae and negligibly low in five day old mosquitoes and seven day old flies. Elevation of temperature in *C. quinquefasciatus* larvae (Ayana Gayathri and Evans, 2018) and transgeneration effect of BA in *D. ananassae* (Anuji, 2021) caused increase of pigmentation in adults and elevation of both enzyme activities. In Monarch butterfly *Danus plexipuss,* infection by a protozoan caused abnormal melanisation and elevation of PO activity (Cecilia *et al*., 2021). All these reports prove the active role of these enzymes in cuticle development and inhibition of TlSP and PO activities in the inhibition of cuticle development.

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A checklist of Indian Epipaschiinae (Lepidoptera, Pyraloidea, Pyralidae)

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ABSTRACT: A systematic checklist for the species of Epipaschiinae (listed alphabetically) occurring in India, listing 80 species under 18 genera is presented. The report accounted for 10.85 per cent of species and 19.15 per cent of genera from India as compared to global record (737 described species under 94 genera). Further, the valid name, type genus, type species, type locality, synonyms, host range and distribution of each species under each genus are provided.

KEY WORDS: Type genus, species, synonyms, host range, distribution, India

INTRODUCTION

The Epipaschiinae was erected by Meyrick in 1884 with type genus: *Epipaschia* Clemens, 1860. It can be characterized by: males with upturned and pointed third segment of the labial palpi, ventrally curved phallobase of the male usually extends beyond the ductus ejaculatorius, the weakly sclerotised tegumen (Nuss *et al*., 2003-2022) and the uncus arms extended to 110 degrees or more from the longitudinal uncus axis (Solis, 1999; Solis and Mitter, 1992). It is the economically important subfamily of Pyraloidea, comprises of 737 described species under 94 genera from all over the world (Singh *et al*., 2022; Nuss *et al*., 2003-2022). Epipaschiinae comprises of crop pests as leaf rollers, leaf tiers and leaf miners on crops like corn, mango, guava, sapota, jamun and forest trees like mahogany. Species like *Lepidogma* sp., *Lamida moncusalis* Walker and *Orthaga exvinacea* (Walker) are considered as major pests of jamun, cashew and mango, respectively.

Checklist is a skeletal classification of groups listed by the taxa for a quick reference. It provides a synopsis for the species occurring in certain region and also gives the current status of that particular species. Previously, Mathew (2006) reported 74 species under 15 genera from India. Of these, 27 species are currently invalid. Later, Shankaramurthy *et al*. (2015) prepared a preliminary checklist of agriculturally important Pyraloidea of India which included 4 species of Epipaschiinae under 2 genera. Similarly, Nagaharish

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(2016) provided the checklist for Epipaschiinae associated with horticultural crops in zone 1 and 2 of Karnataka which comprises only two species. Despite of these works, there is no complete information (number of species and genera, valid names, synonyms, distribution and host range) of Epipaschiinae occurring in India. In this context, an effort was made to provide the comprehensive checklist for the species of Epipaschiinae occurring in India which provides base for further taxonomic research on this economically important group.

MATERIALS AND METHODS

The information for checklist of Epipaschiinae was collected from various primary and secondary sources of publications. The present study mainly referred the works of Hampson (1896a, 1896b); Cashatt (1968), Munroe and Solis (1999), Arora (2000); Mathew (2006), Liu *et al*. (2016), Wang *et al*. (2017a, 2021), Singh *et al*. (2022) and Nuss *et al*. (2003-2022). The present paper includes all the species of Epipaschiinae occurring in India till date with comprehensive information on valid name, synonyms, type genus, type species, type locality, distribution and host range.

RESULTS AND DISCUSSION

The checklist prepared in the present paper for the Indian Epipaschiinae, included 80 species classified under 18 genera. The synoptic checklist of Indian Epipaschiinae is provided here.

Subfamily: **EPIPASCHIINAE Meyrick, 1884; TG: *Epipaschia* Clemens, 1860**

= *Pococerinae* Hampson, 1918; TG: *Pococera* Zeller, 1848

Genus: ***Arcanusa*** Wang, Chen & Wu in Wang, Chen, Zhu & Wu, 2017; TS: ***Arcanusa apexiarcanusa*** Wang, Chen & Wu, 2017

Arcanusa sinuosa (Moore, 1888) (***Scopocera***); TL: India (Darjeeling)

Distribution: Arunachal Pradesh, East Himalayas (Hampson, 1896a), Sikkim (Hampson, 1896b) and West Bengal (Darjeeling) (Solis, 1992)

Host range: Unknown

Genus: ***Canipsa*** Walker, 1866; TS: ***Canipsa suspensalis*** Walker, 1866

= *Sarama* Moore, 1888; TS: *Sarama atkinsonii* Moore, 1888

= *Scopocera* Moore, 1888; TS: *Scopocera pyraliata* Moore, 1888

Canipsa atkinsonii (Moore, 1888) (***Sarama***); TL: India (Darjeeling)

Distribution: Sikkim (Hampson, 1896a) and West Bengal (Darjeeling) (Solis, 1992)

Host range: Unknown

Canipsa pyraliata (Moore, 1888) (***Scopocera***); TL: India (Darjeeling)

Distribution: Sikkim (Hampson, 1896a) and West Bengal (Darjeeling) (Solis, 1992)

Host range: Unknown

Canipsa suspensalis Walker, 1866; TL: Malaysia, Borneo and Sarawak

Distribution: Khasias (Hampson, 1903b)

Host range: Unknown

Genus: ***Coenodomus*** Walsingham, 1888; TS: ***Coenodomus hocking*** Walsingham, 1888

= *Alippa* Aurivillius, 1894; TS: *Alippa anomala* Aurivillius, 1894

= *Dyaria* Neumoegen, 1893; TS*: Dyaria singularis* Neumoegen, 1893

Coenodomus aglossalis (Warren, 1896) (***Scopocera***); TL: India (Khasi hills and Meghalaya)

Distribution: Assam, Khasias (Wang *et al*., 2017a), Meghalaya and North West Himalayas

(Hampson, 1896b)

Host range: Unknown

Coenodomus fumosalis Hampson, 1903; TL: India (Khasi hills and Meghalaya)

Distribution: Khasias (Wang *et al*., 2021; Hampson, 1903b)

Host range: Unknown

Coenodomus hockingi Walsingham, 1888; TL: India (Punjab and Kangra Valley)

= *Alippa anomala* Aurivillius, 1894; TL: Indonesia, Java

= *Dyaria singularis* Neumoegen, 1893; TL: USA (Bangor, Maine)

Distribution: Andhra Pradesh (Visakhapatnam), Himachal Pradesh (Shimla), Kangra valley,

Punjab (Wang *et al*., 2017a), Sikkim and West Bengal (Darjeeling) (Hampson, 1896b)

Host range: Unknown

Coenodomus rotundinidus Hampson, 1891; TL: India (Tamil Nadu (Nilgiris)).

Distribution: Nilgiri Plateau (Hampson, 1896a; Wang *et al*. 2017a)

Host range: Unknown

Coenodomus rubrescens (Hampson, 1903); TL: India (Sikkim)

Distribution: India (Wang *et al*., 2017a)

Host range: Unknown

Genus: *Lamida* Walker, 1859; TS: ***Lamida moncusalis*** Walker, 1858

= *Allata* Walker, 1863; TS: *Allata penicillata* Walker, 1862

Lamida mediobarbalis (Hampson, 1916) (***Macalla***); TL: Sikkim

Distribution: Sikkim (Solis, 1992)

Host range: Unknown

Lamida moncusalis Walker, 1859; TL: India (Hindustan)

= *Allata penicillata* Walker, 1858; TL: India (Hindustan)

Distribution**:** Kerala (Calicut) (Mathew and Menon, 1984), Manipur, Nagas (Hampson,

1896b), North India (Rose and Dhillon, 1980), Sikkim (Hampson, 1896b) and West Bengal

(Darjeeling) (Snellen, 1890)

Host range: *Anacardium occidentale* and *Mangifera indica*

Lamida obscura (Moore, 1888) (***Orthaga***); TL: India (Darjeeling)

= *Macalla sordidalis* Hampson, 1916; TL: India (Sikkim)

= *Macalla sordidalis* subsp. *proximalis* Caradja, 1925; TL: China (Guangdong, Guangzhou)

Distribution: Sikkim (Solis, 1992) and West Bengal (Darjeeling) (Moore, 1888)

Host range: Unknown

Genus: ***Lepidogma*** Meyrick, 1890; TS: ***Hypotia tamaricalis*** Mann, 1873

= *Asopina* Christoph, 1893; TS: *Asopia obatralis* Christoph, 1877

= *Precopia* Ragonot, 1891; TS: *Hypotia atomalis* Christoph, 1887

Lepidogma ambifaria (Hering, 1901) (***Stericta***); TL: Indonesia, Sumatra

= *Stericta dubia* Wileman & South, 1917; TL: Taiwan (Kanshirei)

Distribution: Andaman Islands (Rao and Sivaperuman, 2020)

Host range: Unknown

Lepidogma obatralis (Christoph, 1877) (***Asopia***); TL: Turkmenbasy (Krasnowodsk, Turkmenistan)

Distribution: Punjab (Hampson, 1896b) and North West India (Hampson, 1896a)

Host range: Unknown

Lepidogma olivalis (Swinhoe, 1895) (***Hypsopygia***); TL: India - (Maharashtra, Mahabaleshwar)

= *Ulotrichodes novalis* Warren, 1896; TL: India (Mumbai)

Distribution: khasis (Hampson, 1903b), Maharashtra (Mahabaleshwar) (Solis, 1992) and

Western India (Hampson, 1896a)

Host range: Unknown

Lepidogma tamaricalis (Mann, 1873) (***Hypotia***); TL: Italy (Tuscany, Livorno)

= *Hypotia vafera* Swinhoe, 1884; TL: Pakistan (Karachi)

Distribution: India (Wang *et al*., 2021)

Host range: *Tamarix* sp.

Genus: ***Lista*** Walker, 1859; TS: ***Lista genisusalis*** Walker, 1859

= *Belonepholis* Butler, 1889; TS: *Belonepholis striata* Butler, 1889

= *Belenopholis* Solis, 1993

= *Craneophora* Christoph, 1881; TS: *Craneophora ficki* Christoph, 1881

= *Paracme* Lederer, 1863; TS: *Paracme insulsalis* Lederer, 1863

Lista ficki (Christoph, 1881); TL: Amur area (Amurgebiet)

= *Belonepholis striata* Butler, 1889; TL: Japan

Distribution: Himachal Pradesh (Dharmashala) (Solis, 1993)

Host range: Unknown

Lista haraldusalis (Walker, 1859) **(*Locastra)*;** TL: Malaysia, Borneo, Sarawak

= *Lista genisusalis* Walker, 1859; TL: Malaysia, Borneo, Sarawak

Distribution: Himachal Pradesh (Shimla, Kulu, Dharmashala), Sikkim (Hampson, 1896b),

Himalayas (Hampson, 1896a) and West Bengal (Darjeeling) (Sanyal *et al*., 2012)

Host range: Unknown

Lista insulsalis (Lederer, 1863) (*Paracme)*; TL: Malaysia, Borneo, Sarawak

= *Lista genisusalis* Walker, 1859; TL: Malaysia, Borneo, Sarawak

Distribution**:** India (Wang *et al*., 2017b)

Host range: Unknown

Lista variegata (Moore, 1888); TL: India (Darjeeling)

Distribution: Sikkim and West Bengal (Darjeeling) (Solis, 1992)

Host range: Unknown

Genus: ***Locastra*** Walker, 1859; TS: ***Locastra maimonalis*** Walker, 1859

= *Taurica* Walker, 1866; TS: *Taurica muscosalis* Walker, 1865

Locastra crassipennis (Walker, 1857) (*Eurois*); TL: Bangladesh (Silhet)

= *Locastra maimonalis* Walker, 1859; TL: Malaysia, Borneo, Sarawak

Distribution: Assam (Hampson, 1896a), Naga Hills and Sikkim (Snellen, 1890)

Host range: Unknown

Locastra mizo Ranjan, Singh & Kirti, 2022; TL: India (Mizoram (Kanhmun))

Distribution: Mizoram (Ranjan *et al*., 2022c)

Host range: Unknown

Locastra muscosalis (Walker, 1866) (*Taurica*); TL: North China

= *Locastra cristalis* Hampson, 1893; TL: Sri Lanka (Ceylon), Wattegama

= *Taurica sikkima* Moore, 1888; TL: India (Darjeeling)

Distribution: Arunachal Pradesh, Assam, Mizoram, Nagaland, Tamil Nadu, Andaman and Nicobar Islands (Chandra *et al*. 2018), Sikkim, Nagas (Hampson, 1896b) and West Bengal (Darjeeling) (Sevastopulo, 1948).

Host range: *Malus pumila* and *Pistacia chinensis*

Locastra viridis Rong & Li, 2017; TL: China (Xiajinchang)

Ditribution: Sikkim (Dodak) (Ranjan *et al*., 2022c)

Host range: Unknown

Genus: ***Noctuides*** Staudinger, 1892; TS: ***Noctuides melanophia*** Staudinger, 1892

= *Anartula* Staudinger, 1893; TS: *Noctuides melanophia* Staudinger, 1892

= *Arnatula* Hampson, 1896

= *Parorthaga* Hampson, 1896; TS: *Balanotis euryptera* Meyrick, 1894

Noctuides melanophia Staudinger, 1892; TL: China/Russia (Amur region (Amurgebiet))

= *Balanotis euryptera*; TL: Indonesia (Sambawa)

Distribution: Kerala (Alex *et al*., 2021)

Host range: Unknown

Genus: ***Odontopaschia*** Hampson, 1903; TS: ***Odontopaschia virescens*** Hampson, 1903

Odontopaschia virescens Hampson, 1903; TL: India (Khasi Hills)

Distribution: Khasis (Hampson, 1903b; Solis, 1992)

Host range: Unknown

Genus: ***Omphalota*** Hampson, 1899; TS: ***Omphalota chlorobasis*** Hampson, 1899

Omphalota chlorobasis Hampson, 1899; TL: India (Himachal Pradesh (Shimla))

Distribution: Himachal Pradesh (Shimla) (Hampson, 1899; Solis, 1992)

Host range: Unknown

Genus: ***Orthaga*** Walker, 1859; TS***: Orthaga euadrusalis*** Walker, 1859

= *Edeta* Walker, 1859; TS*: Edeta icarusalis* Walker, 1859

= *Hyperbalanotis* Warren, 1891; TS: *Glossina achatina* Butler, 1878

= *Pannucha* Moore, 1888; TS: *Pannucha aenescens* Moore, 1888

= *Proboscidophora* Warren, 1891; TS: *Pyralis tritonalis* Walker, 1859

Orthaga achatina (Butler, 1878) (*Glossina*); TL: Japan (Yokohama)

Distribution: India (Wang *et al*., 2021)

Host range: *Cinnamomum camphora*

Orthaga aenescens (Moore, 1888) (***Pannucha***); TL: India (Darjeeling)

= *Pannucha vicinalis* Snellen, 1890; TL: India (West Bengal (Darjeeling) and Sikkim)

Distribution: Nagas, Sikkim (Hampson, 1896b), West Bengal (Darjeeling) (Solis, 1992) and

West Himalaya (Sanyal *et al*., 2018)

Host range: *Cinnamomum sp.* and *Litsea glutinosa*

Orthaga auroviridalis Hampson, 1896; TL: India (Sikkim), Bhutan

Distribution: Sikkim (Hampson, 1896b) and South India (Hampson, 1896a)

Host range: Unknown

Orthaga basalis (Moore, 1888) (***Pannucha***); TL: India (Darjeeling)

Distribution: Kerala (Mathew, 2009), Sikkim, Nagas (Hampson, 1896b) and West Bengal

(Darjeeling) (Snellen, 1890)

Host range: Unknown

Orthaga euadrusalis Walker, 1859**;** TL: Malaysia, Borneo, Sarawak, Sri Lanka

= *Orthaga acontialis* Walker, 1863; TL: Malaysia, Borneo, Sarawak

Distribution: Andhra Pradesh (Kavitha *et al*., 2005), Arunachal Pradesh, Andaman and Nicobar Islands, Maharashtra (Chandra *et al*., 2019), Sikkim (Hampson, 1896b), West Bengal (Darjeeling and Kolkata) (Snellen, 1890; Sanyal *et al*., 2012)

Host range: *Mangifera indica* (Kavitha *et al*., 2005)

Orthaga exvinacea Hampson, 1891 (***Balanotis***); TL: India (Tamil Nadu (Nilgiri))

Distribution: Andhra Pradesh, Andaman and Nicobar Islands (Rao and Sivaperuman, 2020),

Kerala (Das *et al*., 2020a), Maharashtra, West Bengal and Tamil Nadu (Nilgiris) (Hamspon, 1891)

Host range: *Mangifera indica* (Gamit *et al*., 2018)

Orthaga mangiferae Misra, 1932; TL: India

Distribution: India (Misra, 1932; Mathew, 2006)

Host range: *Mangifera indica*

Orthaga molleri Hampson, 1896; TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896b)

Host range: Unknown

Orthaga olivacea (Warren, 1891) (***Hyperbalanotis***); TL: Japan

= *Stericta shisalis* Strand, 1919; TL: China, Taiwan (Shisa)

Distribution: India (Wang *et al*., 2021)

Host range: Unknown

Orthaga onerata (Butler, 1879) (***Bleptina***); TL: Japan

= *Orthaga grisealis* Wileman, 1911; TL: Japan (Yokohama)

Distribution: North East India (Hampson, 1896a)

Host range: Unknown

Orthaga rhodoptila (Meyrick, 1932) (***Balanotis***); TL: Sri Lanka (Galle)

Distribution: Andaman and Nicobar Islands (Veenakumari *et al*., 1997)

Host range: Unknown

Orthaga roseiplaga Hampson, 1906; TL: India (Mumbai)

Distribution: Maharashtra (Mumbai) (Hampson, 1896b; Solis, 1992)

Host range: Unknown

Orthaga rudis (Walker, 1862) (***Locastra***); TL: India (Hindustan)

Distribution: Tamil Nadu (Nilgiri) (Hampson, 1896a)

Host range: Unknown

Orthaga vitialis (Walker, 1859) (***Pyralis***); TL: Sri Lanka

= *Pyralis altusalis* Walker, 1859

= *Pyralis helvialis* Walker, 1859; TL: Unknown

Distribution: Tamil Nadu (Nilgiri) (Hampson, 1891)

Host range: *Cinnamomum verum* (Rajapakse and Kumara, 2007; Khan *et al*., 2020)

Genus: ***Pseudocera*** Walker, 1863; TS: ***Pseudocera inconcisa*** Walker, 1863

Pseudocera rubrescens (Hampson, 1903) (*Stericta*); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1903b; Solis, 1992)

Host range: Unknown

Genus: *Salma* Walker, 1863; TS: ***Salma recurvalis*** Walker, 1863

= *Balanotis* Meyrick, 1884; TS: *Salma recurvalis* Walker, 1863

= *Calinipaxa* Walker, 1866; TS*: Calinipaxa validalis* Walker, 1866

= *Enchesphora* Turner, 1913; TS: *Enchesphora poliophanes* Turner, 1913

= *Exacosmia* Walker, 1865; TS: *Exacosmia rubiginosa* Walker, 1865

= *Heterobella* Turner, 1904; TS: *Heterobella triglochis* Turner, 1904

= *Orthotrichophora* Warren, 1891; TS: *Bertula syrichtusalis* Walker, 1859

= *Parasarama* Warren, 1890; TS: *Locastra cuproviridalis* Moore, 1867

= *Pseudolocastra* Snellen, 1890: TS: *Locastra inimica* Butler, 1879

= *Pseudolocastra* Warren, 1891; TS: *Locastra inimica* Butler, 1879

= *Sultania* Kocak, 1987; TS: *Macalla lophotalis* Hampson, 1900

Salma atricinctalis (Hampson, 1916) (***Macalla***); TL: India (Kerala (Travancore, Pirmad)

Distribution: Kerala (Travancore) (Solis, 1992)

Host range**:** Unknown

Salma carbonifera (Meyrick, 1932) (***Lamida***); TL: India (Uttarakhand (Dehradun))

Distribution**:** Uttarakhand (Dehradun) (Solis, 1992) and West Bengal (Kolkata) (Sevastopulo, 1948)

Host range**:** Unknown

Salma cuproviridalis **(**Moore, 1867) (***Locastra***); TL: India (Darjeeling)

Distribution**:** Sikkim (Hampson, 1896a), Tamil Nadu (Nilgiris) (Hampson, 1891) and West

Bengal (Darjeeling) (Solis, 1992)

Host range: Unknown

Salma derogatella (Walker, 1863) (***Acrobasis***); TL: Malaysia, Sarawak

= *Salma derogatella formosibia* (Strand, 1919) (*Macalla derogatella* var.); TL: China, Taiwan, Kosempo, Alikang

= *Salma derogatella scurtata* (Caradja, 1925) (*Macalla derogatella* subsp.); TL: China,

Zhejiang, Mount Mogan, Guangdong, Guangzhou (Canton)

Distribution: Himachal Pradesh (Shimla), Nagas, Sikkim (Hampson 1896b) and Uttarakhand (Nainital) (Smetacek, 2008).

Host range: Unknown

Salma dimidialis (Snellen, 1890) (***Pannucha***); TL: India (Darjeeling)

Distribution: Sikkim (Hampson, 1896a) and West Bengal (Darjeeling) (Snellen, 1890)

Host range: Unknown

Salma eumictalis (Hampson, 1912) (***Macalla***); TL: Sri Lanka (Maskeliya)

Distribution: Kerala (Calicut) (Mathew and Menon, 1984; Mathew *et al*., 2005)

Host range: Unknown

Salma exrufescens (Hampson, 1896) (***Macalla***); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896a; Solis, 1992)

Host range: Unknown

Salma hypnonalis (Hampson, 1899) (***Macalla***); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896a)

Host range: Unknown

Salma hypoxantha (Hampson, 1896) (***Macalla***); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896a)

Host range: Unknown

Salma metasarica (Hampson, 1903); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1903b)

Salma nubilalis (Hampson, 1893) (***Stericta***); TL: Sri Lanka (Colombo)

Distribution: Kerala (Mathew *et al*., 2005) and Tamil Nadu (Das e*t al*., 2020a)

Host range: Unknown

Salma rufibarbalis (Hampson, 1903) (***Macalla***); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1903a; Solis, 1992)

Host range: Unknown

Salma rufitinctalis (Warren, 1896) (***Parasarama***); TL: India (Khasi hills, Meghalaya)

Distribution: Khasis (Warren, 1896b)

Host range: Unknown

Salma scoporhyncha (Hampson, 1896) (***Macalla***); TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896b)

Host range: Unknown

Salma syrichtusalis (Walker, 1859) (***Bertula***); TL: Malaysia, Borneo, Sarawak

= *Orthaga pyralisalis* Walker, 1863; TL: Malaysia, Borneo, Sarawak

Distribution: Sikkim and Nagas (Hampson, 1896b)

Host range: Unknown

Salma validalis (Walker, 1866) (***Calinipaxa***); TL: Malaysia, Borneo, Sarawak

= *Scopocera minor* Moore, 1888; TL: India (West Bengal (Darjeeling))

= *Peucela fumosalis* Warren, 1896; TL: India (Khasi Hills)

= *Pyralis costimacula* Wileman & South, 1917; TL: China, Taiwan (Formosa)

Distribution: Sikkim, Khasis, Nagas (Hampson, 1896b) and West Bengal (Darjeeling) (Bhattacharya, 2000)

Genus: ***Stericta*** Lederer, 1863; TS: ***Glossina divitalis*** Guenee, 1854

= *Glossina* Guenée, 1854; TS: *Glossina divitalis* Guenée, 1854

= *Matalia* Walker, 1866; TS: *Matalia conscisella* Walker, 1866

= *Oncobela* Turner, 1937; TS: *Oncobela philobrya* Turner, 1937

= *Phialia* Walker, 1866; TS: *Phialia gelechiella* Walker, 1866

Stericta asopialis (Snellen, 1890); TL: India (West Bengal (Darjeeling), Sikkim)

Distribution: East Himalayas (Hampson, 1896a), Sikkim and West Bengal (Darjeeling) (Snellen, 1890; Solis, 1992)

Host range: Unknown

Stericta carneotincta Hampson, 1896; TL: India (Sikkim)

Distribution: Sikkim (Hampson, 1896b)

Host range**:** Unknown

Stericta divitalis (Guenee, 1854) (***Glossina***); TL: Central India

= *Locastra phereciusalis* Walker, 1859; TL: Malaysia, Borneo, Sarawak

Distribution: Assam (Hampson, 1896b), Chhattisgarh, Madhya Pradesh, Uttarakhand, Uttar

Pradesh (Solis, 1992) and North East India (Hampson, 1896a)

Host range: Unknown

Stericta olivialis Hampson, 1903; TL: Bhutan, India (Khasi Hills)

Distribution: Khasis (Hampson, 1896b)

Host range: Unknown

Genus: ***Teliphasa*** Moore, 1888; TS: ***Teliphasa orbiculifer*** Moore, 1888

Teliphasa albifusa (Hampson, 1896) (***Macalla)***; TL: India (Nagaland, Sikkim)

= *Macalla shishana* Strand, 1919; TL: China, Taiwan, Shisa

Distribution**:** Kerala (Rai, 1984), Nagas and Sikkim (Hampson, 1896b; Liu *et al*., 2016)

Host range**:** Unknown

Teliphasa dodaki Ranjan, Singh & Kirti, 1896; TL: India (Sikkim (Dodak))

Distribution: Sikkim and Uttarakhand (Ranjan *et al*., 2022a)

Host range**:** Unknown

Teliphasa erythrina Wang & Li, 2016; TL: Mengla Country, China (Yuvan Province)

Distribution: Meghalaya (Ranjan *et al*., 2022a)

Host range: Unknown

Teliphasa hamata Liu, Wang & Li, 2016; TL: China (Yunnan Province), Tengchong

Distribution: Sikkim and Uttarakhand (Ranjan *et al*., 2022a)

Host range**:** Unknown

Teliphasa nubilosa Moore, 1888; TL: West Bengal (Darjeeling)

= *Macalla formisibia* Strand, 1919; TL: Taiwan

Distribution: Kerala (Das *et al*., 2020a), Meghalaya (Ranjan *et al*., 2022a) and West Bengal (Darjeeling) (Moore, 1888)

Host range**:** Unknown

Teliphasa orbiculifer Moore, 1888; TL: West Bengal (Darjeeling)

Distribution: West Bengal (Darjeeling) (Moore, 1888)

Host range: Unknown

Teliphasa similalbifusa Liu, Wang & Li, 2016; TL: China (Guangxi Zhuang)

Distribution: Arunachal Pradesh, Uttarakhand, West Bengal (Chandra *et al*., 2019) and Sikkim (Ranjan *et al*., 2022a)

Host range: Unknown

Teliphasa spinaejuxta Ranjan, Singh & Kirti, 2022; TL: India (Sikkim (Golitar))

Distribution: Sikkim and Uttarakhand (Ranjan *et al*., 2022a)

Host range: Unknown

Genus: *Termioptycha* Meyrick, 1889; TS: ***Termioptycha cyanopa*** Meyrick, 1889

= *Sialocyttara* Turner, 1913; TS: *Sialocyttara erasta* Turner, 1913

Termioptycha albifurcalis Hampson, 1916; TL: India (Kerala (Travancore))

Distribution: Andaman and Nicobar Islands, Kerala (Hampson, 1916) and Maharashtra (Sondhi *et al*., 2021)

Host range: Unknown

Termioptycha almae Ranjan, Singh & Kirti, 2022; TL: India (Sikkim (Deorali))

Distribution: Sikkim (Deorali) (Ranjan *et al*., 2022b)

Host range: Unknown

Termioptycha bilineata (Wileman, 1911) (***Macalla***); TL: Japan (Settsu)

Distribution: Sikkim (Deorali) (Ranjan *et al*., 2022b)

Host range: Unknown

Termioptycha conjuncta (Warren, 1911) (***Parasarama***); TL: India (Khasi Hills, Meghalaya)

Distribution: Meghalaya and Khasias (Warren, 1896a)

Host range: Unknown

Termioptycha cornutitrifurca Rong & Li, 2017; TL: China (Yunnan (Mengla (Bubang))

Distribution: Karnataka, Meghalaya, Mizoram and Sikkim (Ranjan *et al*., 2022b)

Host range: Unknown

Termioptycha gnathospina Ranjan, Singh & Kirti, 2022; TL: India (Sikkim (Dodak))

Distribution: Sikkim (Dodak) (Ranjan *et al*., 2022b)

Host range: Unknown

Termioptycha inimica (Butler, 1879) (***Locastra***); TL: Japan

Distribution: Sikkim and West Bengal (Darjeeling) (Snellen, 1890)

Host range: Unknown

Termioptycha margarita (Butler, 1879) (***Locastra***); TL: Japan (Yokohama)

= *Locastra lativitta* Moore, 1888; TL: West Bengal (Darjeeling)

= *Macalla* (*Parasarama*) *margarita shisana* Strand, 1919; TL: China, Taiwan (Shisa)

Distribution: Kerala (Bhattacharya, 2000), Khasis, Tamil Nadu (Nilgiris) and Sikkim (Hampson, 1896b)

Host range: Unknown

Genus: ***Titanoceros*** Meyrick, 1884; TS: ***Titanoceros cataxantha*** Meyrick, 1884

Titanoceros malefica (Meyrick, 1934); TL: India (Kerala, Nilambur)

Distribution: Kerala (Meyrick, 1934)

Host range: Unknown

Genus: ***Trichotophysa*** Warren, 1896; TS: ***Trichotophysa olivalis*** Warren, 1896

Trichotophysa jucundalis (Walker, 1866) (***Bleptina***); TL: Sri Lanka

= *Trichotophysa olivalis* Warren, 1896; TL: India (Khasi hills, Meghalaya)

Distribution: Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab, Uttarakhand, Uttar Pradesh (Das *et al*., 2020b), Khasis (Hampson 1896b) and North East India (Hampson, 1896a)

Host range: Unknown

The current study is the first effort to consolidate a comprehensive checklist for all the species of Epipaschiinae occurring in India, which includes 80 described species under 18 genera. This study provides a base for further taxonomic research.

