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Contents

Short Commuinication

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A new species to the genus *Neopheosia* Matsumura, 1920 (Lepidoptera, Notodontidae) from India

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ABSTRACT: A new species of notodontid moth, *Neopheosia melaniata* **sp. nov.** is described with illustration. This new species is closely allied to *N. fasciata* Moore, 1888 (type species) and completely conforms to the characterization of genus *Neopheosia* Matsumura. The wing coloration, distinct discal spot on forewing and genitalic features make it distinct. The taxonomic account of *N. fasicata* Moore is included. © 2024 Association for Advancement of Entomology

KEY WORDS: *Neopheosia melaniata,* taxonomic account, characterization, genitalic features

INTRODUCTION

The genus *Neopheosia* was established as a monotypic genus by Matsumura (1920) with *N. fasciata* Moore, 1888 as its type species. Gaede (1930) added another species *N. albiplaga* under this genus. Kiriakoff (1968) also considered *Neopheosia* Mataumura as a valid genus. Cai (1979) and Wu and Fang (2002) discussed only one species i.e., *N. fasciata* Moore, 1888 from China. Holloway (1983) described *N. fasciata* Moore, 1888 from Borneo. Schintlmeister and Pinratana (2007) and Schintlmeister (2008) described three species i.e., *N. fasciata* Moore, 1888; *N. mandschurica* Oberthur, 1911 and *N. atrifusa* Hampson, 1897 from Thailand and Palaearctic region. Schintlmeister and Pinratana (2007) treated *Hemifentonia* Kiriakoff, 1967 as a junior synonym of *Neopheosia* Matsumura, 1920 on the basis of Y-shaped uncus. Later, Kobayashi and Nonaka (2016) revived genus *Hemifentonia* Kiriakoff, 1967 as a distinct genus on both phenetic and phyletic classification with *mandschurica* Oberthur, 1911 as its type species. They further remarked about distinct genitalic features, particularly the presence of a very unique formation i.e., ventral process at the base of uncus in *Neopheosia fasciata* (Moore, 1888), while it has no ventral process on its base in *Hemifentonia mandschurica* (Oberthür, 1911). Schintlmeister (2008, 2013, 2020) considered five species namely *fasciata* (Moore, 1888); *atrifusa* (Hampson, 1897); *mandschurica* (Oberthür, 1911); *albiplaga* Gaede, 1930 and *mariae* Schintlmeister, 2013 under genus *Neopheosia*. While reporting a new species from China, Zhang *et al*. (2022) followed the same placement. They further placed three species *N. mandschurica* (Oberthür, 1911), *N. atrifusa* (Hampson, 1897) and *N. mariae* Schintlmeister, 2013 under one group on the basis of lack of ventral process at the base of uncus in male genitalia and another three species i.e., *N. fasciata* (Moore, 1888); *N. albiplaga* Gaede, 1930

* Author for correspondence

and *N. pseudofasciata* Zhang *et al*., 2022 under second group with distinct ventral process at the base of uncus. With the addition of new species i.e., *N. melaniata* from India, presently this genus is represented by seven species from Oriental and Palearctic regions.

MATERIALS AND METHODS

The adult representatives of notodontid moths were collected from different localities in the States of North-West and North-East India by using vertical sheet method. The collected moths were killed, stretched and preserved in Lepidoptera Lab, Punjabi University, Patiala. The external morphological characters were studied from the stretched specimens. The dissections were carried out to explore the male and female genitalic features (Robinson, 1976). The permanent slides of fore and hind wings were prepared to study wing venation (Zimmerman, 1978). The terminology for naming various genitalic parts used by Klots (1970) was followed in the present studies.

Abbreviations

1A	: First anal vein
2A	: Second anal vein
AED	: Aedeagus
ANT.APO	: Anterior Apophyses
CRN	: Cornuti
CRP.BU	: Corpus Bursae
CU_1	: First cubital vein
CU_2	: Second cubital vein
DU.BU	: Ductus Bursae
GN	: Gnathos
JX	: Juxta
M_1	: First Medial vein
M_2	: Second Medial vein
M_3	: Third Medial vein
R_1	: First Radial vein
R_2	: Second Radial vein
R_3	: Third Radial vein
R_4	: Fourth Radial vein
R_5	: Fifth Radial vein
Rs	: Radial sector
Sc	: Subcosta
$Sc+R_1$	: Subcosta and first radial vein
TG	: Tegumen
UN	: Uncus
VES	: Vesica
VIN	: Vinculum
VLV	: Valva

RESULTS AND DISCUSSION

Genus *Neopheosia* Matsumura

Neopheosia Matsumura, 1920, *Zool. Mag. Tokyo*, 32: 147; Gaede, 1930, *GroBschmett. Erde*, 10: 638; Kiriakoff, 1968, *Genera Insectorum Fasc.*, 217C: 182; Cai, 1979, *Economic Insect Fauna*, 16: 78; Holloway, 1983, *Moths of Borneo*, 4: 69; Wu and Fang, 2002, *Fauna Sinica*, 31: 419; Schintlmeister and Pinratana, 2007, *Moths of Thailand*, 5: 159; Schintlmeister, 2008, *Palaearctic Macrolepidoptera*, 1: 196.

Type species: *Pheosia fasciata* Moore

Distribution: India: North-India; China; Indonesia; Japan; Korea; Myanmar; Nepal; Pakistan; Philippines; Russia; Taiwan; Thailand.

Diagnosis: Medium sized moths; ochreous or greyish in colouration. Labial palpi porrect. Antennae bipectinate, pectination along two-third length of the flagellum. Forewing triangular; vein M_3 from lower angle of cell; M_2 near middle of discocellulars; M_1-R_2 stalked from upper angle of cell; areole absent. Hindwing with fuscous tornus. Legs hairy; fore-tibia having an epiphysis; mid-tibia with one pair of tibial spurs; hind-tibia with two pairs of tibial spurs. Male genitalia with long and bifid uncus; a pair of long and slender projections representing gnathos; valva with sclerotized costal process; aedeagus of moderate length, vesica with

a patch of cornuti. Female genitalia with membranous corpus bursae; signum elongated.

Key to the studied species of genus *Neopheosia* Matsumura:

Forewing pale-ochreous with indistinct fuscous discal spot. Male genitalia with uncus gradually narrowing towards distal end, bifurcated arms shorter; valva with costal process well developed. Female genitalia with pear-shaped corpus bursae .....***Neopheosia fasciata* (Moore)**- Forewing brown-ochreous with distinct fuscous discal spot. Male genitalia with uncus narrow along entire length, bifurcated arms longer; valva with costal process very small. Female genitalia with globular corpus bursae .....……..... ....…***Neopheosia melaniata* n. sp.**

***Neopheosia fasciata* (Moore)**

(Plate 1, Figs. 1-8)

Pheosia fasciata Moore, 1888, *Proc. Zool. Soc. Lond.*, 1888: 401; Kirby, 1892, *Syn. Cat. Lep. Het.*, 1892: 607; Hampson, 1892, *Moths India*, 1: 160.

Neopheosia fasciata Moore: Matsumura, 1920, *Zool. Mag. Tokyo*, 32: 147; Kiriakoff, 1968, *Genera Insectorum Fasc.*, 217C: 182; Cai, 1979, *Economic Insect Fauna*, 16: 78; Holloway, 1983, *Moths of Borneo*, 4: 69; Wu and Fang, 2002, *Fauna Sinica*, 31: 419; Schintlmeister and Pinratana, 2007, *Moths of Thailand*, 5: 160; Schintlmeister, 2008, *Palaearctic Macrolepidoptera*, 1: 196.

Type locality: North-West India (Kangra)

Diagnosis: Head with vertex and frons greyish. Labial palpi slight and porrect; dressed with brownish. Antenna bipectinate, pectinations along two-third length of the flagellum; scape covered with greyish scales; flagellum brown. Thorax, collar and tegula clothed with greyish scales; two prominent black spots on thorax; thorax underside fringed with pale and reddish-brown scales. Legs hairy, reddish-brown, fringed creamish scales; fore-tibia with an epiphysis; mid-tibia with one pair of tibial spurs; hind-tibia with two pairs of tibial spurs. Abdomen smoky black; underside paler with a median rufous streak.

Wing maculation: Forewing with ground colour creamish-ochreous, traversed with brownish, rufous and fuscous streaks; basal area fuscous; costa with brown and fuscous streaks; dark brown apical patch; vein endings with darker scales giving banded appearance to outer margin; anal margin black from base to tornus; cilia black and pale ochreous; underside paler, rusty costal margin and near tornus. Hindwing creamish-white, darker scales near anal margin; outer margin banded with distinct tornal spot; underside creamish.

Wing venation: Forewing with discal cell half the length of wing, closed; 1A+2A from base of wing, reaching tornus; 3A absent; Cu_2 beyond two-third of cell; Cu_1 just before lower angle of cell; M_3 from lower angle of cell; M_2 above middle of discocellulars; M_1-R_2 stalked from upper angle of cell; R_1 beyond three-fourth of cell, not reaching apex; Sc from base of wing, not reaching apex. Hindwing with discal cell slightly more than half the length of wing, closed; 1A from base of wing running parallel to anal margin, not reaching tornus; 2A from base of wing, reaching tornus; 3A absent; Cu_2 well before lower angle of cell; Cu_1 slightly before lower angle of cell; M_3 from lower angle of cell; M_2 just above middle of discocellulars; M_1 and Rs stalked from upper angle of cell; Sc+R_1 from base of wing, not reaching apex.

Wing expanse: Male: 54mm; Female: 60mm

Body length: Male: 23mm; Female: 23mm

Male genitalia: Uncus long, narrow at base, gradually broadening towards distal end, distal end broad, bifid, both arms with rounded apices, dorsally setosed; ventral sclerotized narrow, spine-like structure from base of uncus, less than half the length of uncus, tip blunt; a pair of well sclerotized long processes representing gnathos, both walls highly sclerotized giving dentate appearance, slightly upturned with blunt apices; tegumen V-shaped, walls almost of equal breadth, longer than vinculum; vinculum U-shaped, distal half well sclerotized; saccus absent; juxta flap-like, slightly sclerotized. Valva simple, sacculus differentiated, moderately

sclerotized, setosed; costa having flap-like structure extending upto middle of valva without any projections, mideo-ventrally setosed; distal end of valva simple and setosed. Aedeagus of moderate length, well sclerotized; ductus ejaculatorius entering near proximal end; distal half having a large patch of numerous minute spines representing cornuti.

Female genitalia: Corpus bursae of moderate size, pear-shaped, membranous; distinct oblong signum, centrally placed; ductus bursae long, membranous, one-third guarded by moderately sclerotized genital plate, dorso-ventrally flattened; ductus seminalis originating near anterior end of genital plate; anterior apophysis short, gradually tapering; posterior apophysis narrower and almost 2X length of anterior ones, apices of both pairs membranous; papilla analis sclerotized, deltoid, setosed with unequal setae.

Material examined: Arunachal Pradesh: Dirang, 27.3584°N, 92.2409°E, 01.v.2013, 2♂♂; Sangti, 27.4038°N, 92.3047°E, 02.v.2013, 1♂; Lumla, 27.5298°N, 91.7219°E, 13.v.2011. Himachal Pradesh: Gharat, 31.5168°N, 77.7938°E, 26.vi.2014, 1♂; Nichar, 31.5581°N, 77.9467°E, 25.vi.2014, 1♂. Mizoram: Rabung, 23.6833°N, 93.2021°E, 17.ix.2015, 2♂♂18.ix.2015, 1♂. Sikkim: Dodak, 27.3333°N, 88.2500°E, 06.v.2014, 1♂; Yaksum, 27.3724°N, 88.2230°E 02.v.2014, 1♂. Uttarakhand: Sitlakhet, 29.5939°N, 79.5445°E, 17.vi.2015, 1♂, 1♀.

Distribution: India: North-East and North-West India; China; Indonesia; Japan; Myanmar; Nepal; Pakistan; Philippines; Taiwan; Thailand.

Remarks: This species was originally under genus *Pheosia* Hübner by Moore (1888). Kirby (1892) and Hampson (1892) followed the same nomenclature. Matsumura (1920) erected a new genus *Neopheosia* for its proper placement. Kiriakoff (1968), Cai (1979), Wu and Fang (2002), Schintlmeister and Pinratana (2007), Schintlmeister (2008, 2020), Zhang *et al*. (2022) and in the present studies, its placement in the present genus has been followed.

Neopheosia melaniata sp. nov. Kaleka & Kumar

zoobank.org:act:9E9511F1-D7AC-412F-BD09-1D0ECFBC6A73

(Plate 2, Figs. 9-17)

Diagnosis: Head with vertex and frons grey. Labial palpi straight; dressed with reddish-brown scales. Antenna bipectinate, pectinations along two-third length of the flagellum; scape clothed with creamish scales; flagellum brown. Thorax, collar and tegula grey; underside darker. Legs hairy, reddish-brown, fringed with greyish scales; fore-tibia with an epiphysis; mid-tibia with one pair of tibial spur; hind-tibia with two pairs of tibial spurs. Abdomen fuscous; underside paler, having a rusty medial streak.

Wing maculation: Forewing with ground colour ochreous, with brown and dark brown streaks; costa fuscous interspersed by creamy streaks; a prominent coffee coloured discal spot; a distinct wavy, hazel coloured submarginal line; anal margin coffee coloured; outer margin chequered with light and dark bands; underside with pale and rufous scales. Hindwing filthy white, anal margin fuscous; costal and apical areas darker; fuscous spot on tornus; cilia creamish; underside paler.

Wing venation: Forewing with discal cell less than half the length of wing, closed; 1A+2A from base of wing, reaching tornus; 3A absent; Cu_2 from two-third of cell; Cu_1 just before lower angle of cell; M_3 from lower angle of cell; M_2 above middle of discocellulars; M_1-R_2 well stalked from upper angle of cell; R_1 beyond three-fourth of cell, not reaching apex; Sc from base of wing, not reaching apex. Hindwing with discal cell slightly shorter than half the length of wing, closed; 1A from base of wing, not reaching tornus; 2A from base of wing, reaching tornus; 3A absent; Cu_2 well beyond three-fourths of cell; Cu_1 just before lower angle of cell; M_3 from lower angle of cell; M_2 just above middle of discocellulars; M_1 and Rs stalked from upper angle of cell; Sc+R_1 from base of wing, not reaching apex.

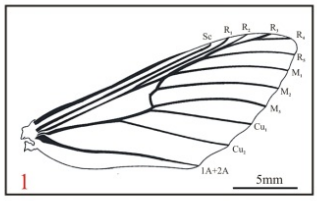
Wing expanse: Male: 50-54mm; Female: 62mm

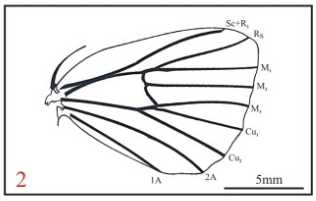
Body length: Male: 22-24 mm Female: 23 mm

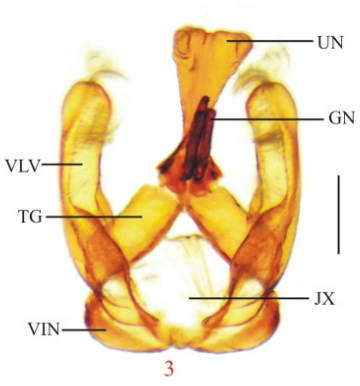
Plate - 1

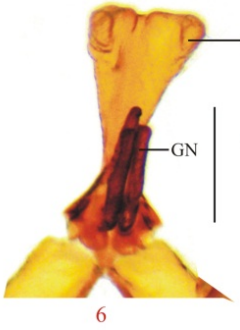
0.88 of Actual Size

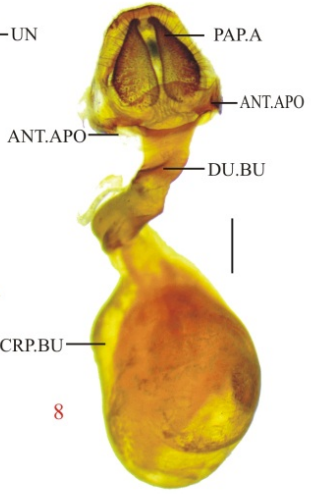
0.9 of Actual Size

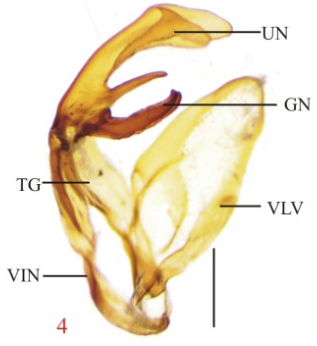


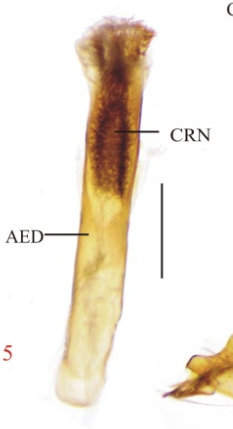












***Neopheosia fasciata* (Moore)**

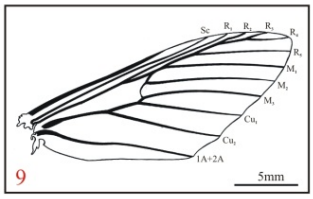
1. Forefwing 2. Hindwing 3. Male genitalia-Dorsal view 4. Lateral view
5. Aedeagus 6. Uncus & Gnathos 7. Valva 8. Female genitalia

(Bar Line = 1mm)

Plate - 2

Actual Size

0.77 of Actual Size



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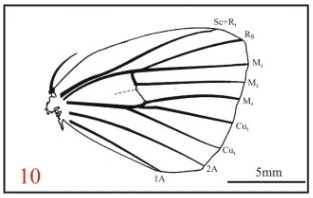

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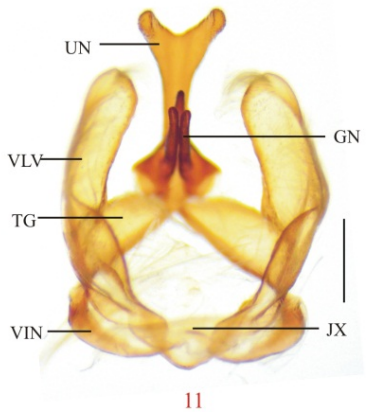

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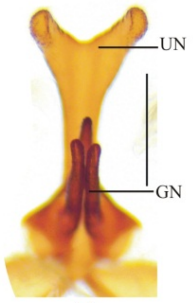

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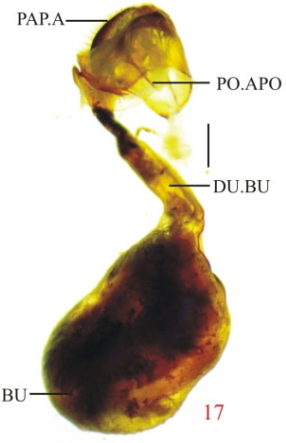

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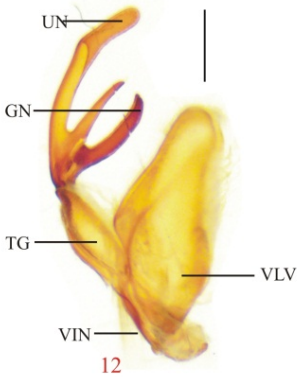

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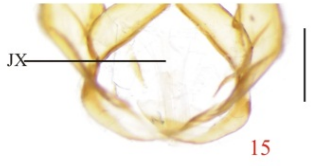

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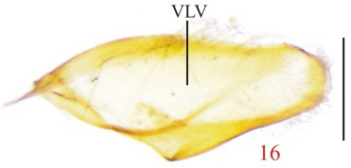

16

Neopheosia melaniata n.sp.

9. Forewing 10. Hindwing 11. Male genitalia-Dorsal view 12. Lateral view
13. Aedeagus 14. Uncus & Gnathos 15. Juxta Enlarged 16. Valva 17. Female genitalia

(Bar Line = 1mm)

Male genitalia: Uncus long narrow, well sclerotized, dorsally setosed with short setae, distal end bifid with rounded apices; ventral sclerotized process with basal one-third part broad, remaining narrow ending into slightly beaked apex, more than half the length of uncus; a pair of well sclerotized projections representing gnathos, basal one-third portion bulbous, remaining narrow ending into rounded tips, slightly apart; tegumen sclerotized, broad, as long as vinculum, narrow at both ends; vinculum sclerotized; saccus absent; juxta V-shaped, broad distally, slightly sclerotized. Valva simple, setosed ventrally upto middle; costa with small sclerotized process; sacculus slightly sclerotized; distal end rounded, broad, setosed. Aedeagus of moderate size, well sclerotized; proximal end rounded flap-like; ductus ejaculatorius entering near proximal end; distal half more sclerotized, distal end flap-like; vesica armed with a longitudinal patch of spines representing cornuti.

Female genitalia: Corpus bursae globular, membranous; signum prominent near middle; ductus bursae long, one-third guarded by moderately sclerotized genital plate, dorso-ventrally flattened; ductus seminalis originating near anterior sclerotized part of ductus bursae; anterior apophysis broad at base, short, tapering; posterior apophysis long, 5X than anterior ones, tapering apices; papilla analis sclerotized, hoof-shaped, setosed with equal sized setae.

Material examined:

***Holotype*:** Mizoram: Hmuifang, 23.4488°N, 92.7590°E, 01.x.2013, 1♂

***Allotype*:** Sikkim: Chungthang, 27.6039°N, 88.6464°E, 12.ix.2013, 1♀.

***Paratype*:** Jammu and Kashmir: Uri, 34.0881°N, 74.0340°E, 27.vii.2014, 1♂; Meghalaya**:** Jowai, 25.4509°N, 92.2089°E, 06.ix.14, 1♂; Riatkhwan, 25.2250°N, 91.4720°E, 03.ix.14, 1♂; Mizoram: Hmuifang, 23.4488°N, 92.7590°E, 01.x.2013, 1♂; Hrangchalkawn, 22.8502°N, 92.7942°E, 03.x.2013, 1♂; Rabung, 23.6833°N, 93.2021°E, 17.ix.2015, 2♂♂.

The material has been deposited in Lepidoptera Lab, Department of Zoology & Environmental Sciences, Punjabi University, Patiala.

Distribution: India: Jammu and Kashmir, Meghalaya, Mizoram, Sikkim.

Etymology: The present species has been named due to its darker colouration i.e., melanism.

Remarks: Though the present species under reference is closely allied to the type species *Neopheosia fasciata* (Moore, 1888), but, its darker general colouration, brown-ochreous forewings with distinct fuscous discal spot and the stalking position of M_1 in forewing makes it distinct externally. As far as genitalic features are concerned, the distinct features include the narrow uncus along its entire length with longer bifurcated arms; ventral process from base of uncus with basal one-third part broad ending into slightly beaked apex and more than half the length of uncus; a pair of well sclerotized projections with smooth walls representing gnathos with basal one-third portion bulbous and ending into rounded tips; valva with costal process very small in male genitalia and corpus burse globular and posterior apophysis almost 5X length of anterior ones in female genitalia.

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Rearing thermal conditions modulate the feeding attributes of *Zygogramma bicolorata* Pallister (Coleoptera, Chrysomelidae)

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ABSTRACT: Effects of temperature on the different parameters of consumption and utilization of food, such as consumption index, conversion of ingested food, absolute digestibility, conversion of digested food and growth rate were investigated by rearing *Zygogramma bicolorata* Pallister at 15°C, 20°C, 25°C, 30°C and 35°C. Death of different life stages, including under-developed adults occurred at 15 and 35°C. Maximum consumption index during the feeding period was observed at 25°C, whereas it was minimum at 30°C. The results revealed that conversion of ingested food was maximum at 20°C and minimum at 25°C. In addition, the conversion of digested food was maximum at 20°C and minimum at 25°C. However, absolute digestibility and relative growth rate increased with increasing temperature from 20 to 30°C.

KEY WORDS: Temperature, life stages, consumption index, conversion efficiency, growth

INTRODUCTION

Like all organisms, insects require an energy balance crucial for their growth, development, reproduction, and survival, depending on the equilibrium between energy acquisition and expenditure in physiological processes (Klepsatel *et al.,* 2019). Energy stored as food reserves determine insect's survival in adverse conditions (Rion and Kawecki, 2007). Insects serves as vital energy transformer, as they are integral components of ecosystems. In terrestrial ecosystems, insect herbivores significantly influence plant biomass (Carson and Root, 2000), species diversity (Bagchi *et al.,* 2014), competition dynamics (Kim *et al*., 2013), and nutrient cycling (Metcalf *et al.,* 2014). As insects are ectothermic, their physiological processes are directly tied to environmental temperature (Fields, 2001). In the last century, Earth's average temperature increased by 1°C and is expected to rise by 0.2°C per decade (Marshall *et al.,* 2020). Studies indicate that temperature significantly influences various aspects of insect such as dispersal, foraging, species interaction (Afaq, 2012; Soga and Gaston, 2018), courtship signaling, mating frequency, species recognition (Larson *et al.,* 2019), movement, recolonization (Fletcher *et al.,* 2018), development, predation, herbivory (McMunn *et al.,* 2019; Owens *et al.,* 2020), initiation, and termination of diapause (Dalin *et al.,* 2010; Tougeron *et al.,* 2020), as well as population growth rate (Miles *et al.,* 2019; Murphy *et al.,* 2020). Additionally, temperature

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plays a role in modulating chemically mediated signals, phenology, life history (Gallinat *et al.,* 2015; Ekholm *et al.,* 2019; Marshall *et al.,* 2020), and changes in voltinism (Van Dyck *et al.,* 2015; Forrest *et al.,* 2019; Kerr *et al.,* 2020) in various insect species.

Zygogramma bicolorata Pallister (Coleoptera, Chrysomelidae) is an effective biological control agent for *Parthenium hysterophorus* L. (Asteraceae), an invasive herbaceous weed with a pan-tropical distribution. The weed affects grass productivity and endemic biological diversity and causes different medical symptoms in humans (Patel, 2011; Jayaramiah *et al.,* 2017). From a biological control perspective, the thermal performance of mass-reared insects facing novel environments upon release in the wild has long been a source of unease (Enserink, 2007; Terblanche and Chown, 2007; Chidawanyika and Terblanche, 2011; Sørensen *et al*., 2013; Terblanche, 2014). Several studies have argued that mass-reared insects, typically kept under constant optimal environments, may struggle under field conditions (Enserink, 2007; Kristensen *et al*., 2008; Chidawanyika and Terblanche, 2011). Therefore, it is imperative to understand the physiological responses to thermal variation in insects used in biological control. It may help optimize rearing and release protocols to enhance field performance (Terblanche, 2014).

Although, studies on consumption and utilization of food by *Z. bicolorata* have been studied by Bhumannavar and Balasubramanian (1998) and Omkar and Afaq (2011) but very few studies have shown the effect of temperature on the feeding efficiency (Afaq, 2012), development and survival (Omkar *et al.,* 2008), mate guarding behaviour (Bhaisare and Chaudhary, 2023), plant mediated effects of temperature and CO_2 on biocontrol (Kumar *et al.,* 2021), effect of temperature and altitude on feeding attributes (Bhusal *et al.,* 2020), and heat tolerance (Chidawanyika *et al*., 2017) of *Z. bicolorata*. Nevertheless, the effect of various rearing thermal conditions on the feeding parameters of *Z. bicolorata* have not been investigated so far. In the present study, investigated the effects of various thermal conditions on the feeding attributes of *Z. bicolorata*, *i.e.*, consumption index, conversion efficiency, digestibility, and growth rate.

MATERIALS AND METHODS

Both sexes of *Z. bicolorata* adults were collected from agricultural fields of Amarkantak (22° 40'N, 81° 45'E), Madhya Pradesh, India. The adults were paired randomly in plastic Petri dishes (9.0×1.5cm) and allowed to mate until natural disengagement and reared under controlled abiotic conditions (i.e. temperature: 25±2°C; humidity: 65±5%; photoperiod: 14L:10D) in BOD incubators (REMI CHM-16 Plus). Beetles were provided with fresh leaves of *P. hysterophorus* daily. Eggs laid were collected daily and used for further experimentation.

Batches of (100 eggs per temperature) eggs were collected from the stock and reared till adult maturity at constant temperatures of 15, 20, 25, 30 and 35°C separately at each temperature regime in the BOD incubator with the same abiotic conditions (humidity 65±5%; photoperiod 14L:10D). Afterward, pre-weighed (Digital weighing balance Model: Aczet-CY223C) adult females were kept with pre-weighed leaves for 24 hours under respective thermal conditions. After 24 hours, the adult was transferred to a fresh Petri dish, concurrently, the adult biomass, the weight of faeces, and remaining unfed *Parthenium* leaves were recorded. Each experiment was replicated ten times and the consumption rate, conversion of ingested food, digestibility, conversion of digested food, and growth rate of the adults at different reared thermal conditions were calculated (Waldbauer, 1968).

Consumption index is the consumption made based on the intake rate relative to the animal's mean weight during the feeding period and was calculated as:=

$$\frac{\text{Fresh or dry weight of food eaten}}{\text{(Duration of feeding period)X(Mean fresh or dry weight of animal during feeding)}}$$

Conversion of ingested food is the efficiency of conversion of ingested food to body substance and was calculated as = $\frac{\text{Weight gained}}{\text{Weight of food eaten}} X100$

Digestibility was calculated as =

$$\frac{\text{Weight of food ingested} - \text{Weight of feces}}{\text{Weight of food eaten}} X100$$

Conversion of digested food is the efficiency with which digested food is converted to body substance and was calculated as: =

$$\frac{\text{Weight gained}}{\text{Weight of food ingested} - \text{Weight of feces}} X100$$

Growth rate =

$$\frac{\text{Fresh mass gain by adults (mg)}}{(\text{Feeding duration}) X (\text{Mean biomass of adults})}$$

The data collected on consumption index, conversion of ingested food, absolute digestibility, conversion of digested food, and growth rate were checked for normality with the help of Kolmogorov-Smirnov's test, which revealed normal distribution. Data were subjected to one-way ANOVA followed by Tukey's post hoc honest test of significance. All statistical analyses were done using MINITAB-16 statistical software (Minitab Inc., State College, Pennsylvania, USA).

RESULTS AND DISCUSSION

At the extreme thermal conditions (15 and 35°C), the death of different immature stages (larvae, pupae and under-develop adults) was observed (Table 1). On the other hand, thermal conditions (20, 25 and 30°C) significantly influenced the consumption index (P=0.040, F=68.47, df=2), conversion of ingested food (P=0.004, F=6.16, df=2), absolute digestability (P=0.002, F=10.38, df=2), conversion of digested food (P=0.025, F=3.96, df=2), and growth rate (P=0.012, F=5.28, df=2). The maximum consumption index (41.28±3.54 mg) was observed at 25°C whereas it was minimum (0.02±0.00 mg) at 30°C (Fig. 1). The conversion of ingested food was maximum (17.52±6.23 mg) at 20°C whereas it was minimum (2.46±1.45 mg) at 25°C (Fig. 2). The conversion of digested food was maximum (41.16±23.29 mg) at 20°C and it was minimum (2.93±1.68 mg) at 25°C (Fig. 4). However, absolute digestibility and growth rate increased with increasing temperature from 20 to 30°C (Figs. 3, 5). In the present study, extreme thermal conditions (15 and 35°C) were not tolerated by the larval and pupal stages and newly emerged adults, leading to death at the immature stages. The intolerance of thermal shock may be because there was a decrease in the number of obligate bacterial endosymbionts, which are responsible for the thermal tolerance of insect host species (Zhang *et al*., 2019). Experimental beetles were collected from a geographical area where temperature ranged from 15°C to 30°C with an average of 20 ± 2°C throughout the year (Malviya and Dwivedi, 2015). So, the beetle might have adopted this temperature range through epigenetic changes. Temperature is one of the factors for changes in the genome at the epigenetic level (Richard *et al*., 2019). The physiology and biochemical activities of the beetles are adversely affected by either a range of positive or negative temperature variations.

Consumption index increased from 20°C to 25°C and then decreased at 30°C. This might be because the energy available for activities other than cellular maintenance, such as movement, feeding, or digestion, drops rapidly at high temperatures. This often lead to lower consumption rates at high temperatures (Somero, 2011). Levesque *et al*. (2002) reported a similar pattern of the consumption index in *Malacosoma disstria*. Apart from this, Lemoine *et al*. (2014) investigated the relationship between food consumption and temperature in phytophagous insects which revealed that food

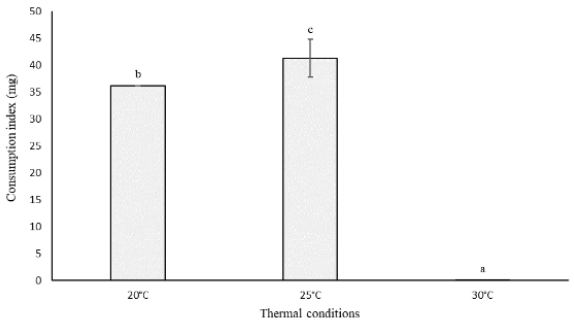

Fig. 1 Effects of temperature on consumption index (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)

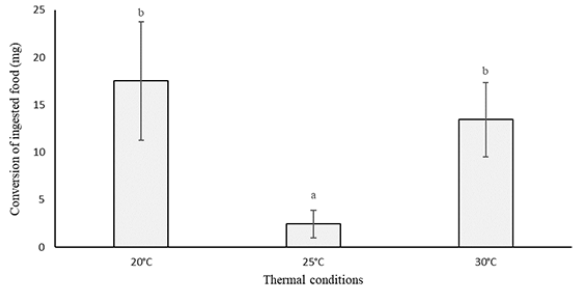

Fig. 2 Effect of temperature on conversion of ingested food (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)

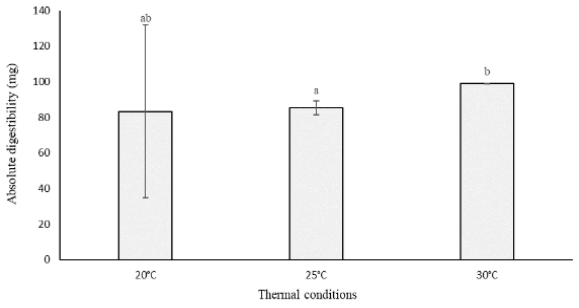

Fig. 3 Effect of temperature on absolute digestibility (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)

intake increased as temperature increased up to a certain range. Many studies reported that consumption rates of insects increase to a certain extent with increasing temperature, and after that, the consumption rates vary according to the fluctuation of the temperature (Niu *et al*., 2003; Yee and Murray, 2004; Rall *et al*., 2010).

Maximum conversion efficiency was recorded at 20°C than other thermal conditions. Several studies also suggested that with increasing temperature, the food conversion efficiency of adults initially increased to an optimal level and then decreased with a further increase in temperature (Bhusal *et al*., 2020). The findings of the present study suggest that 20°C might be the optimal temperature for this beetle. However, temperatures above the optimal levels might induce thermal stress reducing its conversion efficiencies. Similar trends also have been reported in coccinellid beetles (Omkar and Kumar, 2016).

Absolute digestibility increased positively from 20°C to 30°C. This increase in digestibility with temperature might be because of the increase in the metabolic rate of *Z. bicolorata*. Similar results have been documented by Levesque *et al*. (2002) and Hegazi and Schopf (2009) in *Malacosoma disstria* and *Spodoptera littoralis* (Boisd.). In contrast, the conversion of digested food was maximum at 20°C and minimum at 25°C. At 20°C, the beetle might have efficiently converted the food into nutrients needed for physiological functioning. The above finding suggest that this temperature might be optimal for feeding and converting food material. Similar results have also been recorded in the forest tent caterpillar moth and African cotton leafworm (Hegazi and Schopf, 2009; Levesque *et al*., 2002).

Growth rate was negligible at 20°C, but increased in temperature 25°C to 30°C, suggesting that the metabolic rates of *Z. bicolorata* adults increased with the temperature which might have stimulated the growth of the beetles. A similar trend has also been reported in the beetle, *Alphitobius diaperinus*, which grew slower at low temperature and faster at high temperature (Bjorge *et al*., 2018).

In conclusion, the results of the present study suggest that temperature significantly modulated this

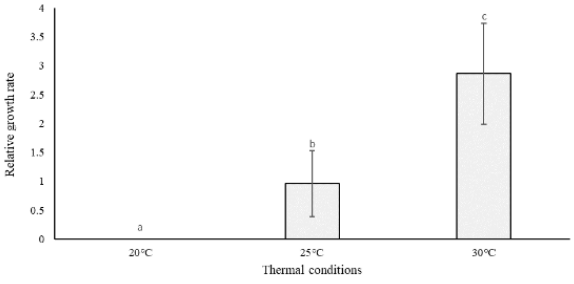

Fig. 5 Effects of different thermal conditions on relative growth rate (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference

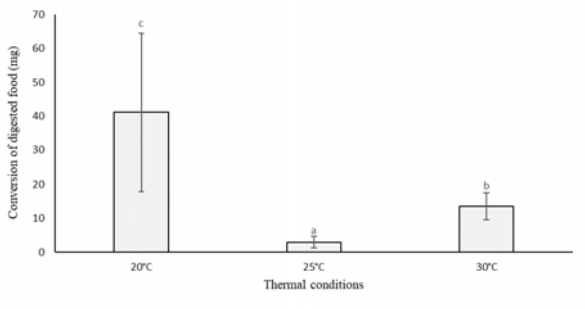

Fig. 4 Effects of different thermal conditions on Conversion of digested food (E.C.D.) (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference

beetle's feeding attributes. Using the nutritional indices consumption index, absolute digestibility, and efficiency of conversion of digested food, the capacity of *Z. bicolorata* to consume and utilize food can be described in three steps: (i) feeding activity, (ii) digestion and (iii) efficacy to assimilate the digested food. This sequence demonstrates the conversion of foodstuff into the body substance of the phytophagous beetles.

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Table 1. Effects of extreme thermal conditions on different developmental stages of *Zygogramma bicolorata*

Stages	Temperature 15 ºC		Temperature 35 ºC	
Larva	 Dorsal view	 Lateral view	 Dorsal view	 Lateral view
Pupa	Mostly pupation did not occur. If occurred, under-developed adults formed		No pupation observed	
Adults	 Under-developed adult		Adults did not emerge	

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Compound eyes of *Camponotus compressus* (Fabricius, 1798) (Hymenoptera, Formicidae) reflects caste specific organisation and adaptation

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ABSTRACT: Caste polymorphs of the ant *Camponotus compressus* (Fabricius, 1798) are distinct by their morphology and life styles; the two distinct castes are largely nocturnal and rely on their visual sensory system to interpret their temporal niches. The compound eyes of the castes were explored through Light and Scanning Electron Microscopy (SEM) in order to delineate cast specific organisation and adaptations of the compound eye. The findings reveal that major workers of *C. compressus* possess a more sophisticated visual system in terms of its morphological features along with optical properties that enhances a better vision, which includes a greater number of ommatidia and high ommatidial density, a higher ommatidial diameter, low inter ommatidial angle and a more efficient pupillary mechanism to counter conditions where ambient light levels are high. This underlines the dependence of scouts (major workers) on visual system and the foragers on olfactory system in the species. © 2024 Association for Advancement of Entomology

KEY WORDS: Ommatidia, caste polymorphs, screening pigments, ommatidial angle

INTRODUCTION

Sensory systems of animals enable them to perceive vital sensory information from their surroundings- a process which is crucial for the success and survival of animals (Rössler, 2023). Social insects - particularly ants offer suitable and amenable systems to address questions about successful sensory adaptations and behaviours in diverse niches (Hölldobler and Wilson 1990). Finding mates, foraging, ovipositing, defending or communicating between conspecifics are notable behaviours of ants that are predominantly mediated by visual cues (Schwarz *et al*., 2011). Features of ommatidia constitute the fundamental design of the compound eye and determine their visual capabilities (Hunt *et al.,* 2018; Narendra *et al*., 2016b). Nocturnal, diurnal and crepuscular ant species have compound eye designs that match their lifestyle and behaviours (Greiner *et al*., 2007; Narendra *et al*., 2016a). Evolutionary, behavioural and ecological aspects of each ant species are reflected in the features of their compound eyes (Klotz *et al*., 1992; Knaden and Graham, 2016).

Camponotus compressus (Fabricius, 1798) (Hymenoptera, Formicidae) is common in evergreen forests, sholas, deciduous forests and plantations and are largely nocturnal. Like other ant societies caste-based division of labour is evident among individuals of these ants. Major (scouts) and minor (foragers) are the prominent caste polymorphs of

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the colony with characteristic morphological features and sizes. Though medium castes are also present in the society they are less in number. Major worker castes are the scouts of the colony and minor workers are foragers. It will be interesting to understand how the compound eyes are organised in the castes of *C. compressus* especially in a context when behaviour of the castes is notably different. Behaviour is driven by visual cues in these ants. In this study the morphological features of compound eyes of major (foragers) and minor (nurses) castes of *C. compressus* were examined to decipher caste specific designs. Castes of this ant species are largely nocturnal and are found to be active in similar temporal niches. Major workers are main defenders of the colony and are involved in foraging with the minors also (Mysore *et al.*, 2009). Foraging and defence are behaviours that put different levels of demands on the sensory systems especially the visual system. This hypothesis is tested by analysing the important optical features of the compound eye of the major workers (scouts) and minor workers (foragers) castes and the distribution pattern of the screening pigments as a reflection of the visual adaptation mechanism within their compound eyes.

