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Note from the Chief Editor

Dear AAE members / Entomologists,

Wishing each one of you a very happy 2025.

I invite you to an overview on the current status of ENTOMON.

During 2024, ENTOMON published articles in agricultural entomology, aquatic entomology, acarology and arachnology, entomophagy, forensic entomology, forest entomology, medical entomology, veterinary entomology and many aspects of apiculture, biodiversity, biological control, biotechnology, botanical insecticides, eco-biology, integrated pest management, morphology, nano technology, new species and reports, physiology, pest outbreaks, pollinators, sericulture, systematics, toxicology and vectors.

We are happy note to that there is an increase in submission of manuscripts to ENTOMON, indicating its wide recognition among the entomologists, and other stakeholders both nationally and internationally. ENTOMON is now in open access and all the published articles from 2014 are freely available online for everyone. All published articles in the ENTOMON issues from 2014 to 2024 (volume 39 to 49, with DOI numbers) are available in the web site.

Digitalisation of past volumes 1 – 37 (1976 - 2012) of ENTOMON (37 volumes with 146 issues) has been completed and the individual issues are uploaded on the Internet Archive. Individual articles and issues are also being uploaded in the web site. All the issues of ENTOMON are as per schedule. ENTOMON volume 50, issue 1 (March, 2025) is presented before you.

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with the comprehensive “480 UGC-CARE List of Journals – Science – 2024” compilation). National Academy of Agricultural Sciences, New Delhi, has given a NAAS score of 5.24 for ENTOMON, with effect from January 2024. ENTOMON is included in the Web of Science Master Journal List (Clarivate). The Journal is indexed in Scopus, Web of Science, EBSCO and CAB International.

SCOPUS, Elsevier’s abstract and citation database, is indexing ENTOMON with effect from 2012. The cite score of ENTOMON is 0.40, and SC Imago Journal Rank (SJR) and the Source Normalized Impact per Paper (SNIP) are 0.155 and 0.212, respectively. ENTOMON is a member of the Committee on Publication Ethics (COPE) and the journal follows the guidelines of COPE.

The increase in the cost of typesetting, page making, proof reading, material cost and other expenses, had warranted a nominal increase in page charges and the membership fees. However, the Executive Committee has decided to reduce the colour page charges. It was also, decided to reduce the page charges to US \$ 4/page for overseas authors from south-east Asia, and underdeveloped economies as per the UN criteria.

The entire team of Executive Committee members render voluntary service for ENTOMON. Valuable contribution of Professor K. Madhavan Nair, as the AAE web site Manager for ENTOMON thankfully acknowledge M/s SB Press, Thiruvananthapuram for the support in bringing out all the issues ENTOMON issues on time.

We express our earnest and deep appreciation for the experts and peer reviewers for their professional expertise, constructive critical comments and suggestions on the referred manuscripts. As the Chief Editor, it is my pleasure to welcome scientific papers which would merit national as well as international interest, and also resourceful backup from entomologists, that would promote the cause of the journal, enabling it to maintain high standards.

With profound sadness we inform the demise of Professor V.K.K. Prabhu, who was the Managing Editor of ENTOMON for three decades. We offer deepest condolences and our prayers for his soul to rest in peace.

“Excellence is a concept that embodies a commitment to continuous improvement and a relentless pursuit of perfection. ENTOMON strives for it”

With sincere regards

*Dr. M.S. Palaniswami
March 31, 2025*

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A new species of the genus *Herpetogramma* Lederer (Lepidoptera, Crambidae) from India

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ABSTRACT: *Herpetogramma viraktamathi* sp. nov. of the subfamily Spilomelinae is described and illustrated in detail. The host plant information and nature of damage caused by the new species is provided. © 2025 Association for Advancement of Entomology

KEY WORDS: Pest, *Eleusine coracana*, feeding damage, Karnataka, Kolar

INTRODUCTION

The genus *Herpetogramma* was established by Lederer in 1863, with the type species *Herpetogramma servalis* Lederer, 1863 from Brazil. There are 104 recorded species worldwide in Global Information System on Pyraloidea, with 37 species recorded from Neotropical, 19 species from Afrotropical, 37 species from Nearctic, 11 species from Australian and 25 species from the Oriental region (Nuss *et al.*, 2003-2024). In India, 15 species of *Herpetogramma* have been recorded so far (Singh *et al.*, 2022). Most adults exhibit varying shades of brown from light to dark, along with distinct wing patterns (Lu *et al.*, 2019). Their genitalia structures are highly conserved, showing only minor differences between species (Handfield and Handfield, 2021; Solis, 2010). In the present study, the description of a new species,

Herpetogramma feeding on finger millet in Karnataka, India along with its feeding damage is provided.

MATERIALS AND METHODS

Field survey was conducted during *kharif* 2017-18 (October) in and around Kolar district (911m, 13° 09' N; 78° 11' E), Karnataka, India to study the incidence of *Herpetogramma* species on finger millet, *Eleusine coracana* L. Field collected larvae were reared to adult stage in the laboratory on the same host. The methods described and terminologies used by Hampson (1896), Clark (1941), Shaffer and Munroe (2003) and Park *et al.* (2016) for studying genitalia and description were closely followed. Adults were photographed using Canon EOS 70D attached with 100 mm macro lens before dissection of genitalia. Adult characters such

* Author for correspondence

as forewing and hindwing, male and female genitalia and wing venation were photographed using Trinocular microscope with auto-montage (Leica M 205C). The wing slides were prepared by closely following procedures of Robinson (1976) and Thomas (2007).

A pair of male and female studied specimens was deposited in each of the following institutions:

IARI - National Pusa Collection, Indian Agricultural Research Institute, New Delhi, India

NBAIR - National Bureau of Agricultural Insect Resources, Bangalore, India

UASR - University of Agricultural Sciences, Raichur, India

UASB - University of Agricultural Sciences, Bangalore, India.

RESULTS AND DISCUSSION

Genus *Herpetogramma* Lederer, 1863

Type species: *Herpetogramma servalis* Lederer, 1863

Herpetogramma Lederer, 1863: 430. TS: *Herpetogramma servalis* Lederer

Pachyzancla Meyrick, 1884: 315. TS: *Botys mutualis* Zeller

(synonymised by Munroe, 1982 and 1995)

Acharana Moore, (1885): 285. TS: *Botys otreusalis* Walker

Stenomeles Warren, 1892: 437. TS: *Botys agavealis* Walker

Piloptyla Swinhoe, 1894: 142. TS: *Piloptyla nigricornalis* Swinhoe

Pantoeocome Warren, 1896: 173. TS: *Pantoeocome deformis* Warren

Macrobotys Munroe, 1950: 228. TS: *Botys aeglealis* Walker

Coremataria Amsel, 1956: 207. TS: *Botys infuscalis* Guenée

Diagnostic characters: Labial palpi porrect, second joint densely rough scaled and third joint

concealed by hairs. Maxillary palpi short and filiform. Hind legs with outer tibial spurs half as long as inner spurs. Forewing with veins Cu_1 , M_3 and M_2 arising from near lower angle of cell; vein R_2 approximated to R_{3+4} and the vein R_1 moderately long and oblique. Hind wing as broad as fore wing where, M_2 and M_3 approximated basally for a short distance. Vein R_s after its origin, anastomose with $Sc+R_1$ beyond one-thirds of its length, disco-cellular cell short.

The genus *Herpetogramma* differs from the genus *Pyrausta* in the hind wings having the discal cell somewhat shorter and veins M_3 and M_2 approximated for a short distance. The outer spurs half the length of the inner spurs.

Remarks: Hampson (1896) distinguished species of the genus *Pachyzancla* Meyrick (Now, *Herpetogramma*) under two sections based on the thickness of the mid femur and its hairiness. The first section characterised by the mid femora of male immensely dilated and clothed with large smooth scales on inner side and included, *H. licarsialis* and *H. phaeopteralis*. The mid femora of male neither dilated nor clothed with large smooth scales on inner side and included the remaining species characterized the other section. The new species described here belongs to the first section.

Key to closely related species of *Herpetogramma* Lederer from India

1. In female, ductus bursae long and sclerotized; signum present.....2

- In female, ductus bursae short and longitudinally folded; signum absent.....

H. phaeopteralis Guenée

2. Forewing without fringe of hairs on underside of costa; fore legs without rough hair clothing near femoro-tibial joint; aedeagus with two cornuti, one rod-shaped with bundle of spinula at basal part, the other bush-shaped and arranged in circular ring at apex

H. viraktamathi sp. nov. (Fig. 1. C, D, E, H)

- Forewing of male with fringe of long thick black hairs on underside of costa in basal half; fore legs

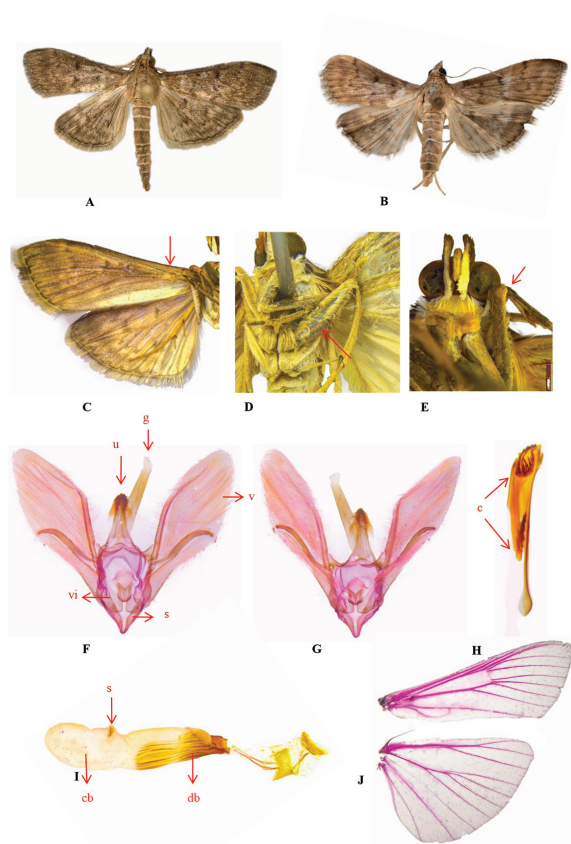


Fig. 1 Morphological and genital characters of adult, *Herpetogramma viraktamathi* sp. nov.