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Morphological and biochemical basis of resistance against the pod borers *Maruca vitrata* F. and *Helicoverpa armigera* (Hübner) in cowpea

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ABSTRACT: For determination of morphological and biochemical basis of resistance in cowpea against the pod borers (*Maruca vitrata* F. and *Helicoverpa armigera* (Hübner)), ten cowpea cultivars were evaluated during *kharif* 2017 and 2018. Results revealed that cultivars having indeterminate growth, yellow flower, light green and short pods as well as long peduncle recorded lower pod borer population. Correlation between different plant morphological characters and the larval population were found non-significant. The cultivars having low protein, high phenol and low total soluble sugars in flowers and immature pods recorded lower larval population. The correlation between biochemical components of flowers and larval population were found to be non-significant. Biochemical components of immature pods revealed that phenol exhibited significant negative relationship with larval population, whereas, correlation of total soluble sugars of immature pods exhibited significant positive association with larval population. Among the cultivars screened, GC-6 and GC-1605 were found to be consistently resistant based on different morphological and biochemical factors and pod borer infestation. Relatively GC-1609, GC-1611 and GC-2 showed lesser pod borer infestation. GC-1606 was found to be highly susceptible.

KEY WORDS: Flower, immature pod, phenol, total soluble sugars, larval population

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important legume crop grown in tropical and sub-tropical habitats both for vegetable and pulse. Cowpea is one of the most important pulse crops, native to central Africa, belongs to family Fabaceae. Cowpea is well adapted to the drier regions of the tropics because of its drought tolerance capacity. In terms of area, it is the second most important food legume crop in the world. Production of cowpea is limited by large number of biotic and abiotic constraints and the productivity remains very low in India. The main reason attributed for the low productivity is the extensive damage caused by insect pests. Among different insect pests, pod borers (spotted pod borer *Maruca vitrata* F. (Lepidoptera, Crambidae) and gram pod borer *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae) damaging the reproductive parts cause

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maximum reduction in yield. Pod borers damaging the reproductive parts cause maximum reduction in yield. Gubbaiah *et al.* (1975) observed 42 to 56 per cent damage to cowpea pods due to pod borer complex. Kumar (1978) observed the damage as high as 94.67 and 78.93 per cent on pods and seeds, respectively by pod borers. The avoidable losses in yield due to insect pests have been recorded in the range of 66 to 100 per cent in cowpea (Pandey *et al.,* 1991). Extensive use of insecticides and pesticides cause serious problems of pesticide residues, pest resurgence and also cause environmental pollution. Therefore it is necessary to use resistant varieties against pod borers. An investigation was carried out to determine the morphological and biochemical factors responsible for resistance in cowpea cultivars against the pod borers.

MATERIALS AND METHODS

An experiment was conducted for two years (kharif 2017 and 2018) to evaluate the basis of resistance in cowpea genotypes/cultivars against the pod borers infesting cowpea, at College of Agriculture, Navsari Agricultural University, Campus Bharuch, Gujarat. Ten genotypes/ cultivars were sown (Table1). The basis of resistance in various cowpea genotypes/ cultivars was determined based on various morphological characters and biochemical parameters. Data on morphological characters such as plant growth habit (determinate/ indeterminate), flower colour (yellow/ violet), pod colour (light green/ dark green) as well as peduncle and pod length of 25 peduncles/ pods of each genotypes/ cultivars were recorded from ten uniformly developed plants. Peduncle and pod length was measured. Biochemical analysis of flowers and immature pods for parameters such as protein, phenols and total soluble sugar were done by following the methods developed by Sadasivam and Manikkam (1996). The data on morphological characters and biochemical components of flowers and immature pods were statistically analyzed using statistical procedure (Steel and Torrie, 1980).

Table 1. Morphological and biochemical parameters of cowpea genotypes/ cultivars

Cultivar/ Genotype	Peduncle length (cm)	Pod length (cm)	Flower			Immature pod		
			Protein (%)	Phenol (mg/g)	sugar (%)	Protein (%)	Phenol (mg/g)	sugar (%)
GC-1605	17.68	9.44	11.75	0.51	1.19	12.40	0.60	1.15
GC-1606	12.68	10.80	11.19	0.46	4.80	20.30	0.21	4.32
GC-1607	19.70	10.10	6.68	0.43	1.65	10.81	0.32	4.42
GC-1609	28.90	8.50	11.64	0.64	1.70	12.99	0.19	4.13
GC-1611	16.90	8.40	10.13	0.45	2.89	13.11	0.42	1.92
GC-2	19.14	11.20	12.45	0.93	4.58	24.22	0.70	1.67
GC-3	16.56	11.90	6.60	0.48	1.43	11.22	0.35	2.96
GC-4	20.04	10.60	11.58	0.37	2.19	10.62	0.26	3.07
GC-5	18.44	9.80	7.18	0.62	2.03	7.34	0.63	4.42
GC-6	23.12	9.60	4.14	0.37	1.45	12.95	0.70	1.20
Mean	19.31	10.03	9.33	0.53	2.39	13.60	0.44	2.93

RESULTS AND DISCUSSION

Morphological characters

The results revealed that determinate genotypes/ cultivars recorded higher larval population of *M. vitrata* and *H. armigera* than indeterminate ones. The genotypes/cultivars having violet coloured flower recorded more larval population than yellow coloured genotypes/cultivars. The genotypes/ cultivars having dark green coloured pods recorded more larval population than light green coloured pods. Genotypes/cultivars having long peduncle recorded lower larval population than short peduncle whereas genotypes/cultivars having long pod recorded more larval population than short pod genotypes/cultivars. Cultivar GC-6 was found to be consistently resistant to the pod borer infestation (Table 1, 2).

Table 2. Impact of different morphological characters on the pod borers in cowpea genotypes/cultivars (Mean of two years)

Plant characters/ Genotypes		Mean larvae /plant	
		M. vitrata	*H. armigera*
Plant growth	Determinate - GC-1607, GC-3, GC-4, GC-5	1.30 ± 0.09	1.13 ± 0.13
	Indeterminate - **GC-1605**, GC- 1606, GC-1609, GC- 611, GC-2, GC-6	**1.05 ± 0.50**	**0.75 ± 0.42**
Flower colour	Yellow - GC-1605, GC-1606, GC-1607, GC-1609, GC-1611, GC-4, **GC-6**	**1.12 ± 0.48**	**0.87 ± 0.45**
	Violet - GC-2, GC-3, GC-5	1.21 ± 0.09	0.97 ± 0.21
Pod colour	Light green – GC-1607, GC-1609, GC-3, **GC-6**	**1.02 ± 0.37**	**0.88 ± 0.41**
	Dark green - GC-1605, GC-1606, GC-1611, GC-2, GC-4, GC-5	1.23 ± 0.42	0.92 ± 0.40
Peduncle length (cm)	Long >19.31*GC-1607, GC-1609, GC-4, **GC-6**	**1.04 ± 0.40**	**0.89 ± 0.42**
	Short <19.31 GC-1605, GC-1606, GC-1611, GC-2, GC-3, GC-5	1.21 ± 0.41	0.91 ± 0.39
Pod length (cm)	Long >10.00*GC-1606, GC-1607, GC-2, GC-3, GC-4	1.40 ± 0.30	1.15 ± 0.23
	Short <10.00 **GC-1605**, GC-1609, GC-1611, GC-5, GC-6	**0.89 ± 0.31**	**0.65 ± 0.34**

*Mean values refer Table 1

Findings in the present investigation are in accordance with the findings of Reddy *et al.* (1983), Lal *et al*. (1986), Kushwaha and Malik (1987), Sharma *et al*. (1999) and Bhadani (2019) who reported higher larval population in determinate genotypes than indeterminate genotypes and more larval population in long pod length genotypes/ cultivars than short pod length.

Correlation of morphological characters and larval population

Plant morphological characters and larval population of *M. vitrata and H. armigera* did not show any significant correlation between them, indicating that the impact of morphological characters on larval population as well flower and pod damage is

negligible. These results are in accordance with findings of Anusha (2013) who reported that the morphological characters did not exhibit any significant relationship with the flower and pod damage in relation to resistance or susceptibility. Singh and Singh (2014) also reported that there were no significant relationship between larval densities and morphological characters of genotypes/cultivars.

Biochemical parameters - Flowers

Flowers with high protein content (> 9.33%) recorded higher larval population of *M. vitrata* than the flowers of cultivars having low protein (<9.33%). However, the protein content did not affect the larval population of *H. armigera.* The genotypes/ cultivars having low phenol content (< 0.53 mg/g) recorded higher larval population (*M. vitrata* and *H. armigera*) than genotypes/cultivars having high

Table 3. Influence of different biochemical components of flower and immature pods on the larval population of pod borers (Mean of two years)

Biochemical components	Category	Genotypes/ cultivars	Larvae /plant	
			M. vitrata	*H. armigera*
Flower				
Protein (%)	High>9.33*	GC-1605, GC-1606, GC-1609, GC-1611, GC-2, GC-4	1.20 ± 0.43	0.90 ± 0.40
	Low <9.33	**GC-1607**, GC-3, GC-5, **GC-6**	**1.06 ± 0.38**	**0.90 ± 0.41**
Phenol (mg/g)	High >0.53*	**GC-1605**, GC-1609, GC-2, GC-5	**1.00 ± 0.26**	**0.71 ± 0.31**
	Low <0.53	GC-1606, GC-1607, GC-1611, GC-3, GC-4,GC-6	1.25 ± 0.46	1.03 ± 0.39
Total Soluble Sugars (%)	High >2.39*	GC-1606, GC-1611, GC-2	1.38 ± 0.46	1.02 ± 0.33
	Low <2.39	**GC-1605**, GC-1607, GC-1609, GC-3, GC-4, GC-5, **GC-6**	**1.05 ± 0.35**	**0.85 ± 0.41**
Immature pods				
Protein (%)	High >13.60*	GC-1606, GC-2	1.51 ± 0.56	1.09 ± 0.44
	Low <13.60	**GC-1605**, GC-1607, GC-1609, GC-1611, GC-3, GC-4, GC-5, **GC-6**	**1.05 ± 0.33**	**0.86 ± 0.38**
Phenol (mg/g)	High >0.44*	**GC-1605**, GC-1611, GC-2, GC-5, **GC-6**	**0.91 ± 0.33**	**0.63 ± 0.33**
	Low <0.44	GC-1606, GC-1607, GC-1609, GC-3, GC-4	1.38 ± 0.33	1.17 ± 0.20
Total Soluble Sugars (%)	High >2.93*	GC-1606, GC-1607, GC-1609, GC-3, GC-4,GC-5	1.35 ± 0.30	1.13 ± 0.20
	Low <2.93	**GC-1605**, GC-1611, GC-2, **GC-6**	**0.84 ± 0.32**	**0.56 ± 0.32**

*Mean values refer Table 1

phenol content (> 0.53 mg/g). The genotypes/cultivars having high total soluble sugar (> 2.39%) recorded higher larval population (*M. vitrata* and *H. armigera*) than genotypes/cultivars having low total soluble sugar content (< 2.39%) (Table 3).

Biochemical parameters - Immature pods

Immature pods with high protein content (> 13.60%) recorded higher larval population (*M. vitrata* and *H. armigera*) than the cultivars having low protein content (< 13.60%). The genotypes/cultivars having low phenol content (< 0.44 mg/g) recorded higher larval population (*M. vitrata* and *H. armigera*) than genotypes/cultivars having high phenol content (> 0.44 mg/g). The genotypes/cultivars having high total soluble sugar (> 2.93%) recorded higher larval population (*M. vitrata* and *H. armigera*) than genotypes/cultivars having low total soluble sugar content (< 2.93%). Among the cultivars screened, GC-6 and GC-1605 were found to be consistently resistant based on different biochemical characters and pod borer infestation. Relatively GC-1609, GC-1611 and GC-2 cultivars also showed resistance to the pod borer infestation. GC-1606 was found highly susceptible cultivar (Table 3).

Cultivars GC-6 and GC-1605 recorded lower pod borers and found to be consistently resistant. Relatively GC-1609, GC-1611 and GC-2 cultivars showed low pod borer infestation indicating these are also have the resistance to the pod borers. GC-1606 was found to be highly susceptible cultivar. The results revealed that genotypes/cultivars having low protein, high phenol and low total soluble sugar in flowers and immature pods recorded lower larval population than genotypes/cultivars having high protein, low phenol and high total soluble sugar in flowers and immature pods. These constituents played vital role in determining the resistance in cowpea genotypes/cultivars against pod borers. These findings are in complete agreement with findings of Jaydeep *et al.* (2006) and Haider and Srinivasan (2007) who reported that susceptible cultivars of mung bean and urd bean had high amount of total sugar, reducing sugar, non-reducing sugar, amino acids and protein where resistant cultivars had high amount of phenol. Singh and Singh (2014) also recorded lower concentration of phenol and higher concentration of total sugar and protein from flowers and immature pods of the susceptible genotypes/varieties of cowpea.

Correlation of biochemical parameters with larval population

Different biochemical components of flowers like protein (%), phenol (mg/g) and total soluble sugars (%) and larval population non-significant correlation between them, indicating there are no significant relationship between pest population and biochemical components of flower. However, the correlation of biochemical components of immature pods with larval population revealed that phenol (mg/g) exhibited significant negative relationship with larval population of *M. vitrata* (r = - 0.650*) and *H. armigera* (r = - 0.728*). Correlation of total soluble sugars of immature pods exhibited significant positive association with larval population of *M. vitrata* (r = 0.688*) and *H. armigera* (r = 0.752*).

According to Jaydeep *et al*. (2006), significant positive correlation exist between total sugar, reducing sugar, non reducing sugar, amino acids and proteins with pod damage, whereas negative correlation existed between phenol contents in pods with pod damage by spotted pod borer in mungbean. Haider and Srinivasan (2007) also reported significant positive correlation between total sugar, reducing sugar, non reducing sugar, amino acids and proteins with pod damage by pod borer, whereas negative correlation prevailed between phenols contents in pod with pod damage in urd bean. Singh and Singh (2014) also reported that phenol content in flower and immature pods have negative correlation with per cent flower and pod damages and larval densities. However, amount of total sugar and protein in flowers and immature pods were correlated positively with infestation of legume pod borer. Based on different biochemical characters and pod borer infestation the cultivars GC-6 and GC-1605 were found to be consistently resistant. Relatively GC-1609, GC-1611 and GC-2 cultivars also showed resistance to the pod borer infestation. GC-1606 was found highly susceptible cultivar.

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Temperature dependent development and population performance of *Tetranychus fijiensis* Hirst (Acari: Tetranychidae) on Papaya

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ABSTRACT: Developmental biology and life table characteristics of *Tetranychus fijiensis* Hirst (Acari: Tetranychidae) on papaya were studied at four distinct constant temperatures (20, 24, 28, and 32°C) and relative humidity (70-85%) conditions. With the increase in rearing temperature conditions, the length of *T. fijiensis* life cycle decreased from 34.46 to 8.58 days. At 20, 24, 28, and 32°C, the Net Reproduction Rate (R_0) was 23.48, 39.06, 33.75, and 23.48 and; the intrinsic rate of population increase (r_m) was 0.0694, 0.2003, 0.2281, and 0.1902 females/ female/day, respectively. The optimum temperature for mite development was observed to be between 24 and 28°C, while favourable temperature for optimal reproduction capacity of the mite was 28°C.

KEYWORDS: Life table, reproduction rate, intrinsic rate, optimum temperature

INTRODUCTION

Papaya (*Carica papaya* L.) is a popular tropical fruit grown in warm climates, primarily for its edible fruit, but also has culinary, medical and industrial benefits. Like most tropical fruits grown in a variety of climates, papaya is attacked by 134 species of arthropods. The Hexapoda accounts for the majority of the species, while the Acarina accounts for 12 of them. Phytophagous mites are frequent secondary pests that cause economic damage, particularly after humans have intervened to control other pests. Spider mites are probably the most persistent arthropod pests and feed more commonly on older leaves, which initially turn yellow on the upper side and silvery on the lower side, followed by necrotic areas and eventually the leaf drops-off. Spider mites do have economic impact on the production of crops in greenhouses and in open fields. There are 1300 species reported so far, with over 100 of them considered as pests and ten are severe pests (Vacante, 2015). The main drivers of a spider mite population growth are their high reproduction potential and rapid development. Among the potential spider mite species, *Tetranychus fijiensis* Hirst is perceived as a serious pest that infests mostly horticultural crops (Anonymous, 2018-20). The major crop plants infested by *T. fijiensis* are coconut palm (Prasad, 1974; Sarkar and Somchoudhury, 1989; Gupta and Gupta, 1994), betel nut palm (Daniel, 1977); papaya (Gupta, 1976; Gupta and Gupta, 1994), white

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mulberry (Bolland *et al*., 1998; Migeon and Dorkeld, 2006-2013), peach and pear (Ehara and Wongsiri, 1975), *Citrus* spp. (Ehara and Wongsiri, 1975; Prasad, 1974 and Gupta, 1992 on *C. aurantium*), sour orange (Gupta and Gupta, 1994), citron, grapefruit, tangerine and calamondin (Othman and Zhang, 2003) and cardamom (Gupta and Gupta, 1994).

Papaya crop is cultivated in varying agroclimatic conditions that would support the development and multiplication of spider mite pests like *T. fijiensis* in a varied manner. The basic information on mite developmental biology and reproduction under ambient temperature conditions has been crucial for understanding the pest population dynamics and for formulating effective management strategies. Temperature is the most crucial environmental factor affecting development and reproduction of poikilothermic organisms like mites, including spider mites. Hence, a study on the mite's biological characteristics at different temperature conditions for the construction of developmental curves were undertaken which can be used for the prediction of developmental time as a function of temperature.

MATERIALS AND METHODS

Life history: Developmental biology of the spider mite, *T. fijiensis* was studied on papaya leaf discs at four constant temperature and humidity conditions (20±1°C, 80-85% RH; 24±1°C, 75-80% RH; 28°C, 70-75% RH and 32±1°C, 75-80% RH with 14h: 10h L: D conditions in a BOD incubator. Initially, a cohort of 30 to 50 eggs laid on 5cm x 5cm host leaf discs were observed periodically for hatching. Soon after hatching, using a fine camel hairbrush, the larvae were individually transferred to 50 separate 1.5 cm x 1.5 cm fresh host leaf discs kept on wet foam in 9" × 6" polyethylene trays. Further, the larvae were observed every 3 to 6 hours under a stereo binocular microscope and the data in respect of development from larva to adult including the quiescent stages (larvochrysalis, nymphochrysalis and teliochrysalis) were recorded. The sex of the emerging adult was also recorded to compute the data for the developmental time of male and female mite, separately.

Reproduction: Female teliochrysalis stages, selected from the mite culture maintained in the laboratory were individually transferred onto 50 (1.5 cm × 1.5 cm) fresh leaf bits. Two male adults were released on to each leaf disc to ensure mating subsequent to the emergence of adult female from the teliochrysalis stage. Further, observations were made every 24 hours to record the pre-oviposition period, the number of eggs laid every day, duration of oviposition (oviposition period), post-oviposition period, fecundity and sex ratio (♂: ♀, as proportion of male and female off-springs) were recorded across different temperature conditions and were compared to know the influence of temperature on these biological attributes.

***Population performance and demography*:** Temperature-wise age specific life table of the mite *T. fijiensis* was constructed separately. Demographic/Life table parameters such as, Mean Generation Time (T), Net Reproduction Rate (Ro), Gross Reproduction Rate (GRR), Finite Rate of Increase (λ), Intrinsic Rate of Natural Increase (r_m) and Doubling Time (DT) were computed following the procedure suggested by Birch (1948) and Atwal and Bains (1974) and the data were analysed (Chidananda, 2016; Pooja, 2018).

Net Reproductive Rate (R_o) = $\sum l_x m_x$

Mean Generation Time (T) = $\frac{\Sigma\ xlxmx}{Ro}$

Finite Rate of Increase in number (λ) = $\text{anti ln}[\frac{\log_e Ro}{T}]$

Intrinsic Rate of Natural Increase (r_m) = ln (λ)

Doubling time, DT = $\frac{\ln 2}{rm}$ where,

l_x = proportion of females alive at age interval x

m_x = number of female off-springs produced by the surviving female at age interval x

$l_x m_x$ = product of the proportion of females live at age interval x and the number of female off-springs per original female produced at the age interval x

Data in respect of development and reproduction parameters were expressed as mean ± SE and the mean data were analysed using one-way ANOVA followed by Tukey's HSD test (P=<0.05) in statistical software SPSS 23 to compare the mean

values across different rearing temperature conditions. Demographic parameters were computed using the relevant formulae and expressed as mean±SE determined by bootstrapping method and subjected to Post Hoc analysis to compare them across different constant temperature conditions.

Determination of thermal constant: Thermal constant of an organism is the temperature heat units required to complete its life cycle. Thermal constant for each stage of development and total development from egg to adult of *T. fijiensis* was determined by making use of the data of duration of corresponding developmental stage in different constant rearing temperature conditions using simple linear regression analysis. Simple linear regression analysis, $\hat{Y} = a \pm b X$ was performed as the relationship between temperature (ºC) and rate of development (1/developmental time in days) to determine the lower developmental threshold or temperature threshold (ºC) (= - a (intercept) / b (regression coefficient) and thermal constant (K) (=1/regression coefficient) according to the rule of constant sum of effective temperature as Thermal constant (b) = (Temperature - Development threshold) X Duration of development (Dent, 1997; Price, 1984).

RESULTS AND DISCUSSION

Temperature threshold and thermal constant

Data in respect of duration of development of *T. fijiensis* at four different constant temperatures were used to determine the mite's temperature threshold (°C) and thermal constant (day-degrees) values. The regression analysis between rearing temperature and rate of development at each stage of development (egg to adult) was carried out. The relationship between rate of development and temperature was expressed in the form of an equation, $\hat{Y} = 0.0065X-0.0859$ ($R^2 = 0.8895$) for female and $\hat{Y} = 0.0073X-0.101$ ($R^2 = 0.8626$) for male (Table 1). Temperature threshold was 13.22 and 13.84ºC for female and male, respectively. The thermal constant value for female was 153.85 degree-days and 136.99 degree-days for male. The temperature showed profound influence on both survival and development of *T. fijiensis*. For survival, male mite required relatively marginally higher temperature of 13.84ºC against 13.22ºC required for female. But male required lower accumulated heat units of 136.99 degree-days compared to 153.85 degree-days necessary for female.

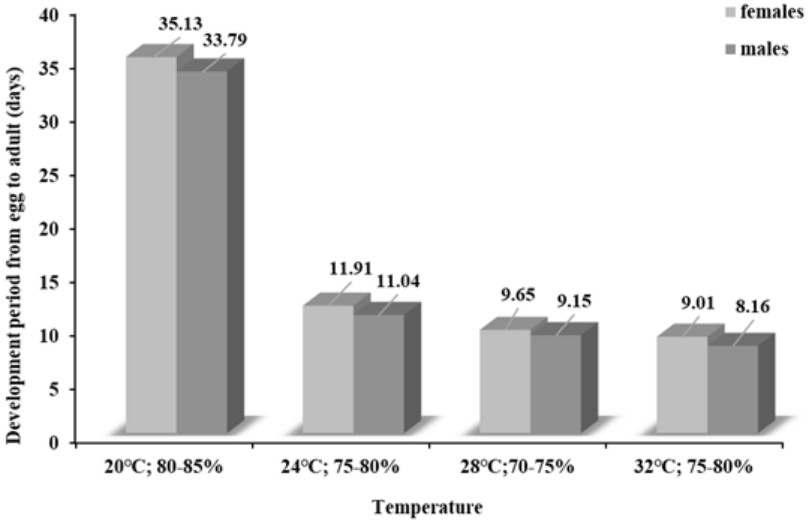

Fig. 1 Development of mite *Tetranychus fijiensis* on papaya at different constant temperature conditions in the laboratory

As the rearing temperature conditions increased from 20 to 32ºC (at the incremental rate of 4ºC), the duration of development for each stage recorded a gradual decrease (Fig. 2). Also, total development of both female and male decreased reasonably (35.13 to 9.01 days for female, 33.79 to 8.16 days for male). Mean developmental duration (female + male) was lowest, 8.58 days at 32ºC. Comparatively higher developmental duration of 34.46 days was recorded at 20ºC.

Data with respect to longevity of mated females at constant temperatures (20, 24, 28 and 32ºC) revealed that the female survived for 19.84, 13.48, 10.96 and 13.50 days. Each mated female laid an average of 25.34, 43.91, 39.43 and 26.62 eggs over 14.06, 11.85, 8.93 and 10.06 days. The fecundity was significantly high at 24ºC i.e. 43.91 eggs, laid over a lower period for 11.85 days (Table 2). The pre-oviposition period, oviposition period, post-oviposition period, female longevity, fecundity respectivelty, and daily egg laying by each female were found significantly affected by temperature factor. *T. fijiensis* had a comparatively longer pre-oviposition period of 4.25 days at 20ºC. The Post-oviposition period was not statistically significant at 20º and 24ºC, while it was only 0.53 days at 28ºC, but it was longer, 1.59 days at 32ºC. The oviposition period was longer, 14.06 days at 20ºC while, it was shorter, 8.93 days at 28ºC. Even though oviposition period was long at 20ºC, but the eggs per female was high, 43.91 at 24ºC. All the egg laying related periods, (pre-oviposition, oviposition and post-oviposition periods), and female longevity were found decreased as the rearing temperature increased; except in respect of total number of eggs/female, which increased up to only certain level of temperature, which further declined at higher temperatures. Fecundity was high at 24°C compared to other temperature levels (Table 2 and Fig. 2).