MATERIALS AND METHODS

Major and minor castes of *C. compressus* were collected from the colonies found in the campus of St Berchmans College, Changanasserry Kerala (9.2702°N; 76.3219°E). Caste polymorphs are distinct by their size. Though queen, major workers media workers and minor workers are present in the colony, selected only major and minor workers. Major workers are larger (14–18mm) than minor workers (6–8mm). Majority of the major workers are foragers. Minor workers are nurses and forage occasionally. They were collected by forceps into a plastic bottle and brought to the laboratory. These ants were cold anesthetized by keeping in the refrigerator for the study.

Haematoxylin and Eosin staining: Heads of different castes of *C. compressus* were decapitated using a razor blade and were fixed in paraformaldehyde (4%) at room temperature in the morning (Decapitated heads of the castes were fixed in the morning and further processed to understand the distribution of the screening pigments in daylight conditions). Heads were then passed through ascending concentrations of alcohol grades (from 35% - 50% - 70%- 80% - 90% - 100%; 15 minutes each in each grade) and cleared in Xylene. The tissues were embedded in paraffin and then were taken onto a rotary microtome to take sections of 5 micrometre thickness. Sections were then stained in hemotoxylin for 3-5 minutes. The sections were then washed in running tap water until sections turned bluish by keeping it for 5 minutes or less and differentiated in 1per cent acid alcohol (1% Hl in 70% alcohol) for 5 minutes; Then washed in running tap water until the sections were again blue by dipping in an alkaline solution followed by tap water wash. Then they were stained in Eosin Y for 10 minutes and washed in tap water for 1-5 minutes and then dehydrated in increasing concentrations of alcohol and cleared in Xylene and mounted on a micro glass slide using DPX for observation under a microscope.

Compound eye replica: The ant was mounted on an insect pin and then the colourless nail polish (Lakme) was applied uniformly as a thick film over the ant eye by placing a small drop of the fast-drying colourless nail polish and quickly allowing it to spread over the eye. The nail polish was out brushed throughout the compound eye area to get a full replica of the compound eye once it is peeled off as a thin layer from the compound eyes. Once it was fully set at the room temperature, a fine insect pin was used to gently lift the replica from the head capsule surrounding the eye. A fine pair of clean forceps was used to lift the replica. The replica was placed on a glass slide for observation. A razor blade was used to trim the replica by carefully removing excess material around the eye. A needle or a pair of forceps was used to prevent the replica from moving. A cover slip was placed gently on the eye replica and sealed the cover slip using very little nail polish on four corners. The slide was imaged on a compound microscope.

Scanning Electron Microscopy (SEM): Heads of the ants were decapitated using a razor blade and fixed with paraformaldehyde (4%) at 40^0C overnight. On the next day, heads were washed

with phosphate buffered saline (PBS) and dehydrated in ascending series of ethanol. The dehydrated heads were then mounted on a stub having a double adhesive carbon tape with the help of a stereo microscope. Heads were coated with gold for three minutes with the help of a sputter coater (Q150RES, quorum Tech). Coated heads were imaged under SEM/ Energy Dispersive Spectrometer (Jeol JS-6390LV/JED-2300) to study the detailed morphological features of the compound eyes of the major and minor workers. All the images obtained were exported to Image J to calculate the various parameters of the eye.

Morphometry: The length (Cl) and width (Cw) of the compound eyes and the thorax length (Thl) of the major and minor castes of *C. compressus* were measured from (the compound eye replicas obtained after applying nail polish). After drying, the polish was removed from their eyes and photographed and observed under light microscope (Magnus MLX) equipped with a digital camera (CatCam E-Series). Images were then digitized in a computer for quantification of the total number of ommatidia (TO), which was obtained from a direct count on a lateral view of the eye. The total surface area of the compound eye (A, μm^2) was calculated using the formula for the area A of an ellipse with length Cl and width Cw (Moser *et al.,* 2004). All measurements in micrometers (µm).

$$A = \pi \left[\frac{Cl}{2} \times \frac{Cw}{2} \right]$$

Yilmaz *et al.* (2014) procedure was followed for obtaining all the optical parameters of the compound eyes; length Cl and width Cw of the compound eye were measured using the Image J software from the images; mean inter ommatidial angle (Õ), which describes the cornea sampling density, mean ommatidial diameter (D) (ìm), which provides a measure of the sensitivity to light (Land, 1997). Ramirez-Esquivel *et al*. (2017) method was adopted to calculate the inter ommatidial angle (Õ). The mean inter ommatidial angle was obtained for each ant (after repeating three times this calculation, in different areas of the compound eye that were chosen randomly). The ommatidial diameter was measured by drawing and measuring a line going through a row of 5–10 ommatidia in the horizontal or the vertical plane, and dividing that length by the number of ommatidia crossed. The mean ommatidial diameter was obtained for each ant (after repeating three times this calculation, in different areas of the compound eye that was chosen randomly). The eye parameter, P gives the relationship between the sensitivity and resolution of the eye. It was calculated as,

$$P = D.\Delta\varphi$$

Where D is the mean ommatidial diameter and " `" is the mean inter ommatidial angle

Morphological data between two groups of castes were compared using a student's unpaired t test with 0.001 as the significance level. At least five samples were used for statistical analysis. Microsoft Excel was used for calculations.

RESULTS AND DISCUSSION

Morphometrics:

Compound eyes of *C. compressus* were elliptical in shape (Fig.1). Important parameters of the eye which are crucial for optics and visual perception, varied significantly between the major and minor worker ants. Compound eyes of the two castes were at varying distances with a significant difference in the number of ommatidia (Major: 900±; minors: 630±). Noticed differences in the shape and size of ommatidia in different areas of the compound eye of both the castes (Figs. 3a-e, 4 a-f). Mean ommatidial diameter and total surface area of the compound eye showed significant variation between major workers and minors. The number of ommatidia per unit area (μm^2) in both the castes varied significantly with the major workers having a significantly high density of ommatidia (399 ommatidial facets/ μm^2). Mean inter ommatidial angles were also found to be less in the major eye in contrast to the minor workers. Ommatidial angles between the ommatidial facets are reliable indices of the resolution of the compound eye. Major worker with a lesser number of ommatidial angle is likely to possess a between the

castes (Table 1). Interestingly the major workers had a high parameter P value relatively high-resolution power of compound eyes. The eye parameter P also varied significantly.

Table 1. External parameters of the compound eye of the workers of *Camponotus compressus*

Workers	Cl (μm)	D (μm)	" `" = angle(^{0})	Cw (μm)	Between the eyes (μm)	TO	A (μm^2)	P (μm.rad)
Minor	21.875	16.75±1.8	5.168	1773.3	1506±2.46	630±0.3	197317 313OF	0.28±.83
Major	23.6	21.5±.2.9*	3.71*	5260	3120*± 1.8	900±0.6*	359868* 399OF*	1.38±.76*

Cl = Length of facet; D = Ommatidial diameter; " `" = Inter ommatidial angle; Cw = Head width; A = TSA of Eye; TO = Ommatidia no. P = Eye parameter; OF = ommatidial facets; mean ± standard deviation; *significant at P= 0.001

Histological studies:

Screening pigments (pigments inside the primary pigment cells and retinula cells) play a crucial role in the pupillary mechanism of the compound eyes of insects. Contrasting results in the distribution of screening pigments in major and minor castes were noted which convey a different mode of sensory adaptation. Major workers had a dense distribution of the pigments in the junction of the crystalline cone and rhabdom area in daylight conditions (Fig 4a), whereas the minor workers had a feeble distribution of the pigments (Fig 4b). Pupillary mechanism is an important adaptation to counter the photons the compound eye encounter when ambient light is more. Being nocturnal ants, they are supposed to possess adaptive measures to counter light abundance in the niches.

Major and minor castes of *C. compressus* were distinct by external features and behaviours, though they have the same genotype. The study indicated that external features that influence optic properties of compound eyes differ significantly between major and minor castes. Major and minor castes of *C.compressus* pereceive light differentially which is evident in their morphological features of compound eyes. Castes of *C. compressus* rely on a a spectrum of olfactory and visual sensory sensory cues in accordance with their contrasting life styles and tasks they are engaged in. The findings indicated that differences in the external and internal features of compound eyes of the castes correlate with their lifestyles. Major workers who are the scouts seems to rely on the visual system more compared to the minor workers, but in such a way that they seem to compromise on some aspects of the visual perception for example the parameter P value of their compound eyes. The study revealed that compound eyes of minor castes were less sophisticated in external visual features compared to major workers. This was evident in the lesser number of ommatidia count they possess, lesser surface area of compound eye with less number of ommatidia per unit area of the compound eye and small ommatidial facet diameter and variations in other crucial parameters inter ommatidial angle and parameter P that are important factors which influence optical properties of the compound eyes (Table 1). A compound eye with less surface area and less number of ommatidial facets is likely to be less efficient in some important aspect of visual perception. Compound eyes with a greater count of ommatidia obviously has more number of lenses for the capture of photons. Ommatidial angles between the ommatidial facets are reliable indices of the resolution capacity of the compound eye. Minor workers with a higher ommatidial angle seemed to have a relatively poor resolution power of compound eyes and their visual capability was further hindered by a lesser number of ommatidia. However it is interesting to note that minor worker posses a low P value which is suggestive of its better optical sensitivity

Further analysis of compound eyes of castes

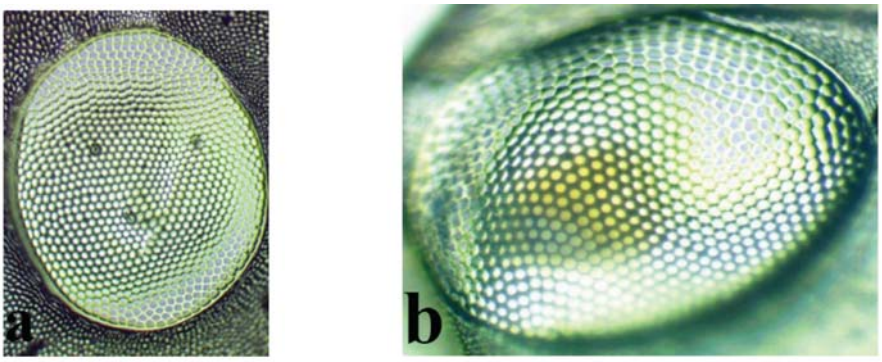

Fig. 1 Light microscopic images of replicas of compound eye of – a) major; b) minor worker of *Camponotus compressus*

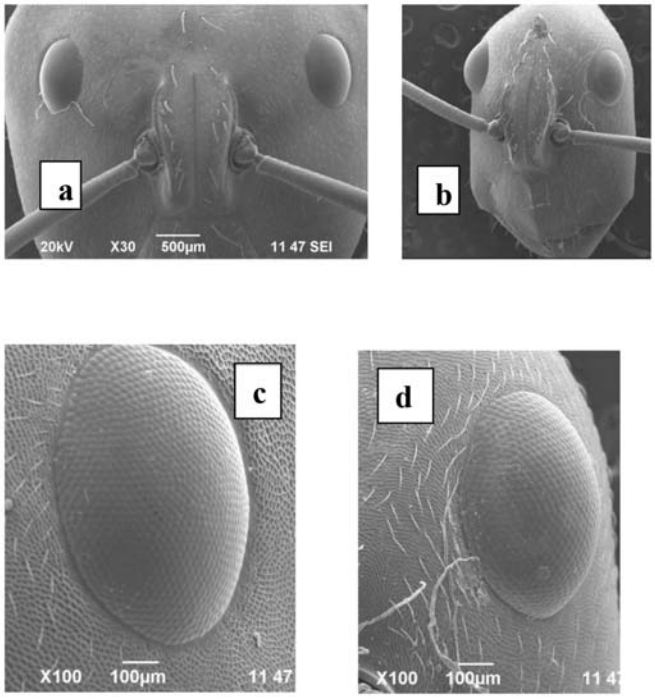

Fig. 2 a-d. SEM images of Compound eyes - a, c major worker; b, d - minor worker

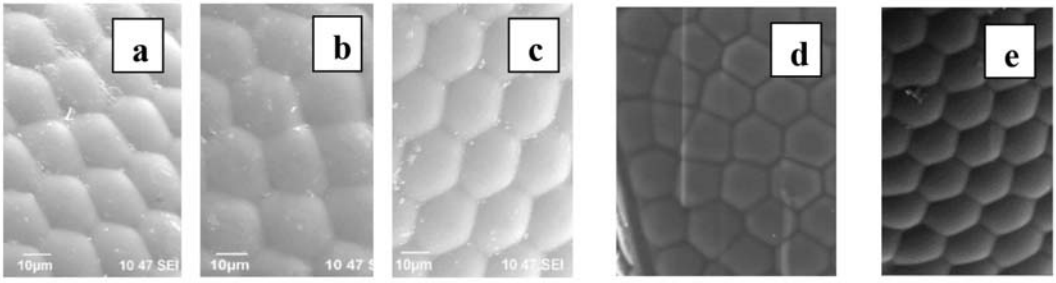

Fig. 3 a) SEM images of different regions of the compound eyes of major workers, showing the differences in the shape of ommatidia at different regions. a-c) middle area, d-e) dorsolateral

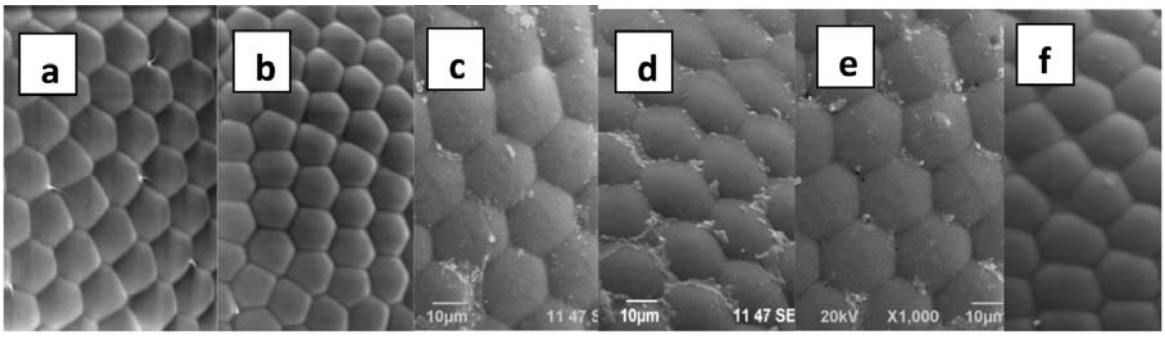

Fig. 4 a –f. SEM images of different regions of the compound eyes of minor workers showing the differences in the ommatidia shape. a-b) central area, c-f) dorsolateral area

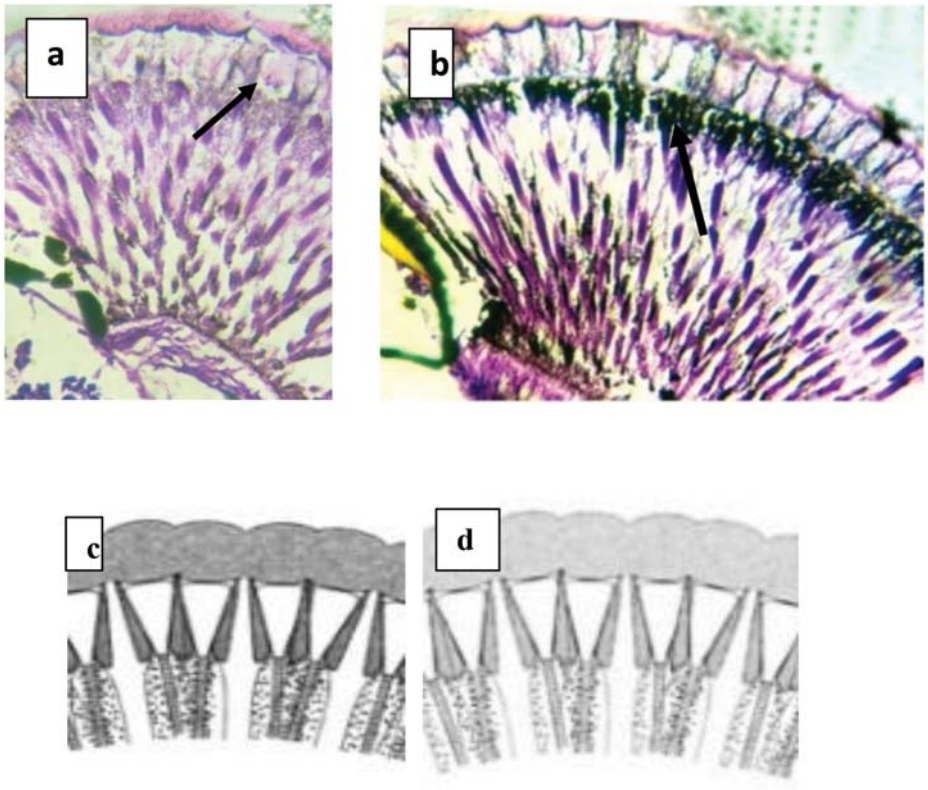

Fig. 4a-d Haemotoxylin and Eosin-stained sections of the compound eyes of (a) major workers, (c) minor workers, (b, d) schematic diagrams of the same sections, the white oval dot indicates the crystalline cone area of the compound eye. Black arrow indicates the screening pigments distribution. Note the difference in the pattern of distribution between the major (a) and minor (b) and schematically represented (c) and (d)

indicated contrastive visual adaptive mechanisms between major and minor workers of *C. compressus*. Visual screening pigments were differentially distributed in the compound eyes of castes; Major castes exemplify radial distribution of visual screening pigments towards rhabdoms in daylight conditions, which seemed to be a typical light adaptation pattern observed in nocturnal ants (where a dense population of screening pigments migrate towards rhabdoms during daylight conditions a phenomenon what is known as pupillary mechanism). The compound eyes of the major workers showed a dense and clustered pattern within the eye at the time of daylight conditions when they were fixed. However a different pattern of distribution was observed in minor castes where primary pigments were diffuse and lesss dense with scarce distribution. Pupillary mechanism is an effective adaptation in insect apposition eyes where primary pigments through the enveloping mechanism cover the rhabdoms and effectively protected the light reception through each lens in ambient conditions, where light is abundant. The findings suggested that minor workers were with comparatively poor pupillary mechanism consistent with lesser amount of time they were active in daylight conditions. Further they were more reliant on the olfactory cues for the foraging duties. Minor workers relied predominantly on olfactory sensory cues for meeting their foraging tasks. Peripheral component of olfactory system, antennal lobes and mushroom body areas of the brain concerned with olfactory sensory processing and perception were found more prominently organised in minor worker castes of *C. compressus* than in majors (Mysore *et al*., 2009) Quite interestingly this a pattern which contradicted the general pattern observed in many studies, where the large sized individuals like the major workers of many ant societies have a more elaborate organisation of sensory systems (Babu *et al*., 2011). The findings are in line with the general trend in that large individuals like major workers possess a more sophisticated compound eye. Contrasting designs of olfactory and visual sensory systems of major and minor castes makes *C. compressus* an interesting insect model system. Minor castes who seem to be more competent in perceiving chemosensory cues possess a more elaborate olfactory system and associated brain areas. However, the study highlights the more superior visual system of major workers.

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Host range and feeding preference of *Basilepta fulvicornis* (Jacoby) adult beetles in the Cardamom Hill Reserves, Kerala, India

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ABSTRACT: Host range and feeding behavior of *Basilepta fulvicornis* (Jacoby) adult beetles were studied on different plant species (25 species from 18 genera and 13 families) in the Cardamom Hill Reserves, Kerala, India. Five new host plants of *B. fulvicornis* adult beetles, viz., *Artocarpus hirsutus* Lam., *Ficus auriculata* Lour., *Anacardium occidentale* L., *Spondias mangifera* Willd., and *Terminalia chebula* Retz., are reported for the first time. Non-preference and non-feeding of *B. fulvicornis* adults on the larval host, *Elettaria cardamomum* (L.) Maton was confirmed. Based on the feeding area, *A. hirstus*, *F. auriculata, Mangifera indica* and *Artocarpus heterophyllus* are the most preferred host species followed by *Terminalia catappa, A. occidentale*, *S. mangifera*, and *T. chebula*. Feeding preference and survival of adult beetles of *B. fulvicornis* on different tree species are indicated with a probable eco-friendly pest management solution.

KEY WORDS: New hosts, adult feeding behavior, survival, larval host

INTRODUCTION

Chrysomelidae is a taxon containing more than 40,000 phytophagous insect species (Jolivet and Hawkeswood, 1995; Futuyma, 2004). Host range and feeding habits will vary greatly among the chrysomelids (Jolivet and Hawkeswood, 1995; Bieñkowski, 2010). Despite the fact that many chrysomelids are monophagous or oligophagous in nature, members of the Eumolpinae, Cryptocephalinae and Clytrinae utilize a wide range of host plants (Fernandez and Hilker, 2007). Eumolpinae is a widely distributed large subfamily of Chrysomelidae that includes more than 500 genera and 7000 species (Jolivet and Verma, 2008). One Asiatic genus, *Basilepta* Baly, under the tribe Nodini (Eumolpinae) is mainly polyphagous (Jolivet and Hawkeswood, 1995). The distribution range of *Basilepta fulvicornis* described by Jacoby (1904) is confined to the states Kerala, Karnataka and Tamil Nadu (Jacoby, 1908), which are the major cardamom growing states in India (Ravindran, 2002).

Small cardamom [*Elettaria cardamomum* (L.) Maton], also known as the "Queen of Spices" is a native to the moist evergreen forests of the Western Ghats of southern India (Ravindran, 2002). In Kerala, the leading producer state, it is cultivated mainly in the Indian Cardamom Hills (ICH),

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covering an area of 1050 km^2 and is designated as Cardamom Hill Reserves (CHR) (Murugan *et al*., 2016). Cardamom crop require perpetual shade, where necessary sunlight is filtered through the overhead canopy of shade trees and these shade trees constitute the major tree flora in Cardamom Hills (Pradip Kumar *et al*., 2012). A survey conducted by Salish *et al.* (2015), identified a total of 99 species of shade trees representing 77 genera and 35 families in the CHR area. Insect pests pose a significant threat to cardamom cultivation in India (Gopakumar and Chandrasekar, 2002). Thrips, shoot borers and root grubs are regular features in all cardamom-growing localities (Thomas, 2001). In contrast to thrips and shoot borers, cardamom root grub adults (*B. fulvicornis*) depend on the foliage of some of the shade trees for their food and survival (Gopakumar *et al*., 1991; Varadarasan, 2001). *B. fulvicornis* (Jacoby) is a subterranean pest that damages cardamom roots and all stages of the grubs have been damaging the feeder roots, resulting in severe yield loss (Varadarasan *et al*., 1988). This pest has been observed in nurseries and plantations in Kerala, Karnataka and Tamil Nadu, and found to harm approximately 64.5 per cent of cardamom clumps in the main field (Varadarasan *et al*., 1988; Thyagaraj *et al*., 1992). Adult beetles are shiny metallic blue, green, brown, bluish green, or greenish brown in color, with color polymorphism in both sexes (Gopakumar *et al*., 1991). They are polyphagous; jack (*Artocarpus heterophyllus*), rose (*Rosa rubiginosa*), Indian almond (*Terminalia catappa*), mango (*Mangifera indica*), guava (*Psidium guajava*), ficus (*Ficus indica, F. bengalensis*), cocoa (*Theobroma cacao*), and dadaps (*Erythrina lithosperma*) are certain recorded hosts of the beetles (Gopakumar *et al*., 1991; Varadarasan, 2001). In this study the feeding habitat of adult beetles, the host range, and survival rate of *B. fulvicornis* among various shade tree species in the CHR system were recorded and analyzed.

MATERIALS AND METHODS

The shade tree species with a previous record as a host of *B. fulvicornis* beetles, related tree species and farmers' preferred shade trees in the CHR system were purposefully selected (Gopakumar *et al*., 1991; Varadarasan, 2001; Nayar *et al*., 2014; Murugan *et al*., 2006, 2022). The identity of the tree species was confirmed with the help of Nayar *et al.* (2014) and Vattakavan *et al.* (2016). The selected trees in the accessible locations were marked and tagged for further leaf collection during the study period. For understanding the feeding preferences of adult beetles, tender leaves (just below the growing tip) were collected and provided as feed. Field active populations of beetles were gathered by using a sweep net from the root grub infested cardamom plantations and the collected beetles were transferred to glass vials prior to transfer into Petri plates to observe the feeding behavior. Feeding preferences of *B. fulvicornis* adult beetles were evaluated in three batches inside the laboratory under room temperature using leaves from different shade trees and the leaves of its larval host, cardamom. In the first batch, beetles were tested with leaves of cardamom (*E. cardamomum*) and six shade trees, *M. indica*, *A. heterophyllus*, *T. cacao*, *E. lithosperma*, *T. catappa* and *P. guajava*. In the second batch, tests were carried out with the most common and farmer's preferred species of shade trees (*Vernonia arborea*, *Persea macrantha*, *Cinnamomum zeylanicum*, *Macaranga peltata*, *Grevellea robusta*, *Toona ciliata* and *Bischofia javanica)* in the CHR system, other than the previously tested identified host species. Related species of previously identified host trees (*Spondias mangifera*, *Anacardium occidentale*, *Artocarpus altilis*, *A. hirsutus*, *Ficus hispida*, *F. auriculata*, *Erythrina indica*, *Terminalia chebula*, *T. bellirica*, *Syzygium aromaticum* and *S. cumini)* found in the CHR area were tested in the third batch. In these three batches, feeding preference and survival of *B. fulvicornis* adult beetles were recorded with leaves of 25 plant species distributed in the CHR area (Table 1).

There were seven, seven and eleven treatments correspondingly in the 1st, 2nd and 3rd batches, with three replications in CRD experimental design. In each treatment, five fresh active beetles from the field were released randomly. Before releasing the adult beetles, the leaves on each plate were weighed

separately using the electronic precision balance SCALETEC SAB 303L. After 24 hours, observations like leaf weight, surface area of the leaves fed by beetles and the number of adult beetles that died were recorded on each petri plate. The surface area fed by the beetles was measured using the graphical method by plotting the fed area on graph paper. The leaves inside the plates were replaced every day (after the readings) with fresh pieces of weighed leaves, and the dead adult beetles were replaced with newly captured ones. Observations were repeated every 24 hours over a period of 10 days. Mortality of adult beetles in each plate was calculated.

$$\text{Mortality \%} = \frac{\text{Number of adult beetles died during the observation period}}{\text{Number of adult beetles released during the observation period}} \times 100$$

Table 1. List of plant species in the CHR system used in the feeding preference

No.	Common name	Scientific name	Family
1.	Small cardamom	*Elettaria cardamomum* (L.) Maton.	Zingiberaceae
2.	Mango	*Mangifera indica* L.	Anacardiaceae
3.	Jackfruit	*Artocarpus heterophyllus* Lam.	Moraceae
4.	Cocoa	*Theobroma cacao* L.	Sterculiaceae
5.	Dadap	*Erythrina lithosperma* Blume ex Miq.	Fabaceae
6.	Indian-almond	*Terminalia catappa* L.	Combretaceae
7.	Guava	*Psidium guajava* L.	Myrtaceae
8.	Vernonia *(Karana)*	*Vernonia arborea* Buch.-Ham.	Asteraceae
9.	Bay tree *(Kulamavu)*	*Persea macrantha* (Nees) Kosterm	Lauraceae
10.	Ceylon cinnamon	*Cinnamomum zeylanicum* Blume	Lauraceae
11.	Macaranga *(Vatta)*	*Macaranga peltata* (Roxb.) Müll.Arg.	Euphorbiaceae
12.	Silveroak	*Grevillea robusta* A. Cunn. ex R. Br.	Proteaceae
13.	Red cedar *(Chandana vembu)*	*Toona ciliat*a M. Roem.	Meliaceae
14.	Bishop wood *(Chorakkali)*	*Bischofia javanica* Blume	Phyllanthaceae
15.	Hog plum *(Ambazham)*	*Spondias mangifera* Willd.	Anacardiaceae
16.	Cashew	*Anacardium occidentale* L.	Anacardiaceae
17.	Breadfruit	*Artocarpus altilis* (Parkinson) Fosberg	Moraceae
18.	Wild jack *(Anjili)*	*Artocarpus hirsutus* Lam.	Moraceae
19.	Rough-leaved fig *(Parakam)*	*Ficus hispida* L. f.	Moraceae
20.	Elephant ear fig	*Ficus auriculata* Lour.	Moraceae
21.	Indian coral tree	*Erythrina indica* Lam.	Fabaceae
22.	Black myrobalan *(Kadukka)*	*Terminalia chebula* Retz.	Combretaceae
23.	Beller ic myrobalan *(Thanni)*	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae
24.	Java plum	*Syzygium cumini* (L.) Skeels	Myrtaceae
25.	Clove	*S. aromaticum* (L.) Merr & L.M. Perry	Myrtaceae

The data obtained were statistically analyzed and interpreted using the Web Agri Statistical Package (WASP). The nature of feeding and feeding scars were also investigated.

RESULTS AND DISCUSSION

Feeding efficiency and death rate of adults on reported host plants (the first batch of the experiment), showed that the average leaf area fed by five beetles per day was significantly greater in *M. indica* (75.90mm^2), followed by *A. heterophyllus* (71.20mm^2) and *T. catappa* (36.10mm^2). Feeding was not significant in *T. cacao* and *P. guajava*, and no feeding was observed in the leaves of its larval host, *E. cardamomum*. In *A. heterophyllus* there was greater drop in leaf weight per day (0.22g) followed by *M. indica* (0.20g) and *T. catappa* (0.19g). Adult mortality was considerably lower on *M. indica* leaves (28.65%), followed by *A. heterophyllus* (31.38%) and *T. catappa* (38.36%) (Table 2a). In common and farmer's preferred shade trees (Table 2b), there was no significant feeding (0.0 - 0.2 mm^2) or reduction in leaf weight (0.10 - 0.12g) among the treatments (in the second batch experiment) and all the leaves from different shade trees exhibited non-significant adult mortality (59.98–61.74%). In the third batch experiment (on related species of reported host plants), maximum feeding area was noticed in *A. hirsutus* (114.77mm^2), followed by *F. auriculata* (98.60mm^2), *A. occidentale* (30.30mm^2), *S. mangifera* (17.17mm^2), and *T. chebula* (15.67mm^2). The feeding was not significant in *E. indica* (1.50mm^2), and no feeding was observed in *A. altilis*, *F. hispida*, *T. bellirica*, *S. cumini* or *S. aromaticum* (Table 2c). The reduction in leaf weight per day was higher in *A. hirsutus* (0.246g), followed by *F. auriculata* (0.239g), *A. occidentale* (0.174g), *S. mangifera* (0.156g) and *T. chebula* (0.150 g). Mortality was lowest in *F. auriculata* (23.55%), followed by *A. hirsutus* (26.83%), *A. occidentale* (49.99%), *S. mangifera* (50.96%) and *T. chebula* (57.35%). *The* beetles were mostly seen on the adaxial side of the leaf lamina and fed on the leaf surface rather than the margin. Feeding punctures were irregular, ranging from 1 to 52mm^2. Feeding causes shot holes in the leaves of *M. indica*, *T. catappa*, *P. guajava*, *S. mangifera*, *A. occidentale*, *F. auriculata* and *T. chebula* (Figs. 1a–g); but as scrapings on the adaxial side, in *A. hirsutus* and *A. heterophyllus* (Figs. 1 h, i).

Shading is essential for the normal growth of cardamom, and differences in shading have a substantial impact on photosynthetic activity, chlorophyll content, chlorophyll fluorescence, and biochemical characteristics (Alagupalamuthirsolai *et al*., 2018). Root grub infestation is most common in cardamom in exposed, warm, and less shaded situations, and an appropriate shade (65-70%) is required in root grub endemic cardamom locations (Prabhakaran Nair, 2006; Murugan *et al*., 2016). Some shade trees, however, provide food for *B. fulvicornis* beetles. As per the previous reports, its known host plants comprise nine species under eight genera belonging to seven families (Gopakumar *et al*., 1991; Varadarasan, 2001). Aside from the previously reported host plants, reporting five new hosts for *B. fulvicornis* beetles: *A. hirsutus*, *F. auriculata*, *A. occidentale*, *S. mangifera*, and *T. chebula* in the CHR system, Kerala. Jolivet and Hawkeswood (1995) noted Eumolpinae larvae feeding on non-related plants of the adult host-pant and its polyphagous nature. All stages of *B. fulvicornis* grubs infest cardamom feeder roots (Varadarasan *et al*., 1988). Varadarasan (2001) also noted that the adults did not feed on cardamom leaves. Larvae of typical Eumolpinae developed in soil, feeding on the roots of the normal host plant of the adults (Jolivet and Verma, 2008). In this study, confirmed the non-preference and non-feeding nature of *B. fulvicornis* adults on its larval host, *E. cardamomum*. Out of the 24 tree species evaluated in three batches, significant feeding was recorded only in eight species: *M. indica*, *A. heterophyllus* *T. catappa, A. hirsutus*, *F. auriculata*, *A. occidentale*, *S. mangifera* and *T. chebula*. These eight species were under three families only, out of the 12 families tested. All the members tested under Anacardiaceae and 60 per cent of the members under Moraceae showed significant feeding rate, indicating an affinity of the *B. fulvicornis* beetles towards these plant families.

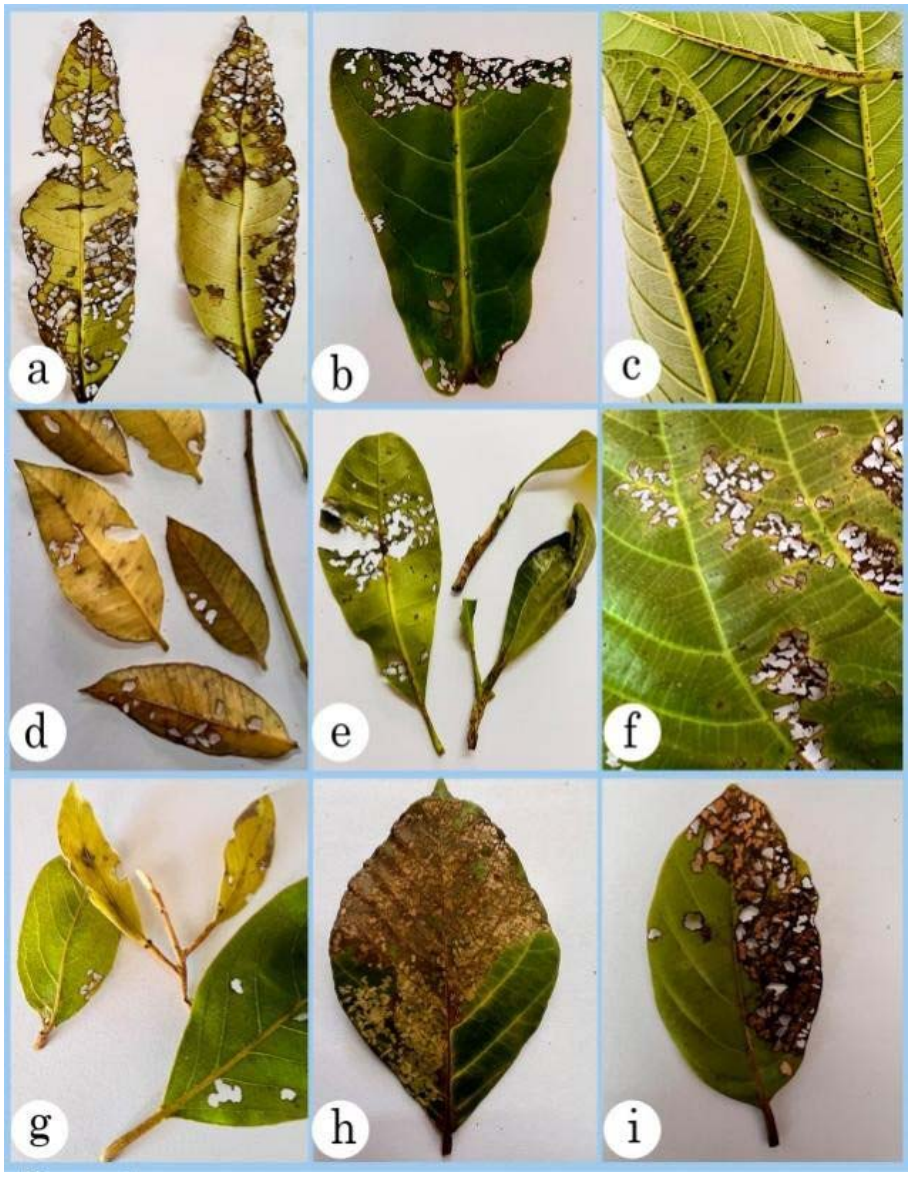

Fig. 1 *B. fulvicornis* beetle feeding marks on the leaves of — a. *Mangifera indica*, b. *Terminalia catappa*, c. *Psidium guajava*, d. *Spondias mangifera*, e. *Anacardium occidentale*, f. *Ficus auriculata*, g. *Terminalia chebula, h. Artocarpus hirsutus, i. A. heterophyllus*

A low mortality rate was also observed in tree species, which supports more feeding. This demonstrates the necessity of some of the shade trees for the survival of the beetles. Tree species like *M. indica*, *F. auriculata* and *A. occidentale* are not widely adopted in young cardamom plantations. But these trees, which are mainly seen in the vicinity of households connected to the CHR system, can act as a shelter and feeding ground for these beetles. Now *S. mangifera, T. catappa* and *T. chebula* were mostly replaced with farmer's preferred tree species in the CHR area. *A. heterophyllus* is one of the dominant species (Salish *et al*., 2015; Murugan *et al*., 2022) in the CHR system due to selective tree felling and replacement. Root grub fecundity was already reported as higher in *A. heterophyllus* (Varadarasan *et al*., 2001). Beetles fed on *A. hirsutus*, an IUCN-red-listed Western Ghats endemic species, have shown heavy feeding and a low mortality rate. The endemicity

Table 2. Feeding efficiency and death rate of *B. fulvicornis* adults (per day)

No.	Host	Leaf area fed (mm^2)	Reduction in leaf weight (g)	Beetles dead (nos.)	Mortality (%)
(a) On reported host plants					
1.	*Elettaria cardamomum*	00.00^{c}	0.09^{d}	1.33^{a}	72.65^{a} (58.48^{a})
2.	*Mangifera indica*	75.90^{a}	0.20^{ab}	0.17^{b}	23.61^{c} (28.65^{c})
3.	*Artocarpus heterophyllus*	71.20^{a}	0.22^{a}	0.20^{b}	27.58^{bc} (31.38^{bc})
4.	*Theobroma cacao*	05.20^{c}	0.10^{cd}	1.13^{a}	68.43^{a} (55.89^{a})
5.	*Erythrina lithosperma*	01.83^{c}	0.10^{cd}	1.30^{a}	72.17^{a} (58.16^{a})
6.	*Terminalia catappa*	36.10^{b}	0.19^{b}	0.33^{b}	38.69^{b} (38.36^{b})
7.	*Psidium guajava*	05.90^{c}	0.12^{c}	1.00^{a}	65.89^{a} (54.32^{a})
(b) Common and farmer's preferred shade trees					
1.	*Vernonia arborea*	0.10	0.12	1.67	76.91 (61.28)
2.	*Persea macrantha*	0.17	0.11	1.60	76.15 (60.77)
3.	*Cinnamomum zeylanicum*	0.00	0.12	1.53	75.40 (60.26)
4.	*Macaranga peltata*	0.20	0.11	1.50	74.96 (59.98)
5.	*Grevillea robusta*	0.00	0.11	1.57	75.72 (60.49)
6.	*Toona ciliata*	0.17	0.10	1.63	76.52 (61.02)
7.	*Bischofia javanica*	0.00	0.11	1.73	77.57 (61.74)
(c) Related species of reported host plants					
1	*Spondias mangifera*	17.17^{d}	0.16^{b}	0.77^{c}	60.32^{bc} (50.96^{ab})
2	*Anacardium occidentale*	30.30^{c}	0.17^{b}	0.73^{c}	58.61^{c} (49.99^{b})
3	*Artocarpus altilis*	0.00^{e}	0.10^{c}	1.70^{a}	77.27^{a} (61.53^{a})
4	*Artocarpus hirsutus*	114.77^{a}	0.25^{a}	0.13^{d}	20.64^{d} ($26.83^{c)}$
5	*Ficus hispida*	0.00^{e}	0.10^{c}	1.70^{a}	77.24^{a} (61.51^{a})
6	*Ficus auriculata*	98.60^{b}	0.24^{a}	0.17^{d}	22.02^{d} (23.55^{c})
7	*Erythrina indica*	1.50^{e}	0.11^{c}	1.63^{a}	76.48^{a} (61.00^{a})
8	*Terminalia chebula*	15.67^{d}	0.15^{b}	1.23^{b}	70.86^{ab} (57.35^{ab})
9	*Terminalia bellirica*	0.00^{e}	0.10^{c}	1.73^{a}	77.48^{a} (61.68^{a})
10	*Syzygium cumini*	0.00^{e}	0.10^{c}	1.70^{a}	77.24^{a} (61.51^{a})
11	*S. aromaticum*	0.00^{e}	0.10^{c}	1.60^{a}	76.08^{a} ($60.73^{ab)}$

In a column means followed by different letters are significantly different otherwise non significant; Values in parentheses are arc sine transformed values

of such a suitable adult host like *A. hirsutus*, larval host *E. cardamomum* and the pest species *B. fulvicornis* sheds light on their co-evolution in the southern Western Ghats.