A. male; B. female; C. fore wing of male without fringe of black hairs on ventral side of costa; D. mid femora of the male immensely dilated and clothed with large smooth scales on inner side; E. fore legs normal, lacking rough hairs near the femoro-tibial joint; Male genitalia - F. dorsal view; G. ventral view; H. aedeagus; I. female genitalia; J. wing venation

clothed with rough hairs near the femoro-tibial joint; aedeagus with two discrete well separated patches of lanceolate spines at apex, one with numerous slender spine.....

***H. licarsisalis* Walker (Fig. 2B, C, D, G)**

***Herpetogramma viraktamathi* sp. nov.**
Shankaramurthy & Nagaraj (Fig. 1A-J)

LSIDurn:lsid:zoobank.org:act:33131465-3001-435D-A5FE-27008E4EC7F5

Adults: Measuring 21-25mm in wingspan. Head ochreous, frons dark yellowish brown dorsally and vertex with brownish erect scales. Antenna

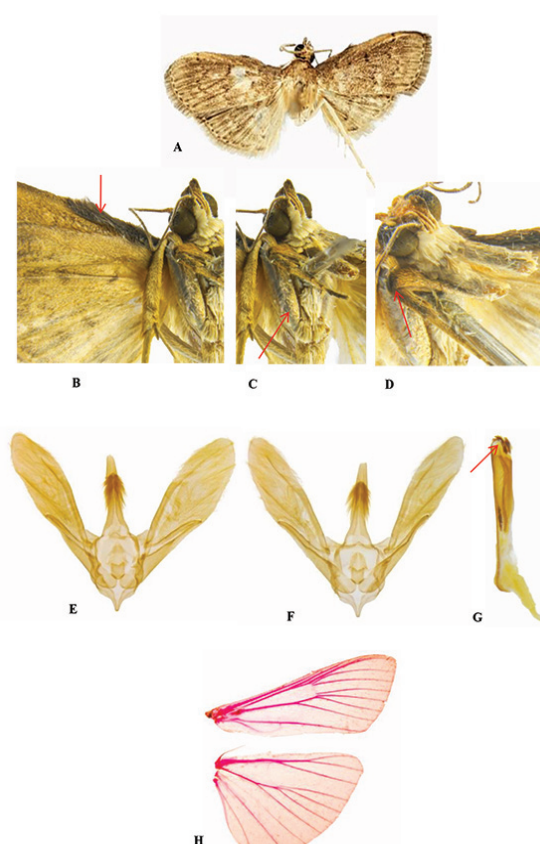


Fig. 2 Morphological and genital characters of adult, *Herpetogramma licarsisalis* Hampson

A. male; B. fore wing of male with fringe of black hairs on ventral side of costa; C. mid femora of the male not dilated and clothed with large smooth scales on inner side; D. fore legs normal, with rough hairs near the femoro-tibial joint; Male genitalia - E. dorsal view; F. ventral view; G. aedeagus; H. wing venation

reaching about 1/2 length of forewing, ciliate, scape and pedicel dark brown; ocelli well developed. Labial palpi with basal region of segment I whitish gray, segments II, III large with fuscous scales and segment III concealed by scales (Fig. 1A, B, 3A). Maxillary palps filiform with dark yellowish-brown scales on outer side and yellowish white on inner side. Proboscis with light yellowish scales at base. Thorax whitish ochreous. Legs long and yellowish white. Fore and hind legs normal, mid femora of male immensely dilated and clothed with large smooth scales on inner side (Fig. 1D, E). Forewing ground colour fuscous yellow, with terminal area dark, markings indistinct and dark brown postmedial



Fig. 3 Infestation of *Eleusine coracana* L. by larvae of *Herpetogramma viraktmathi* sp. nov. (A. adults; B. close up view of larva; C. larva webbing the leaves; D. larva with fresh web; E. larva inside the old web; F. damaged leaves)

line dentate. Forewing of male without fringe of black hairs on ventral side of costa (Fig. 1C).

Wing venation: Forewing: vein Sc straight, unbranched; R_1 and R_2 free; R_{3+4} stalked; R_1 moderately long and oblique; R_2 approximated to R_{3+4} ; R_5 and M_1 free arising below the upper angle of cell; M_2 , M_3 and Cu_1 arise from near lower angle of cell; M_2 , M_3 close to Cu_{1a} ; Cu_{1b} present; 1A distinct, straight, 2A fused at its base with vein 1A; 3A merged with 1A approximately at its midpoint; Hind wing: vein Sc dilated at base anastomosing

with Rs after originating from upper angle of the cell for some distance; M_1 arises from the lower angle of cell; M_{2+3} stalked arises from lower angle of cell; M_2 and M_3 approximated basally for a short distance; Cu_{1a} and Cu_{1b} present; vein CuP present; 1A+2A fused, 3A distinct (Fig. 1J).

Male genitalia: Uncus (u) acuminate, heavily setose. Gnathos (g) membranous and longer than uncus. Valvae (v) elongate, elliptical with lightly sclerotized membranes covered with fine hair-like setae all over the surface, except for the dorsal

margin with a narrow, rod like sclerotized brace angled approximately at $1/3^{\text{rd}}$ of the distance from the base. Gnathos (g) membranous and elongated. Tegumen moderately arched, lateral margins well sclerotized. Juxta (j) prominent, shield-like and well-sclerotized, located centrally between the valvae. (Fig. 1F, G). Vinculum (vi) narrow and saccus (s) moderately developed. Aedeagus rod-shaped, long and slender with two cornuti (c), one rod shaped bundle of spinula at base and other bush shaped arranged in circular ring with several spines at apex (Fig. 1H).

Female genitalia: Papillae anales narrow and setose. Anterior apophyses longer than posterior apophyses. Ostium bursae moderate and membranous. Ductus bursae (db) long, sclerotized anteriorly, slightly membranous posteriorly, twice the length of corpus bursae with longitudinal striations. Corpus bursae (cb) membranous, smooth and balloon-shaped. Signum (s) star-like, sclerotized and arranged horizontally at the centre of corpus bursae (Fig. 1I).

Larva: Grown up larvae is pale cream-colored with brown spots on each segment and a dark, yellowish-brown head (Fig. 3B).

Material examined: *HOLOTYPE* male (1♂♂), INDIA: Karnataka: Kolar, Rajakallahalli, 911m; ASL, 13°09'N; 78°11'E, 04.x.2017, reared on finger millet, coll. Shankaramurthy (UASR).

Paratypes: 8♀, 6♂, same data as *HOLOTYPE* except for date 02-27.x.2017. Further, the specimens 5♀, 2♂ coll. Mahesh (UASB) in the same locality and on the same host from 04-18.x.2017 are also considered as a paratypes.

Etymology: The species epithet, *viraktamathi* named to honour Dr. C.A. Viraktamath, a renowned insect taxonomist globally.

Remarks: The genus *Herpetogramma* formerly comprised 15 recorded species in India (Singh *et al.*, 2022), now increased to 16 with *Herpetogramma viraktamathi*. Externally, it resembles *H. phaeopteralis* Guenee, but differs in the structure of the cornuti in aedeagus and in

the female, a signum is present in the corpus bursae (in *H. phaeopteralis* signum is absent). The new species also resembles *H. licarsisalis* Walker in having similar male genitalia but differing in the detailed structure of cornuti in the aedeagus and the shape of the valva and vinculum, and the new species is without fringe of hair on underside of costa and fore legs are normal, lacking the rough hairs near the femoro-tibial joint.

Host plants and feeding damage: The incidence of this pest was noticed in early stages of finger millet crop (*E. coracana*) and its incidence was preceded by the attacks by a noctuid caterpillar, *Chrysodeixis chalcites* (Lepidoptera, Noctuidae, Plusiinae). The larvae webbed the leaves and fed on leaves staying within the web. Feeding by the larvae was always associated with abundance of webbing strewn with green faecal pellets (Fig. 3C, D, E, F).

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Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin, an entomopathogenic fungi, with selective insecticides for enhanced pest management

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ABSTRACT: Six insecticides, viz. Imidacloprid 17.8 SL, Chlorpyrifos 20 EC, Quinalphos 25 EC, Dimethoate 30 EC, Dichlorvos 76 EC and Fenvalerate 20 EC, at three concentrations [recommended dose - RD, and 0.5 per cent each below RD (BRD) and above RD (ARD)] were used to treat on the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin. Impact on the conidial germination, vegetative growth, and spore production was studied by grading as harmless (<50%), slightly harmful (50-79%), moderately harmful (80-90%), and harmful (>90%). Among all these treatments, imidacloprid and fenvalerate were rated as harmless to *B. bassiana*, whereas chlorpyrifos, was slightly harmful in all three concentrations. In the case of treatment with dimethoate, the toxicity was slightly high in the RD and BRD but moderate at ARD. Quinalphos was moderately toxic in all three concentrations; however, dichlorvos was highly toxic, and there was no mycelial growth in all the three concentrations. Imidacloprid and fenvalerate were found highly compatible with *B. bassiana*. © 2025 Association for Advancement of Entomology

KEY WORDS: Toxicity, conidial germination, vegetative growth, spore production

INTRODUCTION

Synthetic pesticides are the most commonly used crop protection chemicals, particularly in conventional cultivation, but their injudicious use causes uncountable environmental and health hazards. About 99.9 per cent of the pesticide used reaches nontarget organisms and only 0.1 per cent reaches the target organism (Pimentel 1995; Carriger *et al.*, 2006). The biological and biochemical activity of soil microorganisms can influence the effectiveness of even reduced use of synthetic insecticides (Cycon *et al.*, 2006; Vig *et al.*, 2008; Cycon *et al.*, 2010). Entomopathogenic

fungi play an important role in integrated pest management strategies (Amutha *et al.*, 2010). Relying on entomopathogenic fungus as biocontrol agents facilitates the reduction of insecticidal usage, and thereby minimizes the environmental contamination and the incidence of pest resistance to insecticide (Ambethgar, 2009). *Beauveria bassiana*, which can easily be cultured on potato dextrose agar in the laboratory, is a potential biocontrol agent against certain insect pests (Wu *et al.*, 2014). Fungus infected insects develop hyphae that cause mortality of the pests by damaging their internal organs (Bidochka and Khachatourians 1994). Detailed compatibility studies

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are required for the integration of microbial pesticides with chemicals in pest management strategies. Many experiments were carried out, aiming to detect pesticide side effects on entomopathogenic fungi (Clark *et al.*, 1982; Gardner and Storey 1985; Neves *et al.*, 2001).