Bonato *et al*. (1999) studied the effect of mating status on the fecundity and longevity of *T. fijiensis* on *Disoxylum bijugum* (Meliaceae) at a constant temperature of 25±1°C. The median longevity of inseminated females was 16.5 days, more than the longevity on papaya at 24°C in the present study. But fecundity of inseminated females was 49.6 eggs, which is near to the fecundity (43.91 eggs) on papaya at 24°C in the present study. Vatana *et al*. (2001) studied the reproduction of *T. fijiensis* on passion fruit leaf; fecundity of mated female was 20 eggs with male to female sex ratio of 1:8, near similar to the fecundity of 25.34 eggs at 20ºC and sex ratio of 1: 7.47 at 32ºC in our present study. Moro *et al*. (2012) investigated on *T. urticae*

Table 1. Thermal constant and temperature threshold for the development of *T. fijiensis* on papaya

Stage	Sex	Regression equation	R^2	(k=1/b)	(T_0=a/b)
Egg	Female	Ŷ=0.0215X-0.289	0.9108	46.51	13.44
	Male	Ŷ=0.0215X-0.289	0.9108	46.51	13.44
Larva	Female	Ŷ=0.068X-1.153	0.9794	14.71	16.96
	Male	Ŷ=0.076X-1.261	0.9657	13.16	16.59
Protonymph	Female	Ŷ=0.0583X-0.917	0.8125	17.15	15.73
	Male	Ŷ=0.092X-1.692	0.9316	10.87	18.39
Deutonymph	Female	Ŷ=0.0547X-0.781	0.5792	18.28	14.28
	Male	Ŷ=0.082X-1.352	0.9514	12.20	16.49
Total (egg to adult)	Female	Ŷ=0.0065X-0.0859	0.8895	153.85	13.22
	Male	Ŷ=0.0073X-0.101	0.8626	136.99	13.84

Table 2. Reproduction parameters of *Tetranychus fijiensis* on papaya at different constant temperatures in the laboratory

Reproduction attributes	20°C; 80-85% (n=30)	24°C; 75-80% (n=32)	28°C; 70-75% (n=35)	32°C;75-80% (n=30)
Pre-oviposition period (days)	4.25 ± 0.35^{c}	0.71 ± 0.19^{a}	1.50 ± 0.16^{ab}	1.84 ± 0.13^{b}
Oviposition period (days)	14.06 ± 0.98^{b}	11.85 ± 0.79^{ab}	8.93 ± 0.70^{a}	10.06 ± 0.84^{a}
Post-oviposition period (days)	1.53 ± 0.28^{ab}	0.91 ± 0.19^{ab}	0.53 ± 0.13^{a}	1.59 ± 0.44^{b}
Longevity of mated females (days)	19.84 ± 1.03^{b}	13.48 ± 0.80^{a}	10.96 ± 0.72^{a}	13.50 ± 0.86^{a}
Mean no. of eggs/ female	25.34 ± 2.37^{a}	43.91 ± 3.84^{b}	39.43 ± 4.01^{b}	26.62 ± 2.49^{a}
Mean no. of female offsprings	23	38.65	33.96	23.34
Mean no. of male offsprings	2.06	4.94	5.06	3.12
Sex ratio of progeny (@&: B&)	11.15:1	7.18:1	6.70:1	7.47:1

n: number of mites observed; Mean values (± *SE obtained by bootstrapping method*) *with same alphabetical superscript within the row are not significantly different as per Tukey's HSD test ($p<0.05$)*

Table 3. Demography parameters of *Tetranychus fijiensis* on papaya at different constant temperatures in the laboratory

Demographic parameters	20°C; 80-85%	24°C; 75-80%	28°C; 70-75%	32°C; 75-80%
Mean Generation Time (days)	47.30 ± 0.28^{d}	19.09 ± 0.12^{c}	16.56 ± 0.14^{a}	17.93 ± 0.15^{b}
Doubling Time (DT)	10.49 ± 0.07^{d}	3.65 ± 0.02^{b}	3.33 ± 0.03^{a}	4.03 ± 0.04^{c}
Net Reproduction Rate (No. of female offsprings/ female/generation)	29.23 ± 0.09^{a}	58.55 ± 0.14^{c}	61.94 ± 0.22^{d}	37.36 ± 0.14^{b}
Gross Reproduction Rate (GRR)	23.48 ± 0.09^{a}	39.06 ± 0.17^{c}	33.75 ± 0.20^{b}	23.48 ± 0.14^{a}
Finite Rate of Increase (No. of female offsprings/ female/ day)	1.0720 ± 0.000^{a}	1.2231 ± 0.001^{c}	1.2592 ± 0.002^{d}	1.2119 ± 0.002^{b}
Intrinsic Rate of Natural Increase (No. of female off-springs/female/day)	0.0694 ± 0.000^{a}	0.2003 ± 0.001^{c}	0.2281 ± 0.002^{d}	0.1902 ± 0.001^{b}

Mean values (± *SE obtained by bootstrapping method*) *with same alphabetical superscript within the row are not significantly different as per Tukey's HSD test ($p<0.05$)*

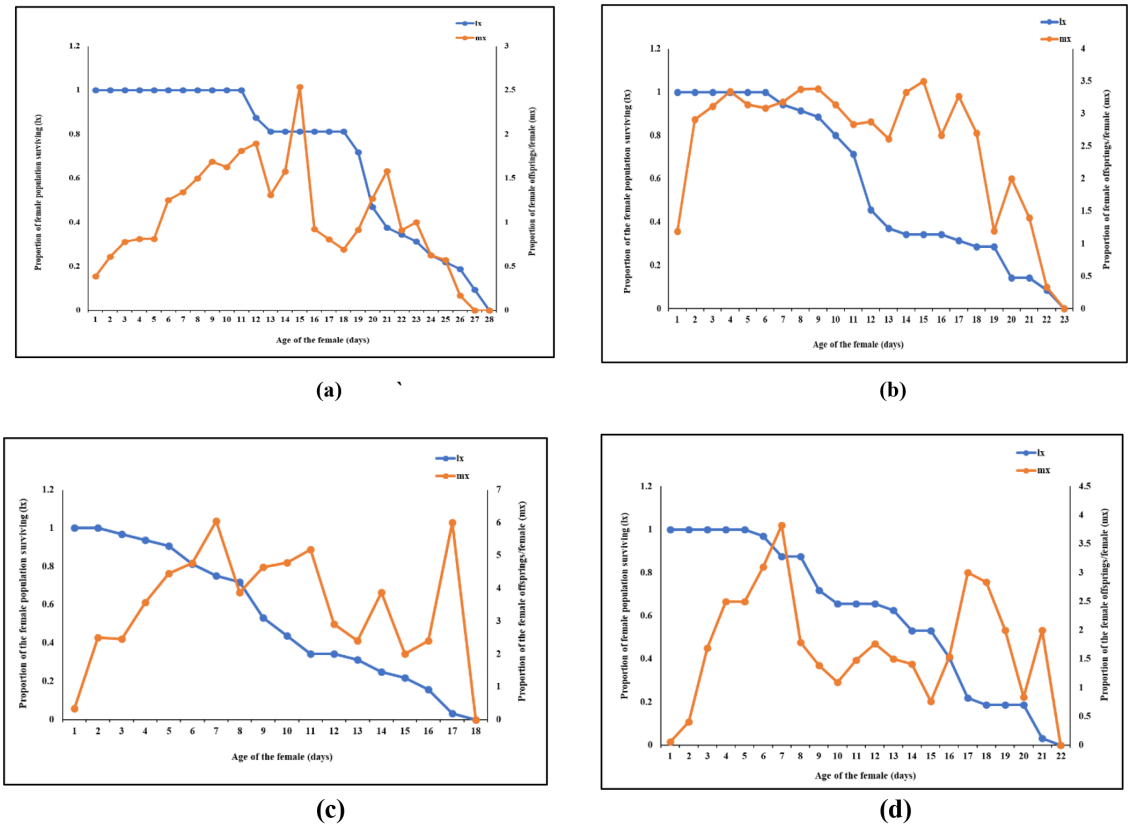

Fig. 2 Age specific survival and fecundity of *Tetranychus fijiensis* on Papaya at different temperature and humidity conditions: (a) 20°C; 80-85% RH, (b) 24°C; 75-80% RH, (c) 28°C; 70-75% RH and (d) 32°C; 75-80% RH

reproduction parameters on commercial papaya cultivars, pre-oviposition duration of females on papaya cultivars ranged from 1.4 to 1.6 days and on cultivar Sunrise, the highest number of eggs (33.9 eggs/female) was recorded, while in the present study, preoviposition of *T. fijiensis* ranged from 0.71-4.25 days and the maximum fecundity of 43.91 eggs/female was recorded. According to Puspitarini *et al*. (2021), *T. urticae* female longevity on papaya was 16.10-17.70 days, while in the present study with *T. fijiensis* it was shorter, 10.96 to 13.48 days (at 24 and 28°C). However, total fecundity was 41.30-43.80 eggs in their study, comparable to the fecundity of 39.43 to 43.91 eggs by *T. fijiensis* in the present study.

As the rearing temperature increased from 20 to 32ºC, the mean generation time and doubling time were found to decrease from 47.30 to 16.56 days. On the contrary, it decreased up to 28ºC and marginally increased thereafter. The higher GRR (61.94) was recorded at 28ºC. The higher R_0, 39.06 females/female/ generation was recorded at 28ºC. The chief demography factor r_m was found increased as the rearing temperature increased and it was highest, 0.2281 at 28ºC indicating favourable population progression of *T. fijiensis* on papaya. Also, this chief demographic parameter, (r_m) (number of female offsprings/female/day) values of the mite differed significantly across the temperatures. Moro *et al*. (2012) studied the population characteristics of *T. urticae* on leaves of four papaya cultivars and the net reproduction rate was highest (106.7 females/female/ generation) on cultivar Sunrise. It was the highest of 39.06 for *T. fijiensis* in the present study on a popular variety of papaya. Puspitarini *et al*. (2021) recorded the intrinsic rate of natural increase of *T. urticae* as 0.1856 to 0.2220 on papaya cultivars under laboratory conditions (26 ± 2°C; RH 72 ± 2% and photoperiod of L12: D12 h) similar to the r_m values of *T. fijiensis* (0.2003 to 0.2281) at 24 and 28°C.

The biological data of spider mite, *T. fijiensis*

generated across various constant rearing temperature and humidity conditions on papaya leaf discs indicated that the optimum temperature range for mite development was 24 to 28°C, while temperature and humidity conditions more favourable for its reproduction and population progression was 28°C and 70 to 75 per cent relative humidity. It may be inferred that this mite species might emerge as an economically important pest of papaya in the event of favourable ambient temperature conditions, necessitating timely control measures or management strategies.

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First record of three exotic whitefly pests (Hemiptera, Aleyrodidae) from Andaman and Nicobar Islands, India

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ABSTRACT: Three exotic whitefly pest species, *Aleurodicus rugioperculatus* Martin, *Aleurothrixus floccosus* (Maskell) and *A. trachoides* (Back) have been recorded for the first time from the Andaman and Nicobar Islands, India. Severe infestation of *A. floccosus* on guava; *A. rugioperculatus* on coconut and *Cinnamomum* and *A. trachoides* on tomato, guava and glory bower was observed. Tomato and glory bower are new host plant records for *A. trachoides*, and *Cinnamomum* for *A. rugioperculatus*. Puparial diagnosis and photographs of habitus are provided to facilitate identification of these species.

KEY WORDS: New hosts, *Aleurodicus rugioperculatus*, *Aleurothrixus floccosus*, *A.trachoides*, SEM, puparium

More than 110 exotic insect species had been reported from India, of which, whiteflies and mealybugs constitute a major part (Mandal, 2011). The immatures of whiteflies are small sized and often overlooked on leaf surfaces, and their accidental introduction with associated host plants is likely in newer areas by anthropogenic activities. Of the eighth exotic whitefly species known from India (Sundararaj *et al*., 2017, 2021) viz., *Aleurodicus dispersus* Russell feeds on more than 500 host plants, *Aleurodicus rugioperculatus* Martin on 40 host plants, *Aleurotrachelus atratus* Hempel on 4 host plants, *Aleurothrixus floccosus* (Maskell) and *Aleurothrixus trachoides* Back on 37 host plants, *Paraleyrodes bondari* Peracchi on 34 host plants, *Paraleyrodes minei* Ęaccarino on 25 host plants and *Tetraleurodes acaciae* (Quaintance) on 5 host plants, the latter *T. acaciae* is not considered as a pest, and *A. floccosus* is reported severely infesting guava only. Among these, *P. bondari* and *P. minei* (Dubey, 2019; Vidhya *et al*., 2019) were reported recently invaded in Andaman and Nicobar Islands (*A. dispersus* was previously recorded). Here, three whiteflies, *A. rugioperculatus*, *A. floccosus* and *A. trachoides* are reported for the first time from the Andaman and Nicobar Islands. All the species of the subfamily Aleurodicinae with Neotropical origin, viz., *A. dispersus*, *A. rugioperculatus*, *P. bondari* and *P. minei* are highly polyphagous, and the latter three extensively feed on monocot hosts, arecanut and coconut (Mohan *et al*., 2019).

The whitefly species belonging to subfamily Aleurodicinae have large sized puparia compared to *Paraleyrodes* species, and possess a single wing vein in adults. Adult whiteflies of the genus *Paralyerodes* Quaintance, 1909 have a median ocellus, an ancestral character described in Cretaceous taxa (Drohojowska and Szwedo, 2015).

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An invasive whitefly of the subfamily Aleyrodinae, commonly known as the woolly whitefly, *Aleurothrixus floccosus* (Maskell) is severely infesting guava only (Fig. 1d) in Andaman and Nicobar Islands, and population of *A. trachoides* was high on *Volkameria inermis* L. (known as the glory bower, is flowering plant in the family Lamiaceae), covering entire ventral surface of the leaves (Fig. 1g), but much less on guava and tomato crops. Adults and immatures of these species secrete copious amount of wax that leads to sooty mold development on leaf surfaces, particularly in coconut, causing yellowing of leaves and fungal growth. The host plants and biology of these species are already given in detail (Selvaraj *et al.,* 2020; Sundararaj *et al*., 2021). Awareness on invasion of exotic whiteflies was brought to notice through several publications, but economic losses caused by these whiteflies have not yet been assessed for India. The host plants list published by Sundararaj *et al.* (2021) clearly indicates low to moderate level of infestation of exotic whiteflies and mostly on the tree species. The objective of this study is to authenticate occurrence of these pest whiteflies in Andaman and Nicobar Islands and provide differential diagnoses for identification purpose. A brief diagnosis for each newly reported exotic whitefly is provided with photographs of puparial habitus on leaves and scanning electron microscope (SEM) microphotographs. Biological observations on habitus are also given for differentiating intermingled co-existing exotic whitefly species. All of these species feed on agricultural or horticultural crops, and fruit crops, which are directly consumed, and use of natural enemies could be better choice than synthetic insecticides. These observations and diagnosis will help in diagnosing invaded whitefly pests and explore their natural enemies through rearing for implementation of pest management practices.

Puparia of the pest whiteflies were collected in zip-lock covers from the Andaman and Nicobar Islands (Dubey and David, 2012). Photograph of the puparial habitus of *A. rugioperculatus*, *A. trachoides* and *P. bondari* on the leaf was taken using Nikon Cool Pix 9 camera. *Aleurothrixus floccosus* images on guava leaves were taken using Redmi 9 pro mobile camera. The identity of the whiteflies was confirmed after mounting of the puparia. SEM images were taken using EVO MA 15 microscope (Carl Zeiss, Jena, Germany) at 180× magnification (20 kV/EHT; 20 Pa; 24 nm; Au–Pa coat). SEM imaging was done from the Zoological Survey of India (ZSI), Kolkata, India, following the procedure given in Dubey and Ramamurthy (2013).

***Aleurodicus rugioperculatus* Martin, 2004** (Figs. 1a-c)

Diagnosis: In life, the puparia have cloudy cuticular pigmentation on dorsal surface. Puparium of this species is identifiable in having atypically narrowly acute lingula with four setae placed close to the apex; lingula reaches near puparial caudal margin, and the band of submarginal pores is interrupted immediately posterior to lingula apex.

Host plants: Sundararaj *et al*. (2021) provided a list of host plants. *Cinnamomum malabatrum* (new record).

Distribution: India: Karnataka, Kerala (Sundararaj *et al*., 2021); Andaman and Nicobar Islands (new record).

Material examined: INDIA- Andaman and Nicobar Islands,2 puparia, on coconut, 9.viii.2019, Kishori Nagar, 13°16.268' N; 92°57.573' E (ZSI/ ANRC/ T/13552); 5 puparia Karnataka, Kerala (Sundararaj et al. (2021), 1.iii.2021, Port Blair, 10°37.241' N; 92°43.353' E (ZSI/ ANRC/T/13359); 2 puparia, on coconut, 18.ii.2022, Haddo, 11°37.241' N; 92°43.353' E (ZSI/ ANRC/T/16180); 7puparia, coconut, 13.iii.2019, Tugapur, 12°49.525' N; 90°50.117' E (ZSI/ ANRC/T/14781); 6 puparia, coconut, 3.ii.2020. Kalipur, 10°36.219' N; 92°31.209' E (ZSI/ ANRC/T/14782); 6 puparia, on *Cinnamomum malabatrum*, 20.i.2022; Port Blair, 11°38.803' N; 92°43.659' E (ZSI/ ANRC/T/15940) (deposited in the ZSI, India).

Remarks: This whitefly is severely infesting underside leaves of the coconut plant (Fig. 1a-c). The copious amount of wax secretion from immature and adults' leads sooty mold development on underside of leaves. The leaves develop yellow chlorotic spots on feeding sites which gradually

Fig. 1 Whitefly infestation. a-c *Aleurodicus rugioperculatus* Martin, 2004 on coconut; d-f *Aleurothrixus floccosus* (Maskell, 1896) on guava; g-h *Aleurothrixus trachoides* (Back, 1912) on glory bower

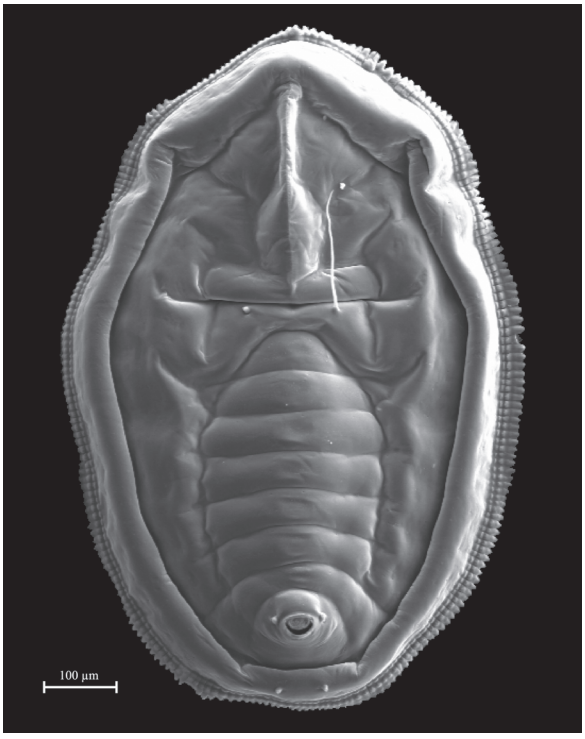

Fig. 2 SEM microphotograph of puparium of *Aleurothrixus floccosus*

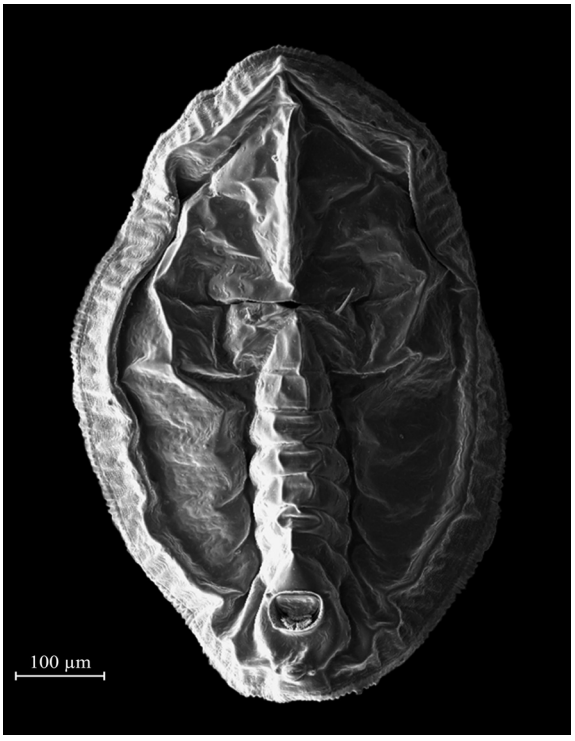

Fig. 3 SEM microphotograph of puparium of *Aleurothrixus trachoides*

increases with population build-up, then weakens and gradually dries. The infestation was more severe on matured leaves, which appears smoky brown to black on underside. This species was observed colonising with other invasive whitefly, *Paraleyrodes bondari* Peracchi (Fig. 1c). Puparia of *P. bondari* can be identified being housed in wax nest made by radiating wax threads' deposits, and the adults have two rows of smoky black spots on forewings.

***Aleurothrixus floccosus* (Maskell, 1896)** (Figs. 1d-f, 2)

Diagnosis: Puparia of this species differ from *Aleurothrixus trachoides* (Back) in having creamy white colour and covered with dull brown covering of wax deposit.

Host plants: Sundararaj *et al.* (2021) provided a list of host plants.

Distribution: India- Kerala, Karnataka, Lakshadweep, Tamil Nadu (Sundararaj *et al*., 2021), Andaman and Nicobar Islands (new record).

Material examined: INDIA- Andaman and Nicobar Islands,7 puparia, 31.i.2020, Hut bay, 16 km, 10°42.449' N; 92°32.946' E (ZSI/ ANRC/T/13758); 10 puparia, 3.ix.2018, R. K. Dam, 10°42.686' N; 92°31.396' E (ZSI/ ANRC/T/13759); 9 puparia, 10.v.2021, Haddo, 10°37.241' N; 92°43.353' E (ZSI/ ANRC/T/13760); 5 puparia, 8.v.2021, Bhatu Basti, 11°36.905' N; 92°45.805' E (ZSI/ ANRC/T/13761); 12 puparia, 2.v.2021, Sippighat, 11°36.669' N; 92°40.502' E (ZSI/ ANRC/T/13762) (deposited in the ZSI, India).

Remarks: This whitefly is known to feed on *Citrus* sp. in the Neotropical region (Malumphy *et al*., 1915), but it is severely infesting only guava leaves in India. The varied feeding preferences for this whitefly in Neotropical *versus* Oriental region is unknown. Puparial population was observed almost entirely covering the underside of the matured leaves (Fig. 1d-f) whereas the tender apical leaves were found infested with eggs and immatures. Immatures and adults population were not found

on the upper side of the leaves. This species is observed co-existing with *A. dispersus* (Fig. 1f) and *A. trachoides* on a single host tree but on separate branches. *Aleurothrixus trachoides* population was much less on guava and found intermingled with *A. dispersus*. Both *A. dispersus* (large sized) and *A. floccosus* (small sized) puparia are pale white and covered with copious amount of wax secretion whereas *A. trachoides* puparia are black with small marginal wax threads.

***Aleurothrixus trachoides* (Back, 1912)**
(Figs. 1g, h, 3)

Diagnosis: Puparia of *A. trachoides* are similar to *A. floccosus* in being black, in having fringe of wax around the body margin, bands of submarginal microtubercles and the abdominal segments are narrowly rhachisform. The bands of submarginal microtubercles are lacking in *A. floccosus* and in life, the puparia are pale white to dull creamy and surrounded with a fine fringe of white wax threads.

Host plants: Sundararaj *et al.* (2021) have provided a list of host plants. *Solanum lycopersicum* L. and *Volkameria inermis* L. (new records).

Distribution: India- Karnataka, Kerala (Sundararaj *et al.*, 2021); Andaman and Nicobar Islands (new record).

Material examined: INDIA- Andaman and Nicobar Islands, 7 puparia, 11.iv.2019, South Andaman, Bhatu Basti, 10°35.368' N, 92°32.115' E (ZSI/ ANRC T-7915); 4 puparia, 20.ii.2019, South Andaman, Port Blair, 11°40.372' N; 92°43.509' E (ZSI/ANRC T-7561); 1 puparia, 12.i.2020, Port Blair, 10°37.241' N; 92°43.353' E (ZSI/ANRC/T/ 10719); 2 puparia, 12.ii.2020, Port Blair, 10°37.241' N; 92°43.353' E (ZSI/ANRC/T/10720); 1 puparium, 2.ii.2020, Little Andaman, Krishna nalah, 10°40.695' N; 92°32.511' E, (ZSI/ ANRC/T/10401); 4 puparia, 3.ii.2020; Little Andaman, Kalipur forest, 10°36.219' N; 92°31.209' E (ZSI/ ANRC/T/10402); 5 puparia, 9.viii.2019, Kishori Nagar, 13°16.268' N; 92°57.573' E (ZSI/ ANRC/T/13553); 12 puparia, on *Capsicum annuum*, 2.xii.2021, Port Blair, 11°38.803' N; 92°43.659' E (ZSI/ ANRC/T/15956); 12 puparia, on *C. annuum*, 2.xii.2021, Port Blair, 11°38.803' N; 92°43.660' E (ZSI/ ANRC/T/15957); 12 puparia, on *C. annuum*, 2.xii.2021; Port Blair, 11°38.803' N; 92°43.658' E (ZSI/ ANRC/T/15958); 4 puparia, on *C. annuum*, 20.ii.2022, Ranchi Basti, 11°38.801' N; 92°43.659' E (ZSI/ ANRC/T/16177); 15 puparia, 18.ii.2022, Haddo, 11°37.241' N; 92°43.353' E (ZSI/ ANRC/T/16181) (deposited in the ZSI, India).

Remarks: This species was first recorded from India by Dubey and Sundararaj in 2015, initially from *Duranta* plant in Bangaluru, Karnataka, which is mainly used for fencing and boundary purposes. Later, it was found colonising on chilli and tomato.This species is reported here colonising on tomato and *V. inermis* in Andaman and Nicobar Islands. It was found severely infesting *V. inermis*, but a few scattered puparia are found on tomato. Since tomato is a seasonal crop and likely to get re-infested from the population build-up on *V. inermis* which is serving as a reservoir host during non-cropping season.

Apart from the three exotic species reported here, the other four invasive whitefly species on record from the Andaman and Nicobar Islands are *A. dispersus*, *Bemisia tabaci* (Gennadius), *P. bondari* and *P. minei*. These whiteflies were observed severely infesting the matured leaves of coconut, guava and tomato, however early stage population build-up was obvious on tender leaves. Leaves fall with maturity, and new population of whitefly pests' builds on fresh leaves that vary with the weather conditions. In these circumstances coupled with occurrence of natural enemies, the economic loss remains non-evaluated. The indirect damage caused by sooty mold development could more harm to plants along with the direct damage by feeding of insects. Considering population of *A. floccosus* on guava and *A. rugioperculatus* on coconut the direct damage could be more serious, particularly on young stage plants. Further, molecular study on mitogenome could be useful in understanding evolution of these pests.

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Studies on external genitalic attributes of two species of genus *Traminda* Saalmüller (Lepidoptera, Geometridae, Sterrhinae) from India

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ABSTRACT: Two species i.e., *mundissima* (Walker) and *aventiaria* (Guenee) belonging to geuns *Traminda* Saalmüller of subfamily Sterrhinae have been collected from different localities of India. The external male and female genitalial structures are studied in detail and a key to the species is given. The diagnosis of genus *Traminda* Saalmüller along with first reference, type species and distribution is also mentioned.