Cardamom cultivation in the CHR system is highly intensive and costly, and pesticides are an inevitable input in an intensive agriculture system (Shetty *et al*., 2008; Murugan *et al*., 2017). Some cultural, mechanical, physical and bio-control methods were also evaluated and developed against *B. fulvicornis*, taking into account the damage

potential of this pest and the sensitive nature of the CHR system (Josephrajkumar *et al*., 2005; Prabhakaran Nair, 2006; Murugan *et al*., 2006, 2016; Naseema Beevi *et al*., 2014; Rashid *et al*., 2016). A clear understanding of the bio-ecology, including the feeding habitats of different life stages of a pest, will help to formulate better eco-friendly pest management strategies in such a complex but unique system as the CHR. Tree species that support more feeding, low mortality and high fecundity for the beetles should be avoided during the establishment of new plantations in root grub endemic areas. Desired shade trees that don't support feeding by the beetles will not invite much severity due to the root grub attack. Non-host tree species of *B. fulvicornis* adults with other desirable characteristics of shade trees can be recommended as one of the strategies to reduce heavy pesticide drenching in CHR soil to sustain the cardamom production system.

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Taxonomic studies on a collection of Chalcididae (Hymenoptera, Chalcidoidea) from Chilika Lake, Odisha, India

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ABSTRACT: The species diversity of family Chalcididae (Hymenoptera) from the islands of Chilika Lake, Odisha, India was studied. Eighteen species under seven genera of Chalcididae were identified with the addition of new distributional records for eight species from Odisha. Distributional data from India and the host details of all the listed species from Chilika Islands were provided.

KEY WORDS: New distributional records, species diversity, host details

INTRODUCTION

Chalcididae is one of the most important and fourth largest parasitic hymenopteran family under the superfamily Chalcidoidea (Aguiar *et al*., 2013), and is the one of the largest in size among the superfamily Chalcidoidea with a body length ranging 1.5 to 15mm. Some Chalcididae species are larval, pupal endoparsitoids of several pest species of Lepidoptera, Diptera and Coleoptera, thus they play significant role in controlling the number of pest in agricultural field by acting as potent bio control agents. They have swollen hind femur rowed with small teeth in the ventral side, oval shaped tegulae, narrow prepectus and simple venation in fore wing without closed cell structure. Most members of the family have black coloured body with white, yellow or red patches on hind femur. The Indian fauna of Chalcididae consists of 225 species under 31 genera (Noyes, 2019). Chilika is the largest brackish water lagoon in Asia spread over 1,100 km^2 area in the districts of Puri, Khordha and Ganjam of Odisha, India. Chilika Lake was designated as the first "Ramsar Site" of India in the year of 1981 for its rich ecosystem and species diversity. This lake provides shelter to several rare and vulnerable species to sustain their life and it also a home to a large number of migratory birds. Sureshan (2009) reported a total of 45 species of Chalcidoidea under Chalcididae, Eurytomidae, Pteromalidae and Torymidae from Odisha which includes nine species of Chalcididae. Noyes (2019) reported 15 species of Chalcididae from Odisha. Islands in Chilika are lying kilometers apart from one another. Salinity of the water also varies in different localities, so the soil too, that determines the growth of vegetation in the islands. Vegetation is poor to minimum in the islands and insect diversity also varies depends on the availability of vegetation. The present study exclusively carried out from the small islands within

* Author for correspondence

the lake and land cover outside lake to find out the species diversity of the family Chalcididae.

MATERIALS AND METHODS

The Chalcididae specimens were collected by net sweeping method from a variety of vegetation of Chilika during the year 2017. The specimens were collected from Balugaon, Grazing Island, Kaliyugeswar, Malati Island and Muggarmukh of Chilika (Fig.19). The collected Chalcididae specimens were mounted on triangular cards as per standard procedure documented by Noyes (1982). Identification of species was done using the dichotomous keys mentioned in Oriental Chalcididae (Narendran, 1989). The specimens were photographed using Leica S8 APO microscope and Leica MC 120HD camera. The voucher specimens are deposited in National Zoological Collections, Zoological Survey of India, Kolkata.

RESULTS AND DISCUSSION

From the present study, 18 species were collected and identified under seven genera of Chalcididae from Chilika. Eight species were found to be new reports from Odisha. The genus *Brachymeria* was most speciose with ten species. Distributional data provided for all the reported species along with the host association wherever available.

Systematic account

Class: Insecta; Order: Hymenoptera; Superfamily: Chalcidoidea; Family: Chalcididae

Genus: *Antrocephalus* Kirby, 1883

***Antrocephalus phaeospilus* Waterston, 1922 (Fig. 1)**

Antrocephalus phaeospilus Waterston, 1922: 22, F. INDIA, Bhim Tal, Kumaon (BMNH)

Distribution: Taiwan (Narendran, 1989), India: Kerala, Uttar Pradesh, West Bengal, Dadra Nagar Haveli (Basak *et al*., 2020), Andhra Pradesh (Rameshkumar *et al*., 2022), Odisha (New Record).

Material examined: 1♀, INDIA: Odisha, Chilika, Balugaon, 19°44’35", 85°12’42", 19.ii.2017, Coll: Rajmohana, K.

Diagnostic characters: Hind femur red in colour and thick, apex of scutellum bilobed, brownish infuscation adjoining marginal vein, eyes slightly pubescent.

Host: Unknown

Genus: *Brachymeria* Westwood, 1829

***Brachymeria apicicornis* (Cameron, 1911) (Fig. 2)**

Oncochalcis apicicornis Cameron, 1911: 3, F. (BMNH)

Distribution: Borneo, Java, Sulawesi (Narendran, 1989), India: Bihar, Uttar Pradesh, Odisha, West Bengal (Sheela *et al*., 2015), Karnataka, Kerala, Tamil Nadu (Noyes, 2019).

Material examined: 1♀, INDIA: Odisha, Chilika, Balugaon, 19°44’35", 85°12’42", 19.ii.2017, Coll: Rajmohana, K.

Diagnostic characters: Base of hind tibia and apex yellow remaining middle portion black, pre and post orbital carinae indistinct, not marked well.

Hosts: *Artona catoxantha* (Lepidoptera, Zygaenidae) (Noyes, 2019).

***Brachymeria burksi* Chhotani, 1966 (Fig. 3)**

Brachymeria burksi Chhotani, 1966:89, F. INDIA, West Bengal (ZSI)

Distribution: India: Kerala, Uttar Pradesh, West Bengal, Dadra Nagar Haveli, Odisha (Basak *et al.,* 2020). Noted only in India.

Material examined: 1♀, INDIA: Odisha, Chilika, Malati Island, 19°38’14.0", 85°10’59.8", 09.ii.2017, Coll: Sheela, S.

Diagnostic characters: Post marginal vein one third of marginal vein, sixth tergite with umbilicate pits, hind femur black with apex, hind tibia base black with sub basal and apical yellow patches.

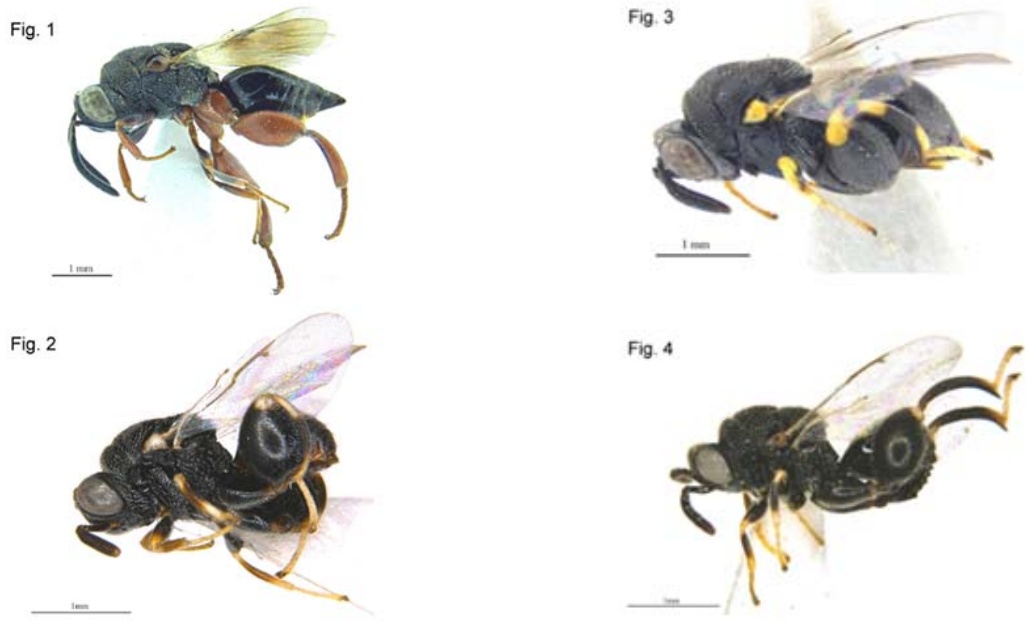

Fig. 1 *Antrocephalus phaeospilus* Waterston, 1922, Lateral view; Fig. 2 *Brachymeria apicicornis* (Cameron, 1911), Lateral view; Fig. 3 *B.* Chhotani, 1966, Lateral view; Fig. 4 *B. carbonaria* (Zehntner, 1906), Lateral view

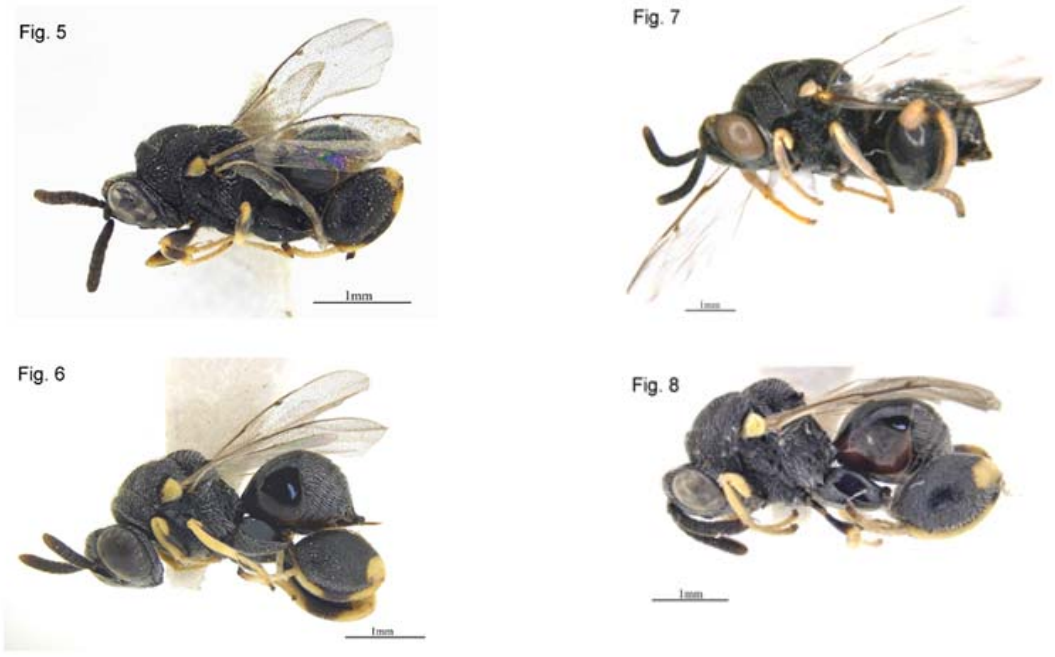

Fig. 5 *Brachymeria excarinata* Gahan, 1925, Lateral view; Fig. 6 *B. hearseyi* (Kirby, 1883), Lateral view; Fig. 7 *B. jambolana* Gahan, 1942, Lateral view; Fig. 8 *B. lasus* (Walker, 1841), Lateral view

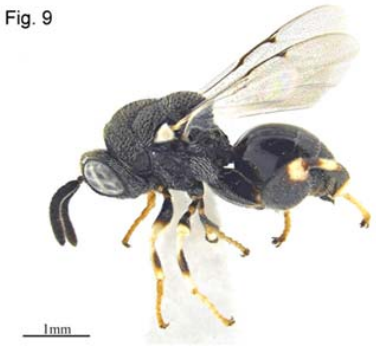

Fig. 9 *Brachymeria minuta* (Linnaeus, 1767), Lateral view; Fig. 10 *Brachymeria podagrica* (Fabricius, 1787), Lateral view; Fig. 11 *Brachymeria taiwana* (Matsumura, 1911), Lateral view; Fig. 12 *Dirhinus anthracia* Walker, 1846, Dorsal view

Fig. 13 *Dirhinus madagascariensis* (Masi, 1947), Dorsal view; Fig. 14 *Epitranus erythrogaster* Cameron, 1888, Dorsal view; Fig. 15 *Epitranus elongatulus* (Motschulsky, 1863), Lateral view; Fig. 16 *Hockeria lankana* Narendran, 1989, Lateral view

Hosts: *Aspidomorpha miliaris* (Coleoptera: Chrysomelidae) (Noyes, 2019).

***Brachymeria carbonaria* (Zehntner, 1906) (Fig. 4)**

Chalcis carbonaria Zehntner, 1906: 164, F.? (BMNH)

Distribution: Java (Narendran, 1989), India: Uttar Pradesh (Sheela *et al*., 2015), Goa, Mizoram, Tamil Nadu (Gowriprakash *et al*., 2018), Odisha (New record).

Material examined: 1♀, INDIA: Odisha, Chilka, Grazing Island,19°42’52.0", 85°24’13.8", 10.ii.2017, Coll: Sheela,S.

Diagnostic characters: First gaster tergite smooth and shiny, hind tibia completely black in colour with apex yellow, hind femur black with a small yellow apical patch, preorbital carina absent.

Host: *Scirpophaga intacta* (Lepidoptera, Pyralidae) (Noyes, 2019).

***Brachymeria excarinata* Gahan, 1925 (Fig. 5)**

Brachymeria excarinata Gahan, 1925: 90, F. PHILIPPINES, Luzon (USNM)

Distribution: Cameroon, China, Egypt, Japan, Iran, Papua New Guinea, Peoples’ Republic of China, Philippines, Taiwan, Vietnam (Noyes, 2019), India: Odisha, Uttar Pradesh, West Bengal (Sheela *et al*., 2015), Andaman and Nicobar Islands, Bihar, Gujarat, Karnataka, Kerala, Tamil Nadu (Noyes, 2019).

Material examined: 2♀, INDIA: Odisha, Chilka, Grazing Island, 19°42’52.0", 85°24’13.8", 10.ii.2017, Coll: Sheela, S., 2♀, Malati Island, 19°38’14.0", 85°10’59.8", 09.ii.2017, Coll: Sheela, S., 2♀, Muggarmukh, 19°41’52.3", 85°25’29.4", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Pre orbital carina well marked, apex of scutellum not emarginate most or less rounded, metasoma completely black in colour.

Hosts: Different species of Arctiidae, Gelechiidae, Hesperiidae, Noctuidae, Oecophoridae, Pyralidae, Tortricidae (Lepidoptera), Braconidae (Hymenoptera) and *Calopepla leayana* (Coleoptera: Chrysomelidae) (Noyes, 2019).

***Brachymeria hearseyi* (Kirby, 1883) (Fig. 6)**

Chalcis hearseyi Kirby, 1883a: 76, F. INDIA, Barrackpore (BMNH)

Distribution: India: Tamil Nadu (Narendran, 1989), Odisha, Uttar Pradesh (Sheela *et al*., 2015), Andaman and Nicobar Islands, Bihar, West Bengal (Noyes, 2019).

Material examined: 1♀, INDIA: Odisha, Chilika, Muggarmukh, 19°41’52.3", 85°25’29.4", 10.ii.2017, Coll: Sheela, S; 3♀, Malati Island, 19°38’14.0", 85°10’59.8", 09.ii.2017, Coll: Sheela, S.

Diagnostic characters: Pre orbital carinae absent, apex of scutellum rounded with some pitted median area, tegulae yellow, scrobe reaching front ocellus.

Hosts: Pupa of Nymphalidae (Lepidoptera) (Narendran, 1989), *Hypsipyla robusta* (Lepidoptera, Pyralidae) (Noyes, 2019).

***Brachymeria jambolana* Gahan, 1942 (Fig. 7)**

Brachymeria jambolana Gahan, 1942: 41, F. INDIA (USNM)

Distribution: Bangladesh, Indonesia (Noyes, 2019), India: Uttar Pradesh (Sheela *et al*., 2015), Andhra Pradesh (Rameshkumar *et al*., 2022), Karnataka, Kerala, Meghalaya, Tamil Nadu (Noyes, 2019), Odisha (New Record).

Material examined: 2♀, INDIA: Odisha, Chilika, Malati Island, 19°38’14.0", 85°10’59.8", 09.ii.2017, Coll: Sheela, S; 3♀, Muggarmukh,19°41’52.3", 85°25’29.4", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Gaster acuminate not at all rounded, ovipositor sheath visible in dorsal view, hind femur black with apical yellow patch, antennal club distinctly shorter than twice the length of preceding segment.

Hosts: *Sarcophaga misera* (Diptera:

Sarcophagidae) and several species of Danaidae, Lymantriidae, Noctuidae, Papilionidae (Lepidoptera) (Noyes, 2019).

***Brachymeria lasus* (Walker, 1841) (Fig. 8)**

Chalcis lasus Walker, 1841: 219, Lectotype F. INDIA, Calcutta (BMNH)

Distribution: Australia, Bangladesh, Fiji, Guam, Indonesia, Iran, Japan, Korea, Malaysia, Pakistan, Palau, Peoples' Republic of China, Philippines, Taiwan, Vietnam (Noyes, 2019), India: Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Punjab, Tripura, Uttar Pradesh, West Bengal (Noyes, 2019), Odisha (New record).

Material examined: 2♀, INDIA: Odisha, Chilika, Muggarmukh, 19°41'52.3", 85°25'29.4", 10.ii.2017, Coll: Sheela, S; 1♀, Kaliyugeswar, 19°44'59.2", 85°14'31.2", 11.ii.2017, Coll: Sheela, S.

Diagnostic characters: Hind coxa black with a distinct ventromesal tooth, hind femur black with apex yellow, first tergite of gaster smooth and shiny not shagreen, apex of scutellum slightly emarginated, scrobe smooth.

Hosts: Different species of Arctiidae, Bombycidae, Danaidae, Gelechiidae, Geometridae, Hesperiidae, Lasiocampidae, Limacodidae, Lycaenidae, Lymantriidae, Lymantriidae, Nymphalidae, Noctuidae, Oecophoridae, Pieridae, Pyralidae, Tortricidae, Zygaenidae (Lepidoptera), Tachinidae (Diptera) and Ichneumonidae (Hymenoptera) (Noyes, 2019).

***Brachymeria minuta* (Linnaeus, 1767) (Fig. 9)**

Vespa minuta Linnaeus, 1767: 952, F.? Europe (?UZM)

Distribution: All over World (Narendran, 1989), India: Odisha (Sheela *et al*., 2015), Andhra Pradesh, Karnataka, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal (Noyes, 2019).

Material examined: 3♀, INDIA: Odisha, Chilika, Malati Island, 19°38'14.0", 85°10'59.8", 09.ii.2017, Coll: Sheela, S.

Diagnostic characters: Hind femur black with yellow apex, sixth tergite shallowly pitted, mesoscutum and scutellum densely pitted in the median portion.

Hosts: Several species of Calliphoridae, Sarcophagidae, Tachinidae (Diptera), Gelechiidae, Hesperiidae, Lasiocampidae, Lymantriidae, Pieridae, Tortricidae, Yponomeutidae (Lepidoptera) (Noyes, 2019).

***Brachymeria podagrica* (Fabricius, 1787) (Fig. 10)**

Chalcis podagrica Fabricius, 1787: 148, M. INDIA, Tamil Nadu Tranquebar (UZM)

Distribution: All over World (Narendran, 1989), India: West Bengal, Odisha (Sheela *et al*., 2015), Andaman and Nicobar Islands, Bihar, Madhya Pradesh, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, Dadra and Nagar Haveli (Basak *et al*., 2020).

Material examined: 1♀, INDIA: Odisha, Chilika, Kaliyugeswar, 19°44'59.2", 85°14'31.2", 11.ii.2017, Coll: Sheela, S.

Diagnostic characters: Hind femur red with apex white or pale yellow on outer dorsal side not on inner side, hind femur nearly twice as long as wide.

Hosts: Several species of Calliphoridae, Muscidae, Sarcophagidae, Tephritidae (Diptera), Lymantriidae, Noctuidae, Psychidae, Yponomeutidae (Lepidoptera) (Noyes, 2019).

***Brachymeria taiwana* (Matsumura, 1911) (Fig. 11)**

Chalcis *taiwana* Matsumura, 1911: 85, Lectotype F. FORMOSA (EIHU)

Distribution: Indonesia, Taiwan, Vietnam (Noyes, 2019), India: Bihar, Karnataka, Kerala, Tamil Nadu (Noyes, 2019), Odisha (New Record).

Fig. 17 *Kriechbaumerella rufimanus* (Walker, 1860), Lateral view; Fig. 18 *Psilochalcis carinigena* (Cameron, 1907), Lateral view

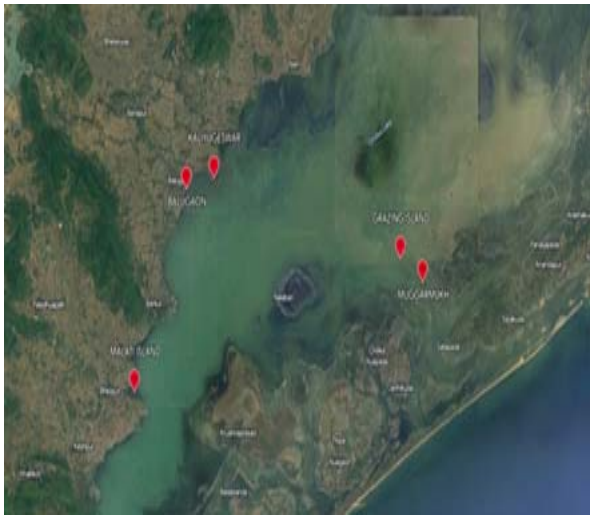

Fig. 19

Fig. 19 Collection localities of Chilika Lake, Odisha

Material examined: 1♀, INDIA: Odisha, Chilika, Grazing Island, 19°42'52.0", 85°24'13.8", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Upper margin of clypeus completely fused with frons, hind tibia yellow with base and ventral margin black, hind femur completely black, apex of scutellum rounded, first tergite smooth, post orbital carinae distinct.

Host: Unknown

Genus: *Dirhinus* Dalman, 1818

***Dirhinus anthracia* Walker, 1846 (Fig. 12)**

Dirhinus anthracia Walker, 1846: 7, 85, M. PHILIPPINES, (BMNH)

Distribution: Australia, Philippines, South Africa, Taiwan, Vietnam, Zambia (Noyes, 2019), India: Kerala, Odisha (Sheela *et al*., 2015); Andaman and Nicobar Islands, Andhra Pradesh, Bihar, Madhya Pradesh, Manipur, Mizoram, Puducherry, Punjab, Tamil Nadu, Tripura, Uttar Pradesh, Dadra and Nagar Haveli (Basak *et al*., 2020).

Material examined: 2♀, INDIA: Odisha, Chilika, Muggarmukh, 19°41'52.3", 85°25'29.4", 10.ii.2017, Coll: Sheela, S; 1♀, Chilika, Balugaon, 19°44'35", 85°12'42", 19.ii.2017, Coll: Rajmohana, K.

Diagnostic characters: Scutellum with a median impunctate strip, each horn in dorsal view broader than scrobal gap, striae on first tergite nearly straight reaching one third of gasteral length, fore and mid legs reddish.

Hosts: Different species of Calliphoridae, Muscidae, Tachinidae, Tephritidae, Sarcophagidae (Diptera), Bombycidae, Noctuidae, Pyralidae, Zygaenidae (Lepidoptera), *Chortoicetes terminifera* (Orthoptera, Acrididae) (Noyes, 2019).

***Dirhinus madagascariensis* (Masi, 1947) (Fig. 13)**

Pareniaca madagascariensis Masi, 1947: 74, Lectotype F. MADAGASCAR (MNHN)

Distribution: Madagascar (Noyes, 2019), India: Uttar Pradesh (Sheela et al., 2015), Karnataka, Kerala, Tamil Nadu, West Bengal (Noyes, 2019), Odisha (New record).

Material examined: 1♀, INDIA: Odisha, Chilika, Grazing Island,19°42’52.0", 85°24’13.8",10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Horn with distinct additional tooth, tip of horn hardly jutting out away from eye margin than frontal tooth, first tergite with strong striation extending more than one third length of tergite.

Host: *Sylepta derogata* (Lepidoptera, Pyralidae) (Narendran, 1989).

Genus: *Epitranus* Walker, 1834

***Epitranus erythrogaster* Cameron, 1888 (Fig. 14)**

Epitranus erythrogaster Cameron, 1888: 119, Lectotype F. JAPAN, Nagasaki (BMNH)

Distribution: Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam (Noyes, 2019), India: Odisha (Sureshan, 2009), Andaman and Nicobar Islands, Bihar, Karnataka, Kerala, Maharashtra, Manipur, Puducherry, Tamil Nadu, Uttar Pradesh, West Bengal (Noyes, 2019).

Material examined: 1♀, INDIA: Odisha, Chilika, Muggarmukh,19°41’52.3", 85°25’29.4", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Malar area hairy, antenna with clava and flagellar segments usually longer, mesosoma black, forewing hyaline without any brown bands.

Hosts: Some species of family Pyralidae (Lepidoptera) (Noyes, 2019).

***Epitranus elongatulus* (Motschulsky, 1863) (Fig. 15)**

Chalcis elongatula Motschulsky, 1863: 40, Lectotype F. SRI LANKA, Mt. Patannas (ZMMS)

Distribution: India: Delhi, Kerala, Tamil Nadu (Noyes, 2019), Odisha (New Record), Japan, Sri Lanka (Noyes, 2019).

Material examined: 1♀, INDIA: Odisha, Chilika, Muggarmukh, 19°41’52.3", 85°25’29.4", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Propodeum without percurrent median portion, incomplete veination, marginal vein completely without colour, seven flagellar segment in female.

Host: Unknown

Genus: *Hockeria* Walker, 1834

***Hockeria lankana* Narendran, 1989 (Fig. 16)**

Hockeria lankana Narendran, 1989: 91, Holotype F. SRI LANKA (BSRI)

Distribution: Sri Lanka (Narendran, 1989); India: Uttar Pradesh (Sheela et al., 2015), Odisha (New Record).

Material examined: 2♂, INDIA: Odisha, Chilika, Grazing Island, 19°42’52.0", 85°24’13.8", 10.ii.2017, Coll: Sheela, S.

Diagnostic characters: Gaster sessile and shorter than thorax, postmarginal vein distinct, marginal vein longer than postmarginal, apex of scutellum bilobed, wings without infuscations in male.

Host: Unknown

Genus: *Kriechbaumerella* Dalla Torre, 1897

***Kriechbaumerella rufimanus* (Walker, 1860) (Fig. 17)**

Halticella rufimanus Walker, 1860: 357, Lectotype M. SRI LANKA (Ceylon) (BMNH**)**

Distribution: Indonesia, Java, Nepal, Pakistan, Philippines, Sri Lanka (Narendran, 1989), India:

Odisha (Sureshan, 2009), Assam, Bihar, Delhi, Karnataka, Kerala, Tamil Nadu, Uttar Pradesh (Sheela *et al.,* 2015), West Bengal (Rameshkumar *et al*., 2020).

Material examined: 1♀, 2♂, INDIA: Odisha, Chilika, Balugaon, 19°44'35", 85°12'42", 19.ii.2017, Coll: Rajmohana, K.

Diagnostic characters: Gaster distinctly shorter than thorax, apex of scutellum bilobed, preorbital carinae present, genotemporal furrow indistinct, gaster smooth and shiny in female, gaster microculptured in male, body colour usually black.

Host: Unknown

Genus: *Psilochalcis* Kieffer, 1905

***Psilochalcis carinigena* (Cameron, 1907) (Fig. 18)**

Coelochalcis carinigena Cameron, 1907b: 579, Lectotype M. INDIA, Gujarat (BMNH)

Distribution: Uttar Pradesh, Odisha (Sheela *et al.,* 2015), Andaman and Nicobar, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Tripura, West Bengal (Noyes, 2019), Taiwan, Vietnam (Noyes, 2019).

Material examined: 2♀, INDIA: Odisha, Chilika, Balugaon, 19°44'35", 85°12'42", 19.ii.2017, Coll: Rajmohana, K.

Diagnostic characters: Lateral part of fore coxa with several well developed rugae, frontogenal carinae complete and distinct, apex of scutellum more or less emerginate, body densely pubescent.

Hosts: *Hyblaea puera* (Lepidoptera, Hyblaeidae) and *Opisina arenosella* (Oecophoridae, Lepidoptera) (Noyes, 2019).

Noyes (2019) reported 15 species of Chalcididae from Odisha but nowhere any species from Chilika Lake were reported. So, this is the first attempt to document the Chalcididae fauna from Islands of Chilika Lake. Altogether 18 species from seven genera of Chalcididae are reported here from Chilika. Out of these 18 chalcididae species, eight species are the first report from the state of Odisha. The genus *Brachymeria* found most dominating with 10 species. The vegetation is very poor in many islands and minimum in many. Only a very few islands have a good vegetational cover. But it is interesting to find the presence of Chalcididae in the small islands with very little grass cover. Collection of specimens in different seasons may provide a clear picture of the Chalcidid diversity in this place with addition of more species.

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Comparative analysis of using housefly maggot, silkworm pupae and earthworm meal-based diets in rohu, *Labeo rohita* (Hamilton, 1822)

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ABSTRACT: A study was conducted in rohu fish, Labeo rohita (Hamilton, 1822) to assess the potential of silkworm pupae, housefly maggot, and earthworm meals as a replacement for soybean meal. In this context, four isonitrogenous and isolipidic diets were prepared, viz., control – SM (30% soybean meal inclusion level), SPM (30% silkworm pupae meal inclusion), HMM (30% housefly maggot meal inclusion level) and EWM (30% earthworm meal inclusion level). The rohu fingerlings (initial average body weight: 5.07 ± 0.01 g) were fed twice daily with the respective experimental diets to reach satiation levels. Specific growth rate (SGR), final body weight (FBW), per cent weight gain (WG), protein efficiency ratio (PER) and feed conversion ratio (FCR) were significantly affected among the experimental groups. SPM and HMM groups had significantly higher FBW, SGR, WG and PER values than the control - SM and EM groups. However, there was no significant difference between the control and EWM groups, and between SPM and HMM groups. FCR values showed a significant reverse trend in respect to WG. Incorporation of SPM and HMM in rohu diets gave greater growth performance and feed utilisation efficiency than SM and EWM-based diets.

KEY WORDS: Fish meal, isonitrogenous and isolipidic diets, growth performance

INTRODUCTION

India's thriving aquaculture and fisheries sectors are not only crucial for feeding the nation but also generate substantial export earnings and provide livelihoods for around 14 million people (FAO, 2020). The growth rate of capture-based fisheries in the world has been relatively static since the late 1980s and worldwide fish production reached its maximum potential (approximately 178 million tons) in the year 2020 (FAO, 2012, 2022). Under these circumstances, the aquaculture sector playing a pivotal role in nutritional security requires lower production costs, efficient production processes, and eco-friendly measures. Fish feed, one of the costliest inputs (accounting for 60 to 70%) of the operating costs in semi-intensive and intensive aquaculture (Singh *et al.,* 2006), needs to be cost-effective, eco-friendly, and nutritionally rich. High fish feed costs due to ingredient shortages threaten to stall fish production despite strong market demand. Replacing conventional protein sources

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with locally available, nutrient-rich options like silkworm pupae, maggot and earthworm meal offers a sustainable and affordable solution to bridge this gap and support future fish farming in line with UNO's Sustainable Development Goals (FAO, 2022). India's sericulture generates approximately 40,000 tons of silkworm pupae yearly, with research highlighting their potential as an aquafeed ingredient (Jayaram and Shetty, 1980). Gohl (1981) and Nandeesha *et al.* (1990, 2000) successfully included varying levels of silkworm meal in carp diets, achieving comparable growth and feed conversion to traditional fishmeal-based diets. Compared to plant-based protein, silkworm meal demonstrated superior performance in carps (Swamy and Devaraj, 1994). Borthakur *et al.* (1998) further supported this, finding similar digestibility of crude protein from silkworm meal compared to fishmeal. Housefly maggots, are emerging as a promising alternative protein source in aquaculture. Processed into "magmeal," these larvae boast high proteins (39 - 61.4% crude protein), lipids (12.5 - 21%), and essential nutrients like phosphorus, B-complex vitamins, and trace minerals (Teotia and Miller, 1973). Studies showed magmeal diets can match the growth performance of fishmeal in *Oreochromis niloticus* fingerlings, while costing significantly less (Fashina-Bombata and Balogun, 1997). This makes magmeal a competitive, sustainable, and potentially cost-effective alternative for fishmeal in aquafeed. Vermicompost, the product of earthworm digestion, serves as a direct organic fertilizer in fish ponds. Studies even suggested dried earthworms (*Eudrilus eugeniae*) as a viable fish meal substitute, boosting carp growth. Similarly, earthworm extract (vermiwash) significantly enhanced fish growth and survival, likely due to its rich vitamin content (provitamin D and B complex) and other beneficial metabolites (Chakrabarty, 2008). Moreover, worm biomass produced during vermicomposting can be directly in Moreover, worm biomass produced during vermicomposting can be directly incorporated into fish feed, further maximizing resource utilization (Tuan, 2010; Tacon *et al.,* 2011). Given the high cost of fish and soybean meal, this study analyzes alternative protein sources (*viz.,* silkworm pupae, housefly maggot, and earthworm meal) substituted fish feed replacing soybean meal on the growth performance of rohu fish *Labeo rohita* (Hamilton, 1822).

MATERIALS AND METHODS

The experiment was carried out at the M/S. Shah Ji Fish Farm (28.1705^0 N, 77.3182^o E), Palwal, Haryana. Four rohu fish feed diets were prepared; defatted soybean meal-based diet as control (SM) and three diets replacing defatted soybean meal with silkworm pupae (SPM), housefly maggot (HMM) and earthworm (EWM) meal (at 30% inclusion levels) separately; and their effect on rohu's growth performance was assessed under a completely randomized design experiment with three replications.

Different feed components (Table 1) were weighed and made into a dough by mixing all the previously dried ingredients (excluding oil and vitamin premix) using water. It was cooked for 30 minutes in a pressure cooker and cooled to room temperature. The calculated proportions of the vitamin premix and oil were then mixed. The dough was compressed through a pelletizer (S.B. Panchal and Company, Maharashtra, India) to obtain pellets of uniform size, allowed to air-dry, packed in zip lock pouches, sealed, and stored at -20 °C until further use. Water temperature and pH in all the experimental tubs were measured using a thermometer (MERCK, Germany) and a digital pH meter (LABINDIA). Dissolved oxygen, carbonate hardness, nitrite, nitrate, free carbon dioxide and ammonia were also analyzed (APHA, 1998).

Proximate composition analysis of the experimental diets *viz.,* estimation of moisture content using oven drying method, nitrogen concentration (crude protein - CP %) using semi-automated Kjeltec technique (Kjeltec Auto Distillation, Sweden), and fibre content using FibroTRON (Tulin equipments, India), and Total ash (TA) content using muffle furnace were done. Soxhlet apparatus was used to estimate crude fat or ether extract (EE) of experimental diets and ingredients using diethyl ether as the solvent.

The following formulae were used to calculate the digestible energy value (DE) and nitrogen free

extract (NFE) ----

DE (kcal/100 g) = [{NFE (%) × 4} + {EE (%) × 9} + {CP (%) × 4}]

NFE (%) =100 – (CP % + CF % + TA % + EE %)

The growth parameters of the experimental feed fed fish as listed below were analysed using samples drawn out at fortnightly intervals. Before being weighed, the fish were fasted overnight.

$$\text{Weight gain (WG\%)} = \frac{\text{Final body weight (FBW) - Initial body Weight (IBW)}}{\text{Initial body weight(IBW)}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e\text{(FBW) - Log}_e\text{(IBW)}}{\text{Experimental duration in days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \times 100$$

The fatty acid content of fish muscle (one sample per tank) was assessed using an Agilent 7820a Series gas chromatograph (Agilent Tech., USA). The fatty acid methyl ester synthesis and gas chromatograph analysis were carried out using the procedures published previously (Tian *et al*., 2014). Total lipids were extracted (Folch *et al*., 1957) from 0.3-0.5g samples in chloroform/methanol (2:1,v/v), filtered, and methanol-extracted. Hexane and potassium hydroxide methanol (0.4 M) were added for methyl esterification, and the upper layer was analyzed using a gas chromatograph with a capillary column and flame-ionization detector. The data represented individual methyl esters as a proportion of total identified fatty acids

The experimental data were subjected to one-way analysis of variance (ANOVA) using SPSS software for Windows (Version 22.0). Duncan's Multiple Range Test was followed for post hoc mean ($p<0.05$) comparisons.

RESULTS AND DISCUSSION

Analysed proximate composition values of all the ingredients used to formulate the experimental diets. Notably, the crude protein contents of the SPM, HMM and EWM were 44.24, 54.13, and 52.48 per cent, respectively; while the crude fat content was 25.32, 22.45 and 3.55 per cent respectively. Digestible energy was 4.87, 4.93, and 4.82 kcal/100 g, respectively, for SPM, HMM and EWM (Table 2). The experimental diets were found isonitrogenous and isolipidic with crude protein (35%) and crude lipid (6%) (Table 3). No significant ($p>0.05$) difference was observed among the water quality parameters during the entire study period (Table 4).

At the end of the experiment, SGR, FBW, FCR, WG and PER were found significantly influenced by the diets. WG, FBW, PER and SGR were significantly higher in SPM and HMM fed groups compared to SM and EWM fed groups. However, no significant ($p>0.05$) difference was found between the SM and EWM fed groups and between SPM and HMM fed groups. FCR values showed a significantly inverse trend, higher in the SM and EWM fed groups than the SPM and EWM fed groups (Table 5). The crude lipid, moisture and total ash content of *L. rohita* were not significantly influenced by the experimental groups. However, feeding insect meal-based diets to *L. rohita* showed significant ($p<0.05$) changes in crude protein and total carbohydrate content. Among the insect meal-based diets, the highest body crude protein deposition was observed in the EWM based diet, followed by the SPM based diet. However, the control diet did not show significant difference from

the SPM and EWM based diets. The total carbohydrate content was significantly higher in the SM and EM meal-based diets than in the SPM and HMM based diets (Table 6).

Fatty acid profile of the fish muscle: Among the muscle fatty acid compositions of different experimental diet fed fish, 16:00, 20:1 (n-9), 18:3 (n-3), and 22:6 (n-3) showed distinct differences. Besides, the sum of total saturated fatty acids was higher in the SM and SPM based diets than the EWM and HMM based diets. Furthermore, there wasn't any observable significant difference in the sum of mono-unsaturated and poly-unsaturated fatty acids (Table 7).