Despite their potential, microbial agents face limitations such as slow pest mortality, environmental susceptibility, and high production costs, which hinder their widespread adoption (Sharma *et al.* 2024). Combining entomopathogenic fungi with low concentrations of chemical insecticides have shown synergistic effects, improved pest control efficacy and reducing pesticide dependency (El-Ashry and Ramadan 2021; Robertson *et al.*, 2017; Thakur, 2022). This integrated approach offers a cost-effective and environmentally sustainable solution, addressing challenges in pest management while maintaining the essential role of synthetic insecticides (Singh, 2016). The objective of this investigation studied the impact of commonly used insecticides at different doses on *B. bassiana*.

MATERIALS AND METHODS

Fungus culture: Isolates of the entomopathogenic fungus, *B. bassiana*, obtained from the ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru were sub-cultured on PDA ($26 \pm 1^\circ\text{C}$; RH 70%), and the conidia produced were used for further investigation. The study was carried out at the ICAR- Central Tuber Crops Research Institute, Thiruvananthapuram.

Insecticides: All the insecticides selected for the current investigation (Table 1) were classified into recommended dose (RD), below recommended dose (BRD) and above recommended dose (ARD). Except for imidacloprid, the concentrations (in %) for other insecticides were 0.10 (BRD), 0.20 (RD), and 0.30 (ARD); whereas it was 0.02 (BRD), 0.03 (RD) and 0.04 (ARD) in the case of imidacloprid.

Conidial germination: An appropriate concentration of each pesticide was added to 50 mL of Potato-Dextrose-Broth (PDB) at 45°C to

reach the required concentration (Table 1). Conidial suspension of *B. bassiana* (1mL) at 10^6 spore/mL, prepared in sterile distilled water amended with 0.02 per cent Tween 20 was added to the above medium; and an aliquot without pesticides was used as control. These were transferred into an incubator ($26 \pm 1^\circ\text{C}$; 12 h photophase), and after 24 h, the germinated spores in 100 conidia per quadrant were observed, both in treatments and control, by using stereomicroscope (Carl Zeiss, Stemi 2000C) under 40X.

Vegetative growth and spore production: The required concentration of the test insecticides was added in to the autoclaved PDA at 40°C , and from this 20 mL was transferred into a Petri plate (90 mm). Medium without insecticides served as the control. After solidification, each plate was inoculated with a PDA plug (4mm dia) with *B. bassiana*, as described by Todorova *et al.* (1998). The plates were incubated at $26 \pm 1^\circ\text{C}$ and growth of the culture was measured on alternative days until 14 days after the treatment (DAT). The experiment was replicated thrice. After 14 d, the conidia were harvested by scraping and suspended in 1 mL of 0.01 per cent Tween 80. The concentration of the conidia was estimated using Neubauer counting chamber, and the percentage growth inhibition of *B. bassiana* by insecticide-treated PDA was estimated by Hokkanen and Kotiluoto (1991).

$$X = \frac{Y - Z}{Y} \times 100$$

Where: X = Growth inhibition (%); Y = Growth diameter (cm) in control; Z = Growth diameter (cm) in treatment.

The insecticides were further categorised into harmless (<50%), slightly harmful (50-79%), moderately harmful (80-90%), and harmful (>90%) and given score from 01 to 04 as classified by Hassan (1989). The data were statistically analysed.

RESULTS AND DISCUSSION

The tested insecticides exhibited varying degrees of inhibition on the germination, vegetative growth and sporulation of *B. bassiana* (Table 1).

Table 1. Effect of insecticides in different doses on sporulation of the entomopathogenic fungus *B. bassiana*

Treatments	Germination		Colony		Number of conidia	
	Total	Reduction (%)	Total area (mm)	Reduction (%)	X *10 ⁶	Reduction (%)
1.1 Effect of insecticides in below recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	74.67±4.53b	13.57	76.33±4.73b	15.19	92.13±9.91b	34.44
Chlorpyrifos	43.13±2.53c	50.08	30.67±3.51c	67.40	52.67 ± 5.27 c	62.52
Quinalphos	16.20±2.58e	81.25	15.67±1.53d	82.60	34.13 5.68d	75.71
Dimethoate	26.27±2.00d	69.59	28.00±2.00c	68.89	30.47±4.76d	78.31
Dichlorvos	4.67±1.15f	94.60	8.67±0.58e	90.37	11.53±1.15e	91.80
Fenvalerate	72.73±5.78b	15.82	78.33±2.52b	12.96	93.33±12.34b	33.58
1.2 Effect of insecticides in recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	65.33±3.50b	24.38	74.33±2.52b	17.77	72.70± 9.82b	48.26
Chlorpyrifos	32.87±2.76c	61.96	28.33±1.53d	68.62	40.00±6.34c	71.54
Quinalphos	12.53±1.75e	85.49	11.67±1.15f	87.04	22.53±6.12d	83.97
Dimethoate	20.00±2.05d	76.85	20.00±2.65e	77.78	21.67 ±6.78d	84.57
Dichlorvos	0.00±0.58f	100	0.00±0.00g	100	0.00 ± 0.00e	100
Fenvalerate	65.07±4.34b	24.69	70.67±1.53c	21.48	73.07±12.71b	48.00
1.3 Effect of insecticides in above recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	54.67±4.67b	36.72	58.33±3.51b	35.17	61.47 ±8.23b	56.25
Chlorpyrifos	24.00±1.78c	72.22	24.67±1.53d	72.59	30.00±5.00c	78.65
Quinalphos	5.33±1.53e	93.83	8.67±0.58f	90.37	14.73 ±5.96d	89.51
Dimethoate	15.40±1.65d	82.17	17.00±2.65e	81.11	14.33± 4.00d	89.80
Dichlorvos	0.00±0.58f	100	0.00±0.00f	100	0.00 ± 0.00e	100
Fenvalerate	55.73±3.88b	35.36	52.00±2.65c	42.22	58.47±10.45b	58.39

X= Conidia in number

Imidacloprid and fenvalerate showed the least inhibition on conidial germination at all three tested concentrations compared with other insecticides. Chlorpyrifos, quinalphos, and dimethoate at BRD, RD and ARD showed >50.00 per cent germination inhibition. The effect of dichlorvos on *Beauveria* germination was statistically on par. However, 100 per cent reduction was observed in RD and ARD where as it was 94.60 per cent reduction in the BRD

In the case of radial growth, imidacloprid in all concentrations and fenvalerate at BRD showed the least inhibition. The vegetative growth inhibition

induced by fenvalerate at RD and ARD was significantly different from BRD. On the other hand, in chlorpyrifos, quinalphos and dimethoate fungal growth inhibition was higher (>65%) in all concentrations. Dichlorvos significantly reduced radial growth at all three concentrations compared to the control, with percentage reductions ranging from 90.37 at BRD to 100 inhibition at RD and ARD.

Concerning spore production, the imidacloprid and fenvalerate formulations showed the best results at the lowest concentrations, relative to the control. Dichlorvos at the three concentrations was poorer

than the other treatments concerning spore production. Dimethoate and quinalphos at the highest concentrations (ARD) also showed significant reduction relative to the control. Considering Hassan's classification scheme (Hassan 1989), imidacloprid and fenvalerate formulations at the three concentrations were compatible with the fungus (Table 2).

Several studies, (Mohamed *et al.*, 1987; Anderson *et al.*, 1989; Castineiras *et al.*, 1991; Oliveira and Neves, 2004; Alizadeh *et al.*, 2007; Abidin *et al.*, 2017) have examined the compatibility of chemical insecticides with the entomopathogens. Hassan and Charnley (1989) identified inconsistent interactions between fungi and insecticides, resulting in varying degrees of inhibition on the conidial germination, vegetative growth and sporulation of *B. bassiana*. The importance of conidial germination in pest management using fungi was emphasized by Batista Filho *et al.* (1998) and De Olivera and Neves (2004). The effect of insecticides on conidia germination is considered a critical aspect of product compatibility in the field, as entomopathogenic fungi primarily infect insects through conidial germination (Anderson and Roberts, 1983; Neves *et al.*, 2001).

Dichlorvos exhibited strong inhibition of *B. bassiana*, while imidacloprid and fenvalerate demonstrated relatively minor fungal inhibition. Boucias *et al.* (1988) demonstrated that the treatment of entomopathogenic fungi conidia with chemical products can neutralize the electrostatic charge of the surface or remove the mucous layer covering conidia, impacting substrate recognition and the transduction of the signal that initiates germination. The role of coat removal in substrate recognition and the transduction of the signal-triggering germ tube formation in *Metarhizium anisopliae* was demonstrated by Leger and Cooper (1987) and Leger *et al.* (1991).

According to James and Elzen (2001) and Alizadeh *et al.* (2007), imidacloprid and fenvalerate exhibited lower toxicity to *B. bassiana*, with imidacloprid showing no adverse effects on the fungus. In the current study, chlorpyrifos demonstrated slight toxicity to *B. bassiana*, although previous reports indicated slight harm to the fungus normal field doses

Table 2. Toxicity scoring index of insecticides according to Hassan's (1989) classification scheme

Treatments	Conc	Germi	Colony	Spore
Imidacloprid	BRD	1	1	1
	RD	1	1	1
	ARD	1	1	2
Chlorpyrifos	BRD	2	2	2
	RD	2	2	2
	ARD	2	2	2
Quinalphos	BRD	3	3	3
	RD	3	3	3
	ARD	4	4	3
Dimethoate	BRD	2	2	2
	RD	2	2	3
	ARD	3	3	3
Dichlorvo	BRD	4	4	4
	sRD	4	4	4
	ARD	4	4	4
Fenvalerate	BRD	1	1	1
	RD	1	1	1
	ARD	1	1	2

Grades: 1: harmless (<50%), 2: slightly harmful (50-79%), 3: moderately harmful (80-90%), 4: harmful (>90%)

BRD- Below recommended dose; RD- Recommended dose; ARD-- above recommended dose

Conc- Concentration; Germi- Germination; Colony- Colony area; Spore- Sporulation

(Ambethgar, 2003). Other studies suggested that chlorpyrifos strongly inhibits the growth and sporulation of *B. bassiana*, even at concentrations lower than the recommended field usage rates (Barbosa and Moreira, 1982; Rao, 1989).