KEY WORDS: *Traminda mundissima* (Walker), *T. aventiaria* (Guenee), genitalic attributes, key

The genus *Traminda* Saalmüller is widely distributed throughout the old world tropics but more diverse in Africa, perhaps particularly associated with Savannah and other vegetation where there is a marked dry season (Holloway, 1997). Holloway (1997) not only recharacterized the genus by inclusion of external male and female genitalic attributes in its diagnosis but also suggested a new combination for one of the Indian species *T. aventiaria* Guenee. The present genus was erected on its type species *T. atroviridata* by Saalmüller in 1891. Warren studied many new species of this genus (1895, 1897, 1899). Likewise, Prout (1916, 1938) also described some new species under genus *Traminda* from different parts of the world including India. The genus is known by 22 species across the globe and out of which only two are reported from India (Scoble, 1999).

During the various collection-cum-survey tours conducted in the Western Ghats of India, only two species i.e., *T. aventiaria* and *T. mundissima* Walker were collected and authentically identified with the help of relevant literature. Both these species were earlier discussed by Hampson (1895) under genus *Timandra* Duponchel. As discussed in the above paragraph, one of the present species has already been shifted by Holloway (1997) under the present genus; the other Indian species was also mentioned and studied under genus *Traminda* by Scoble (1999). Both these species have been described in detail in this manuscript.

The collection of adult Geometrid moths was made with the help of light traps fitted at different places during night time in different localities of Western Ghats of India. Collected and freshly killed specimens were pinned and stretched on stretching boards. Well stretched specimens were preserved in airtight fumigated wooden boxes. The method

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proposed by Common (1970) and advocated by Zimmerman (1978) were followed for the preparation of permanent slides of fore and hind wings. To study external male and female genitalic attributes, the entire abdomen was detached from insect body (Robinson, 1976). The detached abdomen was placed in 10 per cent KOH overnight to soften the chitin and for dissolving away the muscles and other unwanted parts. The abdomen was dissected in 50 per cent alcohol for taking out the male genitalia. After proper dehydration in different grades of alcohol, the genitalic structures were cleared in clove oil and then mounted in Canada balsam on cavity slides. The photography of external genitalic structures was done with the help of image processing unit in the department of Zoology, Punjabi University, Patiala. The terminology given by Klots (1970) has been followed in the study for nomenclature purpose.

Abbreviations:

1A : First anal vein; 2A : Second anal vein; 3A : Third anal vein; AED : Aedeagus, AMP : Ampulla; ANT.APO : Anterior apophyses; CO : Costa; CO.PR : Costal process; CRN : Cornuti; CRP.BU : Corpus bursae; CU : Cucullus; CU_1 : First cubital vein; CU_2 : Second cubital vein; DU.BU : Ductus bursae; DU.EJ : Ductus ejaculatorius; FUR : Furca; GN : Gnathos; HRP : Harpe; JX : Juxta; M_1 : First median vein; M_2 : Second median vein; M_3 : Third median vein; PAP.A : Papilla analis; PO.APO : Posterior apophyses; R_1 : First radial vein; R_2 : Second radial vein; R_2-R_5 : Stalk of R_2, R_3, R_4 and R_5 veins; R_3 : Third radial vein; R_3-R_5 : Stalk of R_3, R_4 and R_5 veins; R_4 : Fourth radial vein; R_4-R_5 : Stalk of R_4 and R_5 veins; R_5 : Fifth radial vein; Rs : Radial Sector; SA : Saccus; Sc : Subcosta; Sc+R_1 : Stalk of Sc + R_1; SIG : Signum; SL : Sacculus; SL.PR : Saccular projection; SO : Socii; TG : Tegumen; UN : Uncus; VES : Vesica; VIN : Vinculum; VLA : Valvula; VLV : Valva.

Genus *Traminda* Saalmüller

Saalmüller, 1891, *Lepid. Madagascar,* **2**: 496.

Type species: *Timandra atroviridata* Saalmüller

Distribution: Old World tropics; Africa.

Diagnosis: Antennae of male bipectinated with the apex simple. Labial palpi porrect, directed upwards, reaching Up to frons and thickly scaled. Forewing with vein R_1 anastomoses with R_2-R_5 to form an areole; R_2-R_5 stalked from before upper angle; Cu_1 from close to lower angle of cell; Cu_2 from middle of cell. Hindwing with Sc+R_1 connected with cell at base; Rs and M_1 shortly stalked from upper angle of cell; M_3 and Cu_1 stalked from lower angle of cell. Hind tibiae dilated with a fold containing tuft of hairs and having two pair of spurs. Male genitalia with uncus strongly built; socii absent; gnathos reduced. Valvae simple, paddle like, narrow at base, broader and rounded towards apex. Aedeagus short, slightly sclerotized and narrow proximally, broader towards distal end; vesica not clearly differentiated. Female genitalia with corpus bursae oblong and membranous; signum absent; ductus bursae short and narrow.

Key to the studied species of genus *Traminda* Saalmüller

1. Forewing with costa red and a grey centred ocellus at end of cell. Male genitalia with juxta simple and flap like…..... ***aventiaria* (Guenee)**

- Forewing with costa not red and without grey centred ocellus at end of cell. Male genitalia with juxta well developed with two arms at its distal end…..... ***mundissima* (Walker)**

Traminda aventiaria (Guenee) (Plate 1)

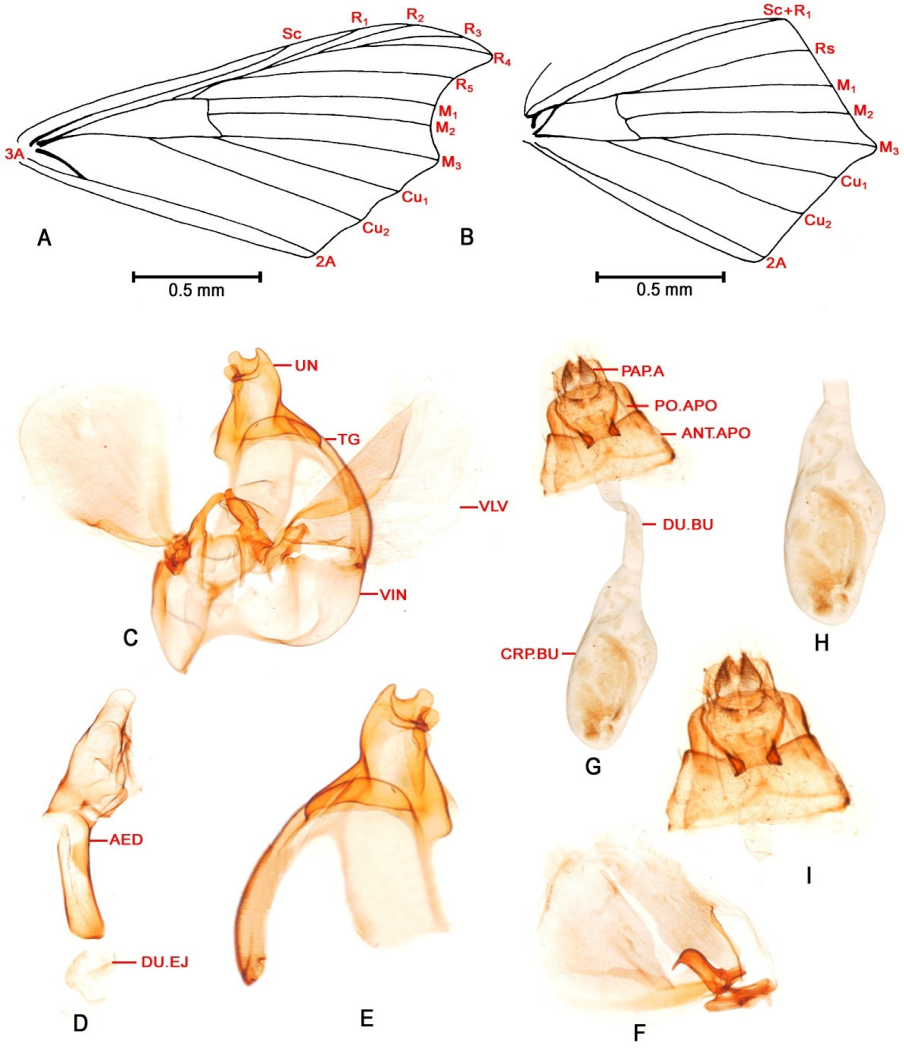
aventiaria Guenee, [1858], *in* Boisduval & Guenee, *Hist. nat. Insectes* (Spec. gen. Lepid), 10: 3.

Timandra molybdias Meyrick, 1899, *Trans. ent. Soc. Lond.,* 1899: 488.

External male genitalia (Plate 1, Figs. C, D, E, F): Male genitalia with uncus strongly built, well sclerotized, broad at base, bilobed apically with subapical projection; socii absent; gnathos reduced; tegumen broad, longer than uncus, inverted v-shaped; vinculum shorter than tegumen, slightly sclerotized, w-shaped; saccus wanting. Valvae simple, paddle like, narrow at base, broader and rounded towards apex, sparsely setosed with setae; strong, sclerotized sacculur projection, with pointed

PLATE- 01

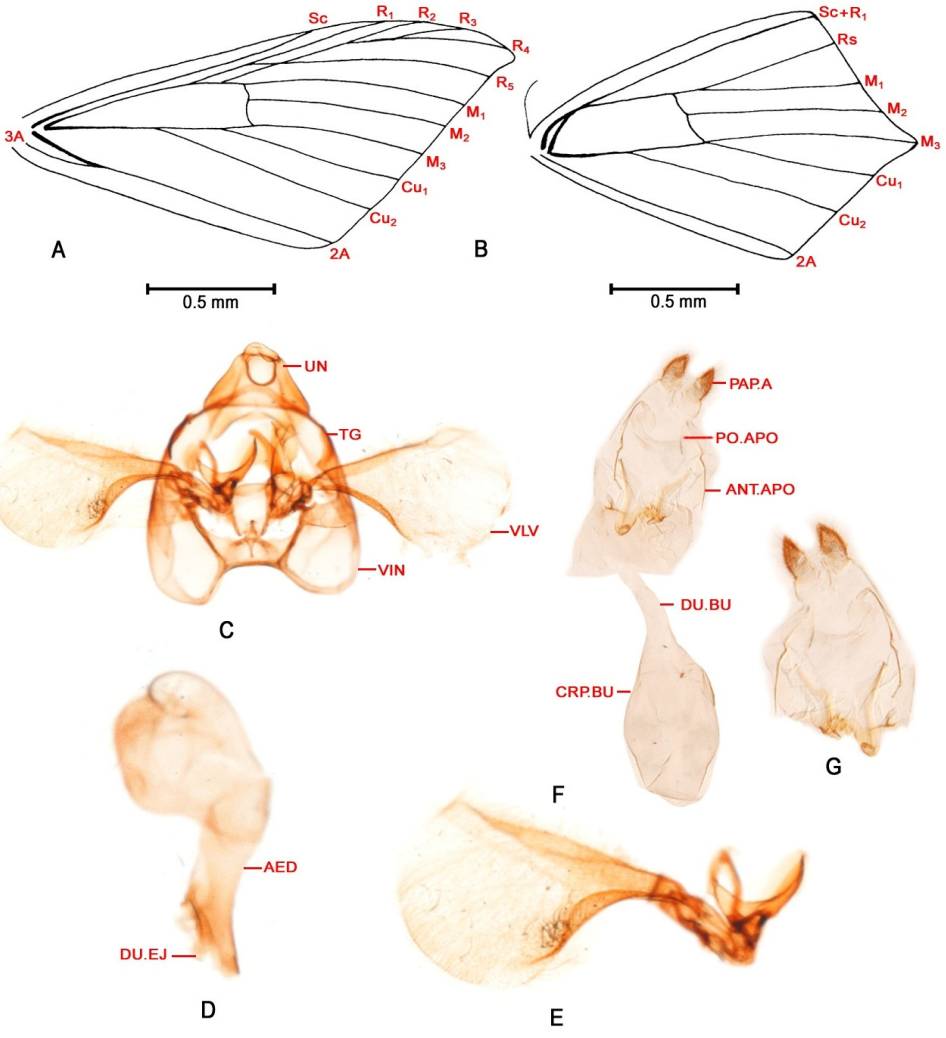
***Traminda aventiaria* (Guenee)**



A. Forewing, B. Hindwing, C. Male genitalia, D. Aedeagus,
E. Uncus with Tegumen (Lateral view), F. Valva (Left),
G. Female genitalia, H. Corpus bursae (Enlarged),
I. Papilla analis with Apophyses (Enlarged)

PLATE- 02

***Traminda mundissima* (Walker)**



A. Forewing, B. Hindwing, C. Male genitalia, D. Aedeagus,
E. Valva (Left), F. Female genitalia,
G. Papilla analis with Apophyses (Enlarged)

tip. Transtilla membranous; juxta simple, flap like. Aedeagus short, slightly sclerotized and narrow proximally, broader towards distal end; vesica not clearly differentiated; ductus ejaculatorius entering subapically.

External female genitalia (Plate 1, Figs. G, H, I): Female genitalia with corpus bursae oblong, membranous, slightly sclerotized at base; signum absent; ductus bursae short, narrow, membranous; ostium bursae well developed, sclerotized; posterior apophyses slightly longer than anterior apophyses; papilla analis well developed, triangular sparsely setosed with few setae.

Wing expanse (Full): Both sexes 36 mm.

Material examined

Karnataka : Dandeli, 16.ix.07-2♂♂, 1♀; 26.x.09- 1♀.

Maharashtra : Panala, 7.ix.07- 1♂.

Old distribution: Formosa; throughout India, Sri Lanka and Myanmar; Java; Australia.

Remarks: Holloway (1997) in his publication 'Moths of Borneo' gave the diagnosis, geographical range and some notes on the biology of species *aventiaria* Guenee and also suggested a new combination for it. Although he gave the photographs of external male and female genitalic structures in his publication he could not describe various genitalic attributes in detail. The genitalic structures of the present species are described and illustrated in detail in the present manuscript.

***Traminda mundissima* (Walker) (Plate 2)**

mundissima Walker, 1861, *List. Specimens lepid. Insects Colln Br. Mus.,* **23**: 795.

External male genitalia (Plate 2, Figs. C, D, E): Male genitalia with uncus well developed, sclerotized, broad at base, narrow towards apex with subapical hood; socii absent; gnathos reduced; tegumen longer than uncus, sclerotized, inverted v-shaped; vinculum as long as tegumen, w-shaped; saccus wanting. Valvae simple, paddle like, narrow at base, broader and rounded towards apex; well sclerotized, strong saccular projections rising from the base. Transtilla well developed; juxta well developed, with two arms at distal end. Aedeagus short, hammer like, narrow proximally, broader towards distal end; vesica not clearly differentiated; ductus ejaculatorius entering laterally.

External female genitalia (Plate 2, Figs. F, G): Female genitalia with corpus bursae oval, membranous; signum absent; ductus bursae short, narrow, tubular, membranous; posterior apophyses longer than anterior apophyses; ostium bursae weakly sclerotized; papilla analis triangular, slightly sclerotized, beset with micro and macro setae.

Wing expanse (Full): Both sexes 26 mm.

Material examined

Gujarat : Waghai, 31.viii.07- 1♀; 1.ix.07- 1♂, 1♀.

Maharashtra : Malshej Ghat, 3.ix.07- 1♀.

Karnataka : Dandeli, 26.x.09- 1♂.

Old distribution: Abyssinia; throughout India, Sri Lanka and Myanmar; Australia.

Remarks: The genitalic features of the present species strictly conform to the characterization of the type species of genus *Traminda* Saalmüller. The species is described and illustrated here in detail for the first time.

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Coptosoma variegatum (Herrich-Schäeffer, 1838) (Hemiptera, Plataspidae) infesting mango in Kerala and Karnataka with redescription of the species

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ABSTRACT: The incidence of *Coptosoma variegatum* (Herrich-Schäeffer, 1838) on mango tree is reported for the first time from south Indian states, Kerala and Karnataka**.** The bugs colonised and fed on tender shoots of mango trees. Even though the extent of damage was low, the insect in association with other pests may affect the vigour of young trees. The species is redescribed and illustrated based on male and female genitalia. © 2023 Association for Advancement of Entomology

KEYWORDS: Heteroptera, host plant, first report, morphology, genitalia

Mango, the most popular fruit in the world, commonly known as 'King of fruits', is attacked by 300 species of insect pests in different parts of the world (Patel *et al*., 2004). Leaf webber, *Orthaga exvinacea* (Hampson), mango leafhoppers, *Amrtitodus* spp. and *Idioscopus* spp., fruit fly, *Bactrocera dorsalis* (Hendel), stem borer, *Batocera rufomaculata* (De Geer), leaf cutting weevil, *Deporaus marginatus* Pascoe and nut weevil, *Sternochetus mangiferae* (Fabricius), are important insect pests. In addition many insects of minor importance are also observed to attack mango trees.

Members of Plataspidae are usually small to medium size, usually orbiculate and coloured black or brown, often shiny with pale stripes or spots and comprise 66 genera and 606 species from world (Rider *et al*., 2018). They are reported as phytophagous (Candan *et al*., 2012). *Coptosoma* was mentioned as the largest genus to effect economic loss to the crops (Schaefer *et al*., 2000). Species of *Coptosoma* occur in India, China and Southeast Asia through Indonesia to New Guinea (Rider, 2010). According to Zhang *et al.* (2012), adults and nymphs feed on tender stems or leaves of plant family Fabaceae leading to defoliation and reduced photosynthesis due to sooty mold which affects development of pods. The incidence of infestation by *Coptosoma variegatum* (Hemiptera, Plataspidae) on mango flowers was reported for the first time in Malaysia (Huda *et al*., 2020). The present study reports its incidence and feeding on the tender shoots of mango trees in Kerala

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(Alappuzha) and Karnataka, along with the redescription of the species based on male and female genitalia.

The bug samples were collected during April, 2022 from mango trees located at Rice Research Station, Moncompu, Alappuzha district, Kerala. The trees did not receive any pesticide application. The bugs were found to colonise new flushes of mango trees (Fig.1) and almost all tender shoots. Besides, as per the observations made by the second author, this species was also found feeding on mango inflorescence in Nelamangala, Bangalore, Karnataka (Fig. 3).

The infested shoots were carefully inserted into finely perforated polythene bags of suitable size and thoroughly shaken to collect insects inside the bag. Uninfested fresh shoots were provided as food for the collected insect's in-order to confirm their feeding. Few of the collected insects were preserved in 90 per cent ethanol to facilitate taxonomic identification. They were identified as the black stink bug, *Coptosoma variegatum* (Herrich-Schäeffer, 1838) (Figs. 4–5) [based on Distant, 1902; Davidivá-Vilímová, 2006].

***Coptosoma variegatum* (Herrich-Schäeffer, 1838) (Figs. 4–15)**

Colouration- Dorsum (Fig. 4) shining black with bright markings as follows: head with mandibular plates in front of compound eyes (except lateral margins), broad and oblique line, intersected by a black line on lateral margins of pronotum (including the moderately dilated anterolateral margins), 1+1 small, oval or roughly round well separated spots medially beneath anterior margin of pronotum, 1+1 small round spots mesad of humeri, sometimes with 1+1 minute spots at each basal angles of scutellum; 1+1 elongate (size variable), transverse spots at basal area of scutellum demarcated by a narrow line, circumference of scutellum (except basal margin) and antennal segments (segments IIb–IV with dark brown sheen), yellow. Ventral side of the body shining black (Fig. 5) with well-defined markings as follows: head, extreme lateral margins of meso and meta pleura, abdominal ventrites III–VII, narrow, tumescent linear spots (with spiracles embedded) mesad of each marginal spots from III–VII, circumference of male genital capsule, yellow. Legs and Labium yellowish with black shade.

Structure- Head declivous, rounded apically, broader (including compound eyes) than long, mandibular plates as long as clypeus, not meeting in front of clypeus; basal region of head accommodated in the middle concavity of anterior pronotal margin. Antennae five segmented with segment II_a shortest; antennal segments from shortest to longest: II_a<I<II_b=III<IV. Pronotum convex dorsally with anterolateral margins slightly explanate and convex, anterolateral angles rounded, lateral margins obliquely straight towards humeri; humeri rounded; scutellum broad, medially convex, basal area developed as a narrow transverse stripe and demarcated from rest of the region with a faint suture. Labium reaching posterior margin of ventrite III; peritreme short, spout-like, extends slightly beyond midmetapleuron

Measurements- Body length: male: 2.4–2.8 mm; female: 3.05–3.10 mm.

Male genitalia (Fig. 6, 8–13) *Genital capsule* subquadrate, ventral rim (vm) shallower than dorsal rim (dm), emarginated medially, ventral rim emargination narrower than dorsal rim emargination; dorsal sinus (ds) of posterior aperture, short and oval, dorsal rim deeply and broadly emarginated; infoldings of dorsal rim impressed laterad of dorsal sinus, deep central impression around posterior aperture; infoldings of ventral rim with a tuft of fine setae at middle. *Paramere* (Figs. 11–12) with crown (cr) slightly broader than stem (st) with apex broadly angulate, crown attached at an oblique angle to stem. *Phallus* short with apical 1/3 moderately sclerotized, remaining 2/3 membranous, basal apparatus sclerotized.

Female genitalia (Figs. 14, 15)- Terminalia (Fig. 14). Laterotergites VIII (lt 8) not separated along midline forming broad, transverse plate posteriad of laterotergites IX (lt9); valvifers VIII (vf8) large elongate more or less triangular, with mesial margins straight. Spermatheca with a prominent spermathecal pump (Fig. 15) and spermathecal duct tube-like; spermathecal pump with apical receptacle

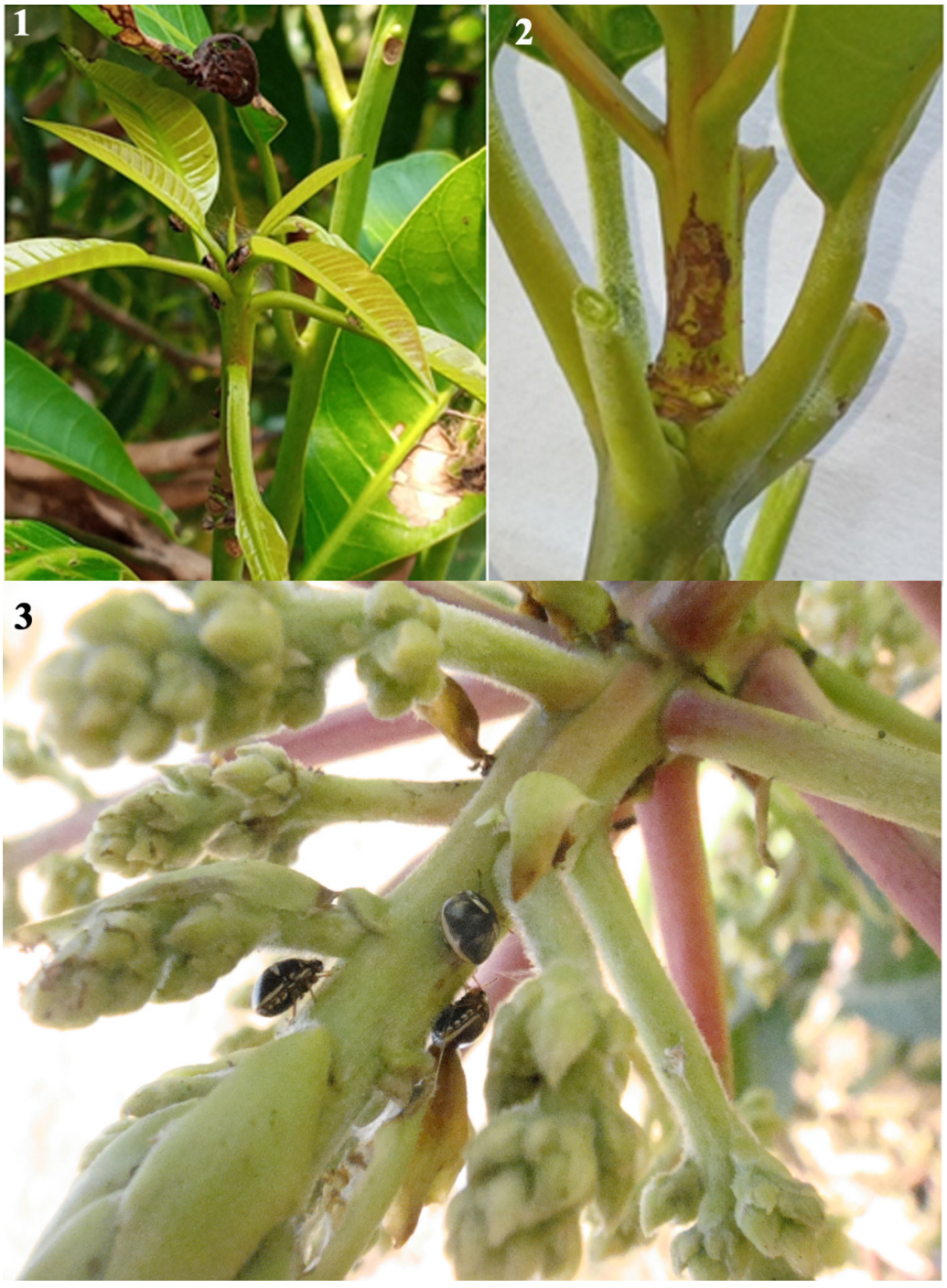
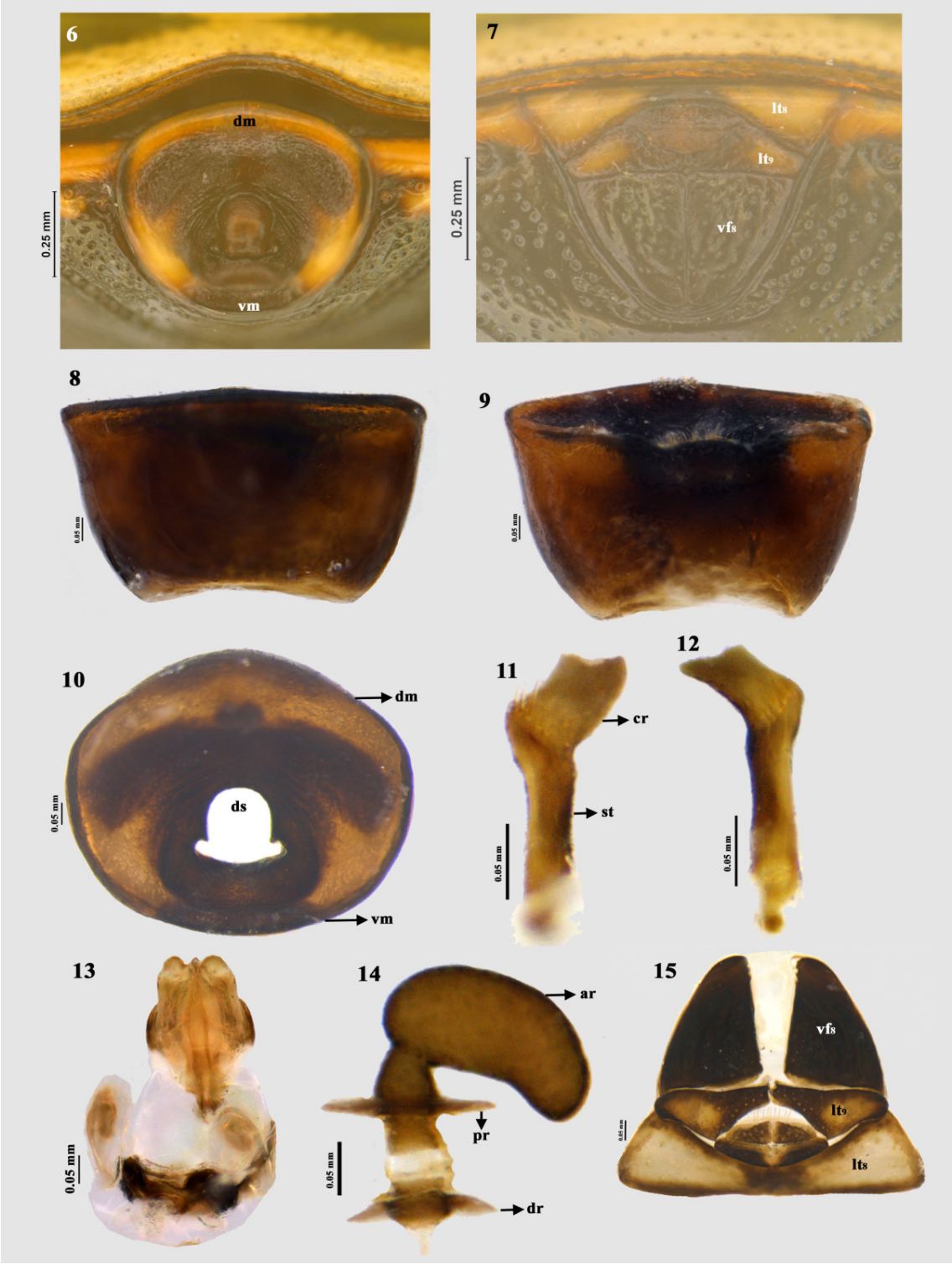


Fig. 1 Colonization of bugs on tender shoots in mango tree, Fig. 2 Feeding marks developed on mango shoots, Fig. 3 Aggregation of bugs on mango inflorescence



Figs. 6–15 *Coptosoma variegatum* (Herrich-Schaeffer) (male and female genitalia). Fig. 6 close up of male genitalia; Fig. 7 close up of female genitalia; Fig. 8 genital capsule (dorsal); Fig. 9 genital capsule (ventral); Fig. 10 genital capsule (caudal); Fig. 11–12 paramere (different planes); Fig. 13 phallus; Fig. 14 spermatheca; Fig. 15 terminalia. Lettering: ar– apical receptacle; cr–crown; dm–dorsal rim; dr–distal flange; ds–dorsal sinus; lt8–laterotergite VIII; lt9– laterotergite IX; pr–proximal flange; st–stem; vf8–valvifers VIII; vm–ventral rim.