Dissolved oxygen, temperature, total hardness, nitrate, ammonia, pH, and nitrite were among the water quality indicators (Debnath *et al*., 2007; Mohapatra *et al*., 2012). The findings on these parameters implied that the water quality indicators had no effect on the assessed parameters across the treatment groups. The proximate composition values of all the feed ingredients assessed were found suitable for feed formulations and was supported by the previous studies (Aniebo *et al*., 2008; Salem *et al*., 2008; NRC, 2011; Mohanta *et al*., 2016). The nutritional contents of the diets were suitable for *L. rohita* and were prepared as per the nutritional requirement (Ngoc *et al*., 2016; Wang *et al*., 2017; Rahimnejad *et al*., 2019; Sahoo *et al*., 2020). In the present study, the overall growth performance of fish fed SPM based diet showed significantly better results than SM based diet, indicating that the SPM can be used as suitable alternative of the SM. Previously studies were mostly focused in common carp (Nandheesa *et al*., 1990, 2000) and rohu (Begum *et al*., 1994) fed with silkworm pupae meal (up to 50%) inclusion ratios instead of fish meal. According to Karthick Raja *et al*. (2019), WG in fish fed with diets replacing fishmeal with 40 or 50 per cent silkworm pupae was significantly lower than in fish fed with diets containing 30 per cent silkworm pupae. When the amount of silkworm pupae in the meal increased, the growth rate was considerably lowered. Similar results were reported by Salem *et al*. (2008) in Nile tilapia and Ji *et al*. (2015) in Jian carp. Further

Table 1. Diet formulations for *Labeo rohita*

Ingredients (%)	SM	SPM	HMM	EM
Insect meal	0	30	30	30
Defatted soybean meal	30	0	0	0
Groundnut oil cake	30	18	30	27
Mustard oil cake	22	8	7	26
De-oiled rice bran	10	23	20	10
Wheat flour	4.5	19.5	12	6
Sunflower oil	2.5	0.5	0	0
Vitamin-mineral mix	1	1	1	1
Total	100	100	100	100

SM control (30% soybean meal inclusion level),
SPM (30% silkworm pupae meal inclusion),
HMM (30% housefly maggot meal inclusion level)
EM (30% earthworm meal inclusion level)

research on fish revealed that higher insect meal substitutes or replacements simultaneously raised chitin levels and impacted lipid and protein digestibility (Kroeckel *et al*., 2012; Longvah *et al*., 2011). In connection to fish fed a control diet, common carp (Gangadhar *et al*., 2017), catla (Umalatha *et al*., 2018), and *Labeo firmbriatus* (Jayaram and Shetty, 1980) demonstrated greater protein digestibility when fish meal was replaced with 30 per cent non-deoiled silkworm pupae. These observations also support the better growth performance of fish fed with SPM based diet in the present study. Salem *et al*. (2008) reported that silkworm pupae meal can be used profitably in Nile tilapia instead of fish meal up to 66.66 per cent due to its favorable impacts on growth, protein efficiency, feed conversion, and economic efficiency. Khatun *et al*. (2005) reported better fish growth when fish meal was replaced with silkworm pupae meal (@ 6 to 8%). However, 25 per cent fish meal protein replacement with *Bombyx mori* in *Clarias gariepinus*, produced best results (Kurbanov *et al.,* 2015). Enzymatic hydrolysates of defatted silkworm pupa can substitute 50 per cent of the fish meal in juvenile mirror carp without having a deleterious impact on growth, according to Xu *et al*. (2018). Shakoori *et al*. (2016) findings

Table 2. Proximate composition of different feed ingredients (% dry matter) in the diets

Composition	Silkworm pupa	Housefly maggot	Earth worm	DSBM	GNOC	MOC	DORB	Wheat flour
Dry matter	93.12 ±0.63^{de}	94.45 ±0.47^{e}	94.63 ±0.58^{e}	90.99 ±0.73^{bc}	92.4 ±0.57^{cd}	91.61 ±0.48^{cd}	89.54 ±0.42^{ab}	88.76 ±0.79^{a}
Crude protein	44.24 ±0.32^{d}	54.13 ±0.4^{g}	52.48 ±0.25^{f}	48.59 ±0.65^{e}	49.21 ±0.74^{e}	39.86 ±0.71^{c}	17.23 ±0.24^{b}	11.7 ±0.16^{a}
Crude fat	25.32 ±0.37^{e}	22.45 ±0.45^{d}	3.55 ±0.12^{b}	1.23 ±0.10^{a}	1.19 ±0.15^{a}	7.47 ±0.27^{c}	1.82 ±0.09^{a}	1.16 ±0.05^{a}
Nitrogen free extract	11.78 ±1.37^{a}	10.26 ±1.36^{a}	18.77 ±0.88^{b}	40.75 ±1.11d	34.14 ±1.43^{c}	34.03 ±1.71^{c}	60.65 ±1.19^{e}	85.33 ±0.36^{f}
Crude fibre	4.56 ±0.17^{c}	6.63 ±0.19^{d}	13.84 ±0.09^{h}	3.67 ±0.16^{b}	9.62 ±0.23^{f}	10.98 ±0.21^{g}	8.43 ±0.25^{e}	1.25 ±0.11^{a}
Total ash	14.1 ±0.51^{e}	6.53 ±0.32^{bc}	11.36 ±0.42^{d}	5.76 ±0.21^{b}	5.84 ±0.31^{b}	7.66 ±0.53^{c}	11.87 ±0.61^{d}	0.56 ±0.04^{a}
Digestible energy	4.87 ±0.27^{c}	4.93 ±0.39^{c}	4.82 ±0.26^{c}	4.81 ±0.22^{c}	4.48 ±0.29b^{c}	4.16 ±0.41^{abc}	3.65 ±0.32^{ab}	3.44 ±0.26^{a}

All values are presented as mean of three. Abbreviation; DORB, Deoiled rice bran, MOC; Mustard oil cake, DSBM; Defatted soybean meal; GNOC, Groundnut oil cake. P= 0.01- 0.001; In a row means followed by different letters are significantly different by DMRT

on rainbow trout's flesh quality, growth, or survival revealed that the fish could be maintained for a period of 60 days while consuming silkworm pupae in proportions up to 10 per cent of fish meal. In *Litopenaeus vannamei*, a Pacific white prawn species, a diet replacing 75 per cent fish meal with silkworm pupae supported growth and immunological indices (Rahimnejad *et al*., 2019). While research on silkworm pupae is abundant, commercially produced and readily available housefly larval meal remains scarce, particularly in developing countries. This scarcity presents an opportunity to leverage the advantages of housefly larvae over earthworms in animal feed formulas, effectively addressing the limitations of the latter. In the present study, the housefly maggot meal-based diet showed a better growth performance than soybean meal and earthworm meal-based diet at 30 per cent inclusion level. This finding is in corroboration with the studies of Fasakin *et al.* (2003), and they observed no difference in growth or nutrient uptake for *C. gariepinus* fingerlings fed with diets containing either 32 per cent sun-dried or 27 per cent oven-dried housefly larvae meal. Similarly, Wang *et al.* (2017) suggested housefly larval meal as a favorable dietary component for Nile tilapia at levels up to 33 per cent. Ogunji *et al.* (2008) even reported no negative impact on growth or nutrient utilization in Nile tilapia fed with diets with up to 68 per cent housefly larval meal. Ngoc *et al*. (2016) found that common carp responded favorably to feeds containing earthworm meal as the predominant protein source, completely replacing fish meal in the diet. Popek *et al*. (1996) on *Carassius auratus* reached the lowest replacement level of 10 per cent, whereas Kostecka and Pczka (2006) on Guppy achieved the greatest replacement level of 100 per cent. However, Popek *et al*. (1996) found that 10 per cent *E. fetida* meal quadrupled the reproductive rate of *C. auratus*. Kostecka and Pczka (2006) discovered that replacing fish food with *E. fetida* meal resulted in enhanced survivability, improved reproduction, and increased biomass in aquarium fish, *P. reticulata*. Vodounnou *et al*. (2016) found that *Parachanna obscura* fingerlings experienced a high SGR of 2.11 g/day when fed with *E. fetida* meal. When *E. fetida* meals were present in amounts greater than 25 per cent, most studies found that fish growth was inhibited. These findings were attributed to the foul-smelling coelom fluid and indigestible chitin, which are known to impair palatability and

Table 3. Proximate composition of different experimental diets (n=6)

Composition	SM	SPM	HMM	EM
Moisture	9.76±0.27	8.84±0.45	9.25±0.19	9.63±0.23
Crude protein	35.17±0.35	35.21±0.29	35.48±0.33	35.24±0.36
Crude fat	6.04±0.19	6.37±0.13	6.22±0.2	6.30±0.18
Crude fibre	6.63±0.28	7.15±0.37	7.56±0.42	7.34±0.46
N free extract*	44.7±1.04	43.46±1.02	43.71±0.96	43.53±0.83
Total ash	7.46±0.14^{b}	7.81±0.19^{c}	7.03±0.09^{a}	7.59±0.17^{bc}

Nitrogen free extract; In a row means followed by different letters are significantly different by DMRT

Table 4. Quality parameters of water filled in experimental tanks for different diets

Parameters	SM	SPM	HMM	EM
Temperature (^{o}C)	27.58±0.29	27.86±0.34	27.48±0.34	27.44±0.35
pH	7.5±0.05	7.52±0.07	7.43±0.07	7.42±0.07
Dissolved O_2 (mg/L)	6.83±0.07	6.82±0.06	6.76±0.08	6.39±0.42
Free CO_2	ND	ND	ND	ND
Hardness (mg/L)	175.89±1.23	176.03±0.68	175.38±0.96	175.19±1.29
Alkalinity (mg/L)	129.54±1.5	128.53±1.64	127.64±1.45	128.25±1.15
Nitrite (mg/L)	0.028±0.001	0.027±0.002	0.027±0.002	0.028±0.002
Ammonia (mg/L)	0.054±0.002	0.044±0.003	0.047±0.003	0.05±0.003
Nitrate (mg/L)	0.024±0.002	0.023±0.001	0.024±0.001	0.025±0.001

All the values are Mean ± SE (n=15). P=0.105-0.943; ND - Not detected;
In a row means followed by different letters are significantly different by DMRT

digestibility (Dedeke *et al*., 2013). Similarly, in the current study, at EW showed significantly lower growth performance in comparison with SPM and HMM based diet. De Chaves *et al*. (2015) found that when *E. fetida* meal given, instead of fish meal, Nile tilapia and common carp experienced slower SGR. Silkworm pupae protein is abundant in critical amino acids such as methionine, phenylalanine, and valine. The essential amino acid content of SWP protein was compatible with the dietary requirements of fish (FAO, 2007). In the present study, the carcass CP content of fish fed with SM and SPM based diets did not differ significantly. Despite this, Salem *et al*. (2008) found no significant differences in the dry matter, CP, EE, and TA of Nile tilapia fed with diets with or without silkworm pupae meal. These findings are comparable with those of Nandeesha *et al*. (2000).

The effectiveness of substituting fish meal in fish diets varies substantially depending on substituting ingredient's nutritional value. According to, the CP content of housefly meal ranged from 42.3 to 60.4 per cent. Protein concentration varies according to the processes employed for processing, drying, storage, and protein measurement, as well as the media components utilized to create housefly maggots (Ogunji *et al*., 2008; Makkar *et al*., 2014).

This might explain why *L. rohita* CP levels were greater when fed with HMM based diet. Ogunji *et al*. (2008) and Idowu *et al*. (2003) found relatable results in *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Wang *et al*. (2017) discovered that a dietary housefly meal had no influence on the muscle proximate composition of Nile tilapia.

Earthworms have a high nutritional value, including lipids and protein, and have been reported as a feasible aquafeed component (Sogbesan and Ugwumba, 2008). According to Dong *et al*. (2010) and Tacon and Metian (2009), the protein content of earthworm meal is equivalent to that of fishmeal. In the current study, the total body protein content of soybean and earthworm meal-based diets was shown to be statistically similar. Pucher *et al*. (2014) discovered a substantial increase in protein content in common carp fed on Em based diet in place of a plant protein. However, there was no significant influence of dietary earthworm meal on the common carp's body proximate composition (Ngoc *et al*., 2016). Fatty acids, or lipids, are the primary form of stored energy in the body, which is crucial during normal cellular metabolic functioning and

Table 5. Nutrient utilization and growth of *Labeo rohita* fed with different diets

Parameters	SM	SPM	HMM	EM
Initial weight (g)	5.06±0.02	5.06±0.02	5.08±0.03	5.1±0.01
Final weight (g) (FBW)	14.58±0.1^{a}	17.21±0.66^{b}	17.8±0.54^{b}	14.4±0.13^{a}
Feed conversion ratio (FCR)	2.11±0.03^{b}	1.85±0.07^{a}	1.85±0.06^{a}	2.14±0.03^{b}
Protein efficiency ratio (PER)	1.35±0.02^{a}	1.54±0.06^{b}	1.54±0.05^{b}	1.33±0.02^{a}
Feed conversion efficiency (FCE)	0.47±0.01^{a}	0.54±0.02^{b}	0.54±0.02^{b}	0.47±0.01^{a}
Specific growth rate (SGR)	1.76±0.02^{a}	2.04±0.07^{b}	2.09±0.04^{b}	1.73±0.02^{a}
Weight gain percentage (WG%)	188.38±3.18^{a}	239.93±13.55^{b}	250.45±9.24^{b}	182.19±2.29^{a}

In a row means followed by different letters are significantly different by DMRT

Table 6. Proximate composition of *Labeo rohita* fed with different diets

Variables	SM	SPM	HMM	EWM
Moisture	74.18±0.2	74.2±0.15	74.15±0.5	74.03±0.16
Crude protein	15.89±0.06^{ab}	16.22±0.13^{b}	16.78±0.15^{c}	15.52±0.21^{a}
Crude lipid	3.66±0.24	3.72±0.03	3.67±0.27	4.06±0.12
Total carbohydrate	3.54±0.11^{b}	2.86±0.1^{a}	2.56±0.29^{a}	3.73±0.14^{b}
Total ash	2.74±0.09	3.01±0.07	2.84±0.12	2.67±0.18

In a row means followed by different letters are significantly different by DMRT

Table 7. Muscle fatty acid composition of *Labeo rohita* fed with different diets

Fatty acids	SM	SPM	HMM	EM
Lauric acid (12:0)	5.35±0.15	5.57±0.21	4.95±0.14	5.19±0.04
Myristic acid (14:0)	6.27±0.12	5.58±0.44	5.92±0.12	6.19±0.09
Pentadecylic acid (15:0)	2.28±0.12	2.4±0.07	2.51±0.18	2.16±0.07
Palmitic acid (16:0)	$11.34±0.15^{c}$	$11.05±0.18^{bc}$	$10.4±0.15^{a}$	$10.49±0.23^{ab}$
Palmitoleic Acid (16:1)	5.02±0.05	4.9±0.12	5±0.03	5.3±0.24
Margaric acid (17:0)	1.18±0.05	1.47±0.2	1.2±0.08	1.2±0.11
Stearic acid (18:0)	9.97±0.19	10.18±0.09	10.44±0.17	9.66±0.3
Oleic acid (18:1, n-9)	6.09±0.13	6.23±0.14	6.63±0.22	6.19±0.04
Linoleic acid (18:2, n-6)	4.2±0.1	4.18±0.18	4.37±0.2	3.74±0.25
Linolenic Acid (18:3, n-3)	$3.95±0.08^{b}$	$3.87±0.06^{b}$	$3.32±0.26^{a}$	$3.07±0.05^{a}$
Arachidic acid (20:0)	8.77±0.25	8.67±0.11	8.16±0.06	8.43±0.16
Gondoic acid (20:1, n-9)	$6.1±0.03^{a}$	$6.66±0.13^{b}$	$6.19±0.05^{a}$	$6.21±0.18^{a}$
Eicosadienoic acid (20:2, n-6)	2.05±0.12	2.26±0.08	2.16±0.02	2.1±0.07
Dihomo-ã-linolenic acid (20:3, n-6)	3.29±0.18	2.93±0.24	3.11±0.26	3.01±0.15
Mead acid (20:3, n-3)	3.11±0.02	3.33±0.16	3.38±0.12	3.42±0.23
Arachidonic Acid (20:4, n-6)	1.27±0.06	1.79±0.13	1.34±0.05	1.68±0.29
Eicosapentaenoic acid (20:5, n-3)	3.05±0.09	2.83±0.2	2.38±0.27	2.22±0.1
Docosahexaenoic acid (22:6, n-3)	$0.81±0.03^{a}$	$0.85±0.05^{a}$	$1.13±0.03^{b}$	$1.02±0.08^{b}$
Nervonic acid (24:1, n-9)	4.14±0.06	4.22±0.15	4.36±0.18	4.47±0.24
∑FA	95.44±0.17	96.2±0.41	93.78±1.12	92.32±1.5
Other	7.19±0.03	7.25±0.35	7.3±0.1	7.07±0.08
∑Saturated FA	$45.16±0.3^{b}$	$44.92±0.57^{b}$	$43.59±0.24^{a}$	$43.31±0.43^{a}$
∑Monounsaturated FA	28.55±0.15	29.24±0.2	29±0.54	28.75±0.63
∑Polyunsaturated	21.73±0.3	22.04±0.48	21.19±0.48	20.26±0.48
∑n-3	7.87±0.09	8.04±0.17	7.82±0.26	7.51±0.22
∑n-6	13.86±0.25	14±0.34	13.36±0.34	12.75±0.28
∑n-3/n-6	0.57±0.01	0.57±0.01	0.59±0.02	0.59±0.01

In a row means followed by different letters are significantly different by DMRT

starvation. In this experiment, the total saturated fatty acid content of SM and SPM diets was substantially greater than that of HMM and EWM. However, there are limited number of studies on the effect of feeding these insect- meal in rohu carp. Moreover, the studies of Cheng *et al*. (2017) and Feng *et al*. (2021) in silkworm pupae-based diets; Lin and Mui (2017) and Hashizume *et al*. (2019) in HMM; and Gunya *et al*. (2016) in EWM revealed that the fatty acid composition and their ratio greatly varies depending upon the ingredients that used to grow the insects. In the present study, insect meals were used in the replacement of SM which contains high amount of saturated fatty acid than fish meal (NRC, 2011). SPM and HMM in rohu diets gave superior performance in terms of growth and feed consumption efficacy and can be used in preparing cost-effective feed for rohu.

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Rice genotypes and the biochemical basis of resistance against brown planthopper, *Nilaparvata lugens* (Stål)

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ABSTRACT: Screening of 50 rice genotypes against brown planthopper infestation was conducted in open field conditions over two consecutive years (Kharif 2018-19 and 2019-20). Based on pest population per hill, rice genotypes IR82475-110-2-2-1-2, Akshyadhan, and MTU-1010 had the least brown planthoppers. TN1, Swarna, MTU 7029, Rajendra Kasturi, Baranideep, and Sahbhagidhan had the highest population and were classified as pest-prone. Rice leaf biochemical characteristics examined in selected genotypes, revealed that the pest population was significantly and positively correlated with total sugar (r = 0.608), crude protein (r = 0.306) and total free amino acid (r = 0.358), but significantly negatively correlated with phenol (r = -0.429), crude silica (r = -0.401), and tannin (r = -0.301). Correlation analysis revealed that susceptible entries contained more total sugar, crude protein, and total free amino acids, whereas resistant genotypes contained significantly more phenol, crude silica, and tannins. This study highlighted the significance of antixenotic properties in rice genotypes against brown planthoppers.

KEY WORDS: Screening, host plant resistance, bio-chemicals, antixenosis

INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family Poaceae, is an internationally vital cereal and a major food source, providing four-fifths of daily energy to more than half of the world's population (Sharma *et al*., 2019). Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera, Delphacidae), is an economically important pest (Sharma *et al.,* 2018). This phloem sap feeder transmits rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV), and has the potential to cause more than 60 per cent economic yield loss under favorable environmental conditions throughout Asia (Wei *et al*., 2019; Kanngan *et al*., 2023). Several chemical insecticides are registered to control rice BPH, but unscientific and injudicious use of these products breaks the natural pest-defender ratio in the field (Sarao and Mangat, 2014; Roy and Chakraborty, 2022). Host-plant resistance is an important factor in developing an integrated pest management system in low-input farming conditions, especially in India (Pal *et al.,* 2021). Insect resistant varieties/genotypes not only reduce

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the insect pest populations, but are also compatible with other methods of eco-friendly pest management (Rani *et al.,* 2020). In several Indian provinces where *N. lugens* outbreaks occur frequently (Andhra Pradesh, Odisha, Haryana, West Bengal, and Punjab), cultivation of susceptible varieties is the main cause of complete crop loss (Anant *et al.,* 2021). Thus, in order to generate promising cultivars that provide persistent and targeted resistance to BPH field populations, novel resistance mechanisms must be identified. Several biochemical constituents in the plant have been identified as causes of resistance. Direct defenses are mediated by plant characteristics have also been shown to reduce insect growth rates by impairing the digestibility and nutritional quality of tissues (Belete, 2018; Golla *et al.,* 2020). For this purpose, there is a continuous search for new resistant genes from diverse sources such as, landraces, wild relatives, induced mutants, and unrelated species. The present study involved systematic phenotyping of rice genotypes for BPH resistance over a two-year period in open field conditions. These studies will facilitate the use of the accessions for the development of rice varieties with durable resistance against BPH.

MATERIALS AND METHODS

Fifty rice genotypes were sown in nursery beds, including two susceptible checks (Swarna and TN1), and screened during Kharif 2018-19 and 2019-20 at the Agricultural Research Farm, Banaras Hindu University, Varanasi, to determine the response of tested genotypes to brown planthopper under open field conditions. All genotypes were obtained from the Department of Genetics and Plant Breeding at Banaras Hindu University. After the 21st day of sowing, the test genotypes were transplanted in three 2 metre rows with a 15cm distance between hills and a 20cm distance between two rows using a Randomised Block Design with three replications. The susceptible check variety was planted after every 30 rows of test genotypes/varieties. For two years, plants were grown under natural open field conditions with no pest protection measures in place.

The total number of brown planthoppers (both nymphs and adults) was counted on five randomly chosen hills from each genotype and variety. To count the number of BPH individuals, each hill was tilted and tapped twice or three times at the base, and the hoppers that fell into the water were counted. The obtained data were transformed appropriately and statistically analysed for interpretation. AICRIP provided a population-based rating for entries (2005). Standard protocols were used to evaluate biochemical constituents in rice leaves of all genotypes. During the crop's booting stage, leaf samples (Leaf blade of leaf sheath) were collected and brought to the laboratory to estimate total sugars, phenols, tannins, and total free amino acids using the methods of Bray and Thorpe (1954) and Moore and Stein (1948). Piper (1945) proposed methods for analysing crude protein, while Yoshida *et al.* (1959) estimated crude silica.

The data from the study of rice germplasm for resistance to brown planthoppers were statistically analysed using analysis of variance (ANOVA). At *p* d" 0.05, the least significant difference test was used to distinguish between the various treatment means. The mean values, ranges, and standard deviations of the previous data were also calculated. The data collected from several tests on biochemical parameters were examined using analysis of variance (ANOVA). Data were square root transformed prior to statistical analysis as needed. Tukey's HSD test (p d" 0.05) was used with SPSS software (version 27.0: Inc., Chicago, IL, USA) to determine the statistical significance of biochemical parameters among tested rice landraces. The relationship between several BPH resistance traits in the tested rice genotypes and varieties was established using SPSS software, pairwise correlation, and Pearson's correlation method.

RESULTS AND DISCUSSION

During 2018-19, all 50 rice genotypes showed significant variation in the mean number of brown planthopper population (nymph and adult) during the crop's vegetative and reproductive stages (Table 1). Anong the 50 rice genotypes, none of the genotypes was free of brown planthopper infestation, and the lowest population of planthoppers was recorded on IR82475-110-2-2-

1-2 (1.07 insects/ hill), which was statistically at par with Akshyadhan (1.67), MTU-1010 (2.12.), and IR-96248-16-3-3-2B (2.28.), followed by Pantdhan-12 (3.52), HUR-917 (3.81), BRRI Dhan-62 (4.05), IR_92978-192-1-2(CR-306) (4.14) and DDR-42 (4.50) which were classified as brown planthopper resistant genotypes. The highest population of brown planthoppers was found on the variety Swarna (35.14) which did not differ statistically from MTU 7029 (26.59), followed by Rajendra Kasturi (24.76) which together were classified as susceptible genotypes/varieties. The susceptible check variety, TN1, had the highest population of planthoppers (41.68) and was classified as a highly susceptible variety against the brown planthopper.

Similarly, in Kharif 2019-20, the mean number of brown planthoppers at the vegetative and reproductive stages differed significantly between the different genotypes (Table 1). The lowest population of planthoppers was recorded in IR82475-110-2-2-1-2 (1.55.), which was statistically comparable to Akshyadhan (1.80.), MTU-1010 (1.98 nos.), and IR-96248-16-3-3-2B (2.58), BRRI Dhan-62 (3.37), IR_92978-192-1-2(CR-306) (3.72), HUR-917 (4.12), DDR-42 (4.54) and classified as resistant genotypes against brown planthopper. On the other hands, the variety Swarna had the highest number of brown planthoppers (32.85), followed by MTU 7029 (27.25), Baranideep (25.34), Sahbhagidhan (25.24.), Sahbhagidhan (25.24) and Rajendra Kasturi (25.20) all of which were classified as susceptible genotypes/varieties, whereas susceptible check variety TN1 had the highest number of planthoppers (42.29 nos.) and was classified as a highly susceptible variety against brown planthopper. Slight variation in the insect infestation was observed during the Kharif 2019–20, which might be due to environmental factors.

The studies on rice resistance to BPH, have been carrying out since long. Various traditional and wild rice varieties were identified as one of the major sources of resistant donors against BPH through mass screening technique (Roy *et al.,* 2022). Subudhi *et al.* (2020), who tested 94 popular elite rice varieties for diverse ecologies in various states against BPH and discovered that eleven of these varieties were moderately resistant. Resistant varieties reported in this study included Balum-2, Megharice-3, Imp sabaramati, GR-7, Karjat-3, MTU 1061, MTU 1075, RTN-3, R-Suwasini, Pravat, and Santepal. Kakde and Patel (2018) tested 18 rice cultivars and reported that GR-104, GR-103, GR-102, and GR-101 were resistant to brown planthopper, Mashuri, IR-28, and GR-11 were classified as susceptible, and Jaya and Gurjari were found to be highly susceptible to brown planthopper infestation. Bhogadhi *et al.* (2015) tested 27 entries, including landraces and improved lines, for resistance to BPH biotype 4. In both field and seedbox screening, entries BM71, ACC5098, ACC2398, MTU1001, and Rathu Heenathi demonstrated resistance to BPH biotype 4. Roy *et al.* (2022) also screened 218 rice landraces in greenhouse and open-field conditions for three years in a row, identifying five landraces, RL4, RL27, RL35, RL42 and RL5 as resistant to BPH. Previous findings were not comparable to the current findings because the current study's screening used different rice genotypes that were not included in the previous research. The geographical variable has an effect on the population, particularly that of the brown planthoppers.

Plant biochemical traits such as total sugars, phenol, crude silica, crude protein, total free amino acid, and tannin content are also useful indicators of resistance/susceptibility of the test lines from germplasm pools (Panda and Khush, 1995). The total sugar content of leaf sheath of different genotypes at the maximum tillering stage of the crop was observed to range from 12.72 to 31.36 mg/g, with significant variability among the genotypes. Akshyadhan had the lowest total sugar content (12.72) and susceptible check TN1 had the highest total sugar content. The total phenol content in the selected rice genotypes at the maximum tillering stage was found to be in the range of 4.17-9.33 mg/100g, with significant variability between genotypes. The amount of total phenol was found to be highest in IR82475-110-2-2-1-2 (9.33), and lowest total phenol content was found in susceptible Sambha SUB-1 (4.17) and TN1 (4.27). Crude silica content in the selected rice genotypes ranged from

Table1 Field evaluation of rice genotypes against brown planthoppers infestation (Mean number per five hills) during Kharif 2018-19 and 2019-20 (Mean of values at vegetative and reproductive stages of the crop)

Genotypes	Kharif 2018-19	Kharif 2018-19	Resis-tance
IR82475-110-2 -2-1-2	1.07 (1.42)	1.55 (1.58)	R
Sahbhagidhan	23.22 (4.88)	25.24 (5.08)	S
PUSA Basmati-1	5.85 (2.60)	6.24 (2.67)	R
UGR-1	21.30 (4.64)	22.13 (4.75)	S
Bansphul	5.37 (2.51)	5.36 (2.52)	R
CGZR-1	22.62 (4.80)	22.22 (4.79)	S
IR 96248-16-3-3-2B	2.28 (1.80)	2.58 (1.89)	R
MTU-1010	2.12 (1.76)	1.98 (1.72)	R
Sathi	5.32 (2.51)	5.34 (2.51)	R
Pantdhan-12	3.52 (2.11)	4.55 (2.35)	R
Akshyadhan	1.67 (1.63)	1.80 (1.67)	R
NDR-359	5.30 (2.50)	6.09 (2.66)	R
Rajendra Kasturi	24.76 (5.02)	25.20 (5.06)	S
Baranideep	23.56 (4.93)	25.34 (5.09)	S
IR-92960-75-1-3	23.92 (4.90)	22.27 (4.73)	S
IR-92978-192-1-2 (CR-306)	4.14 (2.26)	3.72 (2.17)	R
BRRI Dhan-62	4.05 (2.25)	3.37 (2.09)	R
Sambha sub-1	21.50 (4.72)	22.32 (4.82)	S
MTU 7029	26.59 (5.20)	27.75 (5.30)	S
HUR-917	3.81 (2.19)	4.12 (2.26)	R
DDR-42	4.50 (2.34)	4.54 (2.35)	R
Swarna	35.14 (5.95)	32.85 (5.77)	S
TN1	41.68 (6.49)	42.29 (6.57)	HS
S.E. (m)±	0.14	0.12	-
C.D. at 5%	0.42	0.35	-
C.V. %	5.43	6.04	-

*Mean of three replications; **Figures in the parentheses are square root transformed values;Resistance – R- resistant; S - susceptible; HS – highly susceptible

7.06 to 13.87 percent, with significant variability between genotypes. The lowest percentage of silica was found in susceptible check TN-1 (7.06%). However, Akshyadhan (13.87%) was observed with the highest percent of crude silica content. The crude protein content was found to be between 2.16-7.67 mg/g and was significantly lower in the resistant genotype IR82475-110-2-2-1-2 (2.16 mg/g) and higher in the susceptible genotype IR-92960-75-1-3 (7.67 mg/g). Total free amino acids were found to be in the range of 13.02-26.95 mg/g, with the resistant genotype IR82475-110-2-2-1-2 having the lowest content (13.02), while the susceptible genotype Swarna had the highest amount (26.95). Similarly, the tannin content ranged from 0.41 to 5.21 mg/g and was significantly lower in the highly susceptible genotypes IR-92960-75-1-3 (0.41) than in the resistant genotypes Akshyadhan (5.21).

The planthopper population was significantly and positively correlated with total sugar ($r = 0.608$ $P < 0.001$), crude protein ($r = 0.306$ $P < 0.001$), and total free amino acid ($r = 0.358$ $P < 0.001$) (Table 2). The phenol, crude silica, and tannin content had a significant negative correlation with the brown planthopper population ($r = -0.429$, $r = -0.401$, $r = -0.301$, respectively, $p < 0.001$). As a result, total sugar, crude protein, and total free amino acids were found to be related to susceptibility to brown planthopper population because they favoured brown planthopper development and growth, whereas phenols, crude silica, and tannins content in leaves lowered brown planthopper infestation and were likely associated with resistance to brown planthopper in the test genotypes.

Many biochemical variables, such as total sugars, reducing sugars, total phenols, and silica, have been linked to insect resistance (War *et al.* 2012). Padhi (2004) and Chandramani *et al.* (2009) found that the susceptible check TN 1 had more total sugars than the resistant entries. Johnson (2009) reported that various entries with a higher concentration of phenolic compounds make the plant resistant. Rani *et al.* (2020) reported that the higher the sugar content, the higher the occurrence of insect pests, despite the fact that the silica content of vulnerable susceptible varieties such as TN1 and BPT5204, as well as the resistant genotypes C-1247 and C-8

Table 2 Correlation coefficient of brown planthopper population with biochemical constituents of rice genotypes

	BPH	TS	P	CS	CP	TFAA	T
BPH	1.000	0.608**	-0.429**	-0.401**	0.306**	0.358**	-0.301**
TS		1.000	-0.728**	-0.732**	0.413**	0.394**	-0.470**
P			1.000	0.767**	-0.602**	-0.583**	0.655**
CS				1.000	-0.745**	-0.685**	0.761**
CP					1.000	0.951**	-0.935**
TFAA						1.000	-0.944**
T							1.000

BPH - brown planthopper, TS - total sugar, P - phenol, CS - crude silica, CP - crude protein, TFAA - total free amino acid, T - tannins content.

**Significant at 0.01 level

588, were the highest. Similarly, Basanth *et al.* (2017) discovered that the resistant genotypes contained more phenol than the susceptible control variety TN 1. The susceptible genotypes had higher sugar content than the highly resistant genotypes. Likewise, Kumar *et al.* (2021) found that total and reducing sugars, free amino acids were higher in susceptible entries, while total phenols and tannins were significantly higher in resistant genotypes. The phenolic compounds and crude silica content are reported to be the feeding deterrents to BPH in rice and generally have a positive correlation with host plant resistance (Pati *et al.,* 2023). Enhanced silica content in plant defence against rice insect pests has been observed (Han *et al.*, 2015). Rizwan *et al.* (2022) also examined the role of silicon in rice insect pest resistance. These findings were consistent with the results of the current investigation. The resistant genotypes identified against insect pests indicate a reduction in protection costs while maintaining environmental sustainability. Furthermore, such genotypes should be used as donors in a hybridization programme to improve resistance to insect pests.

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Detoxifying enzyme profiles in pesticide tolerant strains of *Trichogramma chilonis* Ishii, a hymenopteran parasitoid

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ABSTRACT: Investigation on enzyme assays were carried out for carboxyl esterase (á and â esterase) and glutathione s-transferase in the susceptible and pesticide tolerant *Trichogramma chilonis* Ishii strains such as TCT1, TCT4, TCT5 and TCCb tolerant to endosulfan, spinosad, lamda-cyhalothrin and indoxycarb respectively. The electrophoretic profile of á-esterase enzyme in the susceptible *Trichogramma* strain showed 3 alleles in comparison with tolerant *Trichogramma* strains TcT5, TCT1, TCCb and TCT4 which depicted 5, 4, 3 and 2 alleles respectively. Similarly, â-esterases profile in the susceptible indicated 4 alleles compared to TCT5, TCT1, TCCb and TCT4 strains which showed 6, 5, 3 and 3 alleles respectively. The results of the quantitative analysis showed an increased enzyme activity in the tolerant strains. The increase in á-esterase activity in the tolerant strains TCT1, TCT4 and TCT5 was 2.07, 1.53 and 1.51 times more than the susceptible one and the mean difference was statistically significant and â-esterase activity in the tolerant strains TCT1, TCT4 and TCT5 was 1.41, 1.69 and 1.16 times more than the susceptible one. In the glutathione s-transferase enzyme activity, the mean value of the susceptible strain was 0.029µM and in tolerant strains, TCT1, TCT4 and TCT5 the values were 0.0243, 0.0289 and 0.023µM respectively indicating that the tolerant strains had lesser activity than the susceptible one. Such an increase in enzyme activity indicated the elevated production of detoxifying enzymes such as carboxylesterase to sustain the parasitoid in the field.

KEY WORDS: Carboxylesterase, glutathione s-transferase, electrophoretic profile, alleles

INTRODUCTION

Trichogramma parasitoids are one of the most important groups of biotic agents employed for the control of several lepidopterus pests in the agricultural field. It has been revealed that continuous exposure to insecticides can lead to increased tolerance and development of resistance by the insects (Ganesh *et al.,* 2002). Selection for insecticide resistance is greatly enhanced when an array of insecticides is widely used in agriculture. In this regard, the most important mechanism evolved by insects is the possession of detoxifying enzymes and the modification of the target sites of insecticide through mutation (Hemingway *et al*., 1998). Biochemical studies provide good evidence about the mechanisms involved in insect resistance development. A perusal of literature showed that

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three major enzyme groups namely esterases, glutathione s-transferase (GSTs) and monooxygenases are responsible for metabolically based resistance to organophosphates, organochlorins, carbamates and pyrethroids (Hemingway *et al*., 1998). Esterases are often involved in organophosphate, carbamate and to a lesser extent pyrethroid resistance. Further, GSTs are dimeric multifunctional enzymes that play a role in the detoxification of a large range of xenobiotics (Prapanthadara *et al.,* 1996). GST also give protection against pyrethroid toxicity in insects by sequestering the insecticide (Enayati *et al.,* 2005). The electrophoretic technique provides considerable promise in relating detoxifying enzymes to resistance and as a means of identifying resistant genotypes in vectors and pests. (Humerus *et al.,* 1990). It has also been possible to detect any genetic change arising through alteration in the nucleotide level because enzymes are direct products and any change or variation in the DNA level will be reflected in the proteins (Pasteur and Raymond, 1996). The isozyme polymorphism as evident from gel electrophoresis could be used as biochemical markers in studying the genetics of insecticide resistance in the absence of any visible markers (Chakraborty *et al.,* 1993). Research on the biochemical and genetics of insecticide resistance related to *Trichogramma* are rather limited regarding resistance development. Earlier isozyme studies are restricted to *Trichogramma* systematics (Coa *et al.,* 1986; Lu *et al*., 1988; Miura *et al.,* 1990; Pinto *et al.,* 1992; Pintureau, 1993a, b, 1999; Zhu *et al*., 2002; Summer *et al.,* 2008). In light of the above information, the present study on enzyme assay related to insecticide detoxification was carried out on qualitative analysis of á and â-esterases and quantitative analysis of á, â-esterases and GST in the pesticide susceptible and tolerant strains of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae).

MATERIALS AND METHODS

Pesticide susceptible and tolerant *Trichogramma* strains were obtained from the ICAR - National Bureau of Agriculturally Important Insects (NBAII), Bengaluru and maintained for up to three generations in the Department of Zoology, University of Mysore, Mysore, India where the present work was carried out. These strains were reared on *Corcyra cephalonica* eggs. The pesticide susceptible along with four tolerant *Trichogramma* strains namely TCT1, TCT4, TCT5 and TCCb tolerant to endosulfan, spinosad, lamda-cyhalothrin and indoxycarb respectively were employed for the investigations.

Qualitative enzyme assay: á-esterase and â-esterase were analysed to establish the differential isozyme profiles in the susceptible and pesticide-tolerant *Trichogramma* strains by polyacrylamide gel electrophoresis (PAGE). A small dual vertical slab (11x11cm) gel electrophoresis system (Broviga make, Chennai, India) was used. Gels of 0.7mm thickness were cast employing Teflon spacers with separating and stacking gels prepared (with 5% and 3.5% acrylamide respectively). Gel was run at a constant power supply (60V) for nearly four hours at 4°C. Twenty adult *Trichogramma* were homogenized in 25µl of 40 per cent sucrose solution in an eppendorf tube using a pestle. An equal volume (15µl) of supernatant was loaded to each well. Gels were transferred to the Petri dish containing the staining solution. Napthyl acetate was used as substrate and stained with fast blue BB salt. Gels were incubated at 37°C in the dark for 20 minutes.

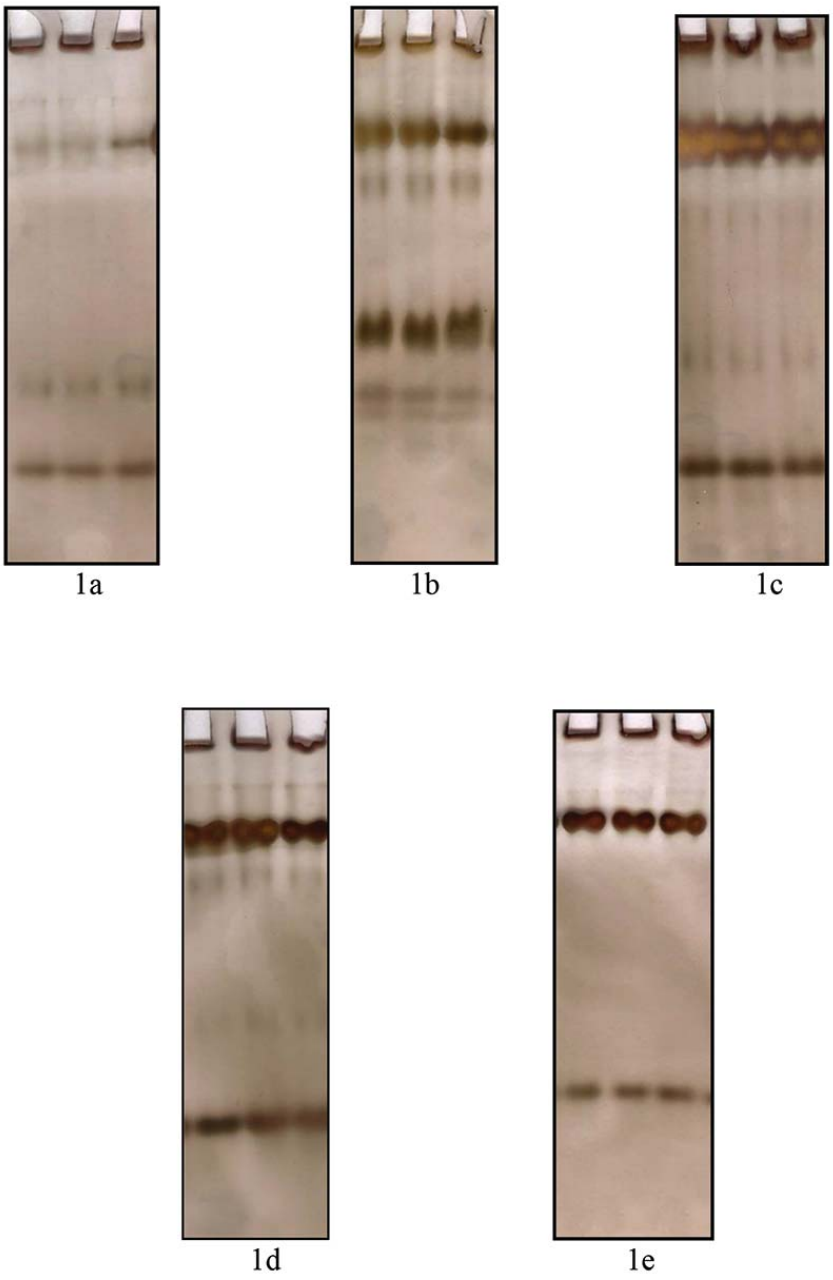
Quantitative enzyme assay: The assay was carried by using the microplate assay method for á-esterase, â- esterase and the spectroscopic method for GST enzyme (Hemingway *et al.,* 1998). Twenty adults *Trichogramma* were homogenised in 200µl of distilled water. Twenty µl of the supernatant was taken and made upto100µl by adding 0.1M KPO_4 buffer (pH 7.2). For establishing esterase activity 100µl of sample was loaded to the wells of the microplate and 100µl of á-naphthyl acetate solution for á-esterase and â-naphthyl acetate for â-esterase were added after 10min incubation in room temperature 100µl of dianisidine - fast blue BB solution was added to each well and kept for 5 min and was read at 620nm for alpha esterase and at 540nm for beta esterase. For GST one ml of sample,1ml of reduced glutathione and 1ml of 1-chloro 2,4-dinitrobenzene

was added and kept for 5 minutes and read at 340nm employing UV spectroscopy. The enzyme activity was reported as µmoles of product formed/minute/mg protein. The paired samples t-test at 0.05 level was employed to compare quantitative values of the enzyme between the susceptible and tolerant *Trichogramma* strains.