The findings highlighted the incompatibility of dichlorvos with *B. bassiana*, suggesting the potential of combined applications of *B. bassiana* with compatible chemical pesticides as a promising strategy for integrated pest management programs. Insecticides such as imidacloprid and fenvalerate were recommended in pest management programmes due to their compatibility with the entomopathogenic fungus *B. bassiana*, commonly utilized as a biocontrol agent.

The study elucidates the differential impact of tested

insecticides on *B. bassiana*, crucial for biocontrol. Imidacloprid and fenvalerate demonstrate consistent compatibility, showing minimal inhibition on conidial germination, radial growth, and spore production. Conversely, chlorpyrifos, quinalphos and dimethoate exhibits substantial inhibitory effects, posing risks to *B. bassiana*. The findings underscore the needful insecticide selection in integrated pest management. Imidacloprid and fenvalerate emerge as favorable choices, while caution is advised with chlorpyrifos, quinalphos, dimethoate and dichlorvos to preserve beneficial fungi efficacy.

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Molecular characterization of *Ceylonitermellus sahyadriensis* Emerson, 1960 (Termitidae, Nasutitermitinae) - a rare species from Kerala part of Western Ghats, India

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ABSTRACT: Molecular characterization of *Ceylonitermellus sahyadriensis* (Termitidae, Nasutitermitinae), using the cytochrome oxidase I (COI) gene was studied, along with its morphological characteristics. The combined molecular analysis with its morphological figures provided a comprehensive species description, the first genetic profile of this species and giving a baseline data for the genus *Ceylonitermellus*. The phylogenetic analysis validated the species' taxonomic position and revealed its evolutionary relationships within Nasutitermitinae. This integrated approach is particularly significant given the genus's rarity and limited distribution, contributing to our understanding of termite diversity in the Oriental region. The molecular data generated will serve as a crucial reference for future systematic studies and species identification of this endemic genus. © 2025 Association for Advancement of Entomology

KEY WORDS: Termite, endemism, cytochrome oxidase, integrated validation, phylogenetic analysis

INTRODUCTION

Ceylonitermellus Emerson, 1960 is a rare genus, which is endemic to oriental region erected by Emerson 1960 (Termitidae, Nasutitermitinae), was initially understood as an endemic to Sri Lanka (Hemachandra *et al.*, 2010). Amina and Rajmohana's later description of *C. periyarensis* from Kerala, India shed light into the distribution of the species (Amina and Rajmohana, 2013). The new species *C. sahyadriensis* was recently described by Renjith *et al.* (2022). Nasutitermitinae is a widely distributed termite subfamily (Sen-sarma and Verma, 1983). The genus is very rare and presumed to be endemic to Sri Lanka till the description of *C. periyarensis* from Kerala, India

(Amina and Rajmonha, 2013; Renjith *et al.*, 2022). In this study, molecular characterization of *C. sahyadriensis* was carried out using COI gene and morphological description was also made. The resulting molecular data were combined with detailed morphological observations, for genetic profile of this. Phylogenetic analysis was studied for its evolutionary relationships within *Nasutitermitinae*.

MATERIALS AND METHODS

Specimens were collected from a tree bark of *bombax ceiba* in Mamalakandam, Idukki District, Kerala State, Southern India. The samples were collected using an aspirator and kept in 80% alcohol.

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Measurements and dissections (80% ethanol) of the specimens were carried out using stereo zoom microscopes, a Labomed Luxeo 4D binocular microscope with an attached camera, and pixelpro software. The magnification range was 8X to 35X. According to Roonwal and Chhotani (1989), Sands (1998) and Chhotani (1997), soldiers and workers were described using morphological terms, measurements, and indices.

DNA extraction, amplification and sequencing:

Soldier termite heads were used to harvest the DNA. Distilled water was used to clean the specimen, then the specimen was then spread out on filter paper to dry completely. Using the Machery-Nagel NucleoSpin® Tissue Kit and according to the manufacturer's instructions, genomic DNA was extracted from the tissues. Agarose gel electrophoresis was used to evaluate the quality of the extracted DNA. The amount of template given to the polymerase chain reaction was calculated based on the concentration of DNA in each sample using a DNA spectrophotometer (Thermo Fisher).

For the polymerase chain reaction amplification, cytochrome oxidase c subunit I (COI) primers are utilized (Sequences, 5' to 3': Forward LCO: GGTCACAAATCATAAAGATATTGG, Reverse HCO: TAAACTTCAGGGTGACCAAAAAA TCA). In a 10 µL reaction volume, individual PCR reactions were conducted using a mixture of 5µL of 2X Sapphire Master Mix, 4µL of D/W, 0.25µL of forward primer chloride, 0.25 µL of reverse primer chloride, and 1µL of DNA template [2-8 ng/l]. PCR reactions were run at 98.0°C for 30 seconds, followed by 10 cycles at 98.0°C for 5 seconds, 45°C for 10 seconds, and 72.0°C for 15 seconds, followed by 30 cycles at 98.0°C for 5 seconds, 45°C for 10 seconds, 72.0°C for 15 seconds, and 72.0°C for 60 seconds (Folmer *et al.*, 1994). The GenElute PCR Clean-Up Kit (Sigma-Aldrich) was used to purify the PCR products, which were then submitted to sequencing using the BigDye Terminator Cycle Sequencing Kit version 3.1. (Applied Biosystems).

Phylogenetic analysis: The chromatograms were edited using the BioEdit software. To collect meaningful data, unclear and skipped bases were

removed. The sequences were then converted to FASTA format for subsequent processing. The generated sequence was compared to similar sequences from the NCBI nucleotide database using the Basic Local Alignment Search Tool (BLAST N) tool (Altschul *et al.*, 1997). Before tree construction, the sequences were aligned using Clustal Omega's multiple alignment technique with the default values. The tree was created using the Maximum Likelihood (ML) technique with the MEGA X software. This technique is based on the uniform rate substitution among sequences approach and the Kimura 2-parameter distance. To ensure the accuracy of the constructed tree, 1000 bootstrap replication phylogenies were examined (Felsen and Stein, 1985). The necessary termite sequences were obtained from the NCBI GenBank database

RESULTS AND DISCUSSION

Family: Termitidae Latreille (1802);

Genus: *Ceylonitermellus* Emerson (1960); *Ceylonitermellus* Emerson. In: Emerson, American Museum; Novitates.

Type species: *Ceylonitermellus hantanae* (Holmgren)

Ceylonitermellus sahyadriensis Emerson

Materials examined: Specimen: 12 soldiers and 10 workers, India, Kerala, Idukki (Mamalakandam), 11/05/22, Edwin Joseph. Colony Code : CMSZ126

Description:

Imago. **Unknown**

Soldier (Fig. 1, Table 1): The species exhibits monomorphic characteristics with a pear-shaped (pyriform) head capsule displaying a golden yellow coloration. The head features sparse pubescence, comprising two prominent hairs and numerous shorter ones, with a slight hump when viewed laterally. The reddish-brown rostrum is slender and cylindrical, matching the head length, and darkens towards the apex where multiple hairs are present. Dorsally, the cephalic gland duct is partially

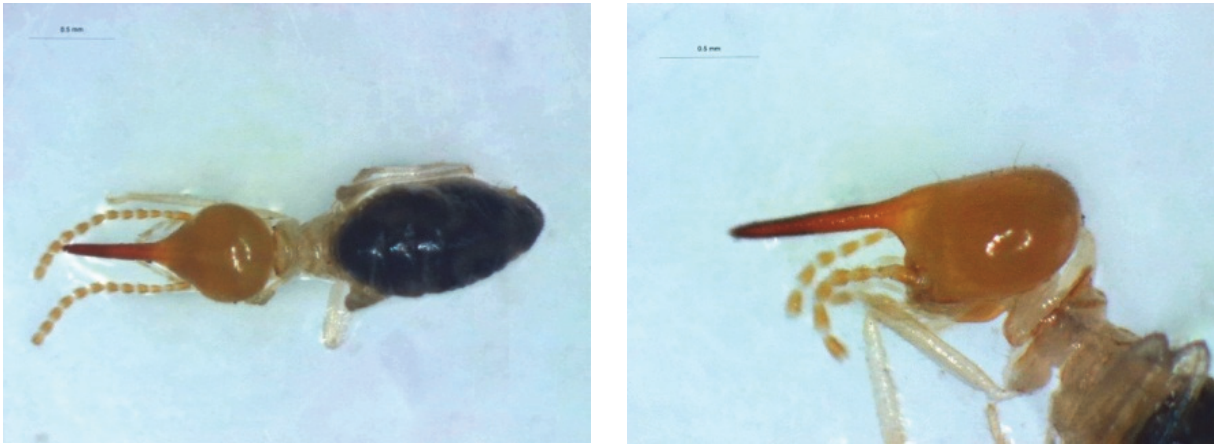


Fig. 1 *Ceylonitermellus sahyadriensis* soldier. (a) Dorsal view (b) lateral view of the head.

observable, extending from the rostrum to the gland reservoir. The 12-segmented antennae show a yellowish-brown coloration, with the second segment being the shortest, while segments three through five are approximately equal in length. The mandibles are reduced (vestigial) and lack spine-like projections. The pronotum exhibits a saddle-shaped structure, appearing lighter yellow dorsally and narrower than the head width. The abdomen demonstrates slight transparency, revealing the internal gut, and is adorned with both short and long bristles. The species possesses small, bi-segmented cerci and legs with a 2:2:2 arrangements of apical tibial spurs, complemented by four-segmented tarsi.

Worker (Fig. 2): The species displays monomorphic characteristics with an unsclerotized, oblong body structure. The sub-circular head capsule reaches its maximum width at the antennal base, exhibiting a pale yellow coloration and dense setation. In lateral view, the postclypeus shows slight swelling with notable flattening, while the clypeus presents a transparent, pale yellowish appearance. Both the epicranial suture and fontanelle are poorly defined, and the labrum maintains a pale yellow coloration. The antennae comprise 13 segments, with the third segment being the shortest. The pronotum demonstrates a saddle-shaped configuration and is significantly narrower than the head width. The oblong abdomen exhibits transparency, revealing visible gut coiling, and features dense pubescence. The species possesses small, two-segmented cerci and legs with four-segmented tarsi, accompanied by apical tibial spurs arranged in a 2:2:2 pattern.