Figs. 4–5 *Coptosoma variegatum* (Herrich-Schaeffer). Fig. 4 habitus (dorsal); Fig. 5 habitus (ventral).

(ar) elongate oval, distal flange (dr) nearly equal size to proximal flange (pr).

Remarks**:** The number and size of spots on pronotum are variable among the collected specimens. The illustration of various specimens shows slight variation especially in the shape of the parameral crown and also in dorsal and ventral rim of the genital capsules as mentioned earlier by Davidivá-Vilímová (2006). Therefore, the observed variability in the present investigation is considered as mere variation unless a thorough revision of this problematic *Coptosoma* genus group is completed. The nomenclature of antennal segments follows Leston (1956).

In the present investigation, bugs colonized tender shoots and sucked plant sap (Fig. 1). The feeding marks later turned into brown patches (Fig. 2). Bugs moved to the tip of the new flushes to continue feeding. The bugs were not present on mature twigs. Though the incidence of *C. variegatum* was not observed to reduce the vigour of the trees, the development of new flushes was affected. The heavy incidence on tender shoots may suppress the vigour of young tress or seedlings. In addition to this, the infestation may also extend to the inflorescence as recoded by Huda *et al*. (2020) from Malaysia. Indirect effects caused by the feeding of *C. variegatum* have to be studied.

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Record of *Onomarchus uninotatus* (Serville, 1838) (Orthoptera, Tettigoniidae) as a pest of jackfruit from Kerala, India

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ABSTRACT: *Onomarchus uninotatus* (Orthoptera, Tettigoniidae), one of the neotropical Pseudophyllinae is recorded on the jackfruit (*Artocarpus heterophyllus*) var. Vietnam Super Early at Thrissur, Kerala, India. This is a new distributional report of the species from Kerala and additional new documentation as a serious pest of jackfruit from India. The nymphs and adults are nocturnal in habit and feed on the leaves resulting in withering and drying of the leaves giving a burned up appearance to the canopy. The species exhibited colour polyphenism with green and brown morphs in the nymphal stages. A detailed description of the nature of damage caused by the pest is given. © 2023 Association for Advancement of Entomology

KEYWORDS: Pseudophyllinae, katydid, distribution, nature of damage

The jackfruit (*Artocarpus heterophyllus* Lam (Moraceae) is an evergreen fruit crop native to tropical Asia and is rightly called "wonder fruit" or "Poor man's food" due to its high nutritional value, and rich source of dietary fiber. Jackfruit is relatively free of any serious pest infestation, though about 38 species of insect pests were reported to attack jackfruit (Tandon, 1998). Shiewei *et al.* (2013) reported *Onomarchus uninotatus* as a serious pest of jackfruit in China. However, no other reports of *O. uninotatus* as a pest of jackfruit from India or any other country have been encountered. Although Srinivasan and Prabhakar (2012) reported the presence of this katydid in India in Arunachal Pradesh and Rajaraman *et al*. (2018) observed *O. uninotatus* on jackfruit in the Western ghats, reports regarding its pest status and nature of the damage in jackfruit is nil. The authors present here the first report of the katydid pest *O. uninotatus* (Orthoptera, Tettigoniidae) from Kerala, India supported by its morphological identification, nature of the damage, and symptoms on jackfruit var.Vietnam Super Early.

During the second fortnight of January 2022, the authors observed a serious pest behavior of *Onomarchus* on jackfruit in the campus of the Academy of Climate Change Education and Research, KAU, Vellanikkara, Thrissur, Kerala (10.3244° N and 76.1627°E), India. The Katydid was found to be occupying the foliage of a new early bearing variety *viz.,*Vietnam Super Early on

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two year old jackfruit. Both nymphs and adults of katydids were collected using a sweep net, transferred to polythene bags and were brought to the laboratory of the Department of Entomology, College of Agriculture, Vellanikkara, Thrissur for identification and further studies. The feeding behavior of the nymphs and adults was observed by closely monitoring them from 9.00 am to 1.00 am. To establish the taxonomic identity, a few male and female adults in the culture were killed by using ethyl acetate and preserved as dry specimens by mounting on the entomological pin. The pinned specimens were subjected to taxonomic studies under Carl Zeiss Stereo Zoom (Stemi 305) microscope and photographed using Axiocam 105 color attached with the Zeiss image analyzer and the morphological characters were studied. Species identity was confirmed with the help of the fifth author and was updated after Orthoptera Species File Online (Cigliano *et al*., 2022).

Katydid feeding on jackfruit was identified as *O. uninotatus* by using the original description and type specimen reference images in the Orthoptera Species File (Cigliano *et al.,* 2022). The katydid species exhibited sexual dimorphism, the females are larger in size compared to the male (Plate 1). Adults are robust and the tegmina resembled a green leaf. The male measured on an average 59.9 mm in length including tegmina and the corresponding measurements for the female is 75 mm. The tegmen is broad and green in colour with a white spot on the base of the angle made by the medial and radial veins. The tegmen is about 65 mm long in females and 52 mm long in males.

The species exhibited colour polyphenism with both green and brown morphs in the nymphal stages. They mimicked the colour of the substrate, where those on dried leaves appeared brown in colour, whereas those on green leaves were green (Plate 2 A, B).

All the stages of the katydid, *viz*., eggs, nymphs, and adults were observed on the plant. Observations in the laboratory revealed that they have nocturnal feeding habits. By dusk, they started moving onto the upper surface of the leaves slowly and then started feeding and were highly active during the night. Both the nymphs and adults caused damage to the plants. The early instars scraped the green matter from the upper surface of the young leaves resulting in skeletonization. The skeletonized area appeared light green initially, which later turned brown and got dried up subsequently. The later instars and adults made notches or cuts and fed on the green leaves, including veins. Heavy population resulted in withering and drying of the outer leaves, which gave a burned-up appearance to the canopy (Plate 3). Johg (1946) reported a species similar to

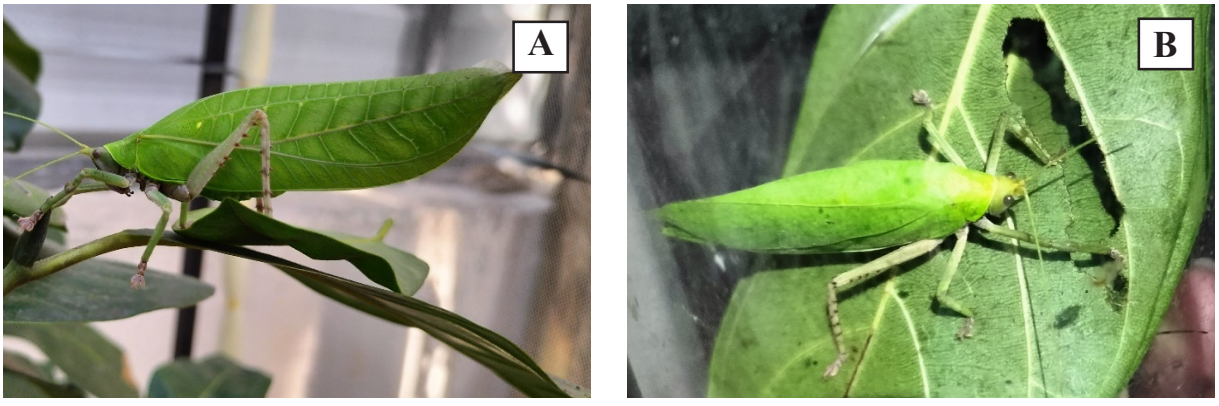

Plate 1 A. Adult female, B. Adult male

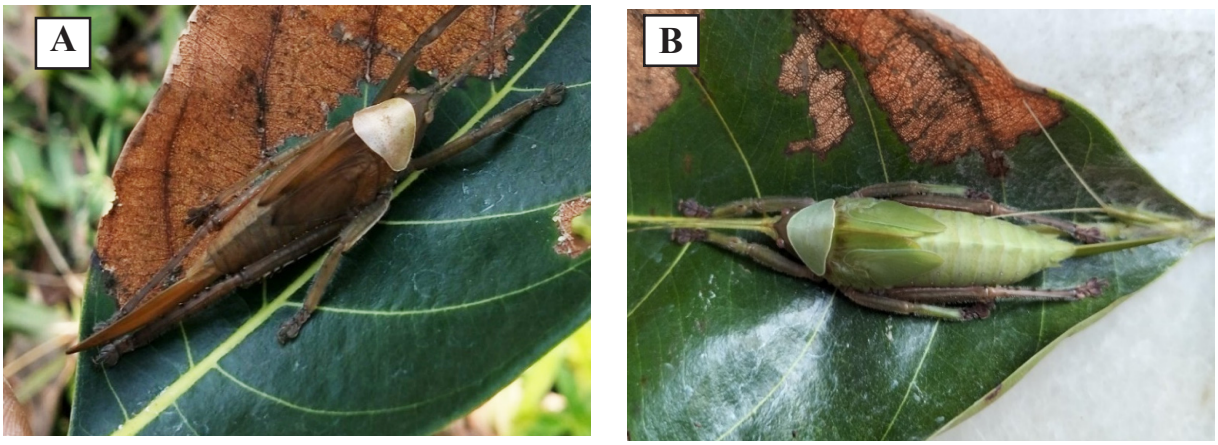

Plate 2 Colour polymorphism A. Brown morph, B. Green morph

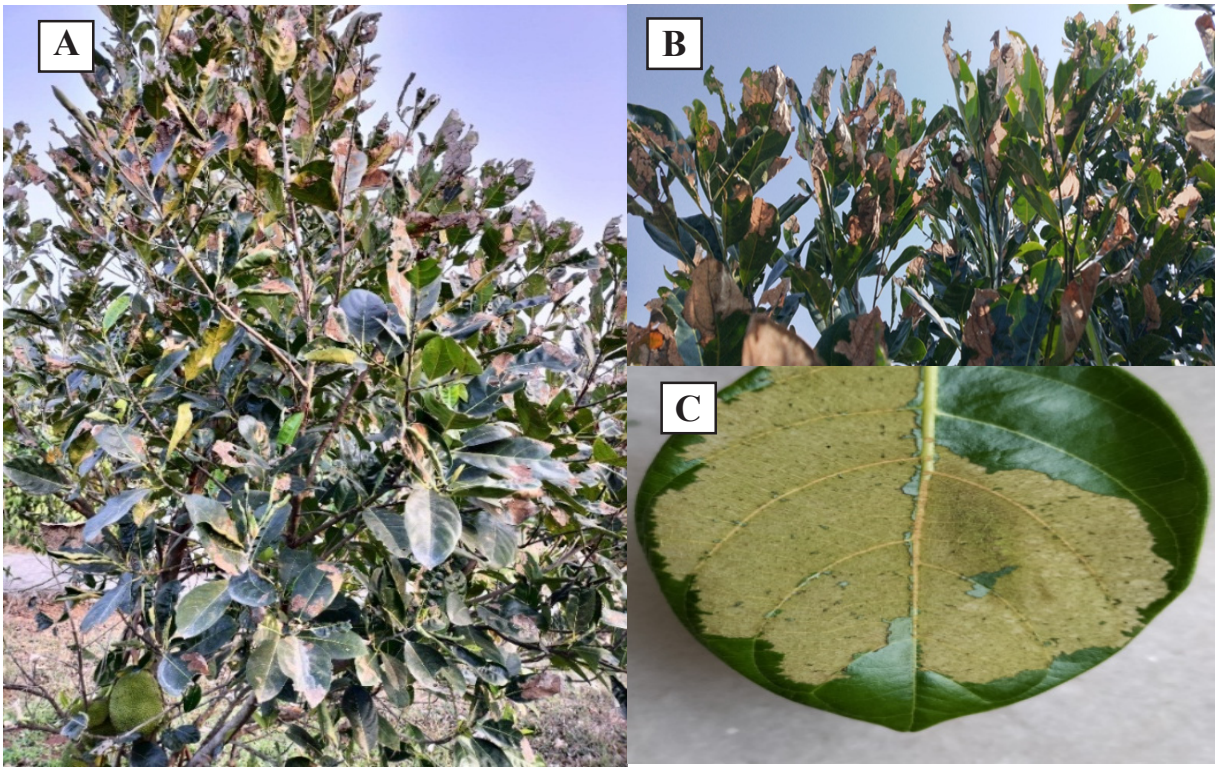

Plate 3 Symptoms of katydid infestation in field. A. Infested jackfruit tree B. Dried leaves due to infestation, C. Skeletonised leaf

O. uninotatus for the first time from Madurai, India. However, the species, *O. uninotatus* (Serville, 1838) was described (female) from Arunachal Pradesh (Srinivasan and Prabakar, 2012). It is one of the model katydids for elucidating novel acoustic signals among tettigoniids (Rajaraman *et al.,* 2015). Though the species has been reported earlier from India, the reports are of either taxonomic importance or as a model insect for acoustic studies. Hence, this report forms the first of its kind as an important pest of young jackfruits in India, and as the first report of the species from Kerala. Intensive surveys need to be initiated to identify the geographical extent to which the new pest has spread and hence, warrants constant monitoring to mitigate the damage caused by the pest.

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Four new records of dragonflies (Insecta, Odonata) from Amboli region of Western Ghats, Maharashtra, India

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ABSTRACT: *Microgomphus souteri* Fraser, 1924, *Macromia flavocolorata* Fraser, 1924, *M. irata* Fraser, 1924 and *Idionyx corona* Fraser, 1921 are reported from Maharashtra State for the first time. The records are the northern most distribution range for the respective species. Brief description with identification characters of the species is provided. © 2023 Association for Advancement of Entomology

KEYWORDS: Genera *incertae sedis*, Gomphidae, Hiranyakeshi, Nangartas, range extension

The Western Ghats mountain range, a global biodiversity hotspot, is divided into three biogeographic regions namely northern, central and southern Western Ghats (Kuriakose and Sebastian, 2016). Fraser (1933, 1934, 1936) and many others (Subramanian *et al.,* 2018; Nair *et al.*, 2021) did comprehensive documentation in this region. A major part of this biodiversity hotspot lies in the State of Maharashtra. Tiple and Koparde (2015) enlisted 134 Odonata species from the Maharashtra State. After that many species have been added to the Odonata list by Joshi and Sawant (2019, 2020), Mujumdar *et al.* (2020), Kalkman *et al.* (2020), Bhakare *et al.* (2021), Koli and Dalvi (2021), Koli *et al.* (2021), Dalvi and Koli (2022), Joshi *et al.* (2022a, b) and Payra *et al.* (2022). Sawant *et al.* (2022) added six more species to the list and revised the total number of Odonata species to 144.

Amboli region is known for high floral and faunal diversity. The landscape around Amboli is varied with patches of evergreen forest, riparian habitats, open lateritic plateaus with grasslands, and moist deciduous forests at mid and low elevations (Sawant *et al.,* 2022). Due to the unique location at the junction of northern and central Western Ghats and drastic variations in habitats, it harbors many odonate species (Subramanyam and Nayar, 1974; Sawant *et al.,* 2022). In this paper, four new State records of dragonflies from family Gomphidae and Genera incertae sedis with their brief diagnosis and distribution map are presented.

Hiranyakeshi (15.95 °N; 74.02 °E, 840 m asl) and Nangartas (15.99 °N; 74.06 °E, 790 msl) area of Amboli village of Sawantwadi Taluka, Sindhudurg District, Maharashtra State was surveyed in June 2022 for documenting odonates. Samples from non-protected areas were collected and photographed for the purpose of identification and their morphological features. Field photographs were taken with DSLR cameras and macro lenses

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(Canon Inc. and Nikon Inc., Japan). Species were identified based the identification keys given in the standard literature (Fraser, 1934, 1936; Subramanian *et al.,* 2018; Joshi *et al.,* 2022a). Systematic arrangement of the species follows Kalkman *et al.* (2020). The general terminology used in description follows Garrison *et al.* (2006).

From the collections of odonates, four species were found to be new records for Maharashtra State. All these observations are in the northern most range extensions of Western Ghats. Taxonomical and distributional notes are follows-

1. *Microgomphus souteri* Fraser, 1924
(Figs. 1A-F)

Locality: Hiranyakeshi, Amboli; Habitat: Hill stream surrounded by trees; 06.vi.2022; 3m, 2f, 15.95°N; 74.02 °E; 840 msl.

Locality: Nangartas, Amboli; Habitat: Hill stream with bushes; 19.vi. 2022; 15.99°N; 74.06°E; 790 m above sea level (m asl).

Identification: Small size, peculiar scissor-like male caudal appendages with medial branch of cerci longer than that of the lateral, mesothoracic collar interrupted at the middle, absent antehumeral spot, lesser markings on abdomen and unmarked segment 9-10.

Notes: Earlier, *Microgomphus souteri* was known from Coorg and South Kanara (Fraser, 1934). Subramanian *et al.* (2018) plotted the distribution from Kerala to Goa State. Recently Dattaprasad Sawant photographed a female of *M. souteri* at Hadpid village, Sindhudurg District, Maharashtra (Joshi *et al.,* 2022b) (Fig. 1E). However the presence of the species was confirmed after the close observation of male caudal appendages found in Amboli, hence extending the distribution range up to the Maharashtra State. Previously, from *Microgomphus* genus only *M. torquatus* Selys, 1854 was reported from Maharashtra (Tiple and Koparde, 2015). Both the *Microgomphus* spp. are unique in terms of small size and scissor-like cerci. *Microgomphus torquatus* has its range till Karnataka and yet to be found in more southern parts of India (Subramanian *et al*., 2018), whereas *M. souteri* now has its range from Maharashtra to Kerala. In Amboli, a collective population of around 20 individuals was found in Hiranyakeshi and Nangartas area. The Gomphid is very shy and gets disturbed by the slightest movement. They tend to perch on small bushes and hop around when they get disturbed.

2. *Macromia flavocolorata* Fraser, 1924 (Fig. 2)

Locality**:** Nangartas, Amboli; Habitat**:** Hill stream with bushes; 20.vi.2022; 1f, 15.99°N; 74.06°E; 790 m asl.

Identification**:** A pair of yellow spots on labrum base, reddish-brown wing base, well defined humeral stripes, segment 7 with large yellow annular ring at base, segment 8 with large basal triangular yellow spot and quadrate spot on each side, vulvar scale with deep cleft forming two conical lobes (Figs. 2D-F).

Notes: Earlier, *M. flavocolorata* was known from Karnataka, Kerala, Tamil Nadu and West Bengal within Indian limits, and from China, Lao People's Democratic Republic, Myanmar, Nepal, Thailand and Viet Nam (Subramanian *et al.,* 2018). A *Macromia* male was photographed in Sadavali village, Ratnagiri District and identified as *M. flavocolorata* on the basis of photographs (Joshi *et al.,* 2022a). However no specimen was observed or collected. Thus, our record confirms its presence in the State and extends its distribution range. *M. flavocolorata* Fraser, 1924 is unique in terms of facial and thoracic markings.

3. *Macromia irata* Fraser, 1924 (Fig. 3)

Locality: Nangartas, Amboli; Habitat**:** Hill stream with bushes; 12.vi.2022; 2m, 2f, 15.99°N**;** 74.06°E; 790 m asl.

Identification**:** Male with diamond shaped yellow spots on dorsum of segment 2, peculiar yellow markings at segment 8, robust spine on dorsum of segment 9, cerci shorter than paraprocts (Figs. 3A-C, E-F) and female having similar markings on segment 2 and 8, brownish pigmentation at the wing base (Fig. 3D).

Fig. 1 ***Microgomphus souteri*** **Fraser, 1924:** A–Male from Hiranyakeshi; B–Close up of male caudal appendages; C–Male from Nangartas; D–Female from Nangartas; E–Female from Hadpid; F–Copula from Nangartas (Photos: A, B– Hemant Ogale; C–F– Dattaprasad Sawant)

Fig. 2 ***Macromia flavocolorata*** **Fraser, 1924 (Female):** A–Lateral habitus; B–Lateral view of head and thorax; C–Dorsal view of head and thorax; D–Front view of head; E–Dorsal view of abdomen end segments; F–vulvar scales (Photos:DattaprasadSawant)

Fig. 3 *Macromia irata* Fraser, 1924: A–Male dorsal habitus; B–Male lateral habitus; C–Front view of head of male; D–Female lateral habitus; E–Dorsal view of male caudal appendages; F–Lateral view of male caudal appendages (Photos: Hemant Ogale)

Notes: *M. irata* is endemic to the Western Ghats and known from Karnataka and Kerala (Subramanian *et al.,* 2018). *M. irata* Fraser, 1924 is unique in terms of caudal appendages and diamond shaped mark on segment 2. These observations extend the distribution range up to Maharashtra. Both *M. flavocolorata* and *M. irata* were found in Nangartas stream hovering at a great speed.

***4. Idionyx corona* Fraser, 1921** (Fig. 4)

Locality: Hiranyakeshi, Amboli; Habitat: Hill stream surrounded by trees; 05.vi.2022; 1m; 15.95°N**:** 74.02°E; 840 m asl. (Figs. 4B-C).

Locality: Hiranyakeshi, Amboli; Habitat: Hill stream surrounded by trees; 09.vi.2022; 2m, 1f; 15.95°N**:** 74.02°E; 840 m asl. (Figs. 4A, D-F).

Fig. 4 *Idionyx corona* Fraser, 1921: A–Male from Hiranyakeshi; B–Male from Hiranyakeshi; C–Male dorso-lateral habitus; D–Female from Hiranyakeshi; E–Dorsal view of male caudal appendages; F–Lateral view of male caudal appendages (Photos: A, D, E, F– Hemant Ogale; B, C– Dattaprasad Sawant)

Identification: caudal appendages with longer paraprocts than cerci and tiny spine-like projections on the upper border of paraprocts (Figs. 4E-F).

Notes: Previously *I. corona* was split into two subspecies namely, *I. corona corona* and *I. corona burliyarensis*, which were distinguished by the shape of spine of paraprocts (Fraser 1924). However *I. corona burliyarensis* is currently considered a synonym of *I. corona* (Kalkman *et al*., 2020; Paulson *et al.,* 2022). Earlier *I. corona* was known from Karnataka, Kerala, Tamil Nadu (Subramanian *et al.,* 2018; Joshi *et al.,* 2022b). This is the second species from the *Idionyx* genus reported from Maharashtra State after *I. saffronata*. These observations are the northernmost record of the species and extend its distribution range. *I. corona* is the second species to be reported from Maharashtra State, after *I.*

saffronata Fraser, 1924 (Sawant *et al.,* 2022). A good number of both species were observed flying high in the skies at Hiranyakeshi throughout the early June. Females of *I. corona* Fraser, 1921 can be easily distinguished even in flight by their dark pigmentation of wings. They prefer tall trees of Hiranyakeshi to perch early in the morning and late in the evening.

Amboli-Chaukul-Parpoli region is one of the most bio-diverse regions in the northern Western Ghats. Being situated at the junction of northern and central Western Ghats, Amboli shares common flora and fauna of these two regions. The area was surveyed for Odonata documentation and it was found to have 93 species with ~16 per cent Western Ghats endemic species (Sawant *et al.,* 2022b). The four species reported are the addition to Odonata fauna of Amboli region as well as Maharashtra State. In the last two years Amboli has revealed other six new State records of odonates and a new species *Burmagomphus chaukulensis* Joshi, Ogale and Sawant, 2022 (Joshi *et al.,* 2022b; Sawant *et al.,* 2022). However, Sawant *et al.* (2022) did not include three species namely, *Archibasis oscillans* Selys, 1877, *Burmagomphus chaukulensis* and *Merogomphus tamaracherriensis* Fraser, 1931 in the list of Maharashtra State. These three species are recorded from the state (Dalvi and Koli, 2022; Joshi *et al.,* 2022; Sawant and Kambli, 2023). Therefore along with the current four records, Maharashtra State now has 152 Odonata species from 13 families. Earlier, except *M. souteri* other three species were known till the southern region of Karnataka. The present observations from Amboli are ~350 km away north from the previously known localities of *M. irata*, *M. flavocolorata* and *I. corona*. *Microgomphus souteri* was known from the neighboring Goa State which is ~100 km south to Amboli region.

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Salad cucumber, *Cucumis sativus* L.: A new host record for *Apomecyna saltator* (Fab.) (Coleoptera, Cerambycidae)

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ABSTRACT: Cucurbit longicorn *Apomecyna saltator* (Fab) (Coleoptera, Cerambycidae) is an economically important pest of cucurbitaceous vegetables. Salad cucumber, *Cucumis sativus L.* grown in a polyhouse in Thrissur, Kerala, India was found infested by *A. saltator.* This is the first report of *C. sativus* as a new host plant for *A. saltator* in India. The grub is an internal feeder and causes damage by tunnelling the vines. A brief note on the nature of damage and symptoms are given.

KEY WORDS: Longicorn, vine borer, first report, damage, symptoms

Pointed gourd vine borer or cucurbit longicorn *Apomecyna saltator* (Fabricius, 1781) (Coleoptera, Cerambycidae) is an economically important pest on many cucurbitaceous vegetables *viz*., ivy gourd (*Coccinia indica* L.), pumpkin (*Cucurbita mohaeta* L.), bottle gourd (*Lagenaria vulgaris* L.), ridge gourd (*Luffa acutangula* L.), sponge gourd (*L. aegyptiea* L.) snake gourd (*Trichosanthes cucumerina L.*), and pointed gourd (*T. dioica* L.) (Biswas and Basak, 1992; Singh *et al*., 2008). The incidence of cucurbit longicorn has now been recorded for the first time on salad cumber grown in polyhouse in Kerala, India, as reported here.

Salad cucumber grown in polyhouse (200 m^2) of the Department of Plant Pathology, College of Agriculture, Vellanikkara, KAU exhibited wilting and drying symptoms in 10 per cent of the plants. The withered vines were collected in polybags and were brought to the laboratory of the Department of Agricultural Entomology, for further studies. Vines were observed for the presence of internal feeders if any and those with immature stages were kept for observation in individual glass jars (15×20×10 cm^3) at ambient room temperature (24–32°C). Adults that emerged were killed and preserved as dry specimens by mounting them on the entomological pin. The pinned specimens were subjected to taxonomic studies under Carl Zeiss Stereo Zoom (Stemi 305) microscope and photographed using Axiocam 105 color attached with the Zeiss image analyzer and the morphological characters were studied. Ten male and female specimens were used for recording the average length of the beetle. The insect was identified on the basis of the taxonomic key to the Indian species

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of the genus *Apomecyna* (Biswas and Basak, 1992).