RESULTS AND DISCUSSION

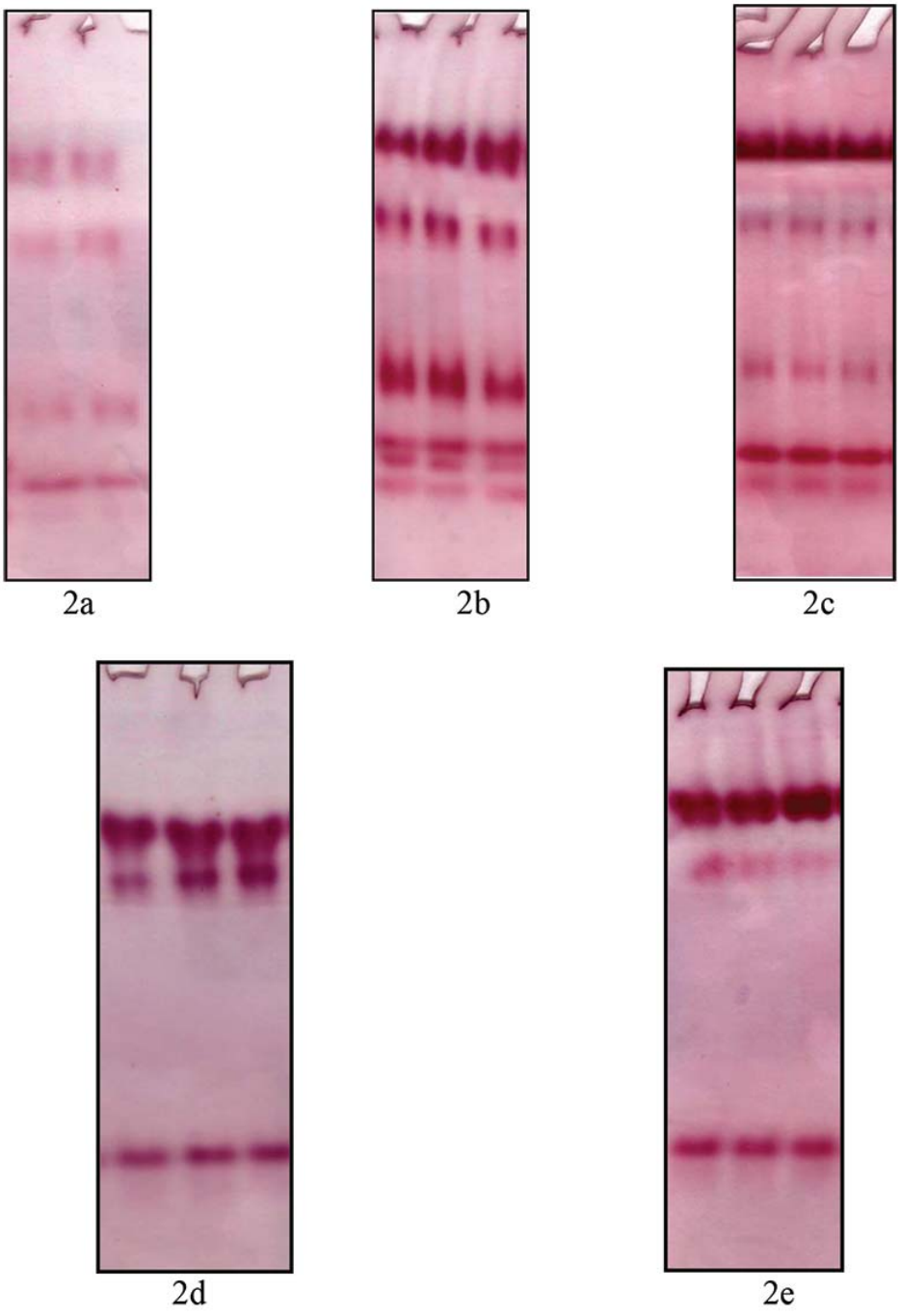
Qualitative enzyme analysis: In the electrophoretic profiles of á-esterase enzyme in the susceptible *Trichogramma* strain (Fig.1a) and in tolerant *Trichogramma* strains TCT5, TCT1, TCCb

Fig. 1 Isozyme profiles of A-esterase in *Trichogramma* strains



Figs. 1a; Susceptible, 1b; lamda cyhalothrin, 1c; endosulfan, d indoxycarb and 1e; spinosad tolerant strains

Fig. 2 Isozyme profiles of B-esterase in *Trichogramma* strains



Figs. 2a; Susceptible, 2b; lamda cyhalothrin, 2c; endosulfan, 2d indoxycarb and 2e; spinosad tolerant strains

Table 1, Allelic variation in the pesticide susceptible and tolerant *Trichogramma chilonis* strains

Enzymes	*Trichogramma chilonis* strains				
	Susceptible	TCT5	TCT1	TCCb	TCT4
No. of alleles	3	5	4	3	2
A – esterase (electromorph)	0.55 - 0.90	0.55 0.65 0.90	0.55 0.65 0.90	0.55 0.65 -	0.60 - -
Common band	1.00 -	1.00 1.03	1.00 -	1.00 -	1.00 -
No. of alleles	4	6	5	3	3
B – esterase (electromorph)	0.55 0.65 0.90 -	0.55 0.65 0.90 0.97	0.55 0.65 0.90 -	0.55 0.65 - -	0.55 0.65 - -
Common band	1.00 -	1.00 1.05	1.00 1.03	1.00 -	1.00 -

Table 2. Differential activity of three detoxifying enzymes in pesticide susceptible and tolerant *Trichogramma chilonis* strains (Mean ± SD)

Enzyme	Susceptible	Strains	Ratio
Á-esterase µmoles napthol produced/min/ mg/protein	0.0153±0.0006	TCT1- 0.0317±0.003*	2.07
		TCT4-0.0235±0.014*	1.53
		TCT5-0.0232±0.003*	1.51
B-esterase µmoles â naptholproduced/min/ mg/protein	0.0305±0.026	TCT1-0.0433±0.004*	1.41
		TCT4-0.0518±0.006*	1.69
		TCT5-0.0261±0.44*	1.16
GST µmoles glutathione produced/ min/mg/protein	0.029±0.0025	TCT1-0.0243±0.0025*	1.19
		TCT4-0.0283±0.003*	1.02
		TCT5-0.0230±0.004*	1.26

* t-value significant at $P<0.05$, n=30, df=29

and TCT4 (Fig.1b, c, d, e respectively), the most common allele is marked as 1.00 and other bands are labelled based on their electrophoretic mobility (Table 1). Two extra alleles in TCT5- lambda-cyhalothrin tolerant (Fig.1b) and one extra allele in TCT1- endosulfan tolerant strains (Fig.1c) were found in comparison with the susceptible strain. There was no extra allele in the TCCb- indoxycarb tolerant strain (Fig.1d) and one allele missing in the TCT4- spinosad tolerant strain (Fig.1e) in comparison with the susceptible strain. Similarly, the isozyme profiles of â-esterase in the susceptible *Trichogramma* strain (Fig. 2a) and tolerant stains TCT5, TCT1, TCCb and TCT4 (Fig. 2b, c, d, e respectively), showed two extra alleles and one extra allele in TCT5 (Fig. 2b) and TCT1 (Fig. 2c) respectively in comparison with susceptible and one allele was missing in TCCb (Fig. 2d) and TCT4 (Fig. 2e) strains.

Quantitative analysis: The mean value of á-esterase activity in susceptible strain was found to be 0.0153µM a-naphthol produced/min/mg protein while it was 0.0317µM in TCT1, 0.0235µM in TCT4 and 0.0232µM in TCT5. The increase in á -esterase activity in the tolerant strain TCT1, TCT4 and TCT5 was 2.07,1.53 and 1.51 times more than the susceptible one and the mean difference was statistically significant (Table 2). Similarly, â-esterase activity in the susceptible strain was 0.0305 µM and in tolerant strains, TCT1, TCT4 and TCT5 activity was found to be 0.0433, 0.0518 and 0.0261 µM respectively. The increase in â-esterase activity in the tolerant strains, TCT1, TCT4 and TCT5 was 1.41, 1.69 and 1.16 times more than the susceptible one. However, in TCT5 the mean value was less than that of the susceptible one which was significantly different. In the GST enzyme activity, the mean value of the susceptible strain was 0.029µM and in tolerant strains TCT1, TCT4 and TCT5 the mean activity values were 0.0243, 0.0289 and 0.023µM respectively which are significantly different. However, the tolerant strains had lesser activity than the susceptible ones.

Studies on insecticide resistance have indicated the specific relevance of esterase to xenobiotic metabolism in several insect species. However, such resistance studies to understand the mechanism in *Trichogramma* are rather limited. The isozyme analysis in *Trichogramma* was carried out to aid in taxonomic identification because of the minute size of the wasp (Coa *et al.,* 1986; Lu *et al*., 1988; Miura *et al.,* 1990; Pintureau, 1993a, b, 1999, Pinto *et al.,* 1992; Zhu *et al.,* 2008; Summer *et al.,* 2008). Esterase isozyme associated with insecticide resistance has been used extensively as a diagnostic tool. For example, elevated esterase banding patterns have been focused on resistant populations of western corn rootworms (Zhou *et al.,* 2002); *Anopheles stephensi* (Ganesh *et al.,* 2002); *Blattella germanica* (Scharf *et al.,* 1997) and *Myzus persicae* (Devonshire *et al.,* 1998). According to Karunarathnae and Hemingway (2001), metabolic resistance to organophosphate compounds in insects is mainly due to qualitative and quantitative differences in the carboxyl esterase. In the present study also qualitative and quantitative changes were characterized in tolerant strains. In the endosulfan (organophosphate) tolerant strain one extra allele and in lambda cyhalothrin (pyrethroid) tolerant strain two extra alleles indicate the elevated activity of this carboxyl esterase compared to the susceptible strains. The result obtained in the present study is in line with that of Ganesh *et al.,* 2002 in *Anopheles stephensi*. There was no extra allele in the indoxycarb tolerant strain and one allele is absent in the spinosad tolerant strain in comparison with the susceptible strain. Further, there was an increase in the intensity of the isozyme bands in indoxycarb tolerant strain. Hence the esterase isozyme may not play any role in the resistance development in the indoxycarb and Spinosad-tolerant strains. However other mechanisms may be involved in the resistance development here. It is also evident from the quantitative results that the activity of carboxyl esterase increased significantly in tolerant strains compared to the control batches. This is in agreement with the results obtained in mosquitoes (Ganesh *et al.,* 2002). However, the increased level of GST enzyme activity in susceptible compared with the tolerant strains is puzzling. Thus, the increased number of esterase enzyme alleles in endosulfan and lambda cyhalothrin tolerant strains

amply proves the onset of the development of resistance in these strains. Maintenance of these pesticide-tolerant *Trichogramma* strains and mass release in the crop fields will help the farmers in containing the pest population. Biological control agents such as *Trichogramma* are perceived to be slow acting in nature and are susceptible to pesticides and other abiotic stresses.

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Effect of photoperiod on the DNA, RNA and protein concentration in the silkgland of tasar silkworm, *Antheraea mylitta* (D) (Lepidoptera, Saturniidae)

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ABSTRACT: In *Antheraea mylitta* (D) (Lepidoptera, Saturniidae), the effect of photoperiod on total DNA, RNA, and protein concentration in the middle silk gland (MSG) and posterior silk gland (PSG) was studied. The photoperiod caused enhancement in the secretory activity and stimulates the sericogenesis process in MSG and PSG. The total nucleic acids and protein concentration of MSG and PSG under 24L:00D condition reached maximum level and then it decreased. Maximum nucleic acids and protein concentration were observed during long-day period and the minimum during short-day period. The photoperiod activated or inhibited the biological clock which acted upon the respective endocrine glands accordingly and control the process of sericogenesis. The larvae underwent significant changes with high DNA, RNA and protein concentration under 24L:00D, which is highly suitable for the silk protein synthesis. © 2024 Association for Advancement of Entomology

KEY WORDS: Sericogenesis, middle silk gland, posterior silk gland, biological clock

INTRODUCTION

Commercially there are four types of silks *viz.*, mulberry, tasar, eri and muga. India is unique in producing all these four varieties of silks. The mulberry silk produced in India, is recognized as one of the major one. Silk is produced from the silkglands which are nothing but the modified labial glands. The silkgland pass through four consecutive phases- the growth phase, the secretory phase, the regression phase, and the degeneration phase. The silkgland complex is well-developed in the last instar larva and differentiated into three regions: anterior, middle, and posterior. The lumen of anterior silkgland (ASG) is empty during the growth phase and filled with protein secretion in middle silk gland (MSG) and posterior silk gland (PSG) during the spinning period. Lumen transports silk secretion from MSG and PSG to the spinneret. As the MSG secretes sericin and the PSG secretes fibroin which later on modifies into true silk fibre oozing out from the spinneret at the time of cocoon spinning (Akai, 1965; Suzuki and Suzuki, 1974; Akai and Kataoka, 1978; Matsumura, 1980; Minagawa, 1980; Akai *et al.,* 1987; Sehnal and Akai, 1990; Motoyuki *et al*., 1993; Ishimuras and Numata, 1994; Minoura *et al*., 1995), these MSG and PSG were used for the estimation of DNA, RNA and protein concentration.

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Tasar silkworm *Antheraea mylitta* (D) (Lepidoptera, Saturniidae), contributes economically to the tasar culture in India (Jolly *et al.,* 1979). The development, cytological structure, and cyclical activity of the silkgland were previously studied (Barsagade and Tembhare, 2000). Further, research on the hormonal activity in relation to silk production, particularly secretion of sericotropic hormones had contributed to understanding medial neurosecretory cells of the insect brain (Tembhare and Barsagade, 2000). Endocrine and nervous factors are involved in insect circadian rhythm (Harker, 1960). The photoperiod acts directly on the brain and regulates the biological clock which determines various physiological and behavioural activities in most of the saturniid silkworms (Williams and Adkisson, 1964). The photoperiodic clock in various saturniid silkworms is known to be confined in the brain,which controls the release or inhibition of some brain neurohormones during diapauses, induction, and termination respectively (Williams, 1969). Jolly *et al.* (1970) studied the influence of temperature and photoperiod on termination of pupal diapauses in *A. mylitta*. The Medial Neurosecretory Cells in the brain also secrete the allatotropic hormone and prothoracicotropic hormone which stimulates the secretion of the juvenile hormone and ecdysone respectively. Prudhomme (1976) discussed the role of these hormones in the sericogenesis and silkgland degeneration in various species of silkworms. Unni and Pant (1985) studied the photoperiodic response on the silkgland of *P. ricini* (eri silkworm) during the fifth instar development and spinning period. The silk protein secretion is also known to be controlled by the brain neurohormone particularly the sericotropic hormone (Kodrik and Sehnal,1991: Tembhare and Barsagade, 2000). There is, however, no report on the effect of photoperiod on silk protein biosynthesis in *A. mylitta*. In India, the species, *A. mylitta* (Drury) occurs in 25 ecotypes or races (Jolly *et al*., 1979). Dabha race has been regularly cropped over the last 20 years at the Central Tasar Research and Training Institute (CTRTI), Basic Seed Multiplication and Training Centre (BSMTC) Dawadipar, Bhandara (Maharashtra State), India. The present research work on the effect of photoperiod on silkgland secretory activity in *A. mylitta*, is reported on the local 'Dabha Race' which is found in the forest zone of Madhya Pradesh, Bihar and Vidarbha Region of Maharashtra.

MATERIALS AND METHODS

The late fourth instar larvae were reared in a laboratory and were kept in specially prepared wire-grid cages (0.75x 0.5 x 0.5m) with top sliding glass covers. Their diet consisted of leaves of *Terminalia tomentosa*. The newly emerged fifth instar larvae were also reared similarly. To study the photoperiodic control of the secretory activity of the silkgland, the 10-15 day-old fifth instar larvae of *A. mylitta* were kept at various photoperiodic regimes such as-

1. 24L:00D (24h Light:00h Dark)
2. 15L:09D (15h Light:09h Dark)
3. 12L:12D (12h Light:12h Dark)
4. 09L:15D (09h Light:15h Dark) and
5. 00L:24D (00h Light:24h Dark)

During the experimental period, larvae were fed fresh leaves of *Terminalia tomentosa*. The cages were cleaned after every 6-hour. The larvae were sacrificed after 24-hours, and MSG and PSG were dissected out and the extracts were subjected to estimate the total concentration of DNA, RNA and protein by Burton's Diphenylamine technique, Dische-Orcinol technique (Lowry *et al.,*1951; Endo, 1970).

Silkgland DNA estimation: Live larvae that were dissected out in ice cold insect Ringer's solution and the silkglands were pulled out from the body. The tracheae and the adhering tissues were removed and the middle and posterior regions of the silkglands were separated. The silkglands were homogenized for 5 minutes at 0°C in different volumes of ice-cold distilled water, Ringer's solution using a pestle and mortar for nucleic acid and proteins respectively. DNA was estimated by Burton's Diphenylamine technique (Searcy and Maclnnis, 1970, 1970a). The standard DNA solution was prepared by dissolving 5mg of Standard DNA calf thymus in 5ml of distilled water(1mg/ml). The blank and unknown tube contains 2ml of water and 2ml of tissue extract respectively. Now 4ml of

Dipheylamine reagent was added to all the tubes. All the tubes were kept in boiling water bath for 10 minutes. The color intensity was observed at 500 nm on the spectrophotometer. The standard calibration curve was prepared with known tubes of standard DNA calf thymus and from this, the actual amount of DNA was determined from the extracted sample.

DNA concentration = 50µg/ml x OD_{500} x Dilution Factor (50)

Where, OD = Optical Density of sample

Silkgland RNA estimation: The silkgland RNA was estimated by Dische-Orcinol technique (Endo, 1970). The standard RNA solution was prepared by dissolving 1mg of commercial yeast RNA in 6ml of distilled water. The solution was assisted by adding 0.1N NaoH (0.166 mg/ml). The blank and unknown tube contains 3ml of distilled water and 3ml of tissue extract respectively. Now 6 ml of acid-Orcinol reagent and 0.4 ml of Orcinol-alcohol reagent was added to all the tubes. All the tubes were kept in boiling water bath for 20 minutes. The colour intensity was observed at 660nm on the spectrophotometer (Milton Roy). The standard calibration curve was prepared with four known tubes of yeast RNA and from this, the actual amount of RNA was determined from the extracted sample.

RNA concentration = 50µg/ml x OD_{660} x Dilution Factor (50)

Where, OD = Optical Density of sample

Silkgland protein estimation: The protein was estimated by Lowry *et al.* method (1951). The standard protein solution was prepared by dissolving 5mg of standard protein bovine serum albumin in 1ml of distilled water. The blank and unknown tube contains 4 ml of distilled water and 4ml of tissue extract respectively. To each test tube, 5.5 ml of reagent C (50ml of 2% sodium carbonate in 0.1N NaoH + 1ml of 0.5% copper sulfate solution in 1% sodium potassium tartarate solution) was added which was kept undisturbed for 10-15min. Then to each test tube, 0.5 ml of Folin-Ciocaltteau reagent with an equal amount of water was added with vigorous shaking and was kept undisturbed for 30 minutes. The colour intensity with blue colour was observed at 650 nm on the spectrophotometer, (Milton Roy). The standard graph was drawn with four known tubes of bovine serum albumin, and from this, the actual amount of protein was determined from the extracted sample.

$\frac{\text{Unknown}}{\text{Standard}}_{x}$ 0.2 x100= mg/ml

Where, Unknown = Optical Density of sample

Standard = Optical Density of standard protein

0.2 = Protein in standard

100 = Dilution Factor

Data were statistically analyzed according to Daniel (2000) and standard deviation, standard error, student-t test, and P-values of the data were derived.

RESULTS AND DISCUSSION

The present studies undertaken to determine the effect of photoperiod on the secretory activity of the silkglands in *A.mylitta,* showed maximum concentration of nucleic acids and proteins, when exposed to maximum day light as compared to night, indicating that the sericin and fibroin in MSG and PSG respectively increased in daylight with maximum photoperiod.

Total DNA concentration: There was a significant gradual decrease in the level of total DNA concentration in MSG. In 24h light condition it measured at 8.00±0.008µg/mg, but it declined gradually to 6.00±0.084, 5.00±0.02 and 1.5±0.14µg/mg respectively in the photoperiods of 15, 12 and 9h light. The concentration decreased to 0.5± 0.1 µg/mg in total dark period. In PSG the total DNA concentration measured at 10.9± 0.008µg/mg in 24h light period. It gradually decreased in the photoperiods of 15, 12 and 9h light to (10.4± 0.084, 10.4±0.02 and 3.00 ±0.14µg/mg respectively). The total DNA concentration was low (2.5 ±0.1 µg/mg in the total dark period (Table 1).

Total RNA concentration: In MSG total RNA concentration was 1.8 ±0.16µg/mg in 24h light condition. It declined in the photoperiods of 15h, 12

Table 1. DNA concentration(µg/mg) at various photoperiods in silk gland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	8.00±0.008*	10.9± 0.008*
15L:09D	8.00 am	6.00± 0.084*	10.4± 0.084*
12L:12D	12.00 pm	5.00± 0.02**	10.4± 0.02**
09L:15D	8.00 am	1.5 ±0.14	3.00 ±0.14
00L:24D	2.00 pm	0.5± 0.1*	2.5 ±0.1*

Each value represents total of one larva, four replicates ± standard error of means (SEM), Significance *P<0.05, **P<0.01.

Table 2. RNA concentration(µg/mg) at various photoperiods in silkgland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	1.8 ±0.16	2.1± 0.16
15L:09D	8.00 am	1.7 ±0.11	2.0± 0.11
12L:12D	12.00 pm	1.6 ±0.141	1.8 ±0.141
09L:15D	8.00 am	0.9 ±0.081*	1.5 ±0.081*
00L:24D	2.00 pm	0.4± 0.2	0.52± 0.2

Each value represents total of one larva, four replicates ± standard error of means (SEM), Significance *P<0.05.

and 9h light (1.7 ±0.11, 1.6 ±0.141 and 0.9 ±0.081µg/mg respectively) and it was low in total dark period (0.4± 0.2µg/mg). RNA concentration in PSG in 24h light period was 2.1± 0.16µg/mg. It was 2.0± 0.11, 1.8 ±0.141 and 1.5 ±0.081µg/mg respectively at 15, 12 and 9h light. It was very low (0.52± 0.2µg/mg) in total dark period (Table 2).

Total protein concentration: In MSG the total protein concentration measured 6.1 ±0.002µg/mg in 24-hour light condition. It declined gradually in the photoperiods of 15, 12 and 9h light and measured 5.5±0.0031, 4.2±0.0025 and 3.0±0.005µg/mg respectively. The concentration decreased to 3.0±0.0058µg/mg in the total dark period. Total protein concentration in PSG in 24h light measured 27.0± 0.002µg/mg. But it low in of 15, 12 and 9h light to 25.0±0.0031, 23.0±0.0025 and 20.0±0.005µg/mg respectively. The concentration decreased to16.9 ±0.0058 µg/mg in total dark period (Table3).

In *A. mylitta* the total protein concentration in MSG and PSG under 24L: 00D (total light photoperiod) condition reached to maximum level along with the total DNA and RNA concentration suggesting that the total light condition is highly suitable for the silk protein synthesis. DNA, RNA and protein concentration in MSG and PSG gradually decreased in 15L:09D (long day photoperiod) and 12L:12D (normal-day photoperiod) conditions and reached the minimum in 09L:15D (short-day photoperiod) and 00L:24D (total dark photoperiod) conditions suggesting that upto 12L:12D photoperiod regime, the MSG and PSG are quite efficient in protein secretion but their activity is declined greatly during the low photoperiod *i.e,* 09L:15D and 00L:24D.

In *A. mylitta* it is well-established that the photoperiod induced and terminated the diapause at the early pupal stage of the third generation (during December - January and June - July respectively). Besides the diapauses, the photoperiod is also known to determine a time regime during the day cycle for the moth-emergence, male-female coupling, egg-laying, and egg-hatching (Jolly *et al.,* 1971, 1979). Unni and Pant (1985) studied the photoperiodic response on the silkgland of *P. ricini* at the time of development of the 5th instar and period of spinning. The photoperiodic effect on larval-pupal characters, fat body, nucleic acids and protein of silkworm *Bombyx mori* L. showed that the nucleic acids and protein contents increased at 24L over other photic regimes (Janarthanan *et al.,* 1994). MSG and PSG are quite efficient in nucleic acid and protein secretion in high photoperiod but their activity declines greatly during the low photoperiod. Studies also shows that long photoperiod promotes and short photoperiod delays development of the silkgland. The study also shows that long day photoperiod promotes the better silk production (Zothanmawii *et al*., 2017). Shewale *et al*. (2019) showed that larval weight, silkgland weight increases in both M5 and V1 mulberry varierty fed larvae when exposed to 18hrs light and decreases when exposed to 18hrs dark. The larvae were exposed to the electric bulb and the cages were covered with black cloth to study the nucleic acids and protein to the light and dark conditions respectively.

Table 3.Protein concentration (µg/mg) at various photoperiods in silkgland

Photoperiod	Hours	MSG	PSG
24L:00D	2.00 pm	6.1 ±0.002*	27.0± 0.002*
15L:09D	8.00 am	5.5± 0.0031*	25.0 ±0.0031*
12L:12D	12.00 pm	4.2± 0.0025*	23.0± 0.0025*
09L:15D	8.00 am	3.0± 0.005**	20.0 ±0.005**
00L:24D	2.00 pm	3.0± 0.0058**	16.9 ±0.0058**

Each value represents the total of one larva, four replicates ± standard error of means (SEM), *P<0.001, **P<0.002.

The effect of photoperiod impact on diapause in saturniid silkworm pupae was however extensively studied (Tanaka, 1951; Williams, 1969; Takada *et al*., 1997). In saturniid silkworms it is now well-established that the photoperiod acts directly on the brain and regulates the biological clock which determines various physiological and behavioral activities (Williams and Adkission, 1964). The present study showed that the photoperiod activated or inhibited the biological clock which acts upon the respective endocrine glands accordingly and controls the process of sericogenesis in *A. mylitta,* causing enhancement in the secretory activity. According to Saunders (1976), the rhythms are used by the organisms to measure the passage of time and establish a biological clock to perform various physiological activities. The 24h light-dark cycle initiates daily rhythms, influencing greatly physiological activities, behavior, body temperature, and hormone production in many vertebrate and invertebrate species (Binkley, 1993; McEachron and Schull, 1993).

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Effect of ethanolic plant extractives on cephalic neuroendocrine system of BmNPV inoculated 5th instar larvae of *Bombyx mori* L. (Lepidoptera, Bombycidae)

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ABSTRACT: In this research 3000, 5000 and 8000ppm concentration of ethanolic extractives of leaves of *Eupatorium odaratum*, *Hyptis suaveolens* and fruits of *Aegle marmelos* were tested on mulberry silkworm fifth instar larvae of PMxCSR2 inoculated with *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV). Extractives of *A. marmelos*, *H. suaveolens* and *E. odaratum* showed promising results against BmNPV of silkworm larvae @8000 ppm. Total improvement occurs in the function of neurosecretory cells (NSC), A1 and A2 cells of median neurosecretory cells (MNC) group. Also found that, NCC I and II, corpus cardiacum (CC) lobes and fine branching of NCA I over corpus allatum (CA) showed strongly stained PF positive granules of NSM due to the subsequent treatment of given ethanolic plant extractives which reduced BmNPV infection about (30 - 40%). © 2024 Association for Advancement of Entomology

KEYWORDS: PMxCSR2, silkworm larvae, *Eupatorium odaratum, Hyptis suaveolens, Aegle marmelos,* neurosecretory cells

INTRODUCTION

Sericulture is agro-cottage based industry. *Bombyx mori* L. (Lepidoptera, Bombycidae), silkworm is susceptible to various diseases causing by viruses and bacteria. Grasserie is one of the important diseases of silkworm due to infection by *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV) which causes more than 35-50 per cent loss (Chitra *et al.,* 1975; Samson, 1992; Ranjith kumar *et al,* 2022). BmNPV infected larvae become restless, crawl on the edges of the rearing trays and exuding white body fluid through skin wounds (Aruga, 1994). The endocrine system regulates the physiological, developmental, reproductive and a behavioural activity in insect has been explored to a great extent (Wigglesworth, 1965; Engelmann, 1970; Gilbert and Kings, 1973; Tembhare, 1988; Bauah and Chinmoyee Kalita, 2020). Fukuda (1940) elucidated the role of prothoracic gland and showed that when both the corpus allatum and prothoracic gland are active, larval ecdysis takes place. When corpus allatum loses its activity, prothoracic gland alone induced active pupation. Fukuda (1944) reported that the brain controls the secretion of sub oesophageal ganglion in the pupal stage, so brain oesophageal ganglion system played an important role in regulating diapause behaviour of eggs in *B. mori*. It is clear that the brains, corpus cardiacum, corpus allatum, sub-oesophageal ganglion are

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important cephalic endocrine organ of the insect. The groups of neurosecretory cells are present in the medial and lateral region of the brain. In *B. mori* they are located in the groups namely two median and two lateral (Kobaayshi, 1957; Tembhare and Barsagade, 2000). As plants contain active secondary metabolites which act as insect growth regulators, phagostimulant, JH analogues, pesticides and antimicrobial agents, the ethanolic extractives of leaves of *Eupatorium odaratum*, *Hyptis suaveolens* and fruits of *Aegle marmelos* were tested on mulberry silkworm fifth instar inoculated with BmNPV.

MATERIALS AND METHODS

Disease free laying of the silkworm $PMxCSR_2$ was incubated and the larvae were reared as per the recommended regimen of Krishnaswami *et al.* (1978, 1979). Appropriate modifications in the rearing techniques were made to suit the local conditions.

Isolation, Purification and Inoculation of BmNPV: During the regular rearing of cross breed (PMxCSR2) race of silkworm, natural infection of grasserie diseases were occurred. The pathogen BmNPV infection was in fourth and fifth instars. The larvae with typical symptoms of grasserie infection [intersegment swelling and look like a 'bamboo like appearance', inactiveness, shiny and translucent skin, bursting body wall, oozing white turbid haemolymph and worms crawling on the edges of the rearing trays], were isolated and individually checked for virus infection by preparing smear for microscope observation and turbidity test. The histological preparation of midgut and salivary glands were stained with Azan stain (Mallory Hiedenhains, 1938) to check the infection of the viral bodies in these tissues. The BmNPV infected fifth instar larvae were collected and Polyhedron Inclusion Bodies (PIBs) obtained directly from the haemolymph of infected larvae to get stock of BmNPV in sterilized double distilled water. The isolated PIBs were purified by repeatedly centrifugation process. The isolated PIBs re-suspended in the sterile distilled water and centrifuged at 5000 rpm for 10 minutes. The centrifugation process was repeated for three times so as to obtained whitish residues of PIBs at the bottom of centrifugation tubes. Then the PIBs were washed in sterilized double distilled water and stored in refrigerator at 4°C until their use.

Inoculation and determination of LC_{50}: Just after completion of fourth moult, about 500 larvae were starved for 6 hours and divided into 10 groups containing 50 larvae each. Each larva from all groups was fed with the piece of mulberry ($1cm^2$) smeared with 10 ìl of each dilution of PIBs suspension. A group of larvae was kept as normal control in which the larvae were fed with mulberry leaves smeared with distilled water and air-dried. Then all the larvae from all the groups were fed on fresh mulberry leaves for a period of six days. The larvae from each group were observed daily and mortality due to BmNPV infection was recorded. The LC_{50} dose was calculated from the observed mortality due to BmNPV infection [LC_{50} = log dilution above 50% + PD x dilution factor]. The observed LC_{50} value 1.413 PIBs/ larva at 10^5 concentrations was used for inoculating the larvae in the further experiments.

Preparation of ethanolic extractives: Leaves of *E. odaratum* and *H. suaveolens* and fruits *of A. marmelos* were collected dried at room temperature and prepared in fine powder. Fine powder of 50g each was macerated separately with 500ml of ethanol. Extracts were evaporated by using speed vacuum evaporator to obtain a thick paste like extracts. These extracts were collected by spatula in glass bottle. The crude extracts (condensed product) were weighed and kept at 4°C prior to test. For the preliminary testing for antimicrobial activity, the stock extracts of these plant parts were dissolved in warm sterile distilled water (40°C) to make a concentration of 3000, 5000 and 8000 ppm. Newly moulted 500 - 600 fifth instar larvae of $PMxCSR_2$ were starved six hours and divided in to 11 groups, each group containing 50 larvae. Except normal groups the larvae of remaining groups were fed with the piece of mulberry leaf ($1cm^2$) coated smeared with 10 µl of $1x10^5$/ml dilution of PIBs suspension of BmNPV. After inoculation, all the groups were fed with

mulberry leaves, which were dipped in 3000, 5000 and 8000ppm concentration of the ethanolic extractives. One group was kept as inoculated control in which the larvae were fed with mulberry leaves smeared with distilled water instead of plant extractives, another control reared on fresh mulberry leaves without any application and it is served as normal control. The treatments were given before morning feeding (9.00am) once in a day. For the studying the effect of BmNPV and the subsequent application of ethanolic plant extractives on the neuroendocrine system of silkworm race multivoltine cross breed PMxCSR2 were utilized. After following the usual mode of inoculation and subsequent application of plant extractives, the larvae from the untreated control (normal control), inoculated control, and the treated groups were used for the preparation of their cephalic neuroendocrine complexes. At least five larvae from each group were dissected for obtaining their neuroendocrine complexes under stereoscopic binocular microscope in the cold insect ringer solution, then the neuroendocrine complexes fixed in a aqueous Bouin's fixative for 24 hours, then they were transferred in 70 per cent alcohol for the removal of yellow colour of Bouin's fluid by giving 4-5 changes of 70 per cent alcohol even after this, if the yellow colour of picric acid persist in the tissue was removed by giving treatment of lithium carbonate in 70 per cent alcohol. After removal of yellow colour, tissues were hydrated by passing through graded series of alcohol, which were utilized in whole mount preparation by using aldehyde fuschin stain (PF) as per the methodology described by Dogra and Tondon (1994). The stained whole mounts observed under the microscope. Observation on the neurosecretory 'A1' and 'A2' cells of MNC groups regarding their staining intensity, size was scored according to the following criteria - unstained stain, slightly stained, moderately stained cell and strongly stained cells. The secretary activity of 'A1' and 'A2' cells and nuclear diameter of these cells and the presence of variable quality of PF positive granules in them (Highnam *et al.,* 1969). The size of the CA was also measured for determining their activity in all the groups during study.

RESULTS AND DISCUSSION

Anatomy of cephalic neuroendocrine complex of 5th instar larva in both the races of *B. mori* showed typically lepidopteran type which comprises of neurosecretory cells of brain, paired corpora cardiaca, paired corpora allata (CA) and aorta (Plate I, Figs. 1, 2). In the 5th instar silkworms, the brain is well distinct bilobed structure situated middorsally in the head capsule, which was connected to the sub oesophageal ganglion by a pair of circum oesophageal connectives. The corpora cardiaca (CC) are the paired elongated structure connected posteriorly to the brain by a pair of nerve corporis cardiaci I (NCC-I) and II and the CA are also paired elongated oval structure which are joined to the lower end of the CC of their respective side by single nervi corporis allati. Thus, corpora cardiaca-allata complex is situated laterally to the oesophagus. Aorta with its cephalic ends terminates behind the brain, which is mid dorsal to the oesophagus. In whole mount by using aldehyde fuschin, staining techniques only PF + ve A type of neurosecretory cells (NSC) are stained and they are seen in the 4 distinct neurosecretory cell groups. All these groups are in paired the A-cells can be further classified into subtypes namingly larger A_1 type and small A_2. The four pairs of NSC groups are named according to their position in the brain tissue (Plate I, Figs. 3):

1. Paired median neurosecretory cells (MNC) (Plate I, Figs. 1, 2)
2. Paired lateral neurosecretory cells (LNC) (Plate I, Figs.1, 2)
3. Paired anterior neurosecretory cells (PANC).
4. Paired posterior NSC groups (PNC).

MNC occupies antero-middorsal region of pars intercerebralis of therotocerebrum of either side of the medial line in each hemisphere of brain. Each group consists of four large A_1 cells measuring 14.12 ± 1.07µm in PM x CSR2 with centrally placed nucleus measuring 7.3± 0.75µm in PM x CSR2. Each MNC group also contains 5 to 6 A_2

cells, which are smaller containing PF + ve granules in their pericaria. These cells measure 11.00 ± 0.98ìm in PM x CSR2 with centrally placed nucleus measuring 5.97 ± 0.45 in PM x CSR2 (Plate I, Figs. 1, 2; Plate II, Figs. 9, 11). The lateral neurosecretory cell group also known as cerebropleural NSC groups located anterior lateral region of protocerebrum lying between the pars intercerebralis and corpora pedunculata. Each lateral neurosecretory cells (LNC) group consists of 5 to 6 A_2 type of cells only and possible number PF –ve (B type) cells (Plate I, Fig. 2, Plate II, Figs. 2, 8). Each anterior group consists of two A_2 types of cells and the posterior group has 3 to 4 A_2 type of cells. PF –ve B cells could not be observed in the whole mount preparation (Plate I, Fig. 3). The axonic pathways of MNC group cross over each other in the centre of the brain and they joined to the axonic pathway of LNC group of the opposite side which emerges from posterior as NCC 1. Axonic pathway of posterior group emerges laterally as NCC-II. The axonic pathway for anterior group could not observe in the present preparation (Plate I, Fig. 3; Plate II, Figs. 5, 7.). NCC I and II enters into to the corpora cardiaca of their sides which do not acts as neurohaemal organ. Axon of the NCC I and II from central axonic core in the corpora cardiaca and emerging from the posterior end as nervi corporis allata- I which forms the fine branching over the anterior surface of the corpus allatum through which NSM is seen to be released in the form of neurosecretory granules in the surrounding haemolymph. (Plate I, Fig.1, Plate II, Figs. 2, 5, 7, 10). Corpora cardiaca paired elongated structure situated on each side of oesophagus covered externally with connective tissue sheath. The centre of each CC lobe is occupied by central axonic core made from NCC I and II peripherally intrinsic and extrinsic secretary cells are present which cannot be stained in whole mount preparation. In the lumen corpora cardiaca NSM in the form of granules is observed (Plate II, Figs. 5, 7, 14). CA is paired elongated oval structure made up from 18 to 26 oval secretary cells, which shows cyclic secretary activity. Externally each CA lobe is covered with connective tissue peritoneal sheath and it is connected to the lower end of which fine axonic barbarization is seen in which granule neurosecretory material is observed (Plate I, Fig. 1; Plate II, Fig. 5 and 7). Somewhat similar findings were reported by Tembhare and Barsagade (2000).

Effect of BmNPV and subsequent treatment of the ethanolic extractives on 5th instar: The infection of BmNPV on the 5th instar produced the pathogenic condition causing severe damage to each and every internal tissue including body wall. The effects infection noted after 3-4 days. Free matured polyhedra were seen in the central nervous system which probably came from the innervating trachea to the central nervous system. Hence tracheal innervation was the possible source for the infection of CNS by BmNPV. Similar observations were reported by Torquaito *et al.* (2006) and Blissard (1996). Through tracheal innervations the BmNPV disseminated into the central system of 5th instar and penetrated in to glial nerve and perineurium cells. The NSC (modified motor nerve cells) must also get infected with the BmNPV due to which their normal function of neurosecretion were altered (Plate II, Fig. 3). Hence the effect of BmNPV infection on neurosecretory cells A1 and A2 type of MNC group was under taken.