The mandibular structure is asymmetrical: the left mandible features an apical tooth exceeding the first marginal tooth in size, with a 45-degree angle between them. A pronounced posterior margin separates the first and second marginal teeth. The third marginal tooth, intermediate in size between the apical and second marginal, is separated by a distinct notch with prominent molar development. The right mandible is characterized by an elongated apical tooth, with the first and second marginal teeth positioned at a narrow angle, lacking the posterior margin separation observed in the left mandible. The molar ridges are prominently developed.

Among Indian *Nasutitermitinae*, three genera are distinguished by their reduced head capsule sclerotization. *Ceylonitermellus* differs from *Aciculitermes* by its longer third antennal segment relative to the second, while both these genera

Table 1. Morphometry of *Ceylonitermellus sahyadriensis* soldier (n = 10)

Characters	Range	SE
Total body Length	3.11-3.64	0.056949
Total Head Length	1.56-1.66	0.010349
Head Length Without rostrum	0.72-0.77	0.008921
Head Width	0.68-0.72	0.005212
Rostrum Length	0.82-0.90	0.007118
Pronotum Length	0.15-0.18	0.002906
Pronotum Width	0.34-0.37	0.003333

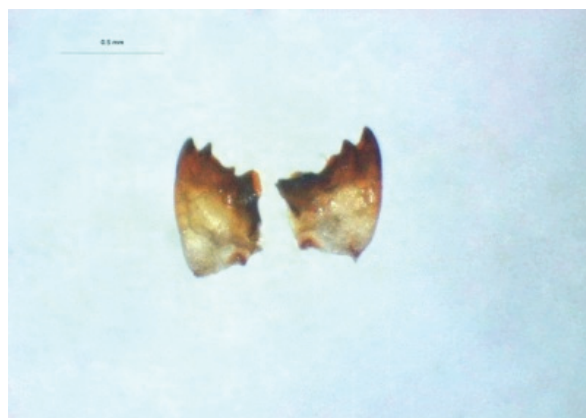


Fig. 2 *Ceylonitermellus sahyadriensis* worker. (a) Dorsal view (b) mandible worker

possess 14-segmented antennae, contrasting with *Emersonitermes*' 12-segmented antennae. For comparison image of the *Ampoulitermes zacharia*, Joseph and Mathew (2024) soldier is provided (Fig. 3), which shows how distinguished the *Ceylonitermellus* from other species in morphology.

Distribution: India: Kerala, Wayanad, Meppadi; Kerala, Idukki, Mamalakkandam as per present study

Molecular analysis: *C. sahyadriensis* sample had a mitochondrial COI gene. DNA fragment of 580 base pair was deposited in NCBI database with accession number OQ471904. The base composition of COI gene fragment presented 33% (A), 30 (T), 15 (G), and 22 (C) with total A+T content 63 per cent. G+C content 37 Per cent. The characteristic property of insect sequence with rich

in A+T content is evident in the current sequence (Navajas *et al.*, 1996). Related termite sequences such as *Bulbitermes laticephalus* (NC_034059.1), 1 *Nasutitermes longirostris* (KY224677.1), *Trinervitermes geminatus* (JF923343.1) and *Cryptotermes dudleyi* (OM415296.1) were accessed from the NCBI data base to construct phylogenetic tree (Fig. 4) using *Cryptotermes dudleyi* as an outgroup. Based on the sequences Evolutionary Divergence between Sequences was calculated (Table 2).

Table 2. Estimates of evolutionary divergence between sequences

Species	1	2	3	4	5
1. <i>Ceylonitermellus sahyadriensis</i>					
2. <i>Bulbitermes laticephalus</i>	0.1009				
3. <i>Nasutitermes longirostris</i>	0.0983	0.1027			
4. <i>Cryptotermes dudleya</i>	0.2462	0.2371	0.2577		
5. <i>Trinervitermes geminatus</i>	0.1059	0.1069	0.1086	0.2398	



Fig. 3 Soldier of *Ampoulitermes zacharia*

The evolutionary history of *C. sahyadriensis* was deduced through the application of the Maximum Likelihood approach and the Kimura 2-parameter model (Kimura, 1980). The illustrated tree is the one with the highest log probability (-1773.92). Next to the branches is the percentage of trees where the related taxa were clustered together. By employing the Neighbor-Join and BioNJ algorithms on a matrix of pairwise distances calculated using

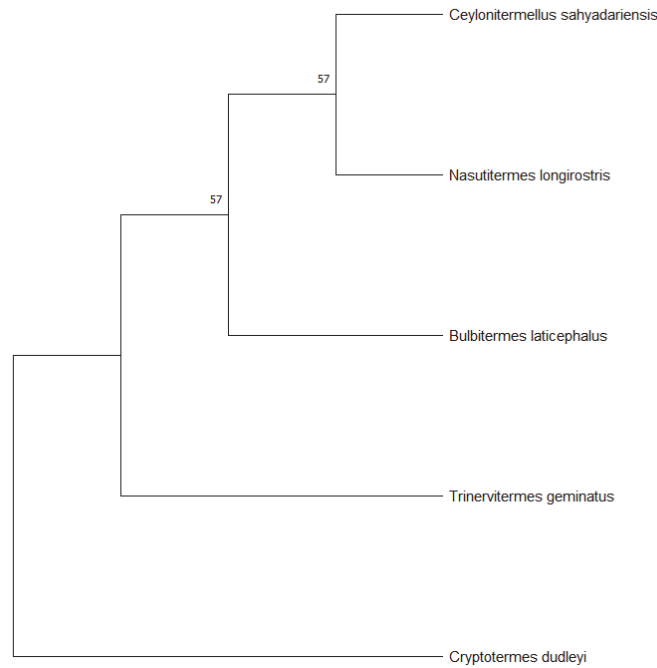


Fig. 4 Phylogenetic tree of *Ceylonitermellus sahyadriensis*.

the Maximum Composite Likelihood (MCL) technique, the initial tree or trees for the heuristic search were automatically generated. The topology with the highest log likelihood value was then chosen. There were five nucleotide sequences in this investigation. Codon positions included were 1st+2nd+3rd+Noncoding. The final dataset contained 580 locations in total. (Kumar et al., 2018). The tree shows *C. sahyadriensis* makes a clade with *Nasutitermes longirostris* showing that they are the most related. The equality of evolutionary rate between sequences A (*Ceylonitermellus sahyadriensis*) and B (*Nasutitermes longirostris*), with sequence C (*Bulbitermes laticephalus*) used as an outgroup in Tajima's relative rate test (Table 3) (Tajima, 1993). The $\div 2$ test statistic was 0.02 ($P = 0.88640$ with 1 degree[s] of freedom) P -value less than 0.05 is often used to reject the null hypothesis of equal rates between lineages. This analysis involved 3 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 580 positions in the final dataset.

Presence of *C. sahyadriensis* in an area other than the type locality first reported here. The presence of the new species and report of the rare species *C. sahyadriensis* highlights the remarkable diversity of the area.

Morphologically the soldiers of the genus *Ceylonitermellus* show similarity with genus *Aciculitermes* and both of these genus show morphological similarity with genus *Emersonitermes* (Renjith et al., 2022). *C. sahyadriensis*, the species under discussion show affinity to *C. kotuae* (Amina and Rajmohana, 2013). Lack of data availability has been a major challenge in molecular studies in India. On the basis of the similar sequences obtained from the data base the phylogeny of the species was studied. *C. sahyadriensis* formed a monophyletic clade with *Nasutitermes longirostris* which thus show highest phylogenetic relation among the species. This clade form a paraphyletic clade with *Bulbitermes laticephalus*. *C. sahyadriensis* has a poly phyletic relation with *Trinervitermes geminatus*. The present study reiterates the diversity of India and also sheds light to the distribution pattern of the

Table 3. Results from the Tajima's test for 3 Sequences

Configuration	Count
Identical sites in all three sequences	501
Divergent sites in all three sequences	4
Unique differences in Sequence A	24
Unique differences in Sequence B	25
Unique differences in Sequence C	26

Sequences A-*Ceylonitermellus sahyadariensis*; Sequences B - *Nasutitermes longirostris*; Sequences C- *Bulbitermes laticephalus*.

rare genus *Ceylonitermellus* and the species *C. sahyadriensis*. This comprehensive study, combining distributional data, morphological analysis, and molecular characterization, significantly advances our understanding of *C. sahyadriensis* and the genus *Ceylonitermellus*. The findings not only expand the known geographic range of this rare species but also provide valuable molecular markers for future taxonomic investigations. This research highlights the substantial knowledge gaps in Indian termite taxonomy and emphasizes the need for continued systematic studies in this biodiversity-rich region. The integration of molecular tools with traditional taxonomy, as demonstrated in this study, offers a robust framework for future investigations into termite diversity, evolution and conservation.

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Age-related comb dimensions of the red dwarf honeybee, *Apis florea* F. (Hymenoptera, Apidae) in the plains of Karnataka, India

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ABSTRACT: Studies were conducted to determine the variations in the dimensions of comb parameters of dwarf honeybee, *Apis florea* F. in relation to comb age. The comb parameters viz comb perimeter, mean comb area, brood, pollen, honey, crest size, and comb thickness were varied with comb age and were found to be relatively high in middle-aged combs compared to old and young combs. Furthermore, the observations showed that the width and depth of the comb wax cells also differed significantly with comb age and maximum size of cell width and depth were found in middle-aged combs.

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KEY WORDS: Brood, middle-aged combs, comb parameters, wax comb

INTRODUCTION

The nests are crucial for the expansion and well-being of insect colonies (Kronauer and Libbrecht, 2018; Gruter, 2020). Combs of the nests are used for food storage and brood rearing for several generations (Taha *et al.*, 2021). Furthermore, nests offer a centralized framework for social interaction, enabling individuals to plan their actions in a way that benefits the colony as a whole (Ireland and Garnier, 2018). *Apis florea* F. (Hymenoptera, Apidae) is renowned for its unique nest architecture and tiny colony size. The feral colonies of *A. florea* build single, exposed combs vertically attached to one or many thin branches of bushes, trees (Hepburn and Hepburn, 2011; Nagaraja, 2020) and piles of dried sticks (Ramyarani and Nagaraja,

2024). The construction of the crown is one of the species-specific characteristics found in *A. florea* (Oldroyd and Pratt, 2015). The crown of the nest was found above the twig acts as a honey storage area and serves as a platform for communication dancing by foragers (Oldroyd and Wongsiri, 2006). Honey bee comb changes colour over time, from whitish to brown and later into darker (Karihaloo *et al.*, 2013). Combs become darker and stiffen with successive brood rearing (Zhang *et al.*, 2010; Smith *et al.*, 2021). The long-term use of combs in beekeeping results in an accumulation of cocoons, pollen, and propolis in the cells. As the comb ages, the cell walls become thicker and the internal cell diameter decreases (Karihaloo *et al.*, 2013).