The cerambycid causing damage to salad cucumber is identified as *A. saltator* Fabricius, 1781. Adults are small (10.0 mm), brown with long antennae measuring 6.95 mm hardly extending up to the middle of the elytra. Elytra elongate, clothed with densely yellowish pubescence, with white irregular spots. Elytral spots together form oblique bands arranged in three evenly V-shaped markings across the elytra. The first band appears between the middle and base of the elytra, the second one appears in the middle of the elytral disc, and the third band, is comprised of four spots which lie close to the elytral apex (Plate1A). The full-grown grubs are cream colored with brown heads. The full-grown larvae pupated within the larval tunnels in fibrous cocoons. The pupa is exarate and brownish in colour. Adult beetle remained in the larval tunnel for 2-3 days after emergence (Plate 1C). Adults emerged by biting their way out of the stem and were less active and remained in hidden places on dried leaves and vines (Plate I C, D). Adults were not found feeding in the field however, they were found gnawing the stem and leaf petioles under laboratory conditions.

The initial symptom, exhibited on the basal portion of the main vines, was reddish brown ooze at the point of infestation (Plate II A). At the advanced stage of infestation, swellings were observed at the nodal region of the vines due to tunneling by the grubs (Plate II B). Feeding tunnels were directed towards nodes that were filled with glutinous waste material. Severe infestation led to the splitting, withering, and drying of vines (Plate II C,D). Moreover, damage led to early senescence of the crop eventually leading to reduction in yield.

Cucurbit longicorn is reported as an important pest of coccinia in South India (Nair, 1975). Additionally, it had been reported in yam (Palaniswami and Pillai, 1982). Among different species of the genus *Apomecyna, A. saltator* is widely distributed in India (Mitra *et al*., 2016). Though it is reported as a pest of irregular appearance on cucurbits in India, its presence in polyhouse on cucumber is alarming. As adults are carried over to the succeeding crops through dried vines and stubbles, the infestation may lead to peak damage at the reproductive phase of the crop. The present finding is valuable information for adopting precautionary strategies against the incursion of this pest into polyhouse as once the pest invades into the poly house, the warm, humid environment, sustained food supply, and absence of natural enemies will provide conducive conditions for their rapid multiplication (Sreeja *et al*., 2018).

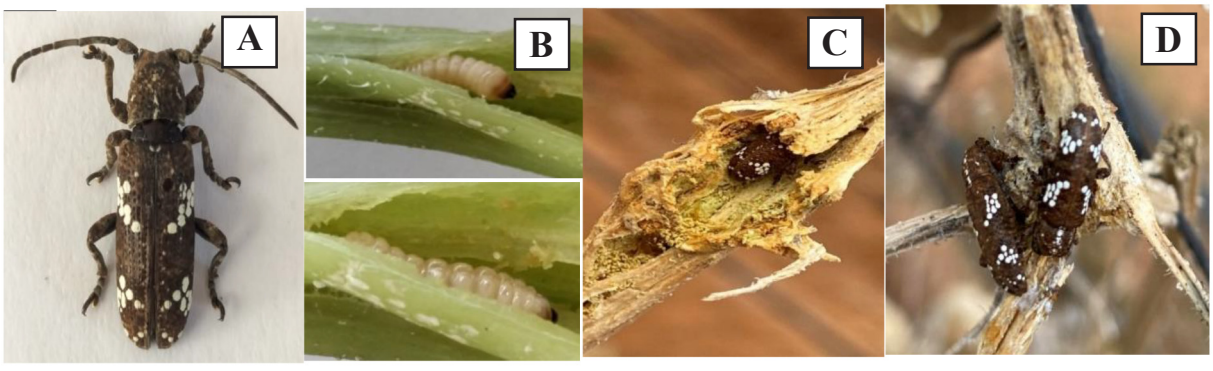

Plate I A. *Apomecyna saltator* (Adult), B. Grub in the larval tunnel, C. Adult in larval tunnel, D. Adults on dried vines

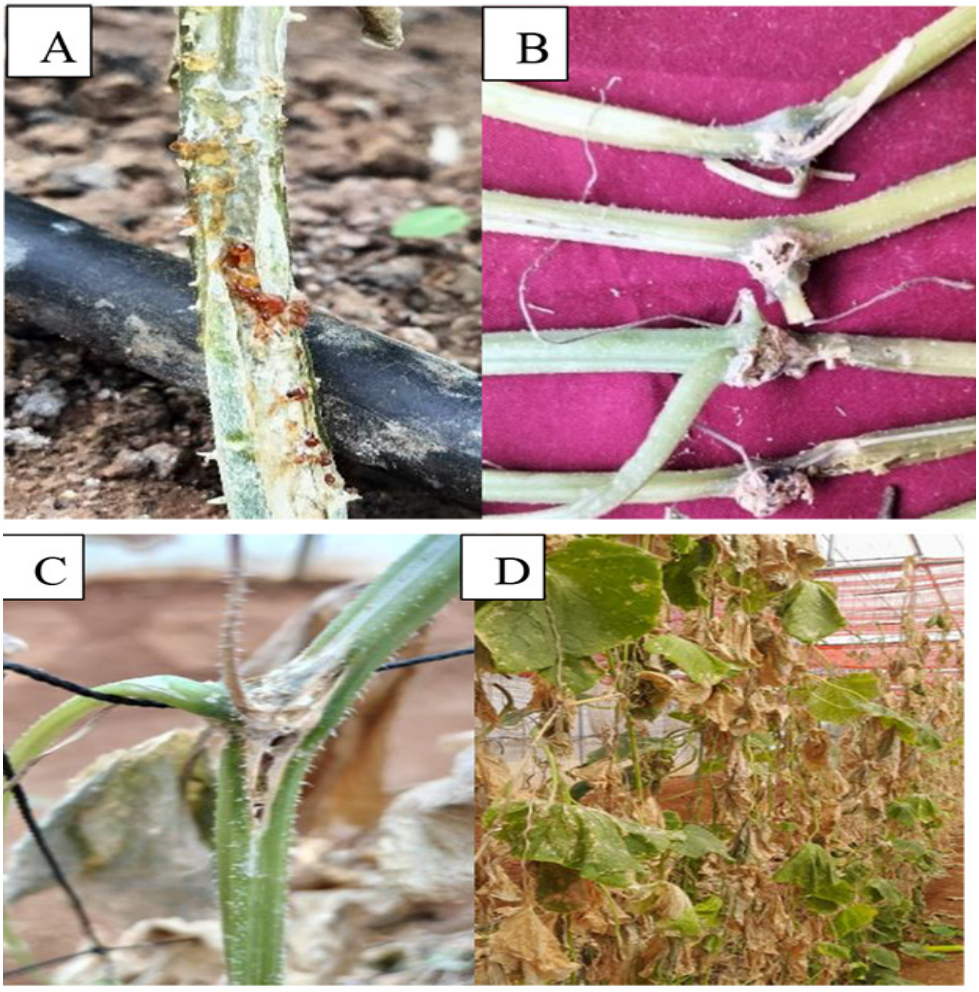

Plate II. A. Gummosis at basal region, B. Swelling in the nodal region, C. Splitting of the vine at nodal region, D. Field view of infestation

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Isolation and characterization of sex specific cuticular hydrocarbons in non-*Drosophila, Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae)

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ABSTRACT: Cuticular hydrocarbons are the major organic compounds synthesized by oenocytes. The CHCs act as sex pheromones, cell communicators, inhibits dissection, and chemotaxonomic cues. The CHCs were isolated from non-*Drosophila* species *Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae) by employing whole body extract through GCMS method. Among the identified organic compounds, a total of 38 compounds were obtained from male flies and 68 organic compounds were isolated from female flies, Majority of the CHCs identified were methyl branched alkanes, some of them were esters and alcohols. Male flies exhibit 15 specific CHCs in which tetradecane, 2, 6, 10-trimethyl was dominant with a relative abundance (15.60%) and cyclopropane tetradecanoic acid was the least found CHCs with a relative abundance (RA) of 0.75 per cent. The flies exhibit 28 female specific CHC's, among them tetradecane was dominant (r value of 19.20%) and Iodomethyl undecane was the least (RA=0.70%). Both male and female flies shared 23 CHCs, but the ratio among them showed great variance. Among the shared CHCs 3-Trifluoroacetoxy dodecane was dominant in female (RA=14.50%), the male exhibited RA of 4.66 per cent. In male flies 3-Trifluoroacetoxy pentadecane was most abundant CHC (RA=10.90%), than female (4.31%). © 2023 Association for Advancement of Entomology

KEY WORDS: Alkanes, GCMS analysis, relative abundance, specific CHCs, tetradecane

The cuticle is the integral part of the exoskeleton of members of the Insecta, Crustaceans, Arachnids, and Myriapoda of phylum Arthropoda. In insects the CHC's acts as chemical signals in inter-specific, inter-colonial, and inter-individual recognition. The role of cuticular hydrocarbons as the pheromones in *Drosophila* was studied way back in 1970s, by Ferveur (1997). The semi-chemical or chemical signal functions of CHCs are attributed to sex attractants, aphrodisiacs, defence secretions, territory marking, species and caste recognition cues, recruitment, kairomones and alarm pheromones (Howard and Blomquist, 1982; Antony *et al.,* 1985; Marisa *et al.,* 2010; Nemoto *et al.,* 1994; Kather *et al.,* 2011). The relative levels of CHCs in the *D. melanogaster* and *D. simulans* male and female flies were described by Ferveur (1997). The CHCs are chemotaxonomic tool for identification of species and show great divergence among different species (Bernier *et al.,* 1998). There are information available regarding the kinds of CHC's in the genus *Drosophila*. Our goal was

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to explore the types of CHC's in non-*Drosophila Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae).

Collection for CHCs profiling: *Phorticella striata* belongs to *Non-Drosophila* genera, the wild *P. striata* were collected from the Tumkur district of Karnataka and maintained in lab to obtain first generation. A total of 300 *P. striata* files were selected from F1 generation with an equal ratio of male to female flies (1:1 ratio). The adult flies with the almost same size and same growth stages were selected and anomalous flies with many variations are discarded in the collection stage only.

Extraction of CHCs using non-polar solvent (Whole body extract): The collected files were directly brought to the laboratory and immobilised using a chill treatment for 5 min at -18 °C. The 50 male and 50 female in three replicates flies were immobilized ("18°C) and then transferred to a desiccators and purged with nitrogen gas to remove surface moisture for 05min. Then the flies groups were transferred to individual 50ml rimmed glass test tubes containing 25ml pure n-hexane and vortexed for 15min to extract CHCs and care taken to keep flies intact without any physical damage or rupture. The extract was filtered off flies and extract was dried with help of nitrogen gas flow. The samples were reconstituted in the minimal amount of n-hexane and made ready for the GC-MS analysis (Antony *et al.,* 1985). Further, the analysis was carried out within 24 hours of post-collection of CHCs (Figs. 1 and 2).

Analysis of CHCs profile in male and female flies: The CHCs were analyzed using a Pekin Elmer Gas Chromatograph Clarus 680 with flame ionization detector (FID coupled with Pekin Elmer Mass Spectrometer Clarus SQ 8C) (PerkinElmer, Inc., Massachusetts, United States) for quantification. Sample of 2 ìl was injected to column Pekin Elmer: Elite-5MS column-30 m long × 0.250 mm id × 1 ìm, (60-350C) with a split mode of 10:1 under a constant Helium gas flow at the flow rate of 2ml min^{-1}.

CHCs profile of adult male and female *P. striata*: A total of 38 different CHC's molecules were observed in the adult male *P. striata* flies. The total 38 organic compounds were obtained in which 18 compounds belong to hydrocarbons and majority of them were methyl branched alkanes. Twenty of them belong to alcohols and the hydrocarbon esters. The tetradecane, 2,6,10-trimethyl were the most abundant CHCs in adult male *P. striata* with a relative abundance (RA) of about 16 per cent, followed by 3-trifluoroacetoxypentadecane and Octadecane, 6-methyl- with RA of about 11 and 10 per cent respectively. About 27 CHCs were present in the range of 5 to 1 per cent. About 7 compounds were less than 1 per cent and cyclopropane tetra decanoic acid and 3-trifluoro acetoxy tetradecane were the least present CHC's with a RA of 0.75 per cent (Table 1).

Similarly in female adult flies a total of 68 compounds were obtained. Among these compounds two compounds were inorganic compounds namely Hydroxylamine (RA 1.24%) and Silane (RA 1.08%). Among the identified 66 CHCs, the tetradecane, 3-trifluoroacetoxydodecane were the most abundant CHCs in adult male *P. striata* with a RA of about 19 and 14% respectively, followed by 3-trifluoroacetoxydodecane and 2,6,10-Trimethyl-3-oxo-12-(tetrahydropyran-2-yloxy)-dodeca-6,10-dienoic acid, methyl ester with about 10 and 8 per cent respectively (Table 2). About 51 CHCs were present in the range of 5 to 1 per cent. About 11 compounds were less than 1 per cent and 3-heptafluorobutyroxypentadecane and 4-(Prop-2-enoyloxy) tetradecane were the least present CHCs with RA of 0.70 per cent. Male and female adult flies shared 23 CHC's common to both sexes. The Octadecane, 6-methyl, 3-Trifluoroacetoxypentadecane, and 3-Trifluoroacetoxydodecane were abundantly common in both sexes with RA of more than 4 per cent in both sexes. Amount the 23 common CHCs, 13 compounds were relatively high in female flies than the male files, 4 compounds were relatively high in male than the female and 6 compounds were present almost equal ratio in both sexes. Collectively as from this study it can be observed that most of the compounds are alkanes and its derivatives. Out of total annotated CHCs observed in both male and

female flies about 45 compounds are alkanes and its derivatives with a contribution more than 60 per cent to total CHCs diversity observed in the experiments.

Jallon (1984) described 7-Tricosene, 7-pentacosene, 7,11-Pentacosadiene and 7,11-nonacosadiene as potential pheromones in *Drosophila* spp., (Z)-7-Tricosene in *D. virilis* (Oguma *et al.,* 1992). But 3-Trifluoroacetoxypentadecane, 3-Trifluoro-acetoxypentadecane and Octadecane, 6-methyl- are the three major CHCs observed in *P. striata*. Due to multivariate nature of the CHCs, smallest alteration of individual CHCs profile dramatically alter the overall composition profile of CHCs during mate selection (Sharma *et al.,* 2012). From the present study, it is evident that the cuticular profile of male and female flies of *P. striata* is unique and implies the species identification, growth stage, or age identification. The shared common CHCs can act as a species-specific biomarker for the identification of the *P. striata* flies. Some of the identified CHCs compounds were unique and are not naturally reported elsewhere in the literature. In several studies the cuticular hydrocarbons of the genera *Drosophila* exhibits all kinds of hydrocarbons viz. alkanes, alkenes and alkynes (Drijfhout *et al.,* 2010; Kather *et al.,* 2011), Since it is *non-Drosophila*, the *P. striata* exhibits only the alkanes. Methods employed in the study can pave the way for the profiling of CHCs in *P. striata* in different stages of the growth and at different ecophysiological conditions.

Table 1. Male specific CHCs and its relative abundance (RA) in adult *P. striata*

No.	CHCs - Compound name	Molecular formula	RA in %
1	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	15.60
2	Decane, 2,3,5,8-tetramethyl	$C_{14}H_{30}$	3.02
3	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$	2.43
4	Hexadecane, 2,6,11,15-tetramethyl	$C_{20}H_{42}$	1.56
5	Tridecane, 4-methyl	$C_{14}H_{30}$	1.56
6	Undecane, 2-methyl	$C_{12}H_{26}$	1.56
7	Decane, 2,4,6-trimethyl-	$C_{13}H_{28}$	1.38
8	Tetradecane, 5-methyl	$C_{15}H_{32}$	1.38
9	Eicosane, 10-methyl	$C_{21}H_{44}$	1.23
10	Dodecanoic acid	$C_{12}H_{24}O_2$	1.17
11	1-Trifluorosilyltridecane	$C_{13}H_{27}F_3Si$	1.13
12	9-Octadecen-12-ynoic acid	$C_{19}H_{32}O_2$	1.13
13	3,6-Octadecadiynoic acid	$C_{18}H_{28}O_2$	0.82
14	3-Cyclopropylcarbonyloxytridecane	$C_{17}H_{32}O_2$	0.78
15	Cyclopropanetetradecanoic acid	$C_{17}H_{32}O_2$	0.75

Table 2. Female specific CHCs and its relative abundance (RA) in adult *P. striata*

No.	CHCs - Compound name	Molecular formula	RA in %
1	Tetradecane	$C_{14}H_{30}$	19.20
2	6-Dimethyl(trimethylsilyl)silyloxytetradecane	$C_{19}H_{44}OSi_2$	3.12
3	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	2.70
4	Dodecane	$C_{12}H_{26}$	2.58
5	5-Dimethyl(trimethylsilyl)silyloxytridecane	$C_{18}H_{42}OSi_2$	2.39
6	2-Bromo dodecane	$C_{12}H_{25}B$	2.18
7	2-Methyltetracosane	$C_{25}H_{52}$	2.11
8	4-trimethylsilylcyclopentane	$C_{16}H_{30}Si$	1.95
9	Tetracosane	$C_{24}H_{50}$	1.78
10	Octacosane	$C_{28}H_{28}$	1.64
11	1-Octadecanamine, N-methyl- 12. 2(1H)-	$C_{19}H_{41}N$	1.61
12	Quinoxalinone	$C_8H_6N_{2O}$	1.61
13	2H-1-benzopyran-2-amine	C_9H_9NO	1.49
14	1-Hexadecanol, 2-methyl-	$C_{16}H_{34}O$	1.34
15	Z, Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	1.34
16	2-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	1.19
17	Decane	$C_{10}H_{22}$	1.19
18	Docosane	$C_{22}H_{46}$	1.19
19	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	1.10
20	Dodecanal	$C_{12}H_{24}O$	1.05
21	Undecane	$C_{11}H_{24}$	1.05
22	3-Heptafluorobutyroxydodecane	$C_{16}H_{25}F_7O_2$	0.82
23	3-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	0.82
24	4-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	0.82
25	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	0.73
26	5-(Prop-2-enoyloxy) pentadecane	$C_{18}H_{34}O_2$	0.73
27	4-(Prop-2-enoyloxy) tetradecane	$C_{17}H_{32}O_2$	0.70
28	Iodomethylundecane	$C_{12}H_{12}I$	0.70

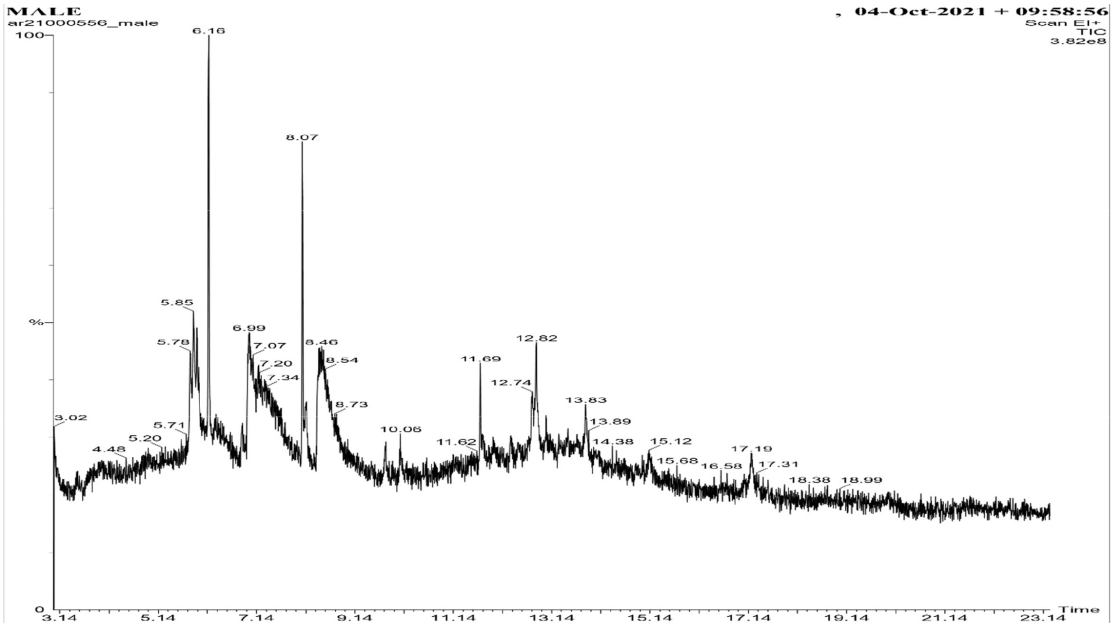

Figure 1 : The GC-MS chromatogram of the adult male *P. striata.* (X-axis- Retention time, Y axis – Relative Abundance)

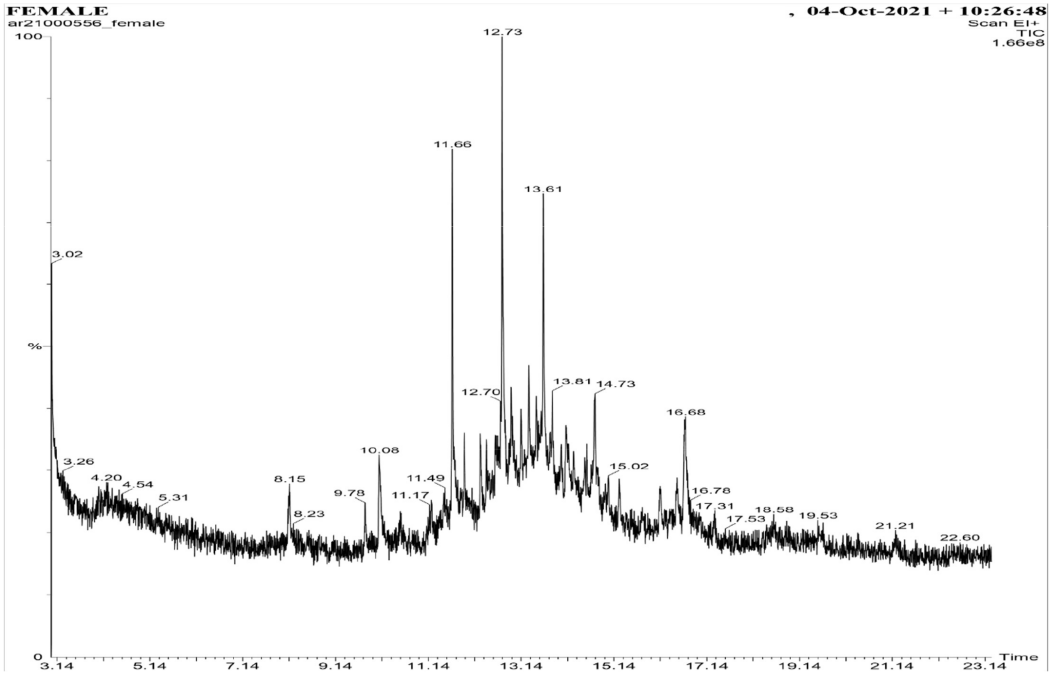

Figure 2: The GC-MS chromatogram of the adult female *P. striata.* (X-axis- Retention time, Y axis – Relative Abundance)

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First record of vesicant beetles: *Paederus nigricornis* Bernhauer, 1911 from south India; *P. extraneus* Wiedemann, 1823 and *P. alternans* Walker, 1858 (Staphylinidae, Paederinae) from Kerala

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ABSTRACT: Three vesicant beetles were reported. *Paederus nigricornis* Bernhauer, 1911 from south India and, occurrence of *P. extraneus* Wiedemann, 1823 and *P. alternans* Walker, 1858 in Kerala. Taxonomic description of each species with remarks is presented. © 2023 Association for Advancement of Entomology

KEYWORDS: Paederus dermatitis, geographical distribution, taxonomic description

The genus *Paederus* is a medically important group of rove beetles and a threat to human beings. Around fifty species of *Paederus* are known to cause Paederus dermatitis. Even though a number of Paederus dermatitis cases have been reported from various parts of India, taxonomic work on this group in India is insufficient. *Paederus* Fabricius, 1775 is a genus of beetles under the subfamily Paederinae of the family Staphylinidae (rove beetles). More than 600 species of *Paederus* have been described so far, among which 50 species of *Paederus* beetles are known to cause Paederus dermatitis (Veraldi *et al*., 2013). *Paederus* beetles produce vesicant toxin, pederin causing skin irritation (Uzunoðlu *et al*., 2017). These beetles are predators and are commonly found in paddy fields and other plantations (Vineesh *et al*., 2022). Most of them live in moist areas, such as at the edges of freshwater lakes, riverine floodplains, marshes and rice fields (Frank and Kanamistu, 1987). They are beneficial to agriculture as they eat crop pests (Mammino, 2011). At the same time they are harmful to humans as they cause Paederus dermatitis. When crushed against the skin, they release the haemolymph toxin Pederin that causes Paederus dermatitis which is also known as spider lick, night burn and dermatitis linearis (Nasir *et al*., 2015). Thirty five species of the *Paederus* are reported from India which includes thirty one species listed by Cameron (1931) and the four species by Löbl and Löbl (2015) and Biswas and Sen Gupta (1982) and this 20 species are from south India. Paederus dermatitis is very common in tropical regions like Kerala (Kambil, 2018). Identification of the dermatitis causing *Paederus* species is necessary, prior to taking preventive measures against them. But taxonomic studies on this genus are inadequate at the subgeneric and species levels (Nikbakhtzadeh *et al*., 2012).

Specimens were collected during the period 2018–2020 using Light traps from the paddy fields at

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Ambalavayal (11.6165° N; 76.2140° E) and Theneri (11.6386° N; 76.1407° E), Wayanad district. Collected beetles were transferred into vials (containing 70% alcohol). All the collected beetles were examined using Stereo Zoom Trinocular Microscope (LABOMED – 200 MAR, CODE:- ZM 45 TM). Specimens were identified using the taxonomic key available in Cameron, 1931 and verified by the photograph given in Sar and Ilango, 2016. Photographs of the specimens were taken with a Leica MC170HD camera attached to a Leica M205C stereomicroscope. Identified specimens are deposited to the insect collections of Zoological Survey of India, Western Ghats Regional Centre, Kozhikode station.

Abbreviations used: Measurements -

TL: Total length of the specimen; AL: Antennal Length; HL: Head length i.e., from the anterior margin of clypeus to posterior margin of head; HW: Head Width including eyes; PL: Pronotal length; PW: Pronotal width; EL: Length of elytra; EW: Width of elytra.

***Paederus nigricornis* Bernhauer, 1911 (Fig. 1)**

Paederus nigricornis Bernhauer 1911: 61; Bernhauer and Schubert 1912: 209; Cameron 1925: 36; id. 1928: 560; id. 1931: 47; Scheerpeltz 1933:1226; Blackwelder 1939: 112; Scheerpeltz 1957: 474; Coiffait 1978: 553; id. 1982: 33; Frank 1988: 123; Thapa 2000: 260; Willers 2001: 21; Löbl and Smetana 2004: 611; Li *et al*., 2013; Nikbakhtzadeh *et al*., 2012: 5; Sar and Hedge 2015: 102; Löbl and Löbl 2015: 987; Sar and Ilango 2016: 234.

Specimen examined: 1♀, 19.ii.2020, Theneri, Wayanad district (11.6386° N; 76.1407° E), Coll. Kavyamol P M, Light trap.

Diagnosis: Measurements: TL: 7.13 mm; AL: 2.26 mm; HL: 0.91 mm; HW: 0.98 mm; PL: 0.93 mm; PW: 0.74 mm; EL: 1.26 mm; EW: 1.22 mm

Colour: Head, first visible and last two segments of the abdomen black in colour; thorax and second to fourth abdominal segments red; elytra blue; antennae black coloured with the first two joints slightly testaceous; palpi and legs, including the coxae black.