Changes in neurosecretory A_1 and A_2 cells of MNC groups: After four days inoculation of BmNPV to 5th instar of PM x CSR_2 race, the inoculated control NSC A_1 and A_2 cell and their nuclei, showed slightly increased size as compared to the normal control (Plate II, Fig. 1), indicating the hypertrophy A1 and A2 cells and their nuclei of infected cell. These cells of inoculated control were practically devoid of PF positive NSM and the same was not observed in NCC I and II as well as in the CC and also in the fine branching NCA I over the CA, indicating that the normal functions of NSC A1 and A2 was disturbed, because BmNPV infected virogenic stroma and polyhedron to the central nervous system. These findings are correlated with the findings of Torquaito *et al.* (2006) and Bauah and Chinmoyee Kalita (2020). The neurosecretory cells probably got infected with BmNPV. As this virus multiply in the nuclei of the infected cells and probably getting into the nuclei of A1 and A2 cells and as the secretion of these cells was in the form of electron dense granules

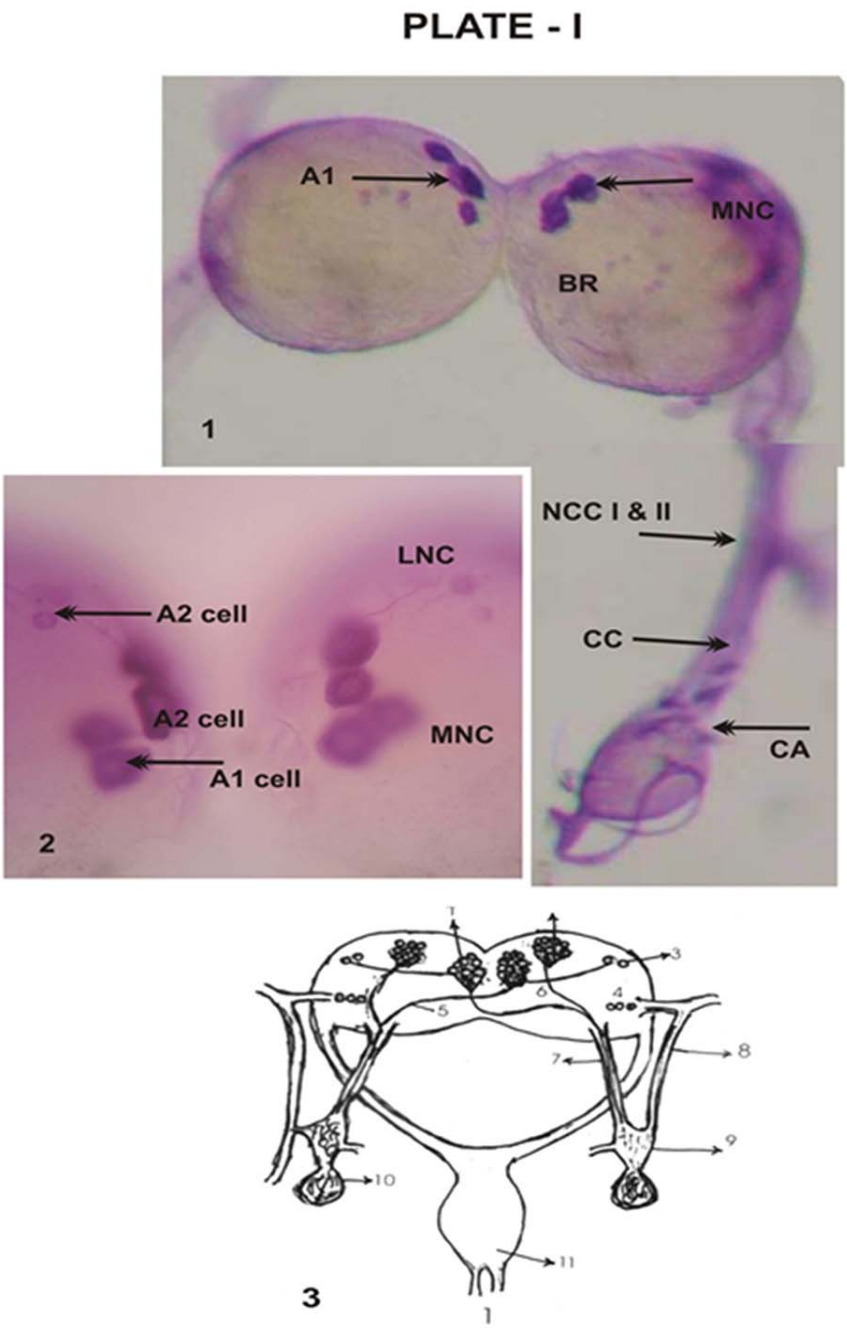

Fig. 1 Whole mount preparation of the brain and CC-CA complex of cross breeds PMxCSR2 showing 4 A1 cells in each MNC group, NCCI and NCCII.

Fig. 2 Whole mount preparation of brain MNC with A1 cells and LNC with A2 cells.

Fig. 3 Diagrammatic representation of cephalic neuroendocrine complex of 5th instar of *B. mori* PMxCSR2 showing- 1. Median Neurosecretory Cell group (MNC), 2. Lateral Neurosecretory Cells (LNC), 3. Anterior Neurosecretory Pathway (ANC), 4. Posterior Neurosecretory Cells (PNC), 5. Median Neurosecretory Pathway (MNSP), 6. Lateral Neurosecretory Pathway (LNSP), 7. Nervi Corporis Cardiaci (NCC I), 8. Nervi Corporis Cardiaci (NCC II), 9. Corpus Cardiacum (CC), 10. Corpus Allatum (CA), 11. Prothoracic Gland (PG), 12. Sub-Oesophageal Gland (SOG)

PLATE - II

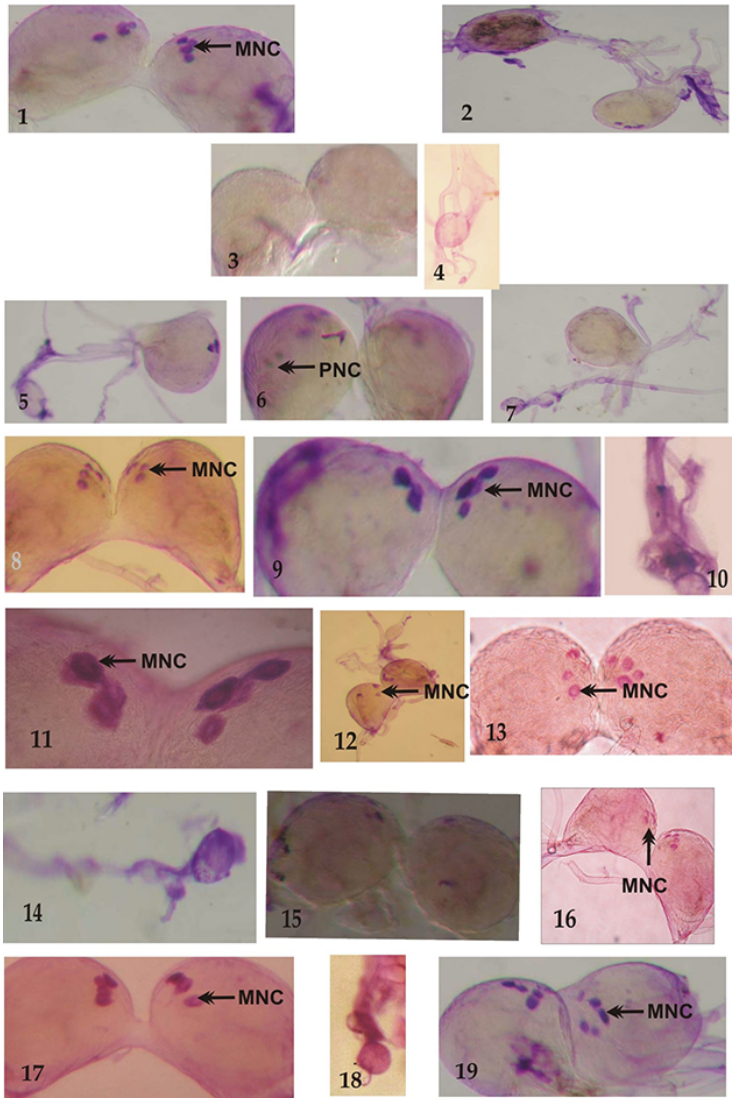

PLATE-II

Fig.1 & 2: Whole mount preparation of endocrine complex of control larvae of PM x CSR2 race showing MNC groups with Al cells containing moderate amount of PG +ve granules (Fig. 1) and NCC I & II and CC showed granular moderate amount of NSM. Fig. 3 & 4: Whole mount preparation of endocrine complex of BmNPV control showing MNC devoid of NSM (Fig. 3) and CC-CA complex with NCC- I and II also devoid of NSM group. Fig. 5 & 6: Whole mount preparation of 3000 ppm concentration of *Aegle marmelos* treated larvae showing slight amount of NSM in Al cells of MNC and in NCCI and II and CC -CA complex. Fig. 7 & 8: Whole mount preparation of 5000 ppm concentration of *Aegle marmelos* treated larvae showing moderate amount of NSM in A1 cells of MNC and in NCC I and II and CC -CA complex. Fig 9, 10 & 11: Whole mount preparation of 8000 ppm concentration of *Aegle marmelos* larvae showing strongly stained similar to the control group of Al cells of MNC group (Fig. 9-11) and increased amount of NSM also observed in NCC I and II and CC -CA complex (Fig. 10). Fig. 12: Whole mount preparation 3000 ppm concentration *Hyptis suaveolens* treated larvae showing slight amount of NSM in Al cells of MNC and in NCCI and II and CC- CA complex. Fig. 13 & 14: Whole mount preparation of 8000 ppm concentration of H. suaveolens *Hyptis suaveolens* treated larvae showing strongly stained similar to the control group of A cells of MNC group (Fig. 13) ad increased amount of NSM also observed in NCC I and II and CC- CA complex (Fig. 14). Fig. 15: The whole mount preparation 3000 ppm concentration of *Eupatorium odaratum* treated larvae showing slight amount of NSM in Al cells of MNC. Fig. 16: Whole mount preparation of 5000 ppm concentration of *Eupatorium odaratum* treated larvae showing moderate amount of NSM in Al cells of MNC Fig. 17, 18 & 19: The whole mount preparation of 8000 ppm concentration of *Eupatorium odaratum* treated larvae showing strongly stained similar to the control group of Al cells of MNC group (Fig. 17 & 19) and increased amount of NSM also observed in NCC I and II and CC -CA complex (Fig. 18).

over 1000A^0 diameter and protein nature which requiring the involvement of nuclei in their synthesis. These cells were devoid of PF positive. NSM indicating that these cells inoculated control were infected by that these cells inoculated control were infected by viral stroma and polyhedra interfering and hampering the synthesis of proteinaceous granular NSM in A1 and A2 cells of MNC groups (Plate II, Fig. 3). These cells had the source of prothroacotrophic, allotropic and allostatatic hormones, hence total process of the metamorphosis getting disturbed. Bauah and Chinmoyee Kalita (2020) reported similar observation. Physiological process leading to the disease condition and finally death of infected larvae occurred in the last two days of 5th instar. However, the NSC $A_1$and A_2 cells of MNC group of plant extract treated larvae showed gradual recovery from the infections. The plant extracts treated group of 3000ppm *A. marmelos*, *H. suaveolens* and *E. odaratum* where in the $A_1$and A_2 NSC of MNC group contain slightly stained granules showed PF positive NSM in three days. The PF positive NSM was quite evident in the NCC II and I CC lobes and in the fine branching of NCA1 over CA indicating the NSC A1 and A2 performing normal functions (Plate II, Figs. 5, 6, 11, 12). The improvement in the functioning of the NSC A_1 and A_2 cells of MNC groups of three days plant extracts treated group of 5000 ppm (where in A-$_1$ & A_{-2} cells, NCC I and II and fine branching of NCA I over CA moderately stained granules of positive materials immediately this treatment) had protective effect and did not allow virus to infect the CNS the size of A_1 and A_2 cells with very negligible hypertrophy (Plate II, Figs. 7, 8, 16). There was total improvement in the function of NSC A1 and A2 cells of MNC group of 3 days plant extractives treated group with 8000ppm concentration, where in A1 and A2 cells of MNC groups, NCCI and II, CC lobes and fine branching of NCA I over CA showed strongly stained PF positive granules of NSM (Plate II, Figs. 9, 10, 11, 13, 14, 17, 19). Thie was more or less similar with normal control groups indicating this treatment had offered total protection to the components of cephalic neuroendocrine system from the infection of BmNPV and these larvae of this groups showed nearly normal feeding behaviour and their body weight were slightly less than the untreated group larvae. The silkworms of PMxCSR2 treated with the ethanolic plant extractives @3000, 5000 and 8000ppm along with the mulberry leaves showed normal behaviour after the 5th day of BmNPV inoculations and were having the more or less similar body weight compared to untreated control groups. Their feeding also was normal and hence they were recovered from the BmNPV infection. Subsequently their feeding rate and the weight gain showed that some factors in plant extractives either growth promoters and phagostimulant or the juvenomimic (terpenoids) compounds which had the effect on the growth of the larvae either due to phagostimulant or by elevating the JH levels in the haemolymph by which, NSC A1 and A2 cells secreting the hormone and activate corpora allata to secrete juvenile hormone. Ranjith Kumar *et al.* (2022) reported similar observation. Bhaisare (2023) reported that the additional consumption of food and the additional growth in larva leading to the improvement in the cocoon characters after the treatment of ethanolic plant extractives of BmNPV inoculated larvae. Plants are having potential of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties (Manimegalai *et al*., 2006; Chanda, 2011, Sithi Jameela Muthu Mohammed *et al.*, 2023). Recently the efforts have been made to promote the use of plant products against the infectious diseases of silkworm as an alternative for chemical control. The present research work, the ethanolic plant extractives showed the significant positive result against viral disease caused by BmNPV of *B. mori*. These are the preliminary observations in search of the bioactive compound having antimicrobial properties and the results obtained were encouraging.

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Meiotic chromosome behaviour of a newly recorded ant-like spider, *Myrmarachne melanocephala* MacLeay, 1839 from Manipur, India

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ABSTRACT: Cytological and chromosomal studies of ant-like spiders *Myrmarachne melanocephala* MacLeay, 1839, were undertaken with 12 males captured alive in the months of January to July 2023 from three habitats. The haploid count of male specimens was observed to be 13: 4 acrocentric (including X chromosome), 6 subtelocentric, 2 submetacentric, and 1 metacentric and showed X0 sex determination mechanisms, so the diploid count of the species was 25 (13+12). The single sex chromosome occupied roughly 4-6 per cent of the nuclear volume prominently in the Interphase – Prophase I stage. The structure of X chromosome in interphase stage was circular-rectangular block. The peculiar shape of rod-shaped X chromosome was maintained from early pre-leptotene stage till the end of the division particularly the Prophase I. The synapsis started early from late leptotene and duration of zygotene was long enough to visualize the perfect ones in late zygotene. There were nine diplotene bivalents with interstitial chiasma.

KEYWORDS: Araneae, Salticidae**,** haploid, prophase, diplotene chiasma, sex chromosomes

The genus *Myrmarachne* MacLeay, 1839 display the marvellous Batesian mimicry, (Wanless, 1978; Yamasaki and Ahmad,2013; Yamasaki and Edwards, 2013). The genus comprised of 195 species (World Spider Catalog version 25.0) with distribution from Australia to Africa and New World (https://en.wikipedia.org/wiki/Myrmarachne). Their habitat consists of leaves and around trees, herbs or shrubs but needs a more intensive look. One such ant-like spider is *M. melanocephala* MacLeay, 1839, a new record from Manipur, mimicking the black ant (https://indiabiodiversity.org/observation/show/15460659). Its ant model is *Tetraponera rufonigra* Jerdon, 1851 (Kumar *et al.,* 2021). The species is distributed from Pakistan to Indonesia (Edwards and Benjamin, 2009; World Spider Catalog version 25.0) with type locality from West Bengal (https://en.wikipedia.org/wiki/Myrmarachne_melanocephala). The species was also newly recorded in Jharkhand (Kumar *et al.,* 2021). Currently cytological works on the genus are rare to find with exception to *M. laurentina* ($2n♂ = 28, X_1X_20$) (Štáhlavský *et al.,* 2020). In the present study meiotic division in *M. melanocephala* MacLeay, 1839 (Araneae, Salticidae), was analysed

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to ascertain the diploid count, chromosomal behaviour and other details. Spiders of *M. melanocephala* were collected from three different places [Khurai Konsam leikai - 24°83'68.54'', 93°97'37.42'' (2 males, 3 females); DM College of Science - 24°82'20.55'', 93°94'15.63'' (3 males, 3 females); DM Community College - 24°82'21.62'', 93°94'15.96'' (7 males, 5 females)] in the months of January to July, 2023 and brought to laboratory for further investigation. Identification of the species was confirmed with Prószyński (2020).

Chromosome preparation: Squashed preparation - The abdomen of the spider was dissected and testes were removed in hypotonic solution, KCl (0.56M) along with yellowish fat and other unwanted materials and exposed to the solution for 15 minutes. Fixative (Carnoy's fluid I) comprising of 1-part glacial acetic acid and 3 parts of methanol by v/v was added and left alone for 10 minutes. The fixed testes were stained with two per cent aceto-orcein for 30 minute and softened with 45 per cent glacial acetic acid. The stained testes were covered with cover slip and squashed by applying thumb pressure after covering the slip with blotting paper (Belling, 1921). Well-spread 50 dividing cells were analysed under microscope for each stage and well spread selected cells were photograph using digital camera attached on Optscopes light microscope. The number of dividing cells in a single testis lobule was fair enough to display all the stages of meiosis division. The details of the chromosomes could be visualised as the chromosomes were fairly big in this species.

Interphase: The single sex chromosome occupied 4-6 per cent of the nuclear volume prominently in the interphase-Prophase I stages. The structure of X chromosome in interphase stage was heavily stained rectangular/circular block. The peculiar shape of rod X chromosome is maintained from early pre-leptotene stage till the end of the division particularly the Prophase I (Figs. 1A, B, C arrow indicates X chromosome).

Leptotene: The condensed pre-leptotene chromosomes were de-condensed in leptotene stage with easily distinguishable chromomeres along the whole length of the chromosomes. The typical rod-shaped X chromosomes are always at periphery of the plate (Figs. 1D, E arrow indicates X chromosome).

Zygotene: The stages were characterised by pairing of homologous chromosomes known as synapsis. The shortened synapsed chromosomes were easily visible in most of the cell plates (Figs. 1F, G). At some point the pairing of the homologous chromosomes initiated preferably at the terminal ends while some elements showed the exposed terminals.

Pachytene: The stage was characterised by thick shortened deeply stained chromosomes with nearly hollow space most likely the remnant of synaptonemal complexes (Fig. 1H).

Diplotene: The characteristic features of the stage were the presence of the chiasma and diplotene loop of the terminal chiasma. There were nine interstitial chiasmatic chromosomes (Fig. 1I). The X chromosomes were characterised by deeply stained (heteropicnotic) rod shape and uniformly dispersed.

Diakinesis: The dispersed chromosomes were coming towards the equatorial region. Here only two interstitial chiasmata could be observed (Figs. 1J, K). The X chromosomes were nearly peripheral.

Metaphase I: The localisation of the chromosomes at the equatorial plate was complete in this stage (Fig. 1 L). The X chromosomes were nearly peripheral.

The remaining stages of meiotic divisions like Anaphase I, Telophase I and Meiotic II stages were classical as in all other organisms (Figs. 1M-R) (Šáhlavský *et al.*, 2020). According to Sharma and Sharma (2014) cytogenetics records of 325 species of Indian spiders exist. Of these 232 species (71.38%) have sex chromosome system of the X_1X_20 type; 48 species (12.92%) have X0 system; 39 species (12%) have $X_1X_2X_30$ system; 1 species (0.3%) has X_1X_2Y type; 4 species (1.23%) have $X_1X_2X_3X_40$ system; 1 species (0.3%) has X_1X_2Y type. The present species is of XO system in accordance with (Araujo and Schneider, 2012) but *M. laurentina* ($2n♂ = 28$, X_1X_20) (Šáhlavský *et*

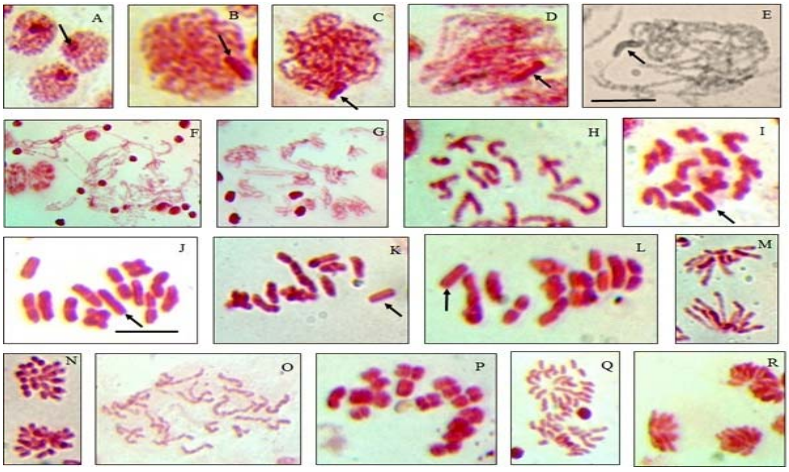

Fig. 1 Meiotic cells of the ant-like spider, *Myrmarachne melanocephala.* A–C: Interphase depicting heteropycnotic sex chromosome, D–E: Leptotene, F-G: Zygotene, H: Pachytene, I: diplotene, J-K: diakinesis, L: metaphase I. M: anaphase I, N: telophase I, O: Spermatogonial pro-metaphase/metaphase II, P: metaphase II, Q: anaphase II, R: telophase II, Scale 10 (micrometer)

al., 2020) was different from the present study.

The X0 system found in *Oxyopes* (metacentric), *Myrmarachne, Misumena* and *Xysticus* (acrocentric) could be derived from X_1X_20 system (Hackman, 1948) in two ways - 1. The metacentric X of the X0 system could have been derived by centric fusion between X_1 and X_2 chromosomes. This mechanism was also employed by several authors (Araujo and Schneider, 2012) to explain the origin of the X0 Sex Chromosomes System (SCS), which involves a metacentric X, in many spider groups. The acrocentric X of the X0 system could have originated through gradual elimination of one X chromosome of the X_1X_20 SCS, as suggested by Suzuki (1952, 1954). The author put forth this proposition based on the fact that some thomisid species with an X_1X_20 system presented gradual differences between the lengths of X_1 and X_2 chromosomes (with both showing the same, slightly different or markedly different sizes). Furthermore, some species even exhibited an X0 system, suggesting that elimination of one X of the X_1X_20 system had taken place in the course of evolution.

It seems that the meiotic Prophase I is lengthy and the zygotene stage is perfect in displaying the homologous pairing or synapsis. It is both terminal and interstitial (Figs. 1E, F, G). As there is no reference for comparing, the haploid count of male specimens with 13 evidences from the diplotene-metaphase I count with four acrocentric (including X chromosome), six subtelocentric, two submetacentric, and one metacentric, is open for further confirmation from the mitotic cells. The diploid count of 25 (13+12) for the species is open for any suggestions too.

The behaviour of the X chromosomes that is peripheral localisation seems to be universal as heteropicnotic particularly in spiders. According to White (1940), the term heteropycnosis was introduced to describe the different levels of condensation and staining that certain chromosomes exhibit in the course of mitosis and/or meiosis. This heteropycnotic pattern can be positive or negative, and it is related to a high or low degree of chromosome condensation, respectively. Manifestation of heteropycnosis is commonly visualised in the sex chromosomes, especially in male meiotic cells; the high level of chromosome condensation in these cells seems to prevent recombination between non-homologous regions of heteromorphic sex chromosomes (McKee and Handel, 1993). In spider spermatogenesis, a heteropycnotic pattern of the sex chromosomes has been recorded for roughly 25 per cent of the species that have been cytogenetically examined, which belong to different suborders (Mygalomorphae and Araneomorphae) and families (Araujo and Schneider, 2012). Regardless of the type of sex

chromosome system, 95 per cent of these spider species showed positively heteropycnotic sex chromosomes in premeiotic interphase and prophase I nuclei (Figs. 1A-C, E-G, I-M) and occasionally, also in metaphase II cells. In late meiotic stages, the sex chromosomes usually appeared to be isopycnotic (Araujo and Schneider, 2012).

The diploid count of the male *M. melanocephala* is 25 (13+12) with X0 system. The haploid karyotype of the species is 4 acrocentric (including X chromosome), 6 subtelocentric, 2 submetacentric, and 1 metacentric. The X chromosome is always peripherally localised and the prophase I is long enough to see the zygotene synapsis and have nine interstitial diplotene elements.

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Correlation of weather parameters on the seasonal incidence of *Helicoverpa armigera* infesting *Cicer arietinum*

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ABSTRACT: Investigation taken up on the seasonal occurrence of *Helicoverpa armigera*, on *Cicer arietinum* L., revealed the presence of *H. armigera* from the 51st to 12th Standard Meteorological Week (SMW). Number of larvae found per plant ranged from 0.14 to 4.28. The larval population began to appear during the 51st SMW, with a mean of 0.66 larvae per plant and steadily increased until reaching its peak during the 6th SMW with a mean of 4.28 larvae per plant. Subsequently, the population declined, reaching a mean of 0.14 larvae per plant by the 12th SMW. The larval population of *H. armigera* displayed a highly significant negative correlation with both the maximum (r = -0.670**) and the minimum temperature (r = -0.665**).

KEY WORDS: Gram, pod borer, seasonal incidence, weather parameters

Gram, *Cicer arietinum* L., is considered the "King of Pulses" and the most important pulse crop grown in India. It belongs to the family Fabaceae and is vulnerable to the attack of more than 60 insect pests right from germination to maturity (Srivastava *et al.,* 2005). Among insect pests infesting gram, the pod borer *Helicoverpa armigeras* (Hubner) is the most serious one (Chhabra, 1980). Besides gram, it can also infest cotton, pigeon pea, tomato, sorghum, cowpea, groundnut, okra, peas, field beans, and soybeans (Subramanian and Mohankumar, 2006; Pimparkar and Raja, 2017). Due to its polyphagous nature, *H. armigera* is also known as the American cotton bollworm, corn earworm, tomato fruit borer, tobacco budworm, and carnation worm. It has been recorded feeding on 181 cultivated and uncultivated plant species belonging to 45 families. It is considered a damaging pest and has assumed as a national pest due to its high fecundity, high adaptability to diverse agro-climatic conditions, migratory behavior, and development of resistance capability against various insecticides (Sarwar *et al.,* 2009; Purabiya *et al.*, 2024). The larvae of *H. armigera* are foliage feeders as early and later instars move to the developing seeds and fruits leading to a drastic reduction in yield. A single larva can consume up to 30-40 pods in its life cycle (Taggar and Singh, 2011). The yield loss in grams due to *H. armigera* is 10 to 60 per cent in normal infestation and up to 85 to 90 per cent during severe infestation. In India, the extent of losses varies from 10 to 40 per cent (Purabiya *et al.*, 2024). The incidence of *H. armigera* has shown violent fluctuations due to

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changes in climatic conditions. The information on the seasonal occurrence of *H. armigera* as influenced by various weather parameters is very useful for developing various strategies to manage this pest. Although the seasonal incidence of *H. armigera* on gram has been reported in different regions of India, such information is limited particularly in south Gujarat. Therefore, the present investigation was carried out to study the seasonal incidence of *H. armigera* and its correlation with weather parameters in this zone.

The present investigation was carried out at the college farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India during the Rabi 2021-22. The farm is situated at 72° 54' East longitude and 20° 57' North latitude and an altitude of 11.98m, above the mean sea level. The soil of the experimental area was heavy black soil. The plot size was 20.1m × 20m and the experimental area was 402m^2. Gram seeds of the variety Gujarat Gram-2 were sown in the 49th Standard Meteorological Week (SMW) (3rd December 2021) at the rate of 60 kg ha^{-1} by dibbling method in the field with row-to-row and plant-to-plant spacing of 30 and 10cm, respectively. Fertilizers were applied at the rate of 20:50:50kg NPK ha^{-1}. All the post-sowing recommended agronomic practices were followed to raise a crop successfully. However, the experimental area was kept free from insecticidal spray throughout the crop season to record the incidence of *H. armigera* on gram. To determine the seasonal incidence of *H. armigera*, fifty plants were randomly tagged from the four quadrates of the experimental area. The infestation of *H. armigera* population was estimated by counting the total number of larvae per plant. The observations were recorded at weekly intervals from tagged plants starting from 15 days after sowing (51st SMW) till the harvest of the crop. The weekly meteorological data on temperature (^{o}C), relative humidity, bright sunshine hours (hrs per day), and wind speed (km per hrs) in different standard meteorological weeks were obtained from the Agro-meteorological observatory, College farm, Navsari Agriculture University, Navsari during the Rabi 2021-22. The simple correlation coefficients were worked out between the population of *H. armigera* in different SMW and various weather parameters.

The results of the present investigation revealed that the activity of larvae of *H. armigera* commenced from 51st SMW and continued till 12th SMW, which ranged from 0.14 to 4.28 larvae per plant. The larval population increased continuously from the 51st to the 6th SMW and then declined up to the 12th SMW. During the 6th SMW, the *H. armigera* larval population showed a peak by recording 4.28 larvae per plant which might be due to formation of seeds during that period. In the subsequent weeks, the population decreased and reached 0.14 larvae per plant during the 12th SMW (Fig. 1). The present findings are similar to the findings of Kumar and Srivastava (2017) who reported that the incidence of *H. armigera* on gram crop commenced from the 51th SMW with 0.5 and 1.0 larvae per five plants, during the years 2001 and 2002, respectively. The larval population showed its peak with 6.50 and 6.25 larvae per five plants in 6th SMW during the years 2001 and 2002, respectively. Similarly, Bhagat and Chandraker (2020) observed that the infestation of *H. armigera* initiated on the crop during 51st SMW with a mean population of 1.65 and 1.72 larvae per meter row during 2016-17 and 2017-18, respectively. The pest touched its peak with a mean population of 4.49 and 4.12 larvae per meter row in the 5th and 6th SMW during 2016-17 and 2017-18, respectively.

The correlation coefficient analysis between the larval population of *H. armigera* and weather parameters (Table 1) revealed that the larval

Table 1. Correlation coefficients between the larval population of *Helicoverpa armigera* on gram and weather parameters from the 51st to 12th SMW during Rabi 2021-22

Weather parameters	r- values
Maximum temperature (TMax) (°C)	-0.670**
Minimum temperature (TMin) (°C)	-0.665**
Morning relative humidity (MRH) (%)	0.530
Evening relative humidity (ERH) (%)	0.253
Wind speed (WS) (km/hr)	0.272
Bright sunshine hours (BSSH) (hrs/day)	0.264

**Highly significant at 1% level of significance

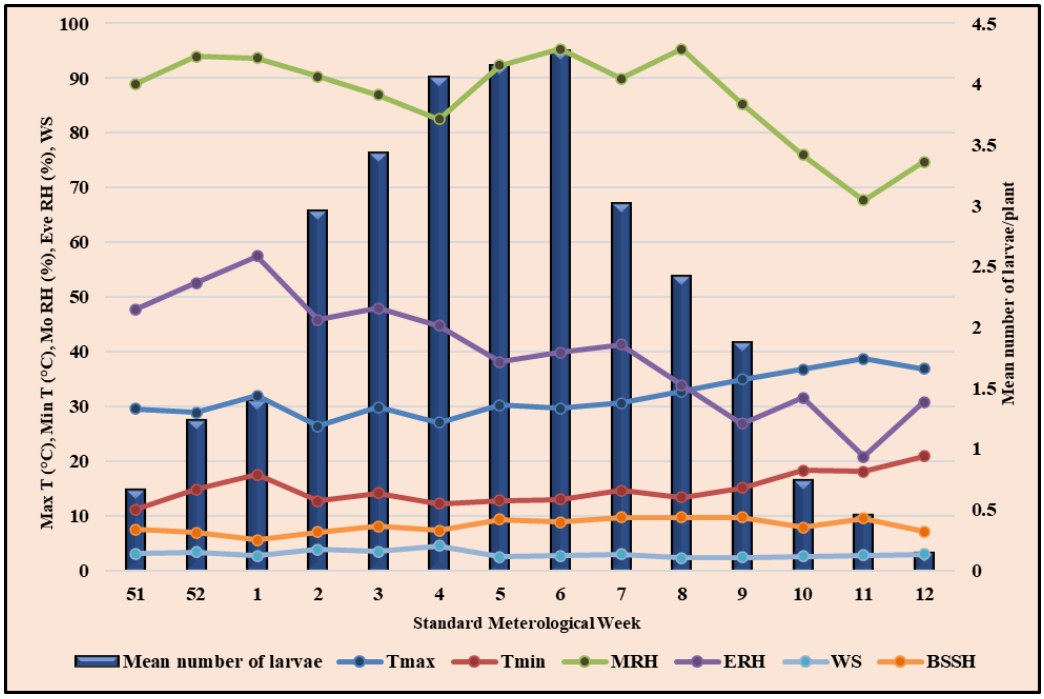

Fig. 1 Seasonal incidence of *Helicoverpa armigera* on gram from the 51st to 12th SMW during Rabi 2021-22; Larvae - Mean number of larvae per plant; TMax - Maximum temperature; TMin -Minimum temperature; MRH - Morning relative humidity; ERH - Evening relative humidity; WS - Wind speed (km/hr); BSSH - Bright sunshine hours (hrs/day)

population of *H. armigera* had a highly significant negative correlation with the maximum temperature (r=-0.670**) and minimum temperature (r=-0.665**). Whereas, the larval population showed a non-significant positive correlation with the morning relative humidity (r = 0.530), evening relative humidity (r = 0.253), wind speed (r = 0.272), and the bright sunshine hours (r = 0.264). The present findings are in line with the earlier reports of Kaneria *et al.* (2018) who reported that there was a highly significant negative correlation between the *H. armigera* larval population with the maximum temperature (r = -0.739**) and minimum temperature (r=-0.725**). Similarly, Chatar *et al.* (2010) observed that there was a highly significant negative correlation of the larval population of *H. armigera* with the maximum temperature (r=-0.751**). Furthermore, Bala (2020) noted that the larval population of *H. armigera* had a non-significant positive correlation with the morning relative humidity, evening relative humidity, and bright sunshine hours. Reddy *et al.* (2009) reported that the larval population of *H. armigera* had a non-significant positive correlation with wind velocity and bright sunshine hours, which also supports the present findings. Based on the above findings, it can be concluded that the larval population of *H. armigera* had a highly significant negative correlation with the maximum temperature and minimum temperature under Gujarat conditions.

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Evaluation of plant oils against angoumois grain moth, *Sitotroga cerealella* (Olivier) infesting stored rice

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ABSTRACT: Angoumois grain moth, *Sitotroga cerealella* Olivier is one of the important insect pests during storages of ricee. Evaluation plant oils against the pest showed that all oils cause mortality over control. Among them neem oil @5ml/kg was found effective followed by pongamia oil as it causes mortality of *S. cerealella*, less weight loss and adult emergence.

KEY WORDS: Neem oil, pongamia oil, storage, mortality, weight loss, adult emergence

Rice, *Oryza sativa* L., is a staple food for a large part of the world's human population. In spite of its potential in providing food for humans and as industrial raw material, rice is not spared by various pests between harvest and storage. The most economically important insect pest of stored rice is the angoumois grain moth, *Sitotroga cerealella* (Olivier) (Ashamo and Khanna, 2006). Infestation by *S. cerealella* starts in the field and may reach serious levels in the store. In many developing countries, insect pests reduce the vigor and viability of infested seeds because they mostly feed preferentially on the germ of the grains (Ivbijaro *et al*., 1985), they cause weight loss and contamination of stored paddy (Ashamo and Odeyemi, 2001). In order to reduce infestation to the barest minimum, various methods such as the use of conventional insecticides, biological control, mechanical control, cultural control, and varietal resistance have been utilized, with chemical control being most effective though having adverse environmental, health, and economic hazards. These include pollution, poisonous residue accumulation in foods, development of resistance by target species, and high cost of insecticide application and reapplication. As alternatives to synthetic insecticides, plant oils have been used to reduce post-harvest losses of cereals including rice. Plant preparations found practical alternative to the increasing insect pest problems and agricultural pest resistance, problems of chemical residues, and environmental safety (Folake *et al*., 2023). However, not much work has been done on the control of *S. cerealella* using plant oils. Therefore, the present work investigated the efficacy of various oils against *S. cerealella* infesting rice in stored conditions.

Male and female adults of *S. cerealella* obtained from the godowns of farmers were used to raise a

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culture on rice, in jars (2litre) capped with a piece of muslin cloth which allowed ventilation but prevented entry or exit of moths, and other insects, as well as foreign materials. The jars were kept in insect cages and the culture was maintained by replacing the infested grains with fresh, uninfested grains. The environment for cultured insects and for experimentation was maintainedat28±2°C and 75±5 per cent relative humidity.

The experiment to evaluate the efficacy of plant oils against *S. cerealella* infesting rice was laid out in the Post Graduate Research Laboratory, Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat in a completely randomized design with ten oil-based treatments (Table 1), replicated thrice. Collected rice grains were sun-dried on the cemented floor for three consecutive days in the month of May. The rice grains were kept treatment wise in plastic jars maintaining one kilogram per jar. One kilogram sterilized rice grains were treated by mixing with different oils thoroughly at 5ml/kg dosages in such a way to get a uniform coating. 100 adults were released in each treatment. Observation on mortality was recorded at respective days i.e. 7, 14, 21 and 28 days after treatment,while after two months of infestation, and weightloss and adult emergence were also recorded.

Effect of different oil based treatments was

Table 1. Effect of plant oils on mortality, weight loss and adult emergence of rice grain moth during 2021-22

Treatments	Per cent corrected Mortality - DAT				Weight loss	Adult emerged-%
	7	14	21	28		
Mustard (5ml/kg)	39.22 (39.99)	39.42 (40.39)	43.48 (47.35)	48.19 (55.56)	26.14 (19.41)	55.67
Sesame (5ml/kg)	32.93 (29.57)	44.53 (49.19)	(51.01) 45.58	50.82 (60.08)	27.18 (20.86)	52.67
Soybean (5ml/kg)	36.66 (35.72)	36.61 (35.64)	38.46 (38.75)	44.64 (49.37)	27.95 (21.97)	57.00
Coconut (5ml/kg)	37.19 (36.55)	34.52 (32.12)	(22.84) 28.53	26.93 (20.60)	25.66 (18.75)	61.67
Groundnut (5ml/kg)	36.24 (34.98)	42.26 (45.22)	42.07 (44.91)	42.49 (45.65)	27.00 (20.62)	60.00
Castor (5ml/kg)	40.56 (42.28)	46.09 (51.90)	(59.99) 50.77	53.26 (64.17)	27.69 (21.59)	64.00
Neem (5ml/kg)	48.75 (56.52)	55.45 (67.83)	59.53 (74.28)	60.48 (75.70)	18.41 (9.98)	33.33
Mahua (5ml/kg)	41.45 (43.82)	36.68 (35.70)	(30.20) 33.33	45.58 (51.01)	24.82 (17.62)	58.00
Pongamia (5ml/kg)	45.42 (50.73)	50.72 (59.90)	53.42 (64.49)	55.78 (68.34)	24.81 (17.61)	46.00
Control	0.81 (0.00)	0.81 (0.00)	0.81 (0.00)	0.81 (0.00)	37.48 (37.04)	91.67
S. Em+	1.07	1.27	1.01	1.09	0.56	0.91
CD at 5 %	3.15	3.76	2.97	3.23	1.65	2.67
CV (%)	5.14	5.70	4.40	4.42	3.77	2.71

Figures in parentheses are retransformed values, those outside are angular transformed values

Table 2. Effect of plant oils on mortality, weight loss and adult emergence of rice grain moth during 2022-23

Treatments/ dose	Per cent corrected Mortality - DAT				Weight loss	Adult emerged-%
	7thDay	14th Day	21st Day	28th day		
Mustard (5ml/kg)	28.32 (22.59)	42.33 (45.45)	43.40 (47.30)	47.57 (54.46)	26.76 (20.28)	56.67
Sesame (5ml/kg)	21.78 (13.79)	39.62 (40.88)	42.21 (45.31)	42.43 (45.54)	27.42 (21.20)	55.00
Soybean (5ml/kg)	20.73 (12.57)	34.79 (32.59)	28.99 (38.66)	38.02 (38.04)	28.12 (22.21)	57.67
Coconut (5ml/kg)	24.78 (17.58)	39.17 (39.90)	42.41 (45.49)	37.43 (37.08)	25.86 (19.03)	62.67
Groundnut (5ml/kg)	23.90 (16.42)	41.68 (44.23)	43.40 (47.21)	41.24 (43.46)	27.20 (20.91)	61.67
Castor (5ml/kg)	26.55 (20.58)	43.63 (47.62)	49.58 (57.96)	38.48 (38.83)	28.10 (22.19)	65.33
Neem (5ml/kg)	48.38 (55.89)	59.24 (73.84)	67.96 (85.79)	71.10 (89.47)	27.20 (20.89)	36.33
Mahua (5ml/kg)	35.83 (34.27)	37.62 (37.30)	42.14 (45.02)	46.33 (52.31)	18.37 (9.94)	59.00
Pongamia (5ml/kg)	43.99 (48.24)	55.44 (67.81)	62.72 (78.96)	64.18 (81.02)	23.81 (16.31)	47.00
Control	0.81(0.00)	0.81 (0.00)	0.81 (0.00)	0.81 (0.00)	43.59 (47.55)	91.33
S. Em+	1.48	1.97	2.31	1.79	0.61	0.88
CD at 5 %	4.36	5.81	6.80	5.28	1.80	2.60
CV (%)	9.31	8.64	9.22	7.25	3.85	2.58

Figures in parentheses are retransformed values, those outside are angular transformed values

evaluated against *S.cerealella* during the year 2021-22 and 2022-23. During the year 2021-22, neem oil recorded the highest mortality of *S.cerealella* i.e., 56.52,67.83,74.28 and 75.70 per cent after 7,14, 21, and 28 days, respectively and was significantly superior over rest of the treatments. The treatment pongamia oil found second best against *S.cerealella* as it recorded 50.73, 59.90, 64.49 and 68.34 percent mortality after 7, 14, 21 and 28 days, respectively and it was at par with the treatment of castor oil. In case of weight loss, the treatment neem oil recorded the lowest weight loss (9.98%), and was followed by the pongamia oil (17.61%). Maximum weight loss was observed in control (37.04%). Adult emergence was maximum (91.67%) in control, whereas in neem oil lowest adult emergence (33.33 nos) was noticed (Table 1). During the year 2022-23, all the treatments effectively reduced the damage of *S. cerealella* in rice over control. The treatment neem oil recorded highest mortality *i.e.*, 55.89, 73.84, 85.79 and 89.47 after 7, 14, 21 and 28 days, respectively but it was at par with pongamia oil after 14 and 21 days. The treatment of pongamia oil found second best treatment against *S.cerealella* as it recorded 48.24, 67.81, 78.96 and 81.02 per cent mortality after 7, 14, 21 and 28 days, respectively and significantly superior over rest of the treatments. Regarding weight loss, neem oil (9.94 %) was most effective and was followed by the pongamia oil (16.31%). The maximum weight loss was recorded in the control (47.55 %). More number of adult emergence (91.33) was noticed in control (Table 2). The two years of pooled over data showed that all oil used as seed protectants was found effective over control. Neem oil was found most effective as it causes 56.20, 70.84, 84.04 and 82.58 per cent mortality after 7, 14, 21 and 28 days, respectively and it was at par with pongamia oil. The minimum weight loss was noticed in neem oil (9.96%) and was followed by pongamia oil (16.96%). The maximum weight loss was observed in the control (42.30 %). In the case of adult emergence, the maximum number of adult emergence was observed in control (91.50 adults) and the minimum in neem oil (34.83 adults) (Table3).