The comb of *A. florea* is a visual treat to know

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how workers build cells with different diameters, small cell sizes for rearing workers, and large cell sizes for drones (Boes, 2010). The comb architecture of cavity-nesting honeybee species viz *A. mellifera* (Crane, 1999; Ruttner, 1988) and *A. cerana* (Deodikar *et al.*, 1977) has been well reported. The comb age affects brood survivorship and colony growth of the nests (Taha, 2021). Rinderer *et al.* (1996) reported the changes in the width of worker and drone cells of *A. mellifera*. Naturally constructed bee combs initially build circular-shaped cells but quickly change to a rounded hexagonal shape (Nazzi, 2016). Information on changes in comb construction is required to know the collective activities of bees engaging in small-scale repetitive tasks, executed by many individuals, which collectively can lead to a multi-purpose structure for the benefit of the colony. The present study aimed to determine the dimensions of comb and wax cell parameters with comb age.

MATERIALS AND METHODS

Studies on comb dimensions of *A. florea* were carried out in and around Bengaluru Rural district (13°27'90" N; 77°23'78" E) India. The study region was rich with agricultural and horticultural crops including different forest bee flora (Ramyarani and Nagaraja, 2024). The colonies of *A. florea* found in a 10 km² radius area on the branches of small trees, thorny bushes, shrubs, hedges, and dried pile sticks at heights between 1 to 3 meters were selected for the study. The bees were removed carefully with a soft brush on both sides of the comb. Each comb of the *A. florea* was photographed using a DSLR camera (Canon EOS 600D with a Nikon 18-55 lens with a settings aperture of 5.6 and a shutter speed: 1/125sec of ISO-100) to measure the comb and cell dimensions. The combs of *A. florea* combs were marked on the day of initiation of comb building after a few days of their arrival at new sites. The combs (N=45) were collected safely without any damage at different age intervals and were categorized into young combs (up to 2 months old) (Free and Williams, 1974), middle-aged combs (2 to 4 months old), and old-aged combs (4 to 6-months old - Fig. 1). The comb metrics viz comb perimeter, and comb area

of each comb were determined by transferring the comb outline onto a transparent plastic sheet. Later, this outline was scanned to produce a digital image. This format was used as an input for the public domain software program "ImageJ". The area occupied by the brood, pollen, and honey on both sides of the comb was measured. The crest size above and below the substratum and the worker and drone comb thickness were also recorded with the help of digital calipers followed by ImageJ software (Rasband, 1997).

To evaluate the differences in cell width and cell depth of worker, drone, pollen, and honey cells concerning comb age, five close-up pictures of the different positions of each comb were taken. The width of cells was measured by wall-to-wall dimensions using ImageJ software (Buawangpong *et al.*, 2014). Similarly, the cell depth of each cell was recorded by a digital caliper. These individual cell-type measurements were used to calculate the means and standard error. The obtained data from the study was analyzed using SPSS software version 22.0. One-way Analysis of variance (ANOVA) was performed to assess differences between comb age and dimensions of different comb parameters. Furthermore, a pairwise comparison with Tukey's HSD test was employed to test the significant differences between cell width and cell depth.

RESULTS AND DISCUSSION

The combs of *A. florea* are incredibly small with hexagon-shaped cells. The young combs were generally creamy white. However, the middle-aged combs were yellowish and the old combs were darkish yellow (Fig. 1). The comb dimensions of 15 each of young, medium, and old combs of *A. florea* colonies showed variation among the ages (Table 1). The observations showed that the comb perimeter of *A. florea* was varied and the maximum comb perimeter size of 41.42 ± 4.84 cm was recorded in middle-aged combs and the minimum size of the comb perimeter of 26.5 ± 4.29 cm was recorded in young combs. The area of comb, brood, pollen and honey was recorded in young, middle-aged and old combs of *A. florea*. The observations revealed that the comb parameters like brood area (297.6 ± 41.53) and comb perimeter (41.42 ± 4.84) in middle-aged

Table 1. Comb dimensions, area of brood and storage of cells in young, middle and old combs of *Apis florea*

Comb Parameters	Comb age		
	Young	Middle	Old
Comb perimeter (cm)	26.5 ± 4.29	41.42 ± 4.84	38.07 ± 4.38
Mean comb area (cm ²)	275.42 ± 21.74	352.01 ± 39.19	345.6 ± 49.41
Brood area (cm ²)	238.85 ± 20.25	297.6 ± 41.53	293.33 ± 33.51
Pollen area (cm ²)	7.78 ± 3.78	19.16 ± 4.34	35.6 ± 3.64
Honey area (cm ²)	36.57 ± 3.95	48.1 ± 18.24	51.33 ± 9.97
Crest size above and below (cm)	3.32 ± 0.65	6.75 ± 2.65	10.36 ± 3.65
	5.17 ± 0.55	9.17 ± 2.83	14.17 ± 3.55
Worker Comb thickness (mm)	15.3 ± 1.95	17.30 ± 2.0	19.51 ± 2.08
Drone Comb thickness (mm)	Nil	Nil	25.22 ± 2.07

N=45; Values are mean ± standard deviation

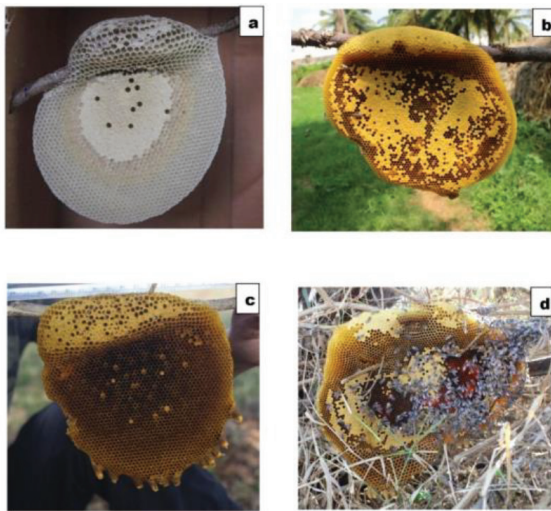


Fig. 1 The young (a), middle-aged (b) and old (c, d) combs of *Apis florea*

combs were greater followed by old combs. The mean comb area (cm²) of young and old combs, was found to be 275.42 ± 21.74 and 345.6 ± 49.41, the brood area at 238.85 ± 20.25 and 293.33 ± 33.51, the pollen at 7.78 ± 3.78 and 35.60 ± 3.64, and the honey at 36.57 ± 3.95 and 51.33 ± 9.97, respectively. The crest size of the bee comb was varied and was relatively larger in old combs compared to young and middle-aged combs. The crest size of *A. florea* combs found above and below the substratum in young, middle-aged and old combs were 3.32 ± 0.65cm (above substratum) and

5.17 ± 0.55 (below substratum), 6.75 ± 2.65cm (above substratum) and 9.17 ± 0.2.83cm (below substratum), and 10.36 ± 3.65cm (above substratum) and 14.17 ± 3.55cm (below substratum) respectively. Furthermore, the thickness of worker and drone combs was larger in old combs and was found to be 19.51 ± 2.08 and 25.22 ± 2.07mm respectively (Table 1).

The cell width of worker brood, pollen and honey cells was recorded in young, middle-aged and old combs of *A. florea*. The observations showed that the cell width of worker brood, pollen and honey cells decreased significantly ($p < 0.05$) with the increase in age of the combs. The maximum cell width of worker brood, pollen and honey cells recorded were 1.91 ± 0.25mm, 1.89 ± 0.30mm and 1.94 ± 0.28mm in young combs. Likewise, in old combs, the minimum cell width size of worker brood, pollen and honey cells recorded were 1.34 ± 0.19, 1.24 ± 0.13 and 1.54 ± 0.20mm respectively (Fig. 2).

The cell depth of worker brood, pollen and honey cells was recorded in young, middle-aged and old combs of *A. florea*. The observations showed that, the cell depth of worker brood, pollen and honey cells were significantly ($p < 0.05$) increased with the increase in age of the combs. The maximum cell depth of worker brood, pollen and honey cells recorded were 8.73 ± 0.14mm, 18.46 ± 0.23mm and



Fig. 2 Cell width of worker, pollen, and honey cells in young, middle and old-aged combs of *A. florea*. Bars having the same letter do not differ significantly between different aged combs at $P < 0.05$ (ANOVA followed by Tukey's test); $N = 20$ cells

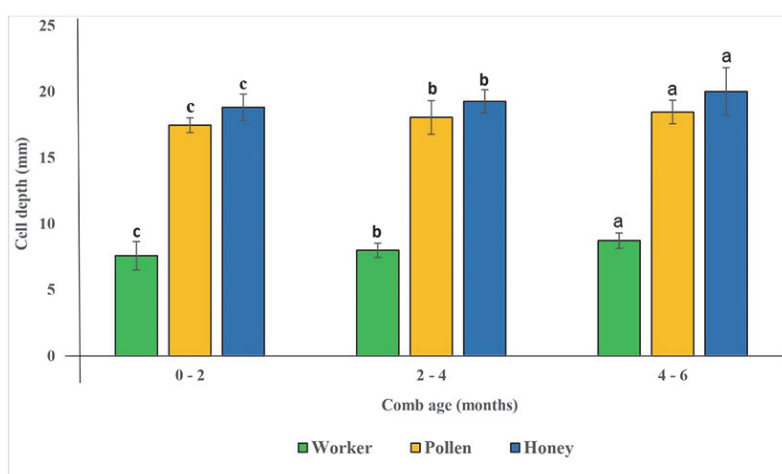


Fig. 3 Cell depth of worker, pollen and honey cells in between different aged combs of *Apis florea*. Bars having the same letter do not differ significantly at $P < 0.05$ (ANOVA followed by Tukey's test) $N = 20$ cells

20.02 ± 0.46 mm in old combs. The minimum size of cell depth was found in young combs and was measured to be 7.58 ± 0.27 mm, 17.4 ± 0.14 mm, and 18.8 ± 0.25 mm respectively (Fig. 3).