Head: Elongate, longer than broad and broader than the thorax. The eyes prominent and the post - ocular region almost straightly converge to the neck. Sculpture consists of big and small punctures, the post-ocular region with a few setae, otherwise smooth.

Thorax: Narrow, oval and longer than broad, impunctate at the middle, the sides sparingly and very finely punctured with a few erect black setae.

Elytra: Slightly longer and broader than the thorax, parallel, rather finely and not very closely punctured, with fairly long, semi-erect, black pubescence.

Abdomen: Closely punctured at the base of the first visible segment, elsewhere sparingly punctured, the pubescence yellow with a few longer black setae.

Distribution: India (Kerala: Wayanad, Theneri; Himachal Pradesh: Simla Hills; Uttarakhand: Chakrata district Garhwal hills; Sikkim; Uttar Pradesh: Allahabad-Ramghat; Mizoram; West Bengal: Darjeeling, Nurbong and Mahanadi Valleys). Elsewhere: Nepal: Sundarijal, Ramechhap: Khimti Khola, Shivalaya; Iran (Mt. Taftan); China (Hubei, Sichuan, Xizang,); Afghanistan; Pakistan.

Remarks**:** First record of dermatitis causing *Paederus nigricornis* from south India.

***Paederus extraneus* Wiedemann, 1823 (Fig. 2)**

Paederus extraneus Wiedemann 1823:13; Dejean 1833: 65; Erichson 1840: 661; Kraatz 1859: 152; Gemminger and Harold 1868: 627; Bernhauer and Schubert 1912: 206; Cameron 1925: 35; id.1931: 41; Scheerpeltz 1933: 1223; Frank 1988: 110; Taneja *et al*., 2013:135; Gopal 2014: 4736.

Specimens examined: 2♂♀, 19.ii.2020, Theneri, Wayanad district (11.6386° N; 76.1407° E), Coll. Kavyamol P M, Light trap.

Diagnosis: Measurements: TL: 6.28 mm; AL: 2.51 mm; HL: 0.92 mm; HW: 0.96 mm; PL: 1.15 mm; PW: 1.05 mm; EL: 1.62 mm; EW: 1.44 mm

Colour: Head black, elytra blue, thorax and first four segments of the abdomen red; antennae and palpi dark at base; first two joints of the antennae slightly yellowish below and dark above, the rest black; palpi dark testaceous, the third joint black and the mandibles black; thorax red and black coloured scutellum; abdomen black with the first four segments red and sternum black; legs with black coxae.

Head: Orbicular and as broad as the thorax, rather convex above, shining, closely and finely punctured.

Thorax: Somewhat oval and almost as broad as the elytra, a little longer than broad, the sides a bit rounded, narrowed behind, convex, closely and finely punctured, middle portion without punctures.

Elytra: Hardly a fourth longer than the thorax, closely, not very strongly punctured, slightly shining and covered with white pubescence.

Abdomen: Abdomen with fine punctures, pubescence long and white.

Distribution: India (Kerala: Wayanad; Theneri; West Bengal; Andhra Pradesh; Karnataka: Manipal). Elsewhere: Nepal.

Remarks: First record of dermatitis causing *Paederus extraneus* from Kerala.

***Paederus alternans* Walker, 1858 (Fig. 3)**

Paederus alternans Walker 1858: 205; Gemminger and Harold 1868: 626; Cameron 1925: 34; id. 1930: 332; id. 1931: 41; Scheerpeltz 1933: 1220; id.1935: 607; id.1965: 103; id. 1978: 196; Biswas and Sen Gupta 1982: 150; Frank 1988:100; Willers 2001: 21; Löbl and Smetana 2004: 614; Li *et al*., 2013: 336; Löbl and Löbl 2015: 986.

Specimens examined: 1♂, 10.iii.2018, Ambalavayal, Wayanad district (11.6165° N; 76.2140° E), Coll. Kavyamol P M, Light trap, 1♀, 19.ii.2020, Theneri, Wayanad district (11.6386° N; 76.1407° E), Coll. Kavyamol P M, Light trap.

Diagnosis: Measurements: TL: 6.37 mm; AL: 2.08 mm; HL: 0.908 mm; HW: 0.95 mm; PL: 0.91 mm; PW: 0.73 mm; EL: 1.3 mm; EW: 1.2 mm

Colour: Head black. Elytra blue. Last two abdominal segments black, thorax and first four visible segments red; antennae black, the first three segments slightly testaceous; palpi with the third joint black; legs black, the coxae and extreme base of the femora testaceous.

Head: Orbicular, the post- ocular region slightly rounded to the neck, a little broader than the thorax, with a few small setiferous punctures.

Fig. 1 *Paederus nigricornis*

Fig. 2 *Paederus extraneus*

Fig. 3 *Paederus alternans*

Thorax: Convex, ovate and longer than broad, not much narrowed behind, smooth along the middle, the sides with fine setiferous punctures.

Elytra: Slightly broader and about a third longer than the thorax, parallel, rather finely and not very closely punctured having fine greyish semi-erect pubescence.

Abdomen: Finely and scarcely punctured, finely and sparingly pubescent with a few long black setae.

Distribution: India (Kerala: Wayanad; Ambalavayal, Theneri; Himalaya; Karnataka: Kanara; Tamil Nadu: Nilgiri Hills). Elsewhere: Sri Lanka (Colombo, Maskeliya); Myanmar (Kawkareik: Pegu, Annam, Tonkin); China (Guangxi, Hainan); Vietnam (Tonkin).

Remarks: First record of dermatitis causing *Paederus alternans* from Kerala.

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Oviposition behaviour of *Callosobruchus chinensis* (L.) (Coleoptera, Chrysomelidae, Bruchinae) on horse gram seed varieties

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ABSTRACT: Oviposition behaviour of *Callosobruchus chinensis* was tested on six varieties of horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) to identify the resistance variety. In no choice test, females of *C. chinensis* laid lesser eggs within 24 h of mating on whole seeds of Urmi variety (11.6 ± 0.41) than the other varieties tested. However, females did not lay eggs when seed coat was removed from the above six varieties of horse gram. In choice test, females laid the lowest number of eggs within 24 h of mating on Urmi (1.11 ± 0.23) than the other varieties. The scanning electron micrograph (SEM) study revealed that there were morphological differences on the seed coat texture of the six horse gram varieties. The seed coat texture of Urmi variety is heterobrochate with some nearly bi-reticulate patches and few unevenly depressed muri, which partially inhibited oviposition on Urmi seeds. Urmi is the less preferred variety for oviposition.

KEY WORDS: Varietal resistance, choice assay, no choice assay, SEM, seed coat texture

Macrotyloma uniflorum Lam. (Verdc.), commonly known as horse gram, is an important pulse crop in India (Reddy *et al.*, 2008; Prasad and Singh, 2015; Agnihotri and Rana, 2021) besides, Australia, Africa, Malaysia, Burma and the West Indies (Kingwell-Banham and Fuller, 2012). Pulses in tropical and subtropical countries are attacked by pulse beetles, C*allosobruchus* species (Coleoptera, Chrysomelidae, Bruchinae) in the fields and storage. In India, *Callosobruchus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.) are reported to cause severe damage of pulse seeds. Among three pulse beetles, *C. chinensis* is the most important bruchid pest in India (Mishra *et al.*, 2013). The larvae of *C. chinensis* complete larval development within horse gram seeds and thus damaged seeds are not suitable for human consumption. Severe attack by this insect pest causes poor germination, low seed weight and no commercial value of horse gram seeds (Divya *et al.*, 2016). Synthetic insecticides are commonly applied to control the insect pests in storage (Jayaram *et al.*, 2022). But applications of synthetic insecticides in stored grains have many harmful effects such as health hazards, toxicity and environmental contamination. Further, insect pests might be resistant to harmful insecticides (Talukder, 2009). So, it will be cost effective and eco-friendly to use resistant horse gram varieties against the attack of *C. chinensis*. The current study was

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undertaken to study the oviposition behaviour of *C. chinensis* on varieties of horse gram seeds to observe the varietal resistance against *C. chinensis*.

Six varieties of uninfested and healthy horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were collected from Kota, Rajasthan, India. Adults of *C. chinensis* were collected from local stores containing cowpea seeds at Burdwan (23°16 N and 87° 54 E) during March 2022. Collected adults were also reared on cowpea seeds for three generations. The insects were not reared on horse gram seeds as the insect might be habituated to horse gram seeds. A pair of newly emerged male and female were kept in a glass jar (20 cm H × 10 cm D) for mating. Newly emerged females mate within 2 hr of emergence. Gravid females were used for oviposition experiments.

In no choice test, twenty seeds of each variety of horse gram (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were placed in a glass jar (20 cm H × 10 cm D) separately. A gravid female was released in each glass jar and observed for 24 hr. The experiment was replicated 35 times for each variety of seeds. In no choice test, seed coats from each variety of horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were removed keeping the seeds intact. Twenty seeds without seed coat from each variety of horse gram were positioned in a glass jar (20 cm H × 10 cm D) separately. The experiment was replicated 35 times for each variety of seeds.

In choice assays, a rectangular glass chamber (35 cm long × 10 cm breadth × 8 cm height) was used, and emery papers (coarse grade) were put along sides and bottom of each glass chamber to prevent oviposition on the floor and walls of glass chamber. Six Petri dishes (5 cm diam) were placed at equal distance along the length of the rectangular glass chamber. Distance between two Petri dishes is 10 cm along the length of the glass chamber. Each Petri dish contained 20 uninfested seeds of a horse gram variety. A gravid *C. chinensis* female was then released in the centre of the rectangular chamber. For the oviposition assay experiment, 35 gravid females were separately examined eliminating those insects that did not oviposit.

The egg laying behaviour of a gravid female for the above two experiments was monitored for 24 h after releasing in the experimental chamber, and when a female began to lay eggs, she was observed until she stopped ovipositing, at which point the replicate was terminated, the number of eggs counted, and the female was discarded. For scanning electron microscope (SEM) study of seed coat, healthy and uninfested whole seeds were broken cautiously in the laboratory to separate the seed coats. The seed coat surface of each variety of horse gram seed sample was passed through graded alcohol (50, 70, 90 and 100 % alcohol, for each grade 15 min) to dehydrate the seed coats. The dehydrated seed coats for each variety were mounted on aluminium holders (stabs) coated with gold-palladium (2 nm thickness) for SEM study. The data in no-choice and choice tests were subjected to one-way ANOVA followed by Tukey test (Zar, 1999).

In no choice assays, number of eggs laid by a gravid female differed significantly among six varieties of horse gram seeds ($F_{5, 204} = 14.221$, $P < 0.0001$). The number of eggs laid by a female within 24 hr of mating was the lowest on Urmi variety (11.6 ± 0.41) among six horse gram varieties, ANK Black (15.06 ± 0.52), AK 42 (16.57 ± 0.53), AK 21 (15.03 ± 0.51), BK 1 (16.34 ± 0.66) and Paiyur 1 (13.26 ± 0.32) (Fig. 1). Among six varieties, Urmi is the least preferred variety for oviposition by *C. chinensis*. However, females did not lay eggs on seeds when seed coat was removed from the above six horse gram varieties, implicating seed coat morphology and surface waxes influenced the oviposition of *C. chinensis* females. A previous study demonstrated that when seed coat was removed from four varieties (BIO L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1) of *Lathyrus sativ*us L. seeds then *C. maculatus* females did not lay eggs on seed coat removed *L. sativ*us seeds (Adhikary *et al.*, 2016a).

In choice assays, number of eggs laid by a gravid female differed significantly among six horse gram

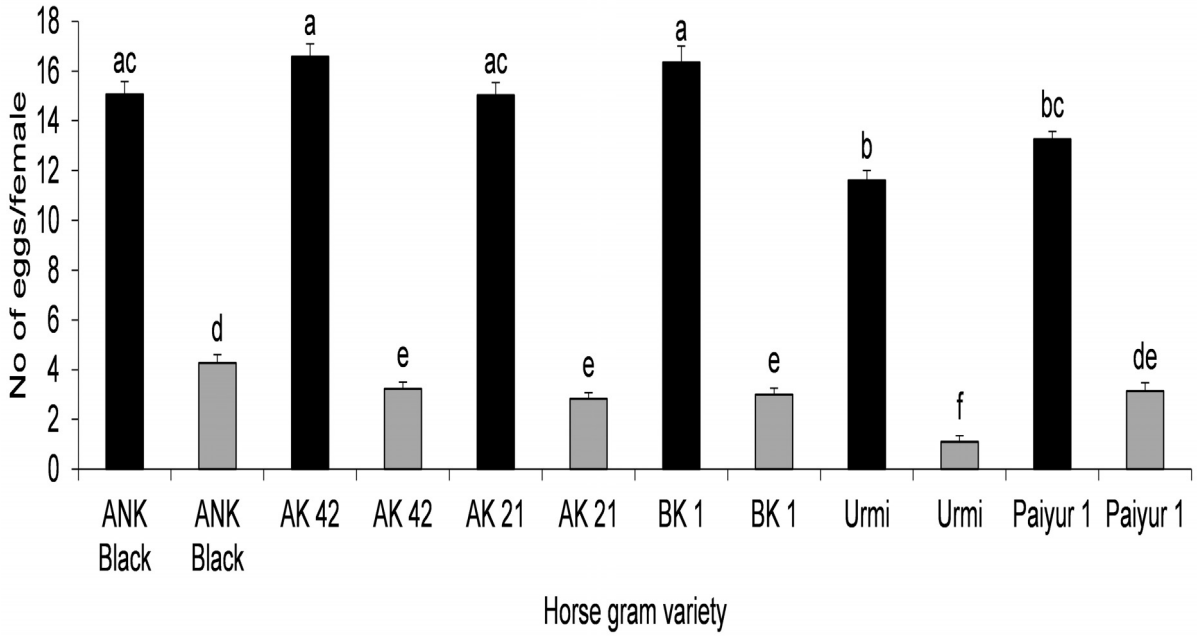

Fig. 1 Oviposition of *Callosobruchus chinensis* within 24 h of mating (n = 35) on different varieties of horse gram seeds in no choice assays (black colour) and choice assays (grey colour). Means followed by different letters are significantly different by Tukey's test at 5% level of significance

varieties ($F_{5, 204}$ = 12.919, $P < 0.0001$). The number of eggs laid by a female within 24 hr of mating was the highest on ANK Black (4.26 ± 0.34) followed by AK 42 (3.23 ± 0.27), BK 1 (3.00 ± 0.26), AK 21 (2.83 ± 0.24) and the lowest on Urmi (1.11 ± 0.23). There is no significant difference between ANK Black and Paiyur 1 (3.11 ± 0.34) (Fig. 1). These above observations revealed that females did not prefer to lay eggs on Urmi, implicating that Urmi is the partially resistant variety for ©*C. chinensis* oviposition.

The SEM study revealed that seed coat of ANK Black was homobrochate reticulate with significantly thicker muri and slightly elevated lumen (Fig. 2a), whereas the seed coat of AK 42 rugulate-reticulate (Fig. 2b). In AK 21, the seed coat was rugulate with few patches showing perforate-rugulate-reticulate and perforation elongated (Fig. 2c), whereas the seed coat of BK 1 was faintly rugulate and sometimes ruptured (Fig. 2d). The seed coat of Urmi was heterobrochate with some nearly bi-reticulate patches and few unevenly depressed muri, but some of these muri were incomplete (Fig. 2e). In Paiyur 1, the seed coat was heterobrochate reticulate with thicker muri (Fig. 2f). These above observations indicate that the seed coat of Urmi variety, i.e., heterobrochate with some nearly bi-reticulate patches partially inhibited egg laying of *C. chinensis*. Different studies demonstrated that seed coat texture influenced oviposition in stored grain insect pests (de Sá *et al.*, 2014; Adhikary *et al.*, 2014, 2015, 2016a, b; Szentesi, 2021).

Host resistance is an effective way of pest management in integrated pest management strategy. Resistance against insect pests is manifested through antibiosis, antixenosis (non-preference) and/ or tolerance. Traits contributing to resistance/ susceptibility of legumes to bruchids include seed color, texture, hardness, size and chemical constituents. In the present study, seeds of six horse gram varieties are having the shape of curved beak and more or less similar in size. The texture and colour of seeds of horse gram varieties are as follows – ANK Black is smooth and shiny black, AK 21 has smooth and creamy brown, AK

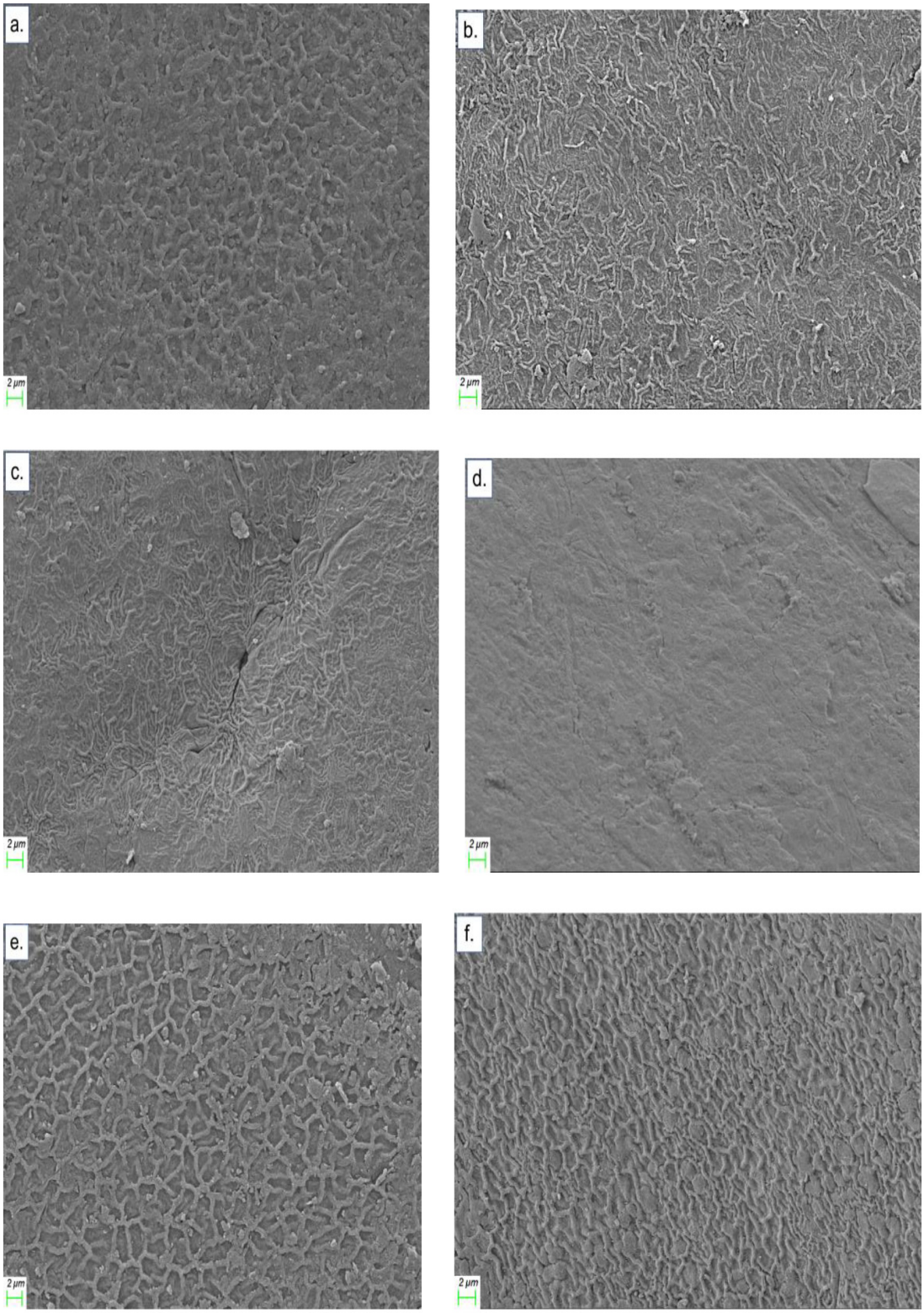

Fig. 2 Scanning electron micrograph of seed coat surface of ANK Black (a), AK 42 (b), AK 21 (c), BK 1 (d), Urmi (e) and Paiyur 1 (f)

42 with smooth and blackish brown, BK1 is smooth and creamy, Paiyur 1 is not so smooth and creamy blackish brown, and Urmi has rough and grey black. The oviposition was low on Urmi variety than the other varieties, suggesting that seed coats with rough texture inhibited egg laying. The uneven and undulating pattern of the rough seed coats of Urmi variety may have provided a stimulus of instability to *C. chinensis* females. Similarly, *C. maculatus* laid more eggs on smooth-coated seeds compared to rough-coated cowpea seeds as smooth-coated seeds allowed firm attachment of the eggs and provided more surface area than rough-coated seeds (Barde *et al.*, 2012). In the present study, ANK Black, AK 21, AK 42, BK 1 and Paiyur 1 were found to be susceptible, while Urmi variety was relatively resistant.

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Trapping analysis of ants (Hymenoptera, Formicidae) in Kurunthamalai, Coimbatore, Tamil Nadu, India

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ABSTRACT: Ant species were collected from three different methods bait trapping (BTM), all-out search method (AOSM), and pitfall trapping method (PTM) method. A total of 14 species belonging to the eight genera and four subfamilies are listed in the Palamalai region. *Monomorium indicum, Solenopsis germinata, Trichomyrmex crinieceps, Trichonymex glaber, Camponotus mendax, C. guticollis, C. irritans, C. parius, C. rufoglaucus, Oecophylla smaragdina,,Diacamma indicum, Leptogenys chinensis,Tetraponera rufonigra and Tetraponera* spp were reported. Shannon Wiener, Simpson diversity, Dominance, Evenness, Menhinick, Margalef, Fisher alpha and Berger- Parker calculations and diversity richness are reported.

KEYWORDS: Myrmecology, *Shannon* Wiener, Simpson diversity, dominance, evenness

Ants are progressively more used for biodiversity studies, and comparison of habitats in different ecosystems (Andersen and Majer, 2004). Sabu *et al*. (2008) estimated the diversity of forest litter inhabiting ants along elevations in the Wayanad region of the Western Ghats. Bharti and Sharma (2009) carried preliminary investigations on diversity and abundance of ants along an elevational gradient in Jammu-Kashmir Himalaya. The environmental significance and bio diversity of ants have lead the ecologists and Myrmecologists to study patterns of ants distribution and species composition throughout the world (Narendra *et al*., 2011). In the tropics level ants play an important ecological role in both natural and agricultural habitats (Delabie *et al*., 2007). The present work was aimed to conduct a survey of ant species inhabited in residential site, industrial site and agricultural site and assess the three different trapping methods *viz.,* bait trapping (BTM), all-out search method (AOSM), and pitfall trapping method (PTM).

Kurunthamalai region, Coimbatore district, is a part of biodiversity hotspot of Western was chosen as ideal site for the study during January 2021 to December 2021 (7.00 am till 7.00 pm). Kurunthamalai region with 11.2503 Latitude and 76.9183 Longitude, is in Karamadai road, Coimbatore district, Tamil Nadu. The survey of insects was undertaken with three different transects (All-out search method, pit fall trap method and bait trap method). In all-out search method (AOSM) ant survey sheet was used. Pit fall trap method (PTM), the pit-fall traps were made by using the 0.4 L plastic cup with an opening of 11 to 12 cm in diameter, covered at ground level. One

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pit-fall trap was placed in each one of the five randomly selected 25m x 25m quadrates of one hectare plots in Kuruthamalai, Palamalai and Maruthamalai of Kurunthamalai. Each glass carried 20–25 mL of diluted ethanol (70%) mixture. The traps were set up between 7 am to 7 pm and the ants were collected after 2- 3 days. Four bait types: egg yolk, fried coconut, sugar or candies, raw rice and chicken waste were used inB and placed in three different areas (bare area, agriculture land and road sides) in Kurunthamalai. The collected samples were kept in the research laboratory, Department of Zoology, Kongunadu arts and Science College, Coimbatore. The collected ants were identified with key (Bingham, 1903; Bolton, 1994, 1995, 2003; Rastogi, 1997; Tiwari, 1999; Varghese, 2004, 2009) and further confirmed by Dr. Himender Bharti, Department of Zoology and Environmental science, Punjabi university, Patiala, Punjab. Diversity indices were worked out as-

Shannon diversity - H= - Sum Pi ln (Pi)

Pi=S/N; S= Number of individuals of one species; N= Total number of all individuals in the sample.

Simpson diversity - Ds=N (N-1)/SUM ni (ni-1)

ni (number of individuals)=total number of a organisms of a given species; N (total number) = Total number organisms of all species.

Evenness - E= H/S

Were, H= Shannon Weiner diversity; S= Total number of species in a sample

Margalef - D=(S-1)/ ln N(2)

Where S = Number of species in sample; N= total individual in data

Menhinick - D Mn= S/ ffi N p,

The number of species (n)/ square root of the total number of individuals (N)

Results of the study indicated prevalence of 14 ant species. A total of 3,005 individual ants, under four subfamilies (Formicinae, Myrmicinae, Pseudomyrmicinae and Ponerine) were collected from the different sampling sites. Totally fourteen species were observed in the study area. *Monomorium indicum, Solenopsis germinate, Trichomyrmex crinieceps and T. glaber* were recorded under Myrmicinae (3 genera and 4 species), followed by Formicinae (*Camponotus mendax, C. augusticollis, C. irritans, C. parius, C. rufoflaucus* and *Oecophylla smaragidna*. Rajagopal *et al*. (2005) reported 25 species in Virudhunagar, Tamil Nadu. Anu and Sabu (2007) documented that Wayanad district, they also documented dominance subfamily is formicine. Subedi *et al*. (2020), documented 48 genera and 128 ant species under eight subfamilies (Amblyoponinae, Dolichoderinae, Dorylinae, Formicinae, Leptanillinae, Myrmicinae, Ponerinae and Pseudomyrmicinae), in Kurunthamalai region Coimbatore, Tamil Nadu. *Solenopsis geminate* (fire ants) was found in human habituated areas. Ponerinae (with *Diacamma indicum* and *Leptogenys chinensis*), and Pseudomyrmicinae (with *Tetraponera rufonigra* and *Tetraponera* spp), Ponerinae and Pseudomyrmicinae were less in diversity. *Componotus mendax* predominated in May (summer season), followed by *C. augusticollis* and *C. parius,* both were abundant in May. Species *C. rufoglaucus* and *C. irrritans* were predominant in April. The ant species reported from different location are in different numbers. Varghese (2009) documented 226 species belonging to 63 genera and 11 subfamilies from Karnataka, Bharti (2012, 2016) reported 40 ant species from Punjab, Azhaguraj (2017) ten species, Sornapriya and Varunprasath (2018) 36 species from Tamil Nadu.

Different species were noted in AOSM, PTM and BTM. Ant collections were rich (n=519) in AOSM (Table 1). *Monomorium indicum* was commonly present in BTM. In pit fall trap (n=433) and bait trap (n=400) compared to the all-out search method, they're less in number because in the trapping some of other insects also trapped like beetles, bugs and some moths. But with the all-out search method, we only concentrated on the ant samples. In three different methods, the all-out search method (AOSM) was (n=519) highly rich

Table 1. Ant species trapped in the different methods

Species	AOSM	PTM	BTM	Total
Monomorium indicum Forel, 1895	57	48	49	154
Solenopsis germinata F., 1894	79	69	89	237
Trichomyrmex crinieceps Mayr, 1875	59	60	71	190
Trichomyrmex glaber Andre, 1883	45	55	65	165
Camponotus mendax Forel, 1895	80	71	92	243
C. angusticollis (Jerdon, 1851)	74	55	36	165
C. irritans (Smith, 1857)	56	69	95	220
C. parius (Emery, 1889)	80	100	90	270
C. rufoglaucus (Jerdon, 1851)	85	87	88	260
Oecophylla smaragdina F., 1775	72	52	49	173
Diacamma indicum (Santschi, 1920)	48	29	10	87
Leptogenys chinensis Mayr, 1870	80	84	79	243
Tetraponera rufonigra (Jerdon, 1851)	68	66	30	164
*Tetraponera spp**	76	86	96	258

AOSM - All out search method

PTM - pitfall trap method

BTM - bait trap method

in our study area. During the all- out search method, we had taken with ant survey sheet.