Table 3. Effect of plant oils on mortality, weight loss and adult emergence of rice grain moth (Pooled 2021-22 and 22-23)

Treatments	Per cent corrected Mortality				Weight loss	Adult emerged-%
	7thDay	14th Day	21st Day	28th day		
Mustard (5ml/kg)	33.77 (31.29)	33.87 (42.92)	43.44 (47.32)	47.88 (55.01)	26.45 (19.84)	56.17
Sesame (5ml/kg)	27.35 (21.68)	33.16 (45.04)	43.90 (48.16)	46.63 (52.81)	27.30 (21.03)	53.83
Soybean (5ml/kg)	28.70 (24.15)	28.67 (34.12)	38.40 (38.70)	41.33 (43.70)	28.04 (22.09)	57.33
Coconut (5ml/kg)	30.98 (27.07)	29.65 (36.01)	35.47 (34.17)	32.18 (28.84)	25.76 (18.89)	62.17
Groundnut (5ml/kg)	30.07 (25.70)	33.08 (44.72)	42.74 (46.06)	41.87 (44.56)	27.10 (20.77)	60.83
Castor (5ml/kg)	33.55(31.43)	36.32 (49.76)	50.18 (58.98)	45.87 (51.50)	27.90 (21.89)	64.67
Neem (5ml/kg)	48.57(56.20)	51.92 (70.84)	63.75 (80.04)	65.79 (82.58)	18.39 (9.96)	34.83
Mahua (5ml/kg)	38.64(39.05)	36.25 (36.50)	37.73 (37.61)	45.95 (51.66)	24.82 (17.62)	58.50
Pongamia@5ml/kg	44.71(49.49)	47.35 (63.86)	58.07 (71.72)	59.98 (68.44)	24.31 (16.96)	46.50
Control	0.81 (0.00)	0.81 (0.00)	0.81 (0.00)	0.81(0.00)	40.54 (42.30)	91.50
S. Em+	3.00	3.90	2.91	4.18	0.98	0.63
CD at 5 %	9.61	9.20	9.31	9.40	3.12	2.64
CV (%)	7.04	7.22	7.43	6.00	3.12	2.64

Figures in parentheses are retransformed values, those outside are angular transformed values

In the past, essential oils from four plant species including *T. vulgaris, S. hortensis, P. roseum*, and *S. aromaticum* showed vapor toxicity during 24 h against female adults of the Angoumois grain moth, *S. cerealella* and found that oils in the volatile concentration caused 25% adults mortality (Ghoorchian *et al*., 2023). Volatile extracts of *P. angolensis* and *P. quadrifolia* was used as alternatives to synthetic chemicals in paddy for the protection against *S. cerealella* and had the insecticidal and repellent effects on *S. cerealella* (Elvis *et al*., 2015). Oil extracted from all the parts of the *Newbouldia laevis* had significant effect on the mortality of the *S. cerealella* moth but the root bark oil extract had the most effective and caused 100% insect mortality within 72h of application at 4% concentration (Ashamo *et al*. 2018). Cooking oils had an insecticidal activity tested against Angoumois grain moth, *S. cereallela,* and found that cooking oils (*Gossypium hirsutum* @ 0.5 ml and *Brassica carinata* @ 0.5 ml per 250 g of maize grains were potent bio-insecticides against *S. cereallela* (Fekadu *et al*., 2013). Bulb extracts of *Allium chinense* G. had significant effect on the developmental period of *S. cerealella* and also showed adverse effect on moth emergence (Rhetso *et. al*. 2020). Garlic essential oil and its active substances viz., diallyl trisulfide (DATS) inhibit oviposition in moths of *S. cerealella* and further the proportion of viable eggs significantly decreased when the moths of *S. cerealella* were treated with diallyl trisulfide (DATS) (Chang *et al*., 2020). Oil of *Cinnamomum camphora* (L.) J. Presl, was found highly effective at 0.05 percent concentration (v/w) against *S. cerealella* and showed that essential oil of *C.camphora* had completely suppressed the development of *S. cerealella* (Geetanjly and Tiwari, 2015). The findings of the above workers, supports thre present findings. The neem oil at 1.0 per cent as the most effective grain protectant against different stored grain pests (Pereira and Wohlgemuth, 1982). The oils and cakes of neem, castor, and mustard as effective to reduce the fecundity, hatching, and adult emergence in *S. cerealella* (Verma *et al*.1983). Neem and karanji oils at 0.25, 0.5, and 1.0 ml/kg as

most effective in reducing the fecundity of pulse beetle on green gram seed during storage. Various oils like castor, mustard, groundnut, sesamum, coconut, and sunflower at 1.0 per cent were found effective against pulse beetle in stored cowpea (Babu *et al.,* 1989). Castor oil at 1.0 per cent was most effective against pulse beetle based on reduced oviposition, egg viability, and adult emergence followed by mustard and groundnut oils (Bhargava and Meena, 2002). Plant products like neem, karanji, clove and lemongrass oils at 1.0 per cent was were found the most effective due to reduced fecundity, adult emergence, longevity, grain damage, weight loss, and prolonged developmental period against *Sitophilus oryzae* L in stored wheat (Yadav *et al.,* 2008). The neem, eucalyptus, sunflower, and castor oil at 0.1 and 0.3 per cent as safest and most effective to minimize the incidence of *C. maculatus* on pigeon pea based on its reduced fecundity, adult emergence and delayed development (Lal and Raj, 2012). Neem oil at 0.20 per cent as highly effective based on the lowest adult emergence of lesser grain borer, *Rhyzopertha dominica* (Fabricius) in stored wheat (Singh *et al.,*2016).

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First record of *Canthesancus gulo* Stal, 1863 (Reduviidae, Stenopodainae) from Maharashtra, India

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ABSTRACT: The brownish castaneous spot assassin bug *Canthesancus gulo* Stal, 1863 is reported from Maharashtra for the first time, as the first species of this genus in the Maharashtra state. The diagnostic characters, colour images, and current geographical distribution of species are given.

KEY WORDS: Assassin bug, castaneous spot, distribution, diagnostic characters

The family Reduviidae is the largest group of predatory Heteroptera, representing of 6250 species and subspecies in 25 subfamilies globally (Maldonado, 1990). The earlier work on the assassin bug from British India was done by Distant (1904, 1910) who recorded 342 species, after that Distant's work, the checklist of the Indian assassin bug was given by Ambrose (2006), which included a total of 464 species belonging to 144 genera and 14 subfamilies, of which 33 species of Reduviidae were reported from Maharashtra by Sharma and Bano (2012). The subfamily Stenopodainae (Heteroptera, Reduviidae) is represented by more than 92 known species distributed in all zoogeographic regions (Maldonado, 1990); it is the fourth largest subfamily in India (Ambrose, 2006). In India, A total of 39 species of the subfamily Stenopodinae under 14 genera have been recorded (Ambrose, 2006). *Canthesancus* Amyot and Serville, 1843 is one of the small genus of the subfamily Stenopodinae. There are 3 species of the genus *Canthesancus* known from India, viz., *Canthesancus picticollis* Stal, 1874, *Canthesancus gulo* Stal, 1843, *and Canthesancus helluo* Stal, 1843. These three species are distributed in the countries like Malaysia, Myanmar, China, India, and Indonesia. Of them, the species *C. picticollis* is endemic to India (Ambrose, 2006). The collected species was identified by using Distant (1904) and confirmed by Dr. Hemant Ghate, Modern College Pune. However, *C. gulo* (Stal) has never been previously reported from the state of Maharashtra, and the current study adds this species to the Reduviidae fauna of Maharashtra state.

***Canthesancus gulo* Stal, 1863 (Figs. 1-5)**

1863. *Canthesancus gulo* Stal, *Ann. Soc. Ent. Fr.*, 4: 44.

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Fig. 1-2 *Canthesancus gulo* Stal, 1863. 1-2. Dorsal and ventral views of male

Fig. 3-5 *Canthesancus gulo*. 3. First segment of all legs with colour, 4. Rostrum, 5. Abdomen

1904. *Canthesancus gulo*, Distant, *Fauna Brit. India*, *Rhynchota*, 2: 235.

1990. *Canthesancus gulo*, Maldonado, *Carribbean J. Sci. (special ed.)*, 496 pp.

Specimens examined: Single male, 25.ix.2022, near the light source, Sonaval village (Dodamarg-Maharashtra), elevation (128 m), coordinates (latitude 15°47' 42'' N and longitude 74°04' 32'' E), Coll. S. More, deposited in ADKS College, Dodamarg, identified by Dr. Hemant Ghate.

Diagnostic characters (male): Head elongat with griseous-ochraceous, anteocular region much longer than postocular region, eyes and ocelli brown in colour; a central black line stretching between the base of the first segment of antennae (or between the apex of antenniferous tubercle) to posterior lobe of pronotum; antennae black to pale ochraceous, the base of first antennal segment pale ochraceous and more than half areas brownish to black, second antennal segment much longer than first antennal segment, with its base pale ochraceous; rostrum pale ochraceous, first segment elongated and incrassate, with slightly longer than the second , second segment brown, distinctly thinner than first segment, third segment dark brownish, slightly curved and pointed, rostrum not reaching to fore coxa and extending more than half portion of gula; the humeral angel of pronotum not rounded, anterior lobe of pronotum shorter than posterior lobe with partially transverse sulcus at the middle, finely transverse sulcus present behind the anterior lobe, anterior and posterior lobe with three pairs of fuscous spines on each lateral margin; scutellum brownish, with a long erect spine; hemelytra brownish-ochraceous, with brownish castaneous irregular spots (the spot smallest at the sub basal area of corium and near the cubitus, the second spot large to the junction between the corium and membrane, the third spot on the disc of membrane); membrane pale castaneous and finely extending beyond the apical segment of abdomen, and lightly mottled with brown in colour; ventrally fuscous, acetabulum coxa and trochanter fuscous, abdomen segments visible with tawny brown; fore femora slightly incrassate than mid and hind femora, with its basal region ochraceous and apical areas fuscous with brownish to yellow fine hairs, tibiae covered with fine hairs with alternative irregular yellow and brownish patches, tarsi brownish yellow.

Measurements (in mm): Length 26.5 mm; breadth between pronotal angles 6.5 mm.

Distribution: India: Assam, Meghalaya, Sikkim and Maharashtra (New record).

Elsewhere: Malay Peninsula, Malaysia, Myanmar and Singapore (Weirauch et al., 2014 and Mukherjee, 2015)

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First report of isolation of opportunistic human pathogenic gut bacteria *Staphylococcus cohnii* from grubs of *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae) from Himachal Pradesh, India

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ABSTRACT: *Staphylococcus cohnii* (Schleifer and Kloos) an opportunistic pathogen for humans, is reported for the first time its presence in grubs of *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae). The cellulolytic gut bacteria were isolated from different populations of *B. coriacea*, collected from different parts of Himachal Pradesh, India. The isolated *S. cohnii* from the grub population of Shillaroo location recorded maximum cellulolytic index. The isolated bacteria were identified using morphological, biochemical, and 16S rRNA gene sequencing approaches.

KEY WORDS: Cellulolytic, pathogen, 16S rRNA

The scarabaeoids are a large and distinct group of highly specialized beetles that are easily recognized by their lamellate antennae and high degree of polyphagy (Mehta *et al*., 2010). While their larvae (white grubs) are among the most destructive soil pests, adults are the most frequent leaf chafers. *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae) an invasive pest of potato and apple agro-ecosystem in north-western India is found in the mid- and high-hill regions of the districts of Mandi, Kullu, Chamba, Kinnaur, Solan, and Sirmaur in the state of Himachal Pradesh. It is a polyphagous pest causeing severe harm by feeding the leaves, fruits of forest trees, their nurseries, vegetables, lawns, and field crops (Chandel *et al*., 1997). It is reported in temperate, subtropical, and tropical regions of Uttarakhand (Singh *et al*., 2003). *Staphylococci* belong to CoNS group (Coagulase-negative Staphylococci), which are opportunistic pathogens in humans, animals and other non-human primates (Kloos and Wolfshohl, 1991). Some of the species are reported to be normal microbiota of human skin and mucous membranes, which are associated with blood stream infection, endocarditis and meningitis (Garza-González *et al*., 2011; Soldera *et al*., 2013).

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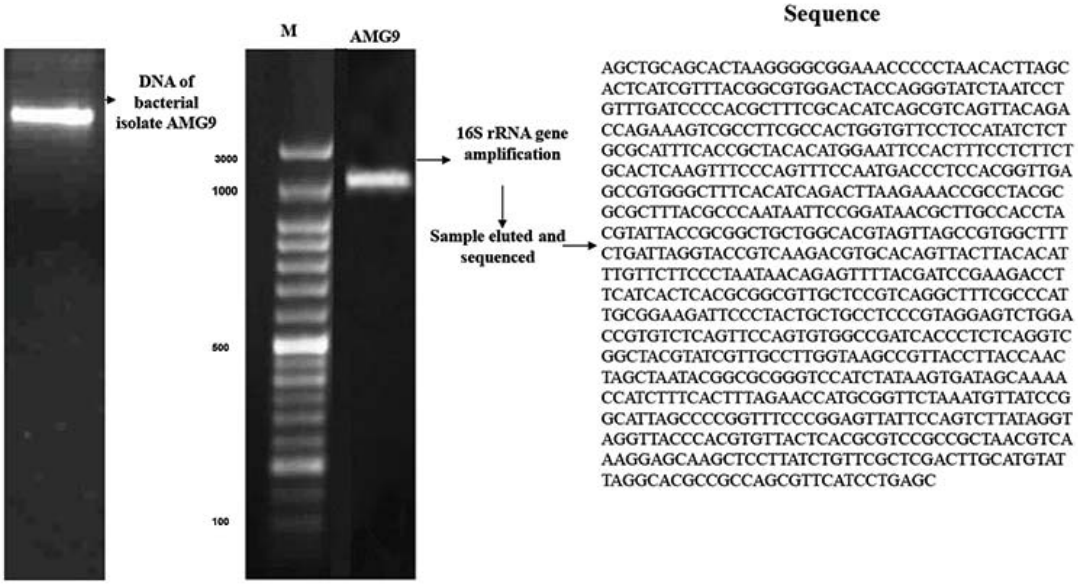

Fig. 1 Molecular identification of bacterial isolate AMG9 based on 16S rRNA amplification (Lane M, DNA marker; Lane1, AMG9)

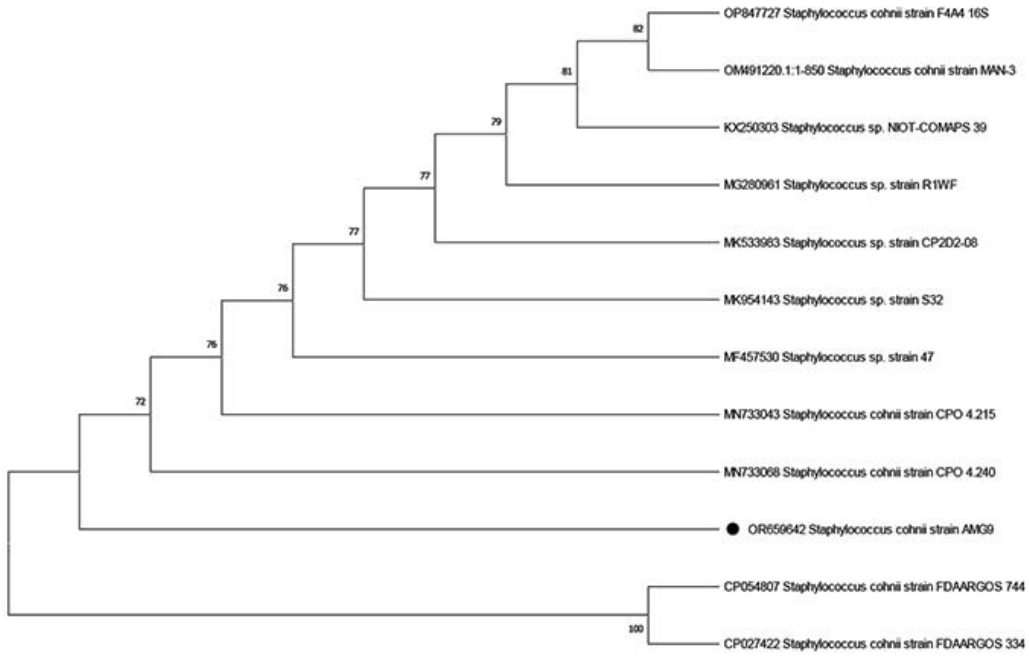

Fig. 2 Phylogenetic tree constructed by using 16S rRNA gene sequences, showing distant relationship of *S. cohnii* strain AMG9 with other strains available from NCBI database (MEGA X)

Some CoNS make biofilm, which is composed of polysaccharides, proteins, and DNA (Götz, 2002).

The grubs of *B. coriacea* were collected from different parts of Himachal Pradesh and identified by examining their raster pattern (Thakur *et al.,* 2022). The grubs were dissected under laminar air flow and bacterial isolation was done on the nutrient agar media. The bacterial isolates were categorized by morphological, biochemical and molecular methods.

The colony morphology of the isolate *S. cohnii* strain AMG9 (where A denotes the location and MG denotes Midgut part of alimentary canal) was circular, convex, entire, and cream in colour. The bacteria were singly arranged cocci, and showed positive results for the gram's reaction. The isolate were able to utilize carbohydrates (Lactose, Maltose, Fructose, Dextrose, Trehalose, Mannose, Inulin, Sodium Gluconate, Glycerol, Salicin, Mannitol, Rhamnose, Cellobiose), perform Esculin hydrolysis and citrate utilization, whereas showed negative results (means not able to utilize) for the Xylose, Galactose, Raffinose, Melibiose, Sucrose, L-Arabinose, Dulcitol, Inositol, Sorbitol, Adonitol, Arabitol, Erythritol, á-Methyl-D-glucoside, Melezitose, á-Methyl-D-mannoside, Xylitol, ONPG, D-arabinose, Malonate, and Sorbose carbohydrates]. *Staphylococcus cohnii* was recorded to be produce halo zones around the colony for starch hydrolysis and not able to produce urease enzyme. The bacteria were identified by direct sequencing of the obtained PCR product (Fig. 1), and by comparing it with the available sequences on the NCBI database. The *Staphylococcus cohnii* strain AMG9 (OR659642) showed 99.98 per cent similarity with the NCBI accession of *Staphylococcus cohnii* strain FDAARGOS-744. Phylogenetic tree was constructed between *Staphylococcus cohnii* strain AMG9 and its closest strains in the GenBank using neighbor-joining method of 16S rRNA sequences (Fig. 2). The dendrogram in figure showed evolutionary relationship of different strains of *S. cohnii*. The bacterial strain AMG9 showed high relatedness with the closest strains at boot-strap value ranged from 75 to 100 per cent at 1000 replicates.

This is a potent cellulose degrading bacterial species which can be used for the degradation of agricultural and biological waste material. In order to manage *B. coriacea*, the bacterial microorganisms were isolated, described, and their potential for insecticidal activity was examined (Sharma, 2013). Mendoza-Otero *et al.,* (2017) reported the draft-genome sequences of two pathogenic strains of *Staphylococcus cohnii* isolated from human. One strain was reported from the blood and the other from a catheter of a male patient.

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Predominance of *Aedes albopictus* in the breeding habitats of Siliguri Sub-division of Darjeeling District, West Bengal, India

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ABSTRACT: To determine the predominant dengue mosquito vector from the Siliguri sub-division of Darjeeling district, a field survey conducted and results showed that *Ae. albopictus* (804 out of 886) was relatively more abundant than *Ae. aegypti* (2 out of 886) in natural and artificial containers. The results from the installed ovitraps also indicated *Ae. albopictus* (1434 out of 1490) as dominant species in the artificial containers than primary vector *Ae. aegypti* (2 out of 1490) in the shared breeding habitats. Larval density of *Ae. albopictus* was remarkably higher than that of *Ae. aegypti* in both the natural-artificial containers and ovitraps.

KEY WORDS: Dengue vectors, natural and artificial habitats, ovitraps, abundance, larval density

Darjeeling is one among the three dengue endemic districts of northern West Bengal and listed as 'high-risk' category in the reports of State Vector Borne Diseases Control and Seasonal Influenza Plan (SVBDCSIP, 2018). Siliguri sub-division alone reports about thousands of dengue cases each year. *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse are the two principal vectors of dengue over the dengue epidemic regions of the world (de Almeida *et al*., 2021). Both, *Ae. aegypti* and *Ae. albopictus* were reported from that area and largely confined to that region (Saha and Saha, 2021). The female *Ae. aegypti* are diurnal biters, mate near the blood-meal host and oviposit exclusively in fresh water (Captain-Esoah, 2020). *Ae. albopictus* mainly occurs in rural and sub-urban regions where they readily oviposit in the natural containers like-tree holes, rotten tree stumps, bamboo stumps but in urban environment they have occupied almost all kinds of artificial containers, especially cemented tanks, different types of plastic containers, glass, metal or earthen pots and even shallow water pools. As breeding habitat for *Ae. aegypti* and *Ae. albopictus* are almost identical, the distribution of both *Aedes* species overlap in many regions (Mbanzulu *et al*., 2022). The present study has been conducted with the aim to report which vector species of *Aedes* genus are most abundant and predominantly occupied the breeding habitats in the surveyed area. Clear understanding about the dengue-mosquito vector will help to know its habitat ecology, design effective vector control measures and dengue prevention.

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The study was conducted over Siliguri sub-division area of Darjeeling district of West Bengal, India, which area falls under the southern foot-plain zone of Darjeeling Himalaya. The area shares international borders with neighbouring nations namely -Bangladesh in south-east and Nepal in west. The sub-division has four blocks, namely - Matigara, Naxalbari, Khoribari and Phansidewa. Larval sampling and installation of ovitraps were done in two sites in each block of the sub-division

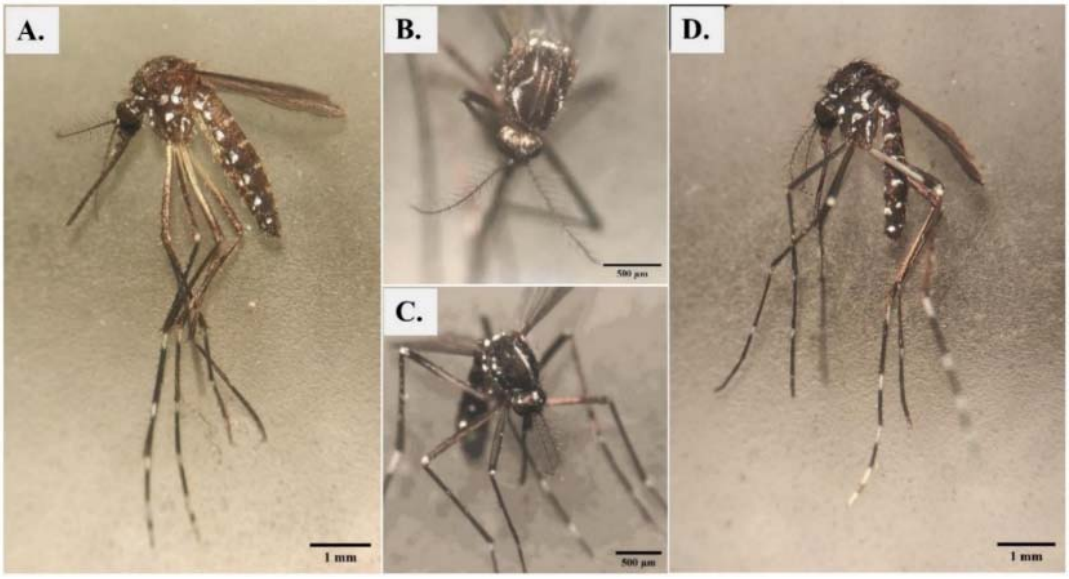

Fig. 1 Image of *Ae. aegypti* (A and B). A. Mesepimeron having two well separated white scale patches; B. Scutum having a pair of sub-median longitudinal lyre-shaped markings; Image of *Ae. albopictus* (C and D). C. Scutum black, having a narrow median longitudinal white stripe; D. Mesepimeron have un-separated V-shaped white patches

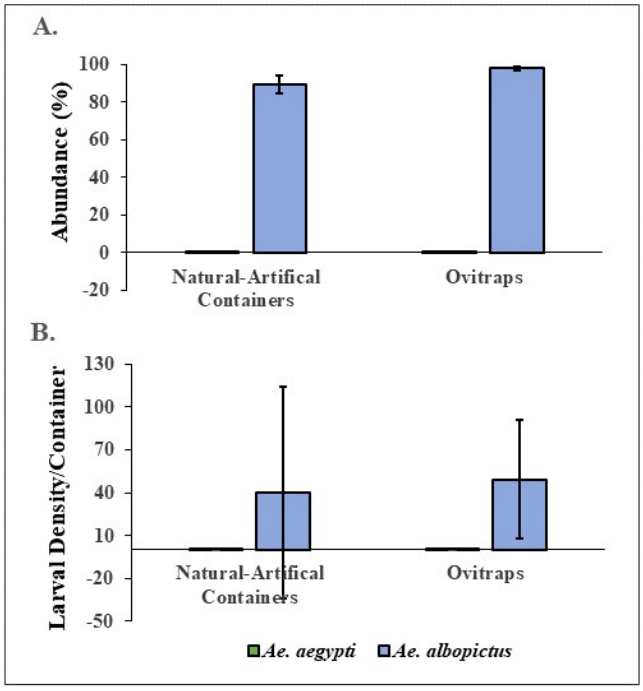

Fig. 2 A. Relative abundance (%) (mean ±SE); B. Larval density (mean ±SD) of *Ae. aegypti* and *Ae. albopictus* in the surveyed water-holding containers and ovitraps from the four blocks of Siliguri sub-divisional area

between October and November, 2022. Ten (5 in each site) water-holding containers in each block (whether natural or artificial) supporting *Aedes* mosquito breeding were randomly inspected for larval sampling. Depending on the habitat size, mosquito larvae and pupae were collected and were brought to the laboratory and reared up to adults (F_0) according to the standard protocol described by Clemons *et al*. (2010). To carry out the entomological surveillance of *Aedes* mosquitoes, standardized ovitraps (oviposition traps) as recommended by CENAPRECE (2015), were installed in each site of the four blocks. Five ovitraps in each site, thus 10 per block were placed with water ($^3/_4$ filled) both in the interior or exterior of the houses as per protocol mentioned by Hernández–Rodríguez *et al*. (2020). A total of 40 ovitraps were installed and the same number of water-holding breeding habitats were inspected for the study. Life history features of fourth-instar larva and all hatched adult mosquitoes were critically analysed under a Magnus Stereoscopic Binocular Microscope, MS-24 for morphological identifications. Systematic identification was done using an *Aedes* based standard morphological key (Rueda, 2005; Tyagi *et al*., 2015). Larval density index (Silver, 2008; Gopalakrishnan *et al*., 2013) and relative abundance (Gopalakrishnan *et al*., 2013; Selvan *et al*., 2015) were calculated to determine the abundance of these mosquito vectors in this region.

Larval density = total no. of individuals of a species/ total no. of positive habitats

Relative abundance (%) = (total occurrence of lavae belonging to a species/total number larvae collected) X 100

Later the mean larval density and relative abundance of the two *Aedes* species among the natural-artificial and ovitrap samples were compared using the independent T-test.

In the survey, 20 out of 40 containers (50%) and 29 out of 40 ovitraps (72.5%) were found as positive breeding habitats of *Aedes* spp. In total, 886 mosquito specimens were collected from the natural and artificial containers in Siliguri sub-division, of which only two individuals were of *Ae. aegypti* and 804 were *Ae. albopictus*. From the ovitraps installed 1490 mosquito individuals were sampled, of which two individuals were *Ae. aegypti* and 1434 were *Ae. albopictus*. Among the natural and artificial containers, larval density (mean ±SD) of *Ae. aegypti* was 0.1 ±0.3 and *Ae. albopictus* was 40.2 ±73.78, whereas in ovitraps larval density of the two mosquitoes were 0.06 ±0.37 and 49.44 ±41.86 respectively. Relative abundance (%) (mean ±SE) of *Ae. aegypti* was 0.72 ±0.55 and *Ae. albopictus* was 89.40 ±4.68 in the natural and artificial containers and in ovitraps were 0.32 ±0.32 and 98.09 ±0.99 respectively. All statistical analysis, was perfomed at a confidence interval of 95 per cent ($p \leq 0.05$). No significant difference in larval density (p= 0.579) and abundance (p= 0.091) of *Ae. albopictus* immatures was found among the natural-artificial containers and ovitrap samples. Similarly, no significant difference in larval density (p= 0.760) and abundance (p= 0.521) of *Ae. aegypti* immatures has found in variance of the natural-artificial containers and ovitrap samples.

Results revealed that *Ae. albopictus* was the more abundant, whereas *Ae. aegypti* was in minimalist proportions in both type of habitats during the two dengue-pick months. In majority of the cases, these two mosquito species were not found in the same habitat although their habitat parameters were identical and they often show sympatry. Rather the mean larval density and abundance of *Ae. aegypti* immatures were significantly in lower side where *Ae. albopictus* has already occupied the habitat. It might be assumed from that, *Ae. albopictus* is replacing *Ae. aegypti* as predominant mosquito-vector in shared breeding containers (whether natural or artificial) of Siliguri sub-divisional area, very similar to the several recent findings that proclaiming the same fact over the globe (Hashim *et al*., 2018; Foster and Walker, 2019; Zhou *et al*., 2022).

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First report of the genus *Mesembrius* Rondani, 1857 (Diptera, Syrphidae) from Kerala

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ABSTRACT: Two hover-flies of the Genus *Mesembrius* Rondai, 1857; *M. bengalensis* (Wiedemann, 1819), and *M. quadrivittatus* (Wiedemann, 1819) are reported for the first time from Kerala along with their diagnosis and key. © 2024 Association for Advancement of Entomology

KEY WORDS: Hover-flies, diagnosis, distribution, key

The genus *Mesembrius* Rondai, 1857 is a widespread Old-World genus with approximately 58 described species altogether from the Oriental Region, Australasian region, the Afrotropical region and Mediterranean Basin of the Palaearctic Region (Jordaens *et al.,* 2021). This genus belongs to the subfamily Eristalinae and Tribe Eristalini. Five species from the genus *Mesembrius* are reported from India namely *M. bengalensis* (Wiedemann, 1819), *M. quadrivittatus* (Wiedemann, 1819), *M. vestitus* (Wiedemann, 1821), *M. tuberculatus* (Brunetti, 1907) and *M. sharpi* Kohli, Kapoor & Gupta, 1988 (Sengupta *et al.,* 2016). in the Brunetti (1923) provided a detailed description of two species, *M. bengalensis* and *M. quadrivittatus* under the genus *Helophilus* Fabricius, 1805 with a key to its species in the 'Fauna of British India'. Male genitalia of *M. bengalensis* are figured by Datta and Chakraborti (1986). Ghorpadé (2014), Mitra *et al.* (2015) and Sengupta *et al.* (2016) discussed the geographical distribution of hover fly species in India and reported *M. bengalensis* from Andhra Pradesh, Assam, Bihar, Chandigarh, Gujarat, Karnataka, Punjab, Tamil Nadu and West Bengal and *M. quadrivittatus* from Andhra Pradesh, Bihar, Chandigarh, Gujarat, Karnataka, Madhya Pradesh, Orissa, Tamil Nadu and West Bengal. *Mesembrius* are marsh dwellers in both the adult and larval stages, well-adapted to live in in marshes and flooded paddy fields throughout the year (Ghorpadé *et al.,* 2011). *Mesembrius* larvae breed in wet fermenting woody pulp (Ghorpadé, 2015), and are regular visitors of orchards and managed gardens (Mira *et al.,* 2008).

Specimens were collected from the Thrissur district, Kerala from flowers of *Boerhavia diffusa* L. and *Premna serrtifolia* L. using a sweep net. The specimens were killed using ethyl acetate and pinned for a thorough examination up to species level was done using Labomed Luxeo 6Z stereo zoom microscope (Brunetti, 1923). Photographs

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were made using a Leica DMC4500 digital camera mounted on a Leica M205 C stereo microscope. The examined specimens were deposited in the insect collection of Shadpada Entomology Research Lab, Kerala, India. The photographs of the host plants were taken and identified with the help of experts. Diagnosis and key to the species under the genera (in Kerala) were prepared using the latest terminology by Van Steenis *et al*. (2023). The species were identified as *M. bengalensis* (Wiedemann, 1819), and *M. quadrivittatus* (Wiedemann, 1819) and are new reportr from Kerala.

Family Syrphidae Latreille, 1897; Subfamily Eristalinae Newman, 1834

Tribe Eristalini Newman, 1834; Genus *Mesembrius* Rondani, 1857

Type species: *Helophilus peregrinus* Loew, 1846

Diagnosis (Based on the known species from Kerala): Eyes bare and holoptic in males and dichoptic in females. Thorax black with yellow vittae. Metafemur strongly incrassate. Wings with cell r_1 narrowly open. Abdomen variably coloured, but usually yellow with black markings.

Key to the species of genus *Mesembrius* of Kerala (Modified from Brunetti, 1923):

Holoptic eyes in males contiguous at least for 5 to 6 facets (Fig. 2A); legs in males with mesofemur suddenly constricted at apex (Fig. 2C); metatibia without any dens (Fig. 2E) ..... ***M. bengalensis*** (Wiedemann, 1819)

Eyes in males not contiguous medially (Fig. 2B); Legs in males with mesofemur gradually narrowed towards apex (Fig. 2D); metatibia suddenly constricted apically, at apex with a dens (Fig. 2F) .....***M. quadrivittatus*** (Wiedemann, 1819)

***Mesembrius bengalensis* (Wiedemann, 1819) (Fig. 1A)**

Eristalis bengalensis Wiedemann 1819.

Eumerosyrphus indianus Bigot 1882.

Eumerosyrphus indicus Bigot 1883.

Diagnosis: Eyes in males contiguous at least for 1/3rd of distance between vertex and frons (5 to 6 facets), occiput black. Thorax with three black vittae, and yellow translucent scutellum. Halteres ochre yellow. Abdominal terga black and orange-yellow, with inverted V marks on 4th abdominal tergite. Legs in males with mesofemur suddenly constricted at apex.

Material examined: 3♂, 17.05.2023, Pullazhi, Thrissur district (10º33' 26.98387'' N; 76º10' 7.56365'' E), Coll. Athul Sankar C., sweep net, host plant: *Boerhavia diffusa* L.

Distribution: Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Karnataka, Tamil Nadu, Himachal Pradesh, West Bengal, Punjab (Ghorpadé, 2019; Mitra *et al.,* 2015). New to Kerala.

Comments: Previously the species were collected from flowers of *Weddelia*, *Tagetes, Mentha, Cucurbita, Rhodiola* and *Sida* spp. (Ghorpadé, 2019; Mitra *et al.,* 2015), while the present study reports it from *Boerhavia diffusa* L.

***Mesembrius quadrivittatus* (Wiedemann, 1819) (Fig. 1B)**

Eristalis quadrivittatus Wiedemann 1819.

Helophilus quadrivittatus Wiedemann: Brunetti 1923.

Mesembrius quadrivittatus Wiedemann: Knutson *et al*. 1975.

Merodon brunetti Sodhi & Awtar 1991.

Diagnosis: Similar to *M. bengalensis,* but differing by the following characters. Eyes in males are not contiguous medially. Legs in males with mesofemur gradually narrowed towards apex, metatibia suddenly constricted apically at apex with a dens. A more extended orange colour on abdomen and black markings on 4th tergum in both sexes. According to Brunetti (1923), the abdominal colour pattern is the only distinguishing feature in the case of female specimens.

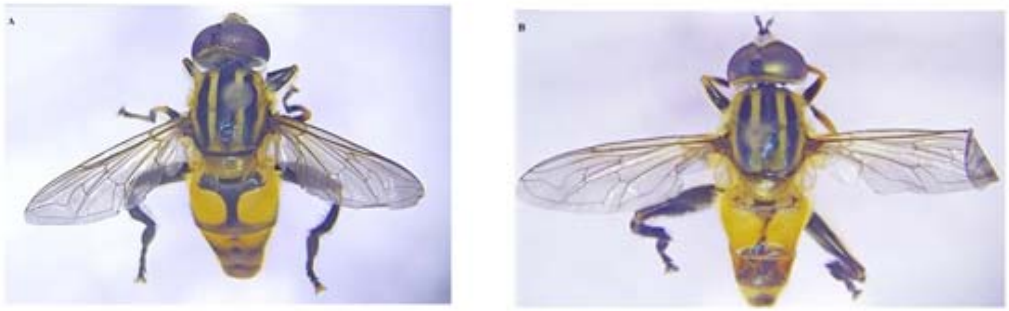

Fig. 1 A - Habitus of male *Mesembrius bengalensis,* B - Habitus of male *M. quadrivittatus*

Fig. 2 (A) *Mesembrius bengalensis* with contiguous eyes at least for 5 to 6 facets, (B) *M. quadrivittatus* without contiguous eyes, (C) *M. bengalensis* mesofemur suddenly constricted at the apex, (D) *M. quadrivittatus* mesofemur gradually narrowed towards the apex, (E) *M. bengalensis* metatibia without a dens, (F) *M. quadrivittatus* apically suddenly constricted mesotibia forming a dens at the appex

Material examined: 3♂, 19.04.2023, Vilangan hills, Thrissur district (10º31' 44.3994'' N; 76º 9' 57.5994'' E), Coll. Athul Sankar C., sweep net, host plant: *Premna serratifolia* L.

Distribution: Andhra Pradesh, Bihar, Chandigarh, Gujrat, Karnataka, Madhya Pradesh, Orissa, Tamil Nadu, West Bengal (Ghorpadé, 2019; Mitra *et al.,* 2015). New to Kerala.

Comments: Previously the species has been collected from flowers of *Weddelia*, *Aegle*, and *Rauvolfia* spp. (Mitra *et al.,* 2008). The present study reported it from *Premna serrtifolia* L.

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A new record of *Rhoenanthus* (*Potamanthindus*) *sapa* Nguyen and Bae, 2004 (Ephemeroptera, Potamanthidae) from India

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ABSTRACT: As part of ongoing exploration of the mayflies in the hill streams of northeast India, *Rhoenanthus* (*Potamanthindus*) *sapa* Nguyen and Bae 2004 was reported as a new record based on larval collections from Meghalaya, India. © 2024 Association for Advancement of Entomology

KEY WORDS: Burrowing mayfly, hill streams, larva, Meghalaya

The burrowing mayfly family Potamanthidae is widely distributed throughout the Holarctic and Oriental regions. The family contains 25 species belonging to four genera worldwide (Bae and McCafferty, 1991; Nguyen and Bae, 2004; Kwanboon *et al.,* 2021; Li and Zhou, 2022). Bae and McCafferty (1991) presented a detailed account of the potamanthid phylogeny and biogeography, and this Laurasian family comprise of four genera, *Rhoenanthus*, *Anthopotamus*, *Potamanthus*, and *Stygifloris. Anthopotamus* is Nearctic, and the others are found in the Oriental region. In India, only three species are recorded: *Potamanthus subcostalis* Navas, 1931 from Maharashtra; *R.* (*Rhoenanthus*) *distafurcus* Bae and McCafferty, 1991 from Kerala; and *R.* (*R.*) *tungaiensis* Balasubramanian *et al.,* 2019 from Karnataka. In the present study, larvae of the subgenus *Potamanthindus* Lestage, 1931, represented by the species *R.* (*Potamanthindus*) *sapa* Nguyen and Bae, 2004 from Meghalaya, were collected from streams and rivers of Meghalaya, by kick-net sampling and hand picking by used brushes. Specimens were stored in ethanol (95%). Photographs were taken with a Leica M205A microscope. Specimens were deposited in National Zoological Collections (NZC) at Zoological Survey of India (ZSI), Kolkata, India.