The combs of honeybees are double-sided hexagonal structures suited ideally for storing food and housing larvae, reducing building materials and space. The observations on the color of *A. florea* combs showed whitish in the beginning later into golden brown followed by darkish brown. Similar observations were made by Karihaloo *et al.* (2013) in the combs of *A. mellifera*. Taha and El-Sanat (2007) opined that colour changes were due to the accumulation of a large number of contaminants absorbed in the wax over time. Nonetheless, the

combs become more fragile and tenuous with increased comb age (Hepburn and Kurstjens, 1988). Brood rearing for several generations made combs darker, the cell walls thickened and the cells became smaller (Winston, 1987; Taha *et al.*, 2010).

The dimensions comb parameters of *A. florea* showed variation with the age of the combs. The maximum comb perimeter was found in middle-aged combs in contrast to the minimum comb perimeter in young and old combs. The middle-age combs provide greater strength to rear the young ones and accommodate their products by enhancing the comb perimeter. In relation to comb age, the areas containing brood, pollen, and honey varied, with the largest areas being found in young and

middle-aged combs, followed by old combs. The findings of our study are consistent with the observations of Abdellatif (1965) who found better brood areas in young and middle-aged combs. The smaller cells in older combs have a negative impact on egg-laying responses by the queen (Berry and Delaplane, 2001), which may eventually result in a lower production of honeybee workers and drones. Free and Winder (1983) found that old wax combs might contain pheromones of brood, which could inhibit the oviposition by queens. The larger pollen area was found in colonies with new combs and was due to the large number of workers collecting pollen during brood-rearing season (Berry and Delaplane, 2001). The crest size of the bee comb was varied and was relatively larger in old combs compared to young and middle-aged combs. The crown is integral to the structural integrity of the nest and supports the protective curtain of worker bees that shields the nest from the elements (Dyer and Seeley, 1991). Furthermore, the thickness of the drone comb was greater than the worker comb.

The cell width of worker brood, pollen and honey cells of *A. florea* were significantly ($p < 0.05$) decreased with the increase in age of the combs. The maximum cell depth of worker brood, pollen and honey cells was recorded in young combs and the minimum cell width in old combs. This is due to the accumulation of debris and each time a larva pupates it spins a silken cocoon that remains in the cell (Shawer *et al.*, 2020). The diameters of the cells become narrow as the age of the comb increases due to the accumulation of silk and faecal materials produced by the developing larvae (Hepburn and Kurstjens, 1988). The study concludes that the middle-aged combs of *A. florea* are more efficient in rearing brood and storing honey and pollen. Furthermore, larger cell width and depth found in young and middle-aged combs pave the way for the development of healthy colonies. Nonetheless, construction of the new combs by abandoning older combs in *A. florea* would be a strategy for better establishment of their colonies.

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Diversity and foraging activity of pollinators of *Foeniculum vulgare* Miller in Bikaner, India

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ABSTRACT: Fennel, a popular winter rabi spice, relies heavily on pollinators for its reproduction. Periodical surveys conducted in fennel fields at weekly intervals, revealed 31 species of flower visitors/pollinators which included 31 insect species from 17 families and 6 orders with Dipterans and Hymenopterans being the most dominant visitors. Hymenoptera (58.21%) was the most common insect order to visit the fennel flowers in terms of percent abundance. *Apis florea* (19.86%), *Lucilia sericata* (17.73%) and *Polistes olivaceus* (17.73%) were identified as the most frequent pollinators. The highest foraging activity was observed at 11:00 a.m (15.09 bees/10 plants/ 5 minutes). The foraging behaviour of these pollinators varied significantly with *A. florea* spending the maximum time per flower (75 sec). *A. florea* (21.24) and *L. sericata* (23.41) had the highest foraging rates. The study revealed that the foraging activity of these pollinators was significantly correlated with weather parameters. *A. florea* exhibited a substantial negative correlation with relative humidity ($r=-0.7224$) and a significant positive correlation with both maximum and minimum temperatures ($r=0.8100$ and 0.7523 , respectively). *A. florea* and *L. sericata* were found to be more sensitive to temperature change, resulting in more dramatic changes in foraging behaviour with warming.

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KEY WORDS: Fennel, entomophylic, flower visitors, diversity, behaviour, weather parameters, warming

INTRODUCTION

Fennel (*Foeniculum vulgare* Miller), also known as saunf, is a popular winter rabi spice grown in northern India. It thrives in dry, cold climates and hence is grown in Gujarat, Rajasthan, Uttar Pradesh, Madhya Pradesh, Karnataka and Punjab (Pruthi, 1976). Plant-pollinator interactions are highly valued mutualism in agricultural food

production because they provide essential ecological activities that promote world biodiversity. Pollinators are group of animal species that transmit pollen in blooming plants (Ollerton, 2017). Pollination insects mainly belong to three orders namely Lepidoptera, Hymenoptera and Diptera (Mitra and Parui, 2002; Mitra *et al.*, 2008). Insects as pollinators play an important role in cross-pollination of fennel which help to create variation, boost hybrid vigour and

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sustain gene flow in the environment, all of which contribute to the preservation of crop varietal diversity. Fennel blossoms are visited by at least twenty-five insect species belonging to fifteen families and five orders, among seven belonging to the order Lepidoptera, nine in Hymenoptera, five in Diptera, three in Coleoptera and one in Odonata. *Apis florea* F., *A. cerana indica* F., *A. mellifera* L. and *A. dorsata* F. were the most common insect pollinators (Bharti *et al.*, 2015). Pollinator diversity has great impact on agricultural production quality. Higher diversity of insect pollinators is very important in maintaining ecological balance. Biodiversity is calculated by various indices which are used to measure the richness and evenness of different species in population. High diversity index is referred as a key indicator of healthy area (Fedor and Zvarikova, 2019). The degree of pollination mainly depends on foraging speed, foraging rate, strength and diversity of pollinators. Foraging ecology is one of important parameter in successful pollination and is variable from one pollinator species to other, even within the species. To know the influence of weather parameters on pollinator's activity and behaviour in fennel, they were correlated with pollinators foraging activity which were helpful to know the ideal weather conditions for pollinator activity.

MATERIALS AND METHODS

The fennel crops were raised under field conditions at research farm, College of Agriculture, Bikaner during 2021-2022. The field area was first divided into four equal plots of 10m width and 10m length with irrigation channels (2m width). The diversity of insect visitors/pollinators on mustard was observed daily on ten randomly selected plants for five minutes at hourly interval from 06.00 to 18.00h throughout the flowering period to document various insect visitors. The insect visiting flowers were collected with sweep nets, mounted and got identified and the diversity was measured using Shannon-Weiner diversity index.

Abundance of insect visitors is checked on ten randomly selected plants for five minutes at hourly interval from 06.00 to 18.00h, using stopwatch.

Further, rhythmic activities of different insect visitors were recorded at the time of blooming of fennel. These observations were started when 50 per cent of the plants came into bloom. The relative abundance of each species/group to the total insect visitors was calculated by formula proposed by Das and Jha (2018). Different aspects of foraging behaviour of most frequent visitors of fennel crop were recorded. Each of different pollinator species were observed for its foraging behaviour for the collection of nectar and/or pollen. The diurnal variations were recorded for 5 min from 06.00h to 18.00h on fennel for major pollinator species on fennel at hourly interval and their variations in foraging activities at different timings were expressed as mean number of pollinators/ 10 plants/ 5 min. The number of flowers visited by foraging species per five minutes was recorded at peak foraging hour of respective forager species during entire flowering period and average number of flowers visited by pollinator species was expressed as number of flowers visited by a pollinator per 5 minutes.

Major pollinators were observed for documenting the time spent by each visitor on flower of fennel at peak activity. The time spent to insert the proboscis and suck up the nectar or brushing/collecting pollen were considered as the time spent per flower, which were recorded with the help of a stopwatch. Pollinators foraging activity was correlated with various weather parameters viz., temperature, rainfall, wind direction and relative humidity to know the activity of different pollinators in relation to various changes in these parameters in fennel. The data collected were statistically analysed by using the one way of ANOVA and and by using the SPSS computer program (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

RESULTS AND DISCUSSION

Diversity of insect visitors/pollinators: Insect visitors of fennel crop revealed that a total of 31 species belonging to 17 families of six orders were recorded from the fennel flowers. The hymenopterans and dipterans were the major and

dominant floral visitors comprising of 10 species each observed from four and five families respectively, followed by floral visitors of two families from Coleoptera (5), three families from Lepidoptera, two from Hemiptera (2) and the least diversified one is the Neuroptera with only one species (Table 1). Earlier, Hymenopterans and dipterans were noticed as dominant among pollinators in mustard bloom by Das and Jha (2018) in Nadia, West Bengal, India and similar records were also provided by Thakur *et al.* (2022) in spice in Pusa and Dholi locations of Bihar, India.

Abundance of insect visitors/pollinators in fennel: The per cent abundance of insect orders visiting the fennel flowers were dominated by Hymenoptera (58.21%) followed by Diptera (32.61%) and the least by Neuroptera (0.72%) (Table 1). Sharma and Meena (2019) and Paikara and Painkra (2020) observed maximum abundance of pollinators from Hymenoptera in spice in Rajasthan (53.05%) and Chhattisgarh (88.17%). Of the total insect visitor population, the family Apidae displayed maximum (28.42%) abundance succeeded by Calliphoridae (24.82%). Ali *et al.* (2024) explained that the honey bee colonies were not only abundant pollinators, but they also increased the number of seeds per umbel, seed size and fruit weight. Muscidae (2.13%) was reported as the minimum pollinators. Among the different insect species *A. florea* (19.86%) was the predominant species visiting the flowers followed by species such as *Lucilia sericata* (17.73%) and *Polistes olivaceus* (17.73%). Vandhi *et al.* (2022) recorded *A. florea* as the most abundant pollinator among all the floral visitors in coriander in Madurai, Tamil Nadu. Four species were observed as least (0.70%) visitors viz., *Uthesia pulcella* L., *Episyrphus balteatus* (De Geer), *Lampides boeticus* L. and *Coccinella transversalis* Fab.