In every day sample collection during the method of AOSM the ant survey sheet played important role in the present study. Myrmicinae ants dominated in the months of March, May and June followed by the Formicinae ants in May. Species diversity and differentiation were higher in AOSM as compared to PTM and BTM, because of trapping make disturbed another some insects, in the site. Gary *et al*. (2010) documented 42 ant species with 15 genera and five subfamilies (Amblyoponinae, Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae) during the survey of Acadia National Park and reported Tuna baits yields

22 species and all other collection methods yielded 15 species or less. Boomsma and Isaaks (1982) estimated that factors like CaCO3, total organic matter, moisture content, temperature of air and soil pH and salinity play an essential role in distribution pattern of ants. Azhagu Raj *et al.* (2017) suggested that AOSM, BTM and PTM were related in ant species differentiation diversity and species abundance in the study area. Lal (1988) used egg yolk, chicken waste and candies in BTM.

Table 2 Diversity indices in different trap methods

Diversity indices	AOSM	PTM	BTM
Individuals	959	931	939
Dominance (D)	0.07385	0.07665	0.08288
Simpson (SID)	0.9261	0.9233	0.9171
Shannon (H")	2.621	2.601	2.542
Evenness (e^H/S)	0.9824	0.9625	0.9079
Menhinick	0.4521	0.4588	0.4569
Margalef	1.893	1.902	1.899
Fisher alpha	2.324	2.337	2.333
Berger-Parker	0.08863	0.1074	0.1022

ASOM - All out search method

PTM - pitfall trap method

BTM - bait trap method

Dominance, Simpson, Shannon, evenness, Menhinik, Margalef, fisher alpha and Berger parker indices were noted. The Shannon wiener (H") index was highest in ASOM (2.621). ASOM had SID and H" as 0.9261, 2.621 respectively, it was followed by PTM (SID - 0.9233; H" - 2.601). In PTM SID was 0.9171 and H" was 2.542. The individual and dominance of ant species were arranged in the ascending order AOSM (n=959) with dominance 0.07985, BTM (n=939) accompanied by D= 0.08288 and PTM (n=931) including D=0.07665. The ant species eveness (e^H/S) ranged from 0.9079 to 0.9824. Menhinick varied from 0.421-0.4588. The Margaelf index arranges with descending order 1.893; 1.899 and 1.902. Fisher alpha (S) ranged 2.324 to 2.337 and Berger- parker ranged 0.08863 - 0.1074 (Table 2). Ramesh *et al.* (2010) reported that diversity in DAE campus Kalpakam, the sample size of five different habitats were compared and Fishers alpha diversity and Shannon diversity indices were calculated as a measure of diversity within the habitat. The calculates values in DAE campus, Kalpakam is Shannon diversity riparian woods area is 2.62, Jungle 2.53, monoculture 2.36, sandy area 2.35 and building area 4.98. In coastal Odisha (Dash *et al*., 2017) reported 27 ant species and Simpson index of diversity and Shannon wiener during the month of April – May 2014 (SID - 0.84 and H - 2.01) and January – February 2015 (SID - 0.90 and H -2.63). It is concluded that, different type of traps were very useful for ant collection and notice the behaviour of ants.

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First record of *Ahmaditermes emersoni* Maiti (Isoptera, Termitidae, Nasutitermitinae) from Meghalaya, India

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ABSTRACT: The nasutiform termite species *Ahmaditermes emersoni* Maiti is reported from Meghalaya, India for the first time. All the studied samples of the species were collected from betel nut plantation in Mawlynnong area of East Khasi Hill district of the state. Morphology of both the soldier and worker castes of the species was studied in detail and the identification was done based on their morphological characteristics. Redescription of morphology and revision of morphometric of both the soldier and worker castes are provided in this article. With the record of this species, the genus *Ahmaditermes* is also reported for the first time from the state. © 2023 Association for Advancement of Entomology

KEYWORDS: Nasutiform species, soldier and worker castes, morphometrics, taxonomy

Termites are eusocial insects that comprise the infraorder Isoptera under the order Blattaria with around 2,933 living species under 282 genera that belong to 9 families that occur world widely across the habitats (Inaward *et al.,* 2007; Krishna *et al.*, 2013a). As new species of termites are being described from different parts of the world, the total number of known species may be more than 3,138 (Mahapatro *et al.*, 2015). Termitidae is the largest family among all the known termite families with about 2,077 species known globally under 7 subfamilies. The subfamily Nasutitermitinae is the second largest in terms of species composition among all the subfamilies of Termitidae with about 596 known species under 77 genera (Krishna *et al.,* 2013a). The genus *Ahmaditermes* is the fourth largest genus among the genera of the subfamily Nasutitermitinae. It comprises 22 species out of which 21 species are known from the Oriental region and one from the Palaearctic region (Krishna *et al.,* 2013a). The Indian region which consists of India, Bangladesh, Pakistan, Sri Lanka, Bhutan, Nepal and Burma harbours only four species of the genus which are found in India, Bangladesh and Bhutan. Two *Ahmaditermes* species are known from India namely *Ahmaditermes emersoni* (also occur in Bhutan) and *Ahmaditermes sikkimensis* which is only known from India from its type locality Sikkim (Chhotani, 1997; Krishna *et al.,* 2013b). The species *Ahmaditermes emersoni* Maiti is reported for the first time from the state Meghalaya, India identified based on the soldier and worker castes morphology. With this species the genus *Ahmaditermes* is also reported for the first time from the state.

The termite samples, collected from a betel nut plantation of Mawlynnong area, Pynursla, East

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Khasi Hills district, Meghalaya, India, were preserved at 80% alcohol for their identification and further taxonomic studies. Some of the collected colonies of the species are deposited in the national repository of Zoological Survey of India (ZSI) at the North Eastern Regional Centre, Shillong (NERCS), Meghalaya, India and few collected colonies are preserved in the research collection of the entomology laboratory, department of Zoology, North-Eastern Hill University (NEHU), Shillong, Meghalaya, India. The samples were studied under Leica S8AP0 stereo zoom microscope fitted with a GT 5.0 camera and the mosaic V.2 photographic software. All the measurements were taken based on the micrograph using the Image J software (latest available version). All morphological measurements, terminologies and indices used here for both the soldier and worker castes are based on Roonwal and Chhotani (1989) and identification is based on Chhotani (1997).

Systematic account

Infra-order: Isoptera Brullé, 1832

Family: Termitidae Latreille, 1802

Subfamily: Nasutitermitinae Hare, 1937

Genus: *Ahmaditermes* Akhtar, 1977

Species: *Ahmaditermes emersoni* Maiti, 1977

Materials examined: ISOP/ENT/ZOO/NEHU/BPM-305: 10 soldiers, 3 workers, 20.xii.19; I/ISOP/ERS/4433: 1 soldier, I/ISOP/ERS/4434: 25 soldiers and 3 workers, ISOP/ENT/ZOO/NEHU/0196: 29 soldiers and 2 workers, 16.x.2021, collected from a tree stump in the betel nut plantation of Mawlynnong area, Pynursla, East Khasi Hill District, Meghalaya, India (25 20¹ 17.183º N; 91 54¹ 40.55º E; Altitude 530 m). Deposited in the Zoological Survey of India, NERCS, Shillong-03 and preserved in the Entomology Laboratory, department of Zoology, NEHU, Shillong 22.

Table 1 Revised morphometric (in mm) of the soldier of *Ahmaditermes emersoni* (in combination of Chhotani, 1997 and the present study)

Characteristics	Chhotani	Study	Revised
Total body length	3.50–4.20	3.04–3.87	3.04–4.20
Head length with rostrum	1.37–1.58	1.36–1.43	1.36–1.58
Head length without rostrum	0.90–1.00	0.88–0.93	0.88–1.00
Head width	0.83–0.93	0.83–0.90	0.83–0.93
Head width / Head length without rostrum	–	0.90–0.98	0.90–0.98
Rostrum length	0.46–0.60	0.46–0.52	0.46–0.60
Rostrum length / Head length without rostrum	0.51–0.53	0.51–0.58	0.51–0.58
Head width at constriction	0.57–0.61	0.53–0.59	0.53–0.61
Head width at constriction / Head width	–	0.60–0.67	0.60–0.67
Head Height	–	0.51–0.62	0.51–0.62
Pronotum length	0.14–0.20	0.13–0.15	0.13–0.20
Pronotum width	0.37–0.53	0.39–0.44	0.37–0.53
Postmentum length	–	0.27–0.28	0.27–0.28
Postmentum width	–	0.28–0.29	0.28–0.29
Posterior head bulge	–	0.30–0.38	0.30–0.38
Posterior head bulge / Head length	–	0.34–0.42	0.34–0.42

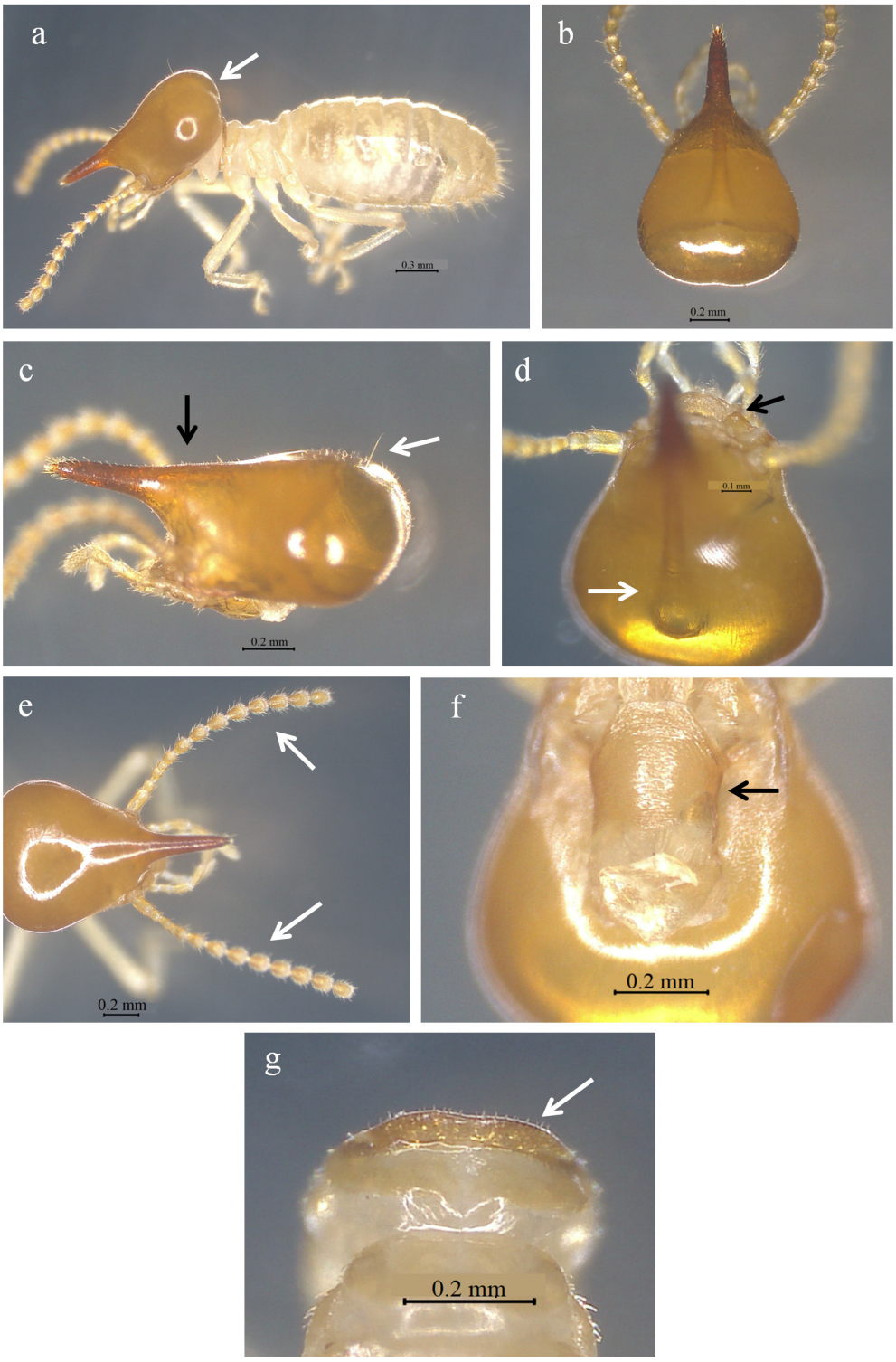

Fig. 1 Soldier of *Ahmaditermes emersoni* Maiti, a) Whole body (Arrow shows emarginated posterior margin of head), b) Soldier head in dorsal view, c) Soldier head in lateral view (white arrow shows long bristle at posterior end of the head and black arrow shows the weak dorsal hump), d) Vestigial mandible without spine like process (black arrowed) and fontanelle tube (white arrowed) of soldier, e) Antennae of soldier (arrowed), f) Postmentum of soldier (arrowed), g) Pronotum of soldier (arrowed).

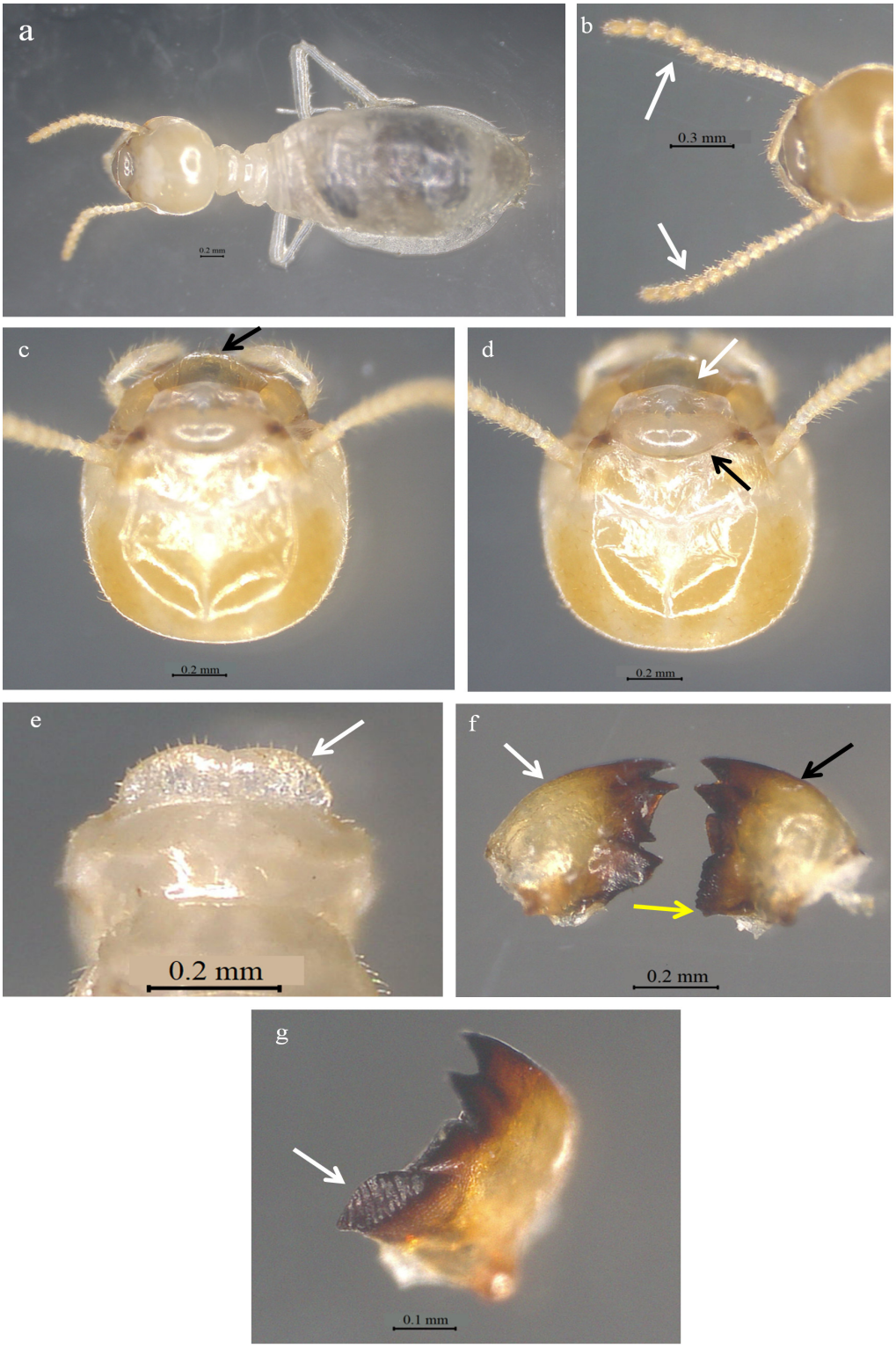

Fig. 2 Worker of *Ahmaditermes emersoni* Maiti, a) Whole body, b) Antennae of worker (arrowed), c) Labrum, d) Worker head in dorsal view showing anteclypeus (white arrowed) and postclypeus (black arrowed), e) Pronotum of worker (arrowed), f) Mandibles of worker. Left mandible (white arrowed) and right mandible (black arrowed). Yellow arrow shows the Cockroach notch, g) Molar ridges of right mandible (arrowed).

Diagnosis:

Imago: Unknown

Soldier (Fig. 1a–g; Table 1): Monomorphic. Head capsule is straw yellow in color and sparsely hairy; pyriform shaped; strongly constricted behind the antenna and widest and rounded at the posterior end with a pair of long bristles on it. Posterior margin is depressed medially. In profile, head capsule is weakly depressed behind the rostrum and with a weak hump. One bristle is prominent on the hump at its end. Rostrum is cylindrical in shape with four slightly longer bristles and many shorter hairs; dark brown in color except the basal portion which is somewhat paler. In length, slightly longer than half of the head length without rostrum. Antennae are yellowish in color and little bit darker distally; 13 segmented, segment 3rd longer than 2nd, 4th shortest. Mandibles are vestigial and without any lateral spine-like mandibular process. Fontanelle gland and tube are prominent. Pronotum are saddle shaped; paler than head in color; anterior lobe weakly raised and darker than the posterior lobe; anterior margin indistinctly emarginated and with minute hairs; posterior margin too indistinctly emarginated with a broad depressed medial line dividing the posterior margin indistinctly. Abdomen are densely hairy and paler than head in color. Legs are yellowish white in color (Adapted from Chhotani, 1997).

Worker (Fig. 2 a–g; Table 2): Monomorphic. Head capsule is subsquarish with distinct epicranial suture; fuscous brown in color with slightly paler anteriorly and fairly hairy. Fontanelle plate is distinct. Antenna 14 segmented; segment 3rd shorter than 2nd; 4th shortest; paler in color than head. Postclypeus swollen and hairy; length is less than half of its width. Pronotum saddle-shaped, weakly emarginate at the anterior margin and indistinctly emarginated at the posterior margin. Abdomen and legs whitish in color and fairly hairy (Adapted from Chhotani (1997)).

Distribution: Oriental region: India: Arunachal Pradesh, West Bengal and Meghalaya: East Khasi Hills, Mawlynnong, betelnut plantation (New records).

Table 2 Revised morphometric (in mm) of the worker of *Ahmaditermes emersoni* (in combination of Chhotani (1997) and the present study)

Characteristics	Chhotani	Study	Revised
Total body length	3.75–4.50	3.05–3.86	3.05–4.50
Head length	0.76–0.88	0.69–0.88	0.69–0.88
Head width	0.92–1.00	0.80–1.00	0.80–1.00
Pronotum Length	0.21–0.23	0.19–0.24	0.19–0.24
Pronotum width	0.50–0.57	0.46–0.54	0.46–0.57
Postclypeus length	0.14–0.18	0.14–0.19	0.14–0.19
Postclypeus width	0.35–0.42	0.34–0.44	0.34–0.42

Remarks: The species *Ahmaditermes emersoni* is recorded only from India and Bhutan till now. Earlier it was described as *Bulbitermes emersoni* by Maiti (1977) (which was actually published in the year 1979) from West Bengal, India. Later, the species was transferred to the genus *Ahmaditermes* by Chhotani (1997) based on the characteristics such as pyriform head with emarginated posterior margin, constriction behind the antennae and mandibles without any lateral spine like processes. Variations in the morphology and morphometrics of few taxonomically important characters in both the soldier and worker castes of the species were observed in comparison to its earlier description in Chhotani (1997). In case of soldiers, rostrum length and head length without rostrum index is slightly more, total body length and head length without rostrum are less than its earlier report. In case of worker caste, total body length, head length and head width are slightly less than its earlier report in Chhotani (1997).

Redescription: In case of soldier caste, the posterior margin of head is either moderately emarginated with two long bristles posteriorly or substraight or indistinctly emarginated without any long bristles. In case of the antennal segments, segment 3 is either longer than 2 or subequal to 2. In case of worker caste, the anteclypeus is either pentagonal or triangular in shape, antenna with the

segment 3rd either shorter than 2nd or subequal. In case of the mandibles, the second marginal tooth of left mandible is slightly longer and prominent and the second marginal tooth of right mandible is slightly protruded and prominent in appearance. Furthermore, the right mandible with seven well developed molar ridges and with or without a weak cockroach notch.

This is first report of the genus *Ahmaditermes* and the species *Ahmaditermes emersoni* from the state Meghalaya, India. It is an addition to the existing termite species and genera of the state.

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First record of *Microcerotermes lahorensis* Akhtar (Blattodea, Isoptera, Termitidae) from India

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ABSTRACT: Reports a subterranean termite species *Microcerotermes lahorensis* Akhtar from Kerala, India, for the first time. The species was only reported from the type locality Lahore, Pakistan, and was endemic to the country. This new record thus changes the endemic status of the species and reaffirms the cosmotropical nature of the genus *Microcerotermes.* © 2023 Association for Advancement of Entomology

KEYWORDS: Distribution, endemism, cosmotropical nature

Microcerotermes is one of the diverse termites genera and the third-largest genus of the infra order Isoptera (Setter and Myles, 2005). About 148 species of this genus are found worldwide (Krishna *et al.,* 2013). Except for the Nearctic region, the genus is cosmotropical in distribution (Chhotani, 1997). Forty-two species of *Microcerotermes* are found in the oriental region, out of which 29 are found in India (Das and Choudhury, 2020). India is one of the diverse countries in the world, harbouring 295 species of termites, and the state of Kerala has around 68 species of termites belonging to 30 genera and studies have reported a new record of termite species in Kerala (Ranjith and Kalleshwaraswami, 2021; Joseph *et al.,* 2022). The present taxonomic study in Kerala reports a new record of *Microcerotermes lahorensis* from India, a species that was endemic to Pakistan and found only in type locality (Chhotani, 1997). This study thus contributes to the diversity of termites in India and shows the peculiar distribution pattern of the *Microcerotermes lahorensis* as it was found only type locality Lahore in 1974 (Akhtar, 1974), and later found in southern part of India after four decades. This new record shows that the species is not endemic anymore.

The samples were collected from a trunk of *Cocos nucifera* (L.) from Neendoor, Kottayam district of Kerala state and were preserved (80 % alcohol). Measurements were under a stereo zoom microscope, Labomed Luxeo 4D binocular microscope with attached camera and Micaps software at magnification of 8–35X. The species identification was done using Chhotani (1997). Specimen was deposited in Zoological Survey of India Western ghats regional centre Kozhikode repository with following registration number ZSIK No. ZSI/WGRC/I.R.-INV.22065.

Systematics:

Family Termitidae Westwood, 1840

Subfamily Amitermitinae Kemner, 1934

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Fig. 1 *Microcerotermes lahorensis* Soldier

Fig. 2 Soldier head dorsal view

Fig. 3 Pronotum of the soldier

Fig. 4 Soldier head ventral view

Genus *Microcerotermes* Silvestri , 1901

Microcerotermes lahorensis (Akhtar, 1972)

Materials examined : CMSZMAI-120, Soldier-1, 08.ii.2022, Neendoor, Kottayam, Kerala, India, 9°41'31.1"N and 76°30'14.7"E , altitude 2 m. coll. Edwin Joseph.

Diagnosis - Soldier (Figs. 1-4, Table 1): Head yellowish brown, slightly darker anteriorly; antennae yellowish; mandibles dark reddish brown; pronotum pale; legs and abdomen dirty white. Head sparsely hairy; postmentum with two pairs of hairs anteriorly. Head-capsule sub rectangular, sides bulging out behind antennae; Segment 3 of the antennae shortest. Clypeus subtrapezoidal. Labrum subpentagonal. Mandibles long, strongly incurved in distal half and coarsely serrated. Postmentum short and club-shaped arched with a strongly incurved waist. Pronotum saddle-shaped; slightly notched anteriorly, posterior margin slightly emarginate.

Distribution: Pakistan: Panjab (Lahore, type-locality), India: Kottayam (New record)

Table 1 *Microcerotermes lahorensis* - soldier morphometric

Characters	mm
Total body length	3.71
Total head length	2.20
Head length without mandible	1.43
Head width maximum	1.14
Mandible length	0.86
Pronotum length	0.32
Pronotum width	0.58
Postmentum length	0.63
Postmentum width maximum	0.38
Postmentum width minimum	0.27
Head width/ Head length Index	0.778

Remarks: According to Chhotani (1997), *the Microcerotermes* genus in the Indian region falls into two categories, species with finely serrated and species with coarsely serrated mandibles. *M. lahorensis* belongs to the category with coarsely serrated mandibles; in this category, the species *lahorensis* is separated for its strongly convexical head-sides behind antennae, but only a single soldier was available to Akhtar at the time of describing the species. The present study also has a single soldier for study, but the unique morphology of the species is very distinct. The number of antennal segments in the original description is 13 with the third segment shortest but in present study, the only specimen available had damaged antennae with incomplete number of segments, but the remaining segments were matching with the original description. The new record of this species depicts the diversity and probability of revealing new species

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BOOK REVIEW

Susthira theneechakrishi (Sustainable Bee Keeping)

Authors:
By Dr. S. Devanesan and Dr. K. S. Pramila
Dev Honey Enterprises, Thiruvananthapuram,
132 pp. (In Malayalam).

Taking insect science to the masses in India is possible only through the regional languages. However, there is dearth of literature on basic as well as applied entomology in Indian languages. The book 'Susthira theneechakrishi (Sustainable Bee Keeping)' by S. Devanesan and K.S. Premila, addressed to the apiculturists in Kerala, is an attempt to bridge this gap.

The book deals comprehensively with apiculture in Kerala, beginning with its historical development in the state. Information on the biology and management of the Indian honey bee and stingless bees is concisely presented. There are separate chapters dedicated to apiculture equipment, honey extraction, honey processing, pests and diseases, crop pollination and honey products. The authors have effectively brought in their expertise and experience of more than two decades in apiculture into this copiously illustrated book. This can be very useful to apiculturists as well as lay people in Kerala.

Dr. T. R. Sharma, DDG (Crop Sciences), Indian Council for Agricultural Research, New Delhi, released the book in the presence of Dr. D.P. Aberol, Dean Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology; Dr. S.C. Dubey, ADG Plant Protection, ICAR, Dr Bal Raj Singh, PC, AICRP Honey, ICAR, in New Delhi, during the annual review meeting of the All India Coordinated Research Project on Honey bees and Pollinators

held during 07 September 2022 at the Sher-e-Kashmir University of Agricultural Science and Technology, Jammu.

The suggestion is that the publication in 'Malayalam' may also be brought out in English and other regional languages, so that the bee farmers/ researchers in other states will also be benefitted.

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I, Dr K. D. Prathapan, Secretary, Association for Advancement of Entomology, here by declare that the particulars given above are true to the best of my knowledge and belief.

Sd/-

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