The larvae of the genus *Potamanthus* were easily distinguished from those of the genus *Rhoenanthus* or *Anthopotamus* by their short tusks, weakly setaceous mouthparts and weakly developed bipectinate-hairlike setae along anterior margin of

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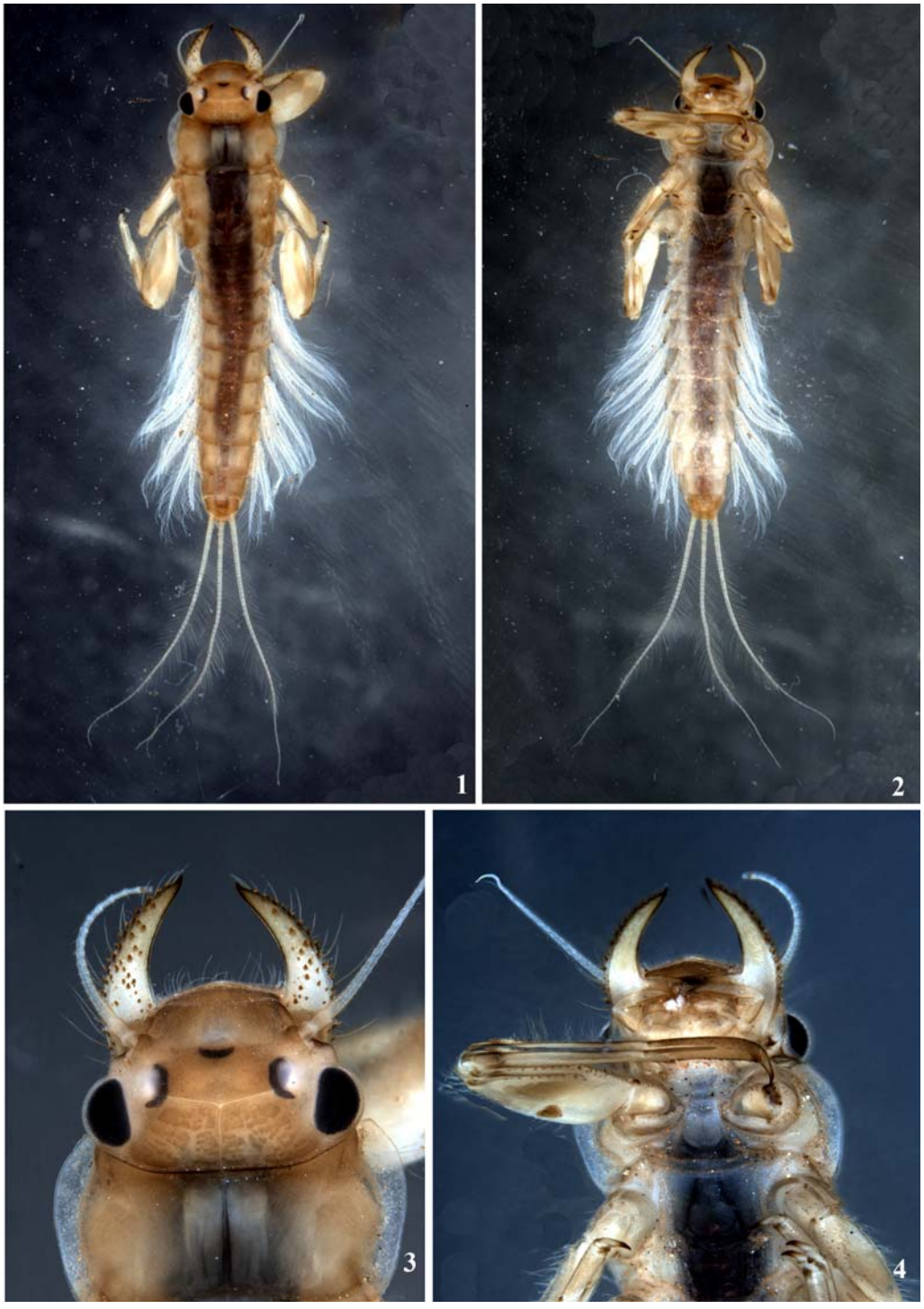

Figs. Larva of *Rhoenanthus (Potamanthindus) sapa* Nguyen & Bae, 2004.
1. Dorsal view, larval habitus; 2. Ventral view, larval habitus; 3. Dorsal view, head and pronotum; 4. Ventral view, anterior portion of body

foretibiae. The larvae of the genus *Rhoenanthus* differentiated from the larvae of the genus *Potamanthus* by their long mandibular tusks and setaceous mouthparts. The larvae of the genus *Rhoenanthus* were easily distinguished from those of *Anthopotamus*, which also possess well-developed tusk, by their relatively long foretibiae and the presence of bipectinate-hair like setae of the dorsal and lateral foretibiae (Bae and McCafferty, 1991). *Rhoenanthus* has two subgenera viz., *Rhoenanthus* s.s. and *Potamanthindus*. Larvae of subgenus *Rhoenanthus* s.s. were distinguished from subgenus *Potamanthindus* by their terminally spined mandibular tusks that appear forked and slender maxillary palpi; the adults were distinguished by MP2 of forewings being basally connected to CuA and by their dorsoventrally flattened, deeply furcated and apically rounded penes. Larvae of subgenus *Potamanthindus* were distinguished from *Rhoenanthus* s.s. by the absence of a large, subapical, lateral spine on mandibular tusks and also possess relatively thick maxillary palpi; the adults were distinguished by MP_2 of forewings being basally connected to MP_1 and by their basally somewhat cylindrical, Y-shaped, and apically notched penes (Bae and McCafferty, 1991). The subgenus *Potamanthindus* consisted of six species viz., *R.* (*Potamanthindus*) *coreanus* (Yoon and Bae, 1985); *R.* (*P.*) *hunanensis* (You and Gui, 1995); *R.* (*P.*) *magnificus* Ulmer, 1920; *R.* (*P.*) *obscurus* Navas, 1922; *R.* (*P.*) *sapa* Nguyen and Bae, 2004; and *R.* (*P.*) *youi* (Wu and You, 1986).

Rhoenanthus* (*Potamanthindus*) *sapa Nguyen & Bae, 2004 (Figs. 1–4)

Materials examined: 2 larvae, INDIA, Meghalaya, East Khasi Hills, Khrang village, Wankwar River, 25.32481 N, 91.77519 E, 1658 m, 02.iii.2016, coll. E. Eyarin Jehamalar (Reg. No. 5143/H13).

Diagnosis: *R.* (*P.*) *sapa* could be distinguished from other species of *Rhoenanthus* by the following combination of characters: In larvae (i) foretibiae relatively short (ca. 1.1x length of fore femora, 1.9x length of fore tarsi) and their filtering setae relatively short and weakly developed (Figs. 2, 4); (ii) gradually attenuated and moderately arched (27.7°) mandibular tusks that possess mixed simple-stout and hairlike setae throughout dorsal and lateral surfaces (Figs. 1, 3); in adults (iii) forewings of female (Figs. 3, Nguyen and Bae 2004) lightly stained brown in costal and central areas and (iv) basal R1 of hind wings strongly bent to costal area and costal projection acute (Fig. 4, Nguyen and Bae 2004). *R.* (*Potamanthindus*) *sapa* was originally described from Vietnam based on larvae and female adult by Nguyen and Bae (2004) and Han *et al.* (2021) reported from China, with details of distribution, habitat, biology and phylogenetic characters of this species. Based on the larval collections of Ephemeroptera from northeast India, a new record of *R.* (*P.*) *sapa* has been established, which is a significant range extension to the distributional range of this species.

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Biology and morphometrics of *Bradysia tritici* (Coq.) (Diptera, Sciaridae) on milky mushroom in Navasari, Gujarat, India

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ABSTRACT: Biology and morphometrics of *Bradysia tritici* (Coq.), a major pest of milky mushroom (*Calocybe indica* P&C), were studied. Adult longevity of male and female was 4.75±1.64 and 6.10±0.91 days, respectively. The female laid an average of 40.45±5.21 eggs in her life period. The eggs were singly laid in clusters of 2 to 3 and hatched in 2.50±0.51days and the viability of eggs were 83.26±5.88 per cent. The larval stage completed in 11.10±1.07 days. The pre-pupal, pupal stages and total life cycle of male and female lasted for 1.60, 2.70.47.0 and 20.15 and 21.50 days, respectively.

KEY WORDS: Sciarid fly, pest, insect, milky mushroom, life stages, life cycle, fecundity

Bradysia tritici (Coq.) (Diptera, Sciaridae) on milky mushroom, is a major pest of milky mushroom *(Calocybe indica* P&C). Sciarid fly larvae causes damage to substrate, compost and casing materials. It causes damage 32 - 75 per cent (Sandhu and Brar, 1980; Khan and Javed, 2002). The biology and morphometrics of sciarid fly were studied at Post Graduate Research Laboratory of the Department of Entomology, N.M. College of Agriculture, Navsari Agriculture University, during May 2022. To raise the initial culture of sciarid fly, the adult flies were collected using aspirator from the mushroom bags procured from the Mushroom Unit. The sciarid fly was reared in the glass jars filled with spawned paddy straw layer of 2 to 3 inches. Some pieces of mushroom were placed over it. Ten adult pairs were transferred into glass jars using aspirator and honey solution (10%) was provided to them as food. Glass jars were covered with black muslin cloth and held in position with help of rubber band. Adults laid eggs on the paddy straw with mushroom, at 26.7±1.66^{0}C temperature and 71.11±2.45 per cent relative humidity. After 24 hours, eggs laid by each female were removed from stock culture using a soft camel brush. The eggs were counted and kept in separate Petri plates to find out the incubation period and hatching per cent. Yong larvae were transferred to the Petri plates containing mycelial colonized paddy straw individually. The moisture was maintained by applying water over paddy straw. Measurement and period of each instar were recorded. Larvae were kept undisturbed for pupation. Pre-pupal and pupal period were recorded and pupae were allowed for adult emergence. The emerged adults were fed with honey solution as food and adult period, pre-

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oviposition, oviposition and post-oviposition period and measurement of male and female were recorded separately. A stereo-trinocular microscope Olympus-SZ (16) equipped with a brand Catcam-130 camera having software power Scope photo was used to measure the length and breadth of various stages of the sciarid fly.

The biology and morphometrics of sciarid fly, *B. tritici* was studied in detail (Table 1, 2, Fig. 1). The female laid eggs singly in clusters of 2 to 3 eggs on the spawned mycelial paddy straw. The freshly laid eggs were pulpy and white in colour. The fertile eggs were transparent and turned brown with the passage of time, while infertile eggs noted as slightly shriveled. Eggs were round to oval in shape and not visible to the naked eyes. At egg eclosion, the larval head become distinct and appeared with black dot in the egg. Length and breadth of eggs varied 0.21 to 0.25 and 0.11 to 0.15 respectively. The similar observation of egg laying pattern, shape and colour were reported by Sandhu and Brar (1980), Brar and Sandhu (1990) and Shivanna *et al.* (2003). The incubation period was 2 to 3 days with an average of 2.50±0.51days.

There were four larval instars. First instar body of freshly emerged maggot was dirty white in colour, transparent with a distinct shining black head which was quite visible to the naked eye. Apodous maggots were cylindrical in shape with visible alimentary canal. This is in accordance with the larval description given by Brar and Sandhu (1980). Length of the first instar ranged 1.32 to1.80, while breadth varied 0.18 to 0.25mm. More or less similar measurement were reported by Lewendowski *et al.* (2004), who reported that length of first instar larvae of *B. tritici* was 0.36 to 1.40mm with average of 0.76±0.22mm. First instar larva completed on 2 to 3 days. Katumanyane (2020) noticed that larval period of first instar lasted for 2 days. Morphologically second instar larva resembled to the first instar but differ in size. Length varied from 2.60 to 4.45 and breadth 0.32 to 0.44mm. This is in agreement with Katumanyane (2020) on *B. impatiens* who reported it as 2.6 to 4.5mm in length and 0.3 to 0.45 mm in width. The second instar lasted for 2 to 3 days. Khanna *et al*. (2017), also similar observation of 2 to 3 days. In third instar the raw food substances could be seen inside their abdomen. Length and breadth varied from 4.30 to 6.30 and 0.43 to 0.63mm. Katumanyane (2020) reported that the length varies from 4.6 to 6.5 mm in length, and 0.46 to 0.65 mm in breadth. The duration of third instar l was 2 to 3 days. Khanna *et al.* (2017) reported that third instar of *B. tritici* lasted for 2 to 3 days with an average of 2.40±0.44 days when reared on *Agaricus bisporus,* while Katumanyane (2020) observed it as 2 days for *B. tritici*.

The fourth instar feeding was voracious and more active than other instars. Fully mature larvae stopped feeding and crawled down to casing material for the pupation. The length of fourth instar ranged between 6.61 to 7.72 and the breadth 0.64 to 0.75mm. Shivanna *et al.* (2003) reported that the average length and width of fourth instar of *B. tritici* as 7.22±0.33 and 0.66±0.06 mm, respectively. The duration of fourth instar larvae varied from 2 to 4 days. Khanna *et al*. (2017) and Katumanyane (2020) reported similar observation. The total larval developmental period of *B. tritici* varied from 9 to 13 days when reared on mycelia of milky mushroom. According to Khanna *et al.* (2017) larval stage of sciarid fly was lasted for 9 to 13 days with an average of 11.10±1.07 days on button mushroom. Shivanna *et al*. (2003) noted that the total larval developmental period of *B. tritici* varied from 9 to 11 days with an average of 9.73±0.05 days.

The full-grown larvae before pupation passed through a pre-pupal stage. In this stage fully mature larvae stopped feeding and reached to the casing material. At this stage larvae became motion less and spun little quantity of silk to form a very loose type of pseudo-cocoon. It is dirty white in color and its length got reduced. Sandhu and Brar (1980) observed that in the pre-pupal stage, the full-grown larvae became sluggish with suspended feeding and movement. Its length got reduced and forms pseudo-cocoon. The length of pre-pupae varied 2.08 to 2.53, while the breadth 0.21to 0.25mm. The length and the breadth of pre-pupal stage was 2.51 and 0.23mm (Khanna *et al.,* 2017). The pre-pupal period

Table 1. Biology of sciarid fly, *B. tritici* on milky mushroom

Particulars	Min.	Max.	Mean ± SE
Incubation period (days)	2.00	3.00	2.50±0.51
Hatching (%)	71.43	92.86	83.26±5.88
First instar (days)	2.00	3.00	2.60±0.50
Second instar (days)	2.00	3.00	2.75±0.44
Third instar (days)	2.00	3.00	2.55±0.51
Fourth instar (days)	2.00	4.00	3.20±0.70
Total larval period (days)	9.00	13.00	11.10±1.07
Pre- pupal period (days)	1.00	3.00	1.60±0.60
Pupal period (days)	2.00	3.00	2.70±0.47
Pre- oviposition period (hrs)	24	48	35.40±9.11
Oviposition period (hrs)	24	36	30±6.16
Post- oviposition period (hrs)	36	48	41.40±6.13
Sex ratio (Male: Female)	1:1.33	1:2.40	1:1.78±0.34
Male longevity (days)	4	6	4.75±1.64
Female longevity (days)	5	7	6.10±0.91
Male- Total life cycle (days)	17	23	20.15±1.79
Female - Total life cycle (days)	19	25	21.50±1.64
Fecundity (eggs/female)	35	52	40.45±5.21

ranged 1 to 3 days during the present study. This is in accordance with Khanna *et al.* (2017), who reported pupal period of *Bradysia* sp as 1.36±0.27 days.

The newly formed pupa was dirty white in color which changed to yellowish brown after 1 to 2 days of pupation. The mature pupa was dark grey to black in colour and at this stage compound eyes and appendages become distinct. The shape of the abdominal tips of male is quite different than female. The last abdominal segment of the male pupae was broader and relatively smaller than female. This is in accordance with the findings of Sandhu and Brar (1980). The pupae measured 2.38 to 2.56 in length, while the breadth 0.51 to 0.56mm. According to Shivanna *et al.* (2003) the length and breadth of pupa ranged between 2.10 to 2.80 with an average of 2.39±0.19 mm and breadth ranged between 0.71 to 1.04 with an average of 0.80±0.07 mm, respectively. The duration of pupal stage varied 2 to 3 days. This is in contrast with the Shivanna *et al.* (2003) who reported the pupal duration was varied from 3 to 5 days with an average of 4.18±0.30 days.

Adults emerged during evening hours and resembled to mosquitoes. Greyish black adults possessed 14 annuli on the antennal flagellum in both the sexes. The flies had elongated abdomen

Fig. 1 Biology of sciarid fly, *Bradysia tritici* on milky mushroom

with long legs and wings. The male and female were similar in appearance except the shape of abdomen and size of the body. In case of male, the abdomen was slender and terminated in double claw like structure known as 'Claspers', while in case of female, the abdomen was swollen and terminated in a pointed ovipositor. The size of male sciarid fly, *B. tritici* was relatively smaller than female. The present findings are in agreement with the findings of Brar and Sandhu (1980), Shivanna *et al.* (2003) and Khanna *et al.* (2017). The length of male fly varied 2.17 to 2.66mm with a wing span ranged from 0.84 to 1.02mm. In female, the length varied from 2.71 to 3.15 with a wing span from 1.27 to 1.75mm. These results are corroborated with the reports of Shivanna *et al.* (2003).

The fecundity of sciarid fly varied from 35 to 52

Table 2. Morphometrics of different stages of *B. tritici*

Stage	Length (mm)			Breadth (mm)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Egg	0.21	0.25	0.23±0.01	0.10	0.15	0.13±0.01
I instar	1.32	1.50	1.57 ± 0.12	0.18	0.25	0.22 ± 0.02
II instar	2.60	4.45	3.80 ± 0.04	0.32	0.44	0.38 ± 0.55
III instar	4.30	6.30	5.46 ± 0.59	0.43	0.63	5.46 ± 0.59
IV instar	6.61	7.72	7.11± 0.34	0.64	0.75	0.68 ± 0.03
Pupa	2.38	2.56	2.49 ± 0.05	0.51	0.56	0.54 ± 0.02
Male	2.17	2.66	2.51 ± 0.17	0.84	1.02	0.40 ± 0.05
Female	2.71	3.15	2.89 ± 0.10	1.27	1.75	1.52 ± 0.15

eggs. Shivanna *et al.* (2003) recorded fecundity of *B. tritici* from 50 to 70 eggs with an average 60.33±6.32 eggs; While, Khanna *et al.* (2017) noted it from 27 to 56 eggs with an average 42.3±6.32 eggs. The pre-oviposition period varied from 24 to 48 hours. Shivanna *et al*. (2003) recorded it as 36 to 48 hours with an average of 42.05±0.09 hours. Oviposition period varied from 24 to 36 hours. Shivanna *et al.* (2003) revealed the oviposition period as 12 to 36 hours with an average of 26.44±0.40 hours. The post-ovipositional period varied from 36 to 48 hours with an average of 39.43±10.83 hours. It varied from 36 to 48 hours with average of 40.45±0.82 hours when fed on white button mushroom (Shivanna *et al.*, 2003). The fecundity of sciarid fly reared on mushroom varied from 35 to 52 eggs with an average of 40.45±5.21 eggs. Shivanna *et al.* (2003) observed fecundity of *B. tritici* varied from 50 to 70 eggs with an average 60.331±6.32 eggs per female. However, slight variation in fecundity may be due to nutritional values of the host. The sex ratio of *B. tritici* was 1:1.78±0.34 (Male: Female). Shivanna *et al.* (2003) reported sex ratio of *B. tritici* as 1:1.32. The total life cycle varied from 17 to 23 days in males, while in females it varied from 19 to 25 days at 26.7±1.66^{0}C and 71.11±2.45 per cent RH. Sandhu and Brar (1980) observed that the mean duration of life cycle was 28 days at 20^{o}C and 23.89 days at 22^{o}C, respectively. The present study will be useful in developing suitable IPM strategies against the pest.

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First report of *Bactrocera dorsalis* (Hendel) (Diptera, Tephritidae) on the white - fleshed dragon fruit *Selenicereus undatus* (Haworth) D.R. Hunt (Cactaceae) in India

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ABSTRACT: The oriental fruit fly *Bactrocera dorsalis* (Hendel) has been reported on the white fleshed dragon fruit *Selenicereus undatus* (Haworth) D.R.Hunt for the first time in India. Symptoms of infestation and the duration of all life stages were observed. Females laid eggs just beneath the mature fruit skin. The tissue surrounding the egg mass turned light yellow. Oviposition punctures were visible on the fruit rind and the feeding of the maggots led to decay of internal contents, foul smell and the fruit turned in to a discolored semi liquid mass**.** Full grown maggots exited the fruit and entered a period of inactivity before pupation in moist soil. The egg, maggot and pupal stages lasted for 1.6± 0.40, 8.0 ± 0.40 and 8.0 ± 0.31 days, respectively under laboratory conditions. Adult longevity was 9.6 ± 0.5 days in the case of females and 6.4 ± 0.50 days for males. © 2024 Association for Advancement of Entomology

KEY WORDS: Pest, symptoms, development, life history, longevity

The white fleshed dragon fruit, *Selenicereus undatus* (Haworth) D.R. Hunt (= *Hylocereus undatus* (Haworth) Britton & Rose) (Cactaceae), native to Mexico, Central and South America, is a trailing, epiphytic cactus widely cultivated in the tropics and subtropics for its fruit. Introduced into India in the 1990s, this exotic fruit is gaining popularity among farmers for its fast growth, suitability for varied agro-climatic conditions including arid and barren soils, high profitability and low input requirement (Nangare *et al*., 2020). Globally dragon fruit is cultivated in 1.12 lakh hectares, with a production of over 2.1 million tons in 2017–18. In India, the crop is cultivated in 3,000–4,000ha and the production is over 12,000 metric tons in 2000 (Wakchaure *et al.,* 2020). The crop is largely free of insect pests in India, except for minor pests such as ants, scale insects and mealy bugs (Wakchuare *et al*., 2020) and the caterpillar *Spodoptera litura* (F.) (Prathapan and Santhoshkumar, 2022).

The Oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera, Tephritidae), originally described from the erstwhile Formosa in 1912 by Friedrich Hendel, is one among the most destructive five fruit flies in the World (Wei *et al*., 2017). Currently it is distributed across 75 countries in four continents *viz*. Africa, Asia, North America and South America and Oceania (Zeng *et al*., 2018). USDA (2016) listed 436 plant species as hosts for *B. dorsalis*. According to Liquido *et al*. (2019), it has been

* Author for correspondence

recorded on 488 species in 215 genera belonging to 80 families. EPPO (2024) provided a list of 475 species of host plants worldwide, belonging to 80 plant families. *Selenecereus undatus,* listed amongst the host plants of *B. dorsalis* (USDA, 2016; EPPO, 2024), infested 76.9 per cent of fruits in the La Reunion in the Pacific Ocean (Moquet *et al*., 2021). The earliest record of *B. dorsalis* in India is that of Fabricius (1764), under the disused name '*Musca ferruginea'*. It was reported on mango at 'Mozafferpore' in June 1890 and July 1891 by E.C. Cotes (1891, 1893) as *Dacus ferrugineus*. The main host of *B. dorsalis* in India is mango followed by many other fruit crops including guava, loquat, pear, fig, persimmon, banana, pomegranate, oranges, avoccade, sapota, rose apple, Singapore cherry, sour cherry and star fruit (Kapoor, 1993). David and Ramani (2011)

Figs 1a"f. Life stages of *Bactrocera dorsalis* and its damage on *Selenicereus undatus:*
a. egg, b. maggot feeding on the decayed fruit, c. pupae d. adult, female, d. oviposition punctures on mature fruit, e. feeding symptoms of maggots

provided check-list and illustrated key to the fruit flies of Peninsular India and the Andaman and Nicobar Islands. *Bactrocera dorsalis* can be differentiated from other common fruit flies by the following characters: abdominal tergites separate; anterior supra-alar seta present; acrostichal seta present; scutum without medial postsutural vitta; costal band of almost uniform width; all femora yellow; scutum predominantly reddish-brown with dark fuscous markings; costal band confluent with R2+3; and lateral postsutural vitta uniform in width and reaches intra alar seta. Males of *B. dorsalis* are attracted to methyl eugenol. Infestation of *B. dorsalis* was noticed on the white-fleshed dragon fruit *S. undatus* in the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala (8Ú 25' 46" N; 76Ú 59' 24" E, 29m above mean sea level) during August, 2023. Two fully mature fruits were found infested. The affected fruits were brought to the laboratory and kept in glass containers till pupation and adult emergence. Two pairs each of the newly emerged adults were released in five plastic containers Honey was provided as food for the adults and mature fruits of *S. undatus* were provided for oviposition. Symptoms of infestation and the duration of all life stages were observed. Voucher specimens of *B. dorsalis* will be deposited in the ICAR - National Bureau of Agricultural Insect Resources, Bengaluru.

Females laid eggs just beneath the mature fruit skin. The tissue surrounding the egg mass turned light yellow. These eggs were seen in clusters just beneath the rind (Fig. 1a). Oviposition punctures were visible on the fruit rind (Fig. 1e) and the feeding of the maggots led to decay of internal contents, foul smell and the fruit turned in to a discolored semi liquid mass. Maggots fed voraciously within the pulp of the fruit, creating tunnels and holes in both the pulp and peel (Figs. 1b, f). Full grown maggots exited the fruit and entered a period of inactivity before pupation in moist soil. The newly formed pupa was yellowish-brown that gradually changed to dark brown over time (Fig. 1c). The egg, maggot and pupal stages lasted for 1.6± 0.40, 8.0 ± 0.40 and 8.0 ± 0.31 days, respectively under laboratory conditions. The adult longevity was 9.6 ± 0.5 days in the case of females and 6.4 ± 0.50 days for males. Moquet *et al*. (2021) reported 76.9 per cent infestation of *B. dorsalis* on cultivated varieties of *S. undatus* in the La Reunion in the Pacific Ocean. *Ceratitis capitata* (Wiedemann) was also reported on dragon fruit in Hawaii (USDA-APHIS, 2006). In Vietnam and elsewhere, *B. dorsalis* and *B. correcta* (Bezzi) are major pests (Nangare *et al*., 2020). This is the first report of *B. dorsalis* on *S. undatus* in India.

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Occurrence of *Antonina pretiosa* (Ferris) (Homoptera, Pseudococcidae) on the inflorescence of *Bambusa bambos* (L.) Voss in Assam, India

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ABSTRACT: *Antonina pretiosa* (Ferris) was reported for the first-time on the inflorescence of *Bambusa bambos* (L.) Voss during its sporadic flowering in Kamrup Rural district of the state of Assam. Egg cases, nymphs and adults of *A. pretiosa* were found in the inflorescence. They were attended by the black ants *Technomyrmex albipes*.

KEYWORDS: Bamboo, mealy bugs, life stages, ants

Bamboos are characterized by two types of flowering, *viz*. sporadic and gregarious (Biswas *et al*., 2016; Das *et al*., 2018). In the former, flowering takes place at a time only in a few culms or a few culms of a population while in the latter, flowering occurs within a brief interval of time amongst all the individuals of a species growing across large areas leading to the mortality of culms after flowering (Janzen, 1976; Xie *et al*., 2016). In India, flowering of *Bambusa bambos* (L.) Voss (=*B. arundinacea*) has been reported from different states *viz*., Assam (Sarma *et al*., 2010; Sharma and Borthakur, 2018), Uttar Pradesh (Malik, 2016) and Uttarakhand (Chandra *et al*., 2022).

Sporadic flowering in two clusters of bamboos has been observed in the Mandakata area of the Kamrup Rural district of Assam (located at 26°13.083' N; 91°44.024' E) during March to May 2023. Egg cases of *Antonina pretiosa* (Ferris) (Homoptera, Pseudococcidae) along with roaming black ants *Technomyrmex albipes* were observed in the inflorescence (Fig. 1 A, B-D, F). These egg cases were 2-3mm long and around 2mm wide, brown-black and covered by white cottony substances (Fig. 1 F-H), distinct long anal wax tube (Fig. 1 F), and around 0.2mm long eggs inside the cases (Fig. 1 I). Adult female insect was around 4mm long (Fig. 1 D-E) and nymphs were 0.4mm long and 0.2mm wide (Fig. 1 J).

Association of insects with bamboos has already been reported from India (Mathew and Varma, 1988; Kazmi and Husen, 1999; Koshy *et al*., 2001;

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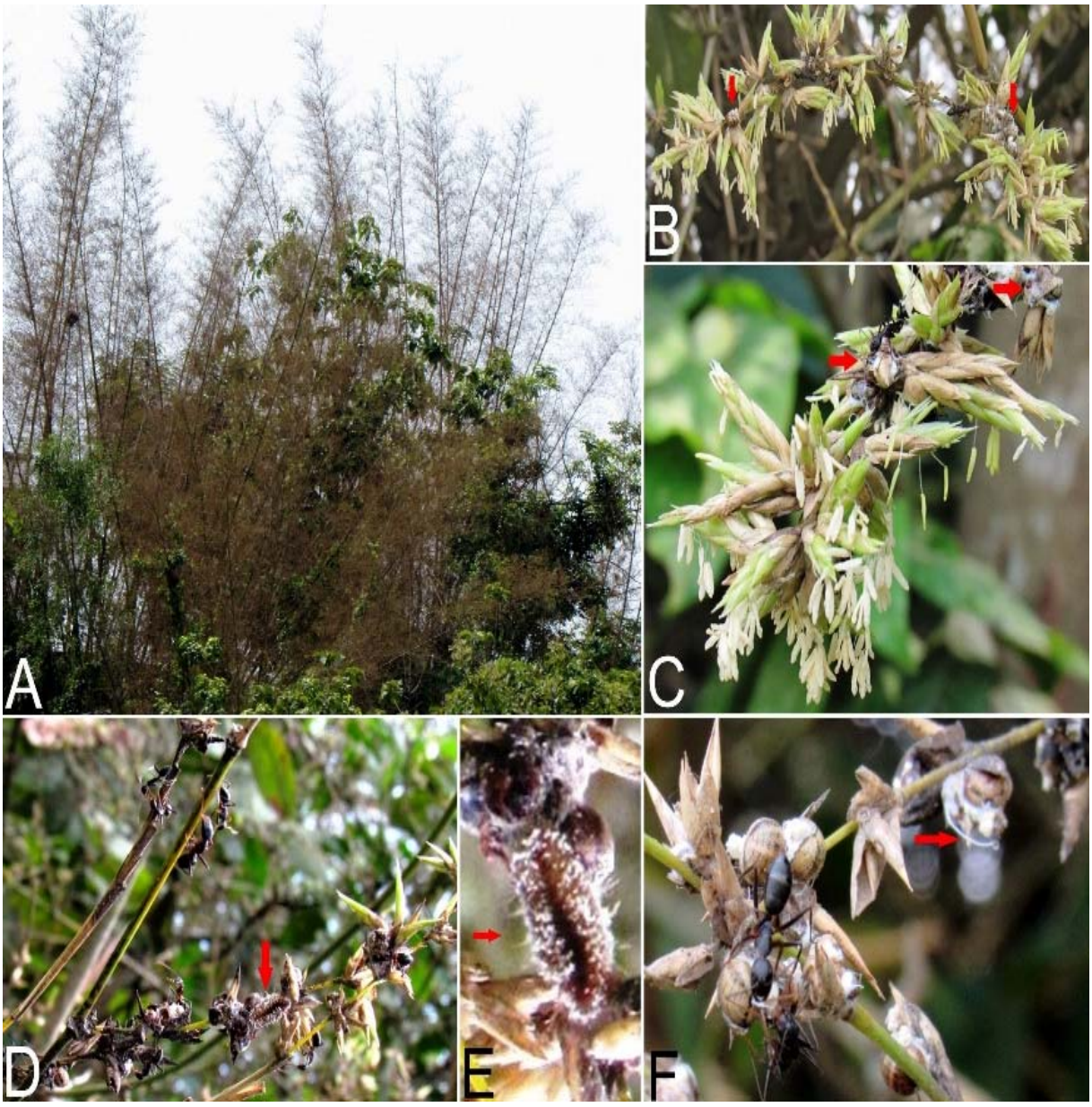
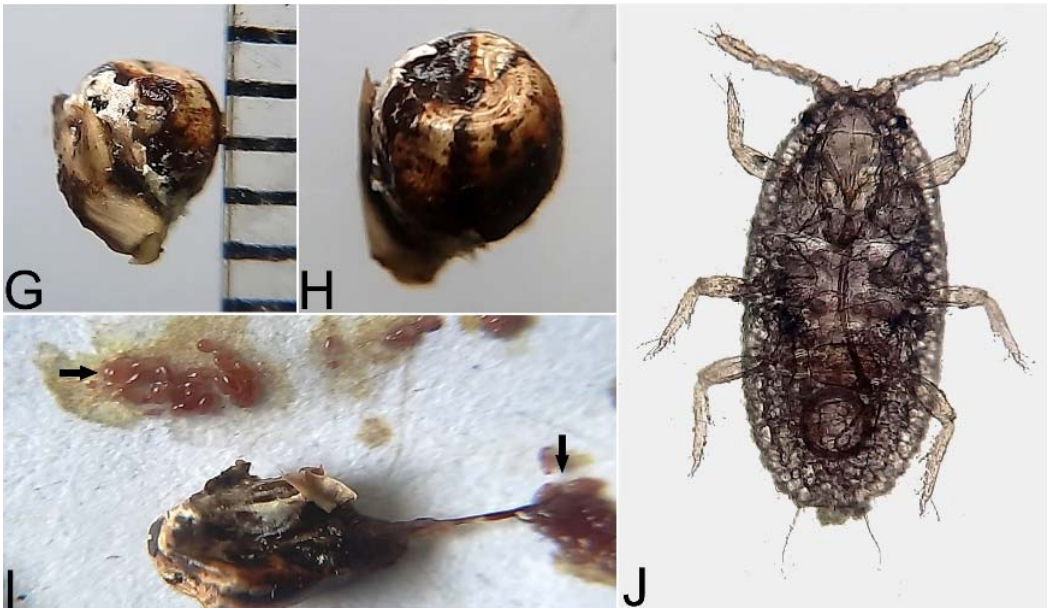


Fig. 1: A. Flowering of *Bambusa bambos.* B-C *Antonina pretiosa* egg cases (arrow) and black ants, D-E. Adult *A. pretiosa* (arrow) in bamboo inflorescence, F. Association of black ants with egg cases of *A. pretiosa* (arrow showing long anal wax tube)



G-H. Egg cases of *Antonina pretiosa,* I. Eggs (arrow) inside the egg case, J. Nymph of *A. pretiosa*.

Joshi *et al*., 2008; Varma and Sajeev, 2015). *Antonina graminis* (Maskell) (=*A. indica*), *A. pretiosa* and *A. zonata* (Green) are common sap suckers on the foliage and culms of different bamboo species in the country (Varma and Sajeev, 2015). Although *A. pretiosa* commonly occurs in the nodes of the stem and under the leaf sheath of bamboo (Ülgentürk *et al*., 2014), its occurrence in the inflorescence of bamboos has not been so far reported.

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OBITUARY

Dr A. Visalakshi

A PIONEERING PESTICIDE RESIDUE ANALYST & ENTOMOLOGIST PASSES AWAY

1939–2024

We are deeply saddened to record the passing of Dr A. Visalakshi, former Professor and Head of the Department of Agricultural Entomology at the College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram on May 31, 2024. She was born to parents of Tamil origin in Thiruvananthapuram, Kerala in 1939. She received her B Sc degree in Agriculture, M Sc in Agricultural Entomology and doctoral degree from the Kerala Agricultural University in 1960, 1963 and 1977, respectively. Her doctoral thesis on 'Dissipation of phorate residues in soil and cowpea plant in relation to pea aphid control and soil microbe population' was the first of its kind in the Kerala Agricultural University.

Soon after graduation in 1960, Dr Visalakshi joined the College of Agriculture, Vellayani, then affiliated to the University of Kerala, as a Research Assistant and was later shifted to the Department of Agricultural Entomology, with which she was associated all through her professional life. Dr Visalakshi is known for her work in toxicology, pesticide residues and insect pests. She established a separate unit for pesticide residue analysis as early as 1974, under the Insect Toxicology section of the Department of Agricultural Entomology. She took the initiative to newly establish a Centre of the All India Coordinated Project on Pesticide Residues, funded by ICAR, at the College of Agriculture, Vellayani despite significant challenges and became the Residue Analyst and in-charge of the Centre in March 1987. Her determination and dedication ensured that Kerala was not left behind in this crucial area of research.

She was the first to report the presence of pesticide residues in various food items from Kerala. As the Principal Investigator, she systematically analysed pesticide residues in food commodities such as milk and milk products, cattle feed, fodder, cereals, pulses, vegetables and fruits collected from markets. Her investigations revealed the presence of HCH residues in breast milk samples, collected from lactating mothers in the Women and Children Hospital, Thiruvananthapuram for the first time in Kerala. The extent of pesticide contamination in various food commodities in the rice and spice growing belts in the state, as revealed through her studies, assisted policymakers and planners in formulating strategies for the safe use of pesticides.

During the early periods of her career as Assistant and Associate Professor, she worked closely with the personnel of the Kerala State Department of Agriculture to find solutions to many problems related to insect pests and pesticide use. She has identified and reported six new insect pests and has worked out control measures against 26

crop pests, of which nine were accepted for inclusion in the Package of Practices Recommendations of the Kerala Agricultural University. As a plant protection extension specialist, she has also trained a multitude of farmers in integrated pest management.

Under her leadership, Team Visalakshi has made significant strides in pesticide residue analysis. They elucidated the persistence and degradation of organophosphate and carbamate insecticides in various crops such as rice, bananas, vegetables and fruit crops. She further extended her focus to spices such as cardamom, pepper, and ginger, where she overcame significant challenges to develop reliable pesticide residue data. She was the first to take up pesticide residue analysis in cardamom and pepper, two high-value spices exported from Kerala.

Dr Visalakshi's commitment and dedication were unparalleled. She travelled extensively throughout the state, including the remote high ranges of Idukki district, to conduct field experiments. She collaborated with other renowned workers in the field, such as Dr A. Regupathy from TNAU, Coimbatore, to analyse samples and generate valuable data. Her research contributed to developing pesticide recommendations for managing serious pests affecting crops such as coconut, bananas, and vegetables.

Dr Visalakshi was an exceptional educator. Her unique teaching style and practical assignments inspired countless students. Her ability to create enthusiasm for entomology and pesticide courses is fondly remembered by her students. She has teaching experience of 27 years, during which she has guided one Ph D and six M.Sc. (Ag) students and served on the Advisory Committee of 27 students.

She was an active member of the Association for Advancement of Entomology and served both on the Executive Committee and the Editorial Board of *Entomon*. Dr Visalakshi was one of the key members instrumental in the revival of *Entomon* during 2013-14 and the Association owes allegiance to her for the staunch support during the struggle. She has also served the Kerala Agricultural University and other institutions in different capacities as Project coordinator of Plant Protection, member of the Board of Studies of the Annamalai University, Resource person for the T&V Project, and Pesticide Residue Expert in several externally funded projects. Besides Tamil, she had impressive literary skills in Malayalam, authoring several books and technical bulletins, aimed at agricultural extension officers as well as students of Agricultural Entomology.

Dr Visalakshi's administrative skills were equally remarkable. She served as the Residue Analyst and Principal Investigator of AICRP on Pesticide Residues from 1988 and later as the Head of the Department of Agricultural Entomology from 1995 till her retirement in 1999. Her capacity for team building was exemplary, leading teams of entomologists, residue analysts, and toxicologists in addressing pest management issues and studying insecticide dynamics.

Dr. Visalakshi's legacy includes 156 research papers published in National and International journals, edited textbooks and conference/symposium proceedings. She had research experience of 35 years in the University. She was passionate about attending scientific meetings, seminars, and conferences across India and abroad, often encouraging her team members to present their work. She has participated in 71 National and 8 International conferences. Her presentation skills, even before the advent of PowerPoint, were renowned for their creativity and effectiveness. In recognition of her research accomplishments, she was awarded the Archana Pallav Gold Medal, instituted by the Association of Environmental Biology in 1990.

Her memory will continue to inspire generations of students and teachers of entomology.

S. Naseema Beevi & Thomas Biju Mathew
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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/-

Vellayani PO, Thiruvananthapuram 695522 — Dr K. D. Prathapan

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