Shannon-Weiner diversity index displayed that *A. florea* as the higher abundance in population (-0.3210) followed by *L. sericata* (-0.3067) and *P. olivaceus* (-0.3067). Shannon biodiversity index of 2.6401 was observed in fennel umbels. A higher Shannon index indicated a greater diversity of species in the ecosystem; if the index is zero, the

community is home to just one species. Similar results of *Apis* sp. dominance were reported by Ranjitha *et al.* (2019) and Thakur *et al.* (2022). It was further observed that, the pollinator population increased with flowering and gradually declined after peak period of flowering. It started with 1.41 insects m⁻² on 24th December, which gradually increased in subsequent weeks to reach its peak on 20th January (3.70 insects m⁻²) synchronising the peak period of flowering. It again decreased towards cessation of the flowering i.e., 6th week onwards (2.39 insects m⁻²) and minimum abundance was observed on 2nd February (0.42 insects m⁻²). The average number of insects recorded during the blooming period was 2.36 m⁻².

Foraging behaviour of frequent insect pollinator in fennel:

The foraging behaviour of three species viz., *A. florea*, *L. sericata* and *P. olivaceus* done on fennel flowers at different hours of the day, showed variations in activity over time (Fig 1). *A. florea* started the foraging activity at 9.00h of the day and its mean number showed an increasing trend up to 11.00h and thereafter declined with maximum foraging activity at 11.00h (15.09 bees/10 plants/ 5 minutes). The study revealed that *L. sericata* started the foraging activity at 9.00h of the day and its mean number showed an increasing trend up to 12.00h and thereafter declined with maximum foraging activity at 12.00h (15.09 insect visitors/ 10 plants/ 5 minutes). However, no bee activity was observed in the morning and after 17.00h. Also, the study revealed that *P. olivaceus* started the foraging activity at 11.00h of the day and its mean number showed an increasing trend up to 14.00h and thereafter declined with maximum foraging activity at 14.00h (9.09 insect visitors/ 10 plants/ 5 minutes). Moderate foraging activity was observed at 12.00h and 13.00h, with 3.73 visitors/ 10 plants and 5.64 visitors/ 10 plants, respectively. However, no bee activity was observed on morning and after 15.00h onwards. The activity of *P. olivaceus* was observed during the midday only. Saha *et al.* (2022) documented that the foraging of *A. florea* was high at 11.00h and low at early morning and late evening. Less activity was observed at morning and evening hours ranging from 4.64 to 1.91 bees per ten plants per five minutes.

Table 1. List and relative abundance of insect visitors / pollinators on fennel during *Rabi*

Order and its abundance (%)	Family and its abundance (%)	Species	Shannon-Weiner index	Species abundance (%)
Hymenoptera (58.21)	Apidae (24.82)	<i>Apis florea</i> Fab.	-0.3210	19.86
		<i>Apis cerana indica</i> Fab.	-0.0604	1.43
		<i>Apis dorsata</i> Fab.	-0.0604	1.43
		<i>Xylocopa auripennis</i> L.	-0.0604	1.43
		<i>Xylocopa fenestrata</i> Fab.	-0.0604	1.43
		<i>Xylocopa aestuans</i> (L.)	-0.1011	2.84
	Halictidae (5.67)	<i>Halictus</i> sp.	-0.1628	5.67
	Megachilidae (2.84)	<i>Megachile</i> sp.	-0.1011	2.84
	Vespidae (21.28)	<i>Polistes olivaceus</i> (DeGeer)	-0.3067	17.73
		<i>Vespa orientalis</i> L.	-0.1184	3.55
Coleoptera (2.85)	Coccinellidae (2.84)	<i>Coccinella septumpunctata</i> L.	-0.0351	0.72
		<i>Coccinella transversalis</i> Fab.	-0.0351	0.70
		<i>Cheilomenes sexmaculata</i> Fab.	-0.0351	0.71
		<i>Epilachna</i> sp.	-0.0351	0.71
	Meloidae (1.42)	<i>Mylabris pustulata</i> Thunberg	-0.0604	1.42
Diptera (32.61)	Calliphoridae (28.42)	<i>Chrysomya megacephala</i> Fab.	-0.1877	7.09
		<i>Lucilia sericata</i> (Meigen)	-0.3067	17.73
	Muscidae (2.13)	<i>Musca</i> sp.	-0.0604	1.42
	Syrphidae (3.07)	<i>Musca domestica</i> L.	-0.0351	0.71
		<i>Eristalis</i> sp.	-0.0604	1.43
	Sarcophagidae (0.70)	<i>Episyrphus balteatus</i> (De Geer)	-0.0351	0.70
		<i>Eupeodes</i> sp.	-0.0351	0.70
		<i>Metasyrphus corollae</i> Fab.	-0.0604	1.42
		<i>Sarcophaga</i> sp.	-0.0351	0.70
	Dolichopodidae (0.70)	<i>Dolichopus</i> sp.	-0.0351	0.70
Hemiptera (2.14)	Pyrrhocoridae (1.43)	<i>Dysdercus cingulatus</i> Fab.	-0.0604	1.43
	Pentatomidae (0.70)	<i>Halyomorpha halys</i>	-0.0351	0.70
Lepidoptera (2.12)	Lycaenidae (0.70)	<i>Lampides boeticus</i> L.	-0.0351	0.70
	Nymphalidae (0.71)	<i>Danaus chrysippus</i> L.	-0.0351	0.71
	Erebidae (0.70)	<i>Uthesia pulcella</i> L.	-0.0351	0.70
Neuroptera (0.72)	Chrysopidae (0.72)	<i>Chrysoperla carnea</i> (Stephens)	-0.0351	0.72
			H'=2.6401	

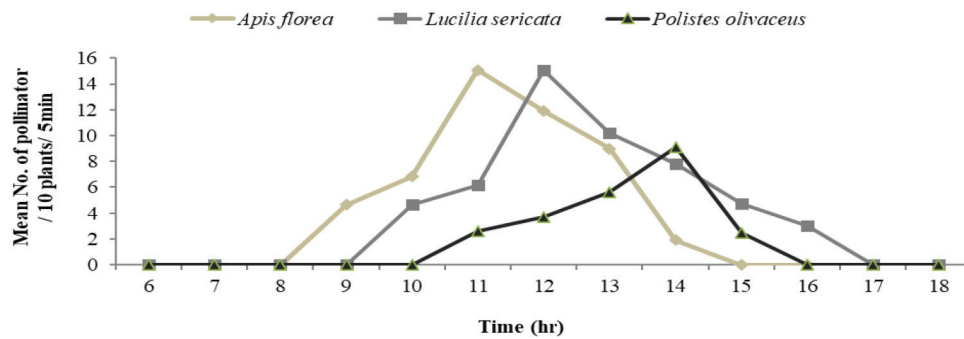


Fig 1. Temporal variations in activity of major insect visitors/pollinators on fennel

However, no bee activity was observed on early morning and after 15.00 h onwards. This is justified with the results of Thakur *et al.* (2022) who observed peak foraging activity during morning hours when the flowers are partially open under favourable weather conditions.

Foraging rate: Foraging rate was different from one insect visitor to other in fennel umbels. The maximum number of umbels were visited by *L. sericata* (23.41) followed by *A. florea* (21.24) and *P. olivaceus* (11.25). The peak foraging hour for these pollinator species with maximum numbers of pollinators were 12.0, 11.0 and 14.0. For all the three insect visitors the peak foraging activity was observed at different hours. The mean number of insect visitors was observed as 15.09, 15.09, 9.09 insect visitors per ten plants per five minutes for *A. florea*, *L. sericata*, *P. olivaceus* respectively. In fennel, *L. sericata*, and *A. florea* made higher visits to flowers. Kumar and Rai (2020) reported that in Bihar, India, among the *Apis* species, *A. florea* recorded the highest foraging rate in Fennel.

Foraging speed: The data showed that *A. florea* spent maximum time (75 sec) with mean foraging

activity of 21.24 sec per flower among the three major insect visitors/pollinators on fennel flowers. This was followed by *L. sericata* which spent maximum time of 48 sec and minimum of 6 sec with mean foraging time 23.41 sec. *P. olivaceus* spent maximum time of 45 sec and minimum time of 8 sec with mean foraging time of 11.25 sec. This was recorded during the peak foraging hour of all the three-insect visitor or pollinator species.

Correlation between foraging activity with weather factors: The data revealed that *A. florea* showed significant positive association with maximum temperature ($r=0.8100$) and minimum temperature ($r=0.7523$), and significant negative association with relative humidity ($r=-0.7224$) and non-significant negative association with wind speed ($r=-0.1327$). According to the report of Thakur *et al.* (2022), the same weather correlation trend was observed with *A. mellifera* in at Pusa and Dholi locations of India. *L. sericata* showed significant positive association with maximum temperature ($r=0.6022$) and minimum temperature ($r=0.7250$) and it showed non-significant negative correlation with relative humidity ($r=-0.4229$) and non-significant positive association with wind speed

Table 2. Correlation between foraging activity of major pollinators and weather parameters in fennel

Pollinators	Temperature - max	Temperature - min	RH (%)	Wind speed (km h ⁻¹)
<i>Apis florea</i>	0.8100*	0.7523*	-0.7224*	-0.1327
<i>Lucilia sericata</i>	0.6022*	0.7250*	-0.4229	0.1165
<i>Polistes olivaceus</i>	0.8712*	0.8388*	-0.5961*	-0.0900

*Significant at 0.05 level

($r = 0.1165$). *P. olivaceus* showed significant positive association with maximum temperature and minimum temperature ($r = 0.8712$ and $r = 0.8388$) and significant negative association with relative humidity ($r = -0.5961$) (Table 2).

Regression models for foraging activity of *A. florea* in relation to weather parameters:

The significant weather factors correlated with activity of insect visitors were taken and regressed to quantify the extent of variability/dependability of insect visitors on these factors. The foraging activity and maximum temperature were regressed to know the variability in *A. florea* foraging activity due to maximum temperature which could be explained by different models. Various models show variability to a extent of 65.6 to 68.9 per cent. The Power model ($y = 2.96x^{1.139}$) variation was 68.9. The variation in linear ($y = 4.569x - 4.773$) was of 65.6, followed by Logarithmic ($y = 36.68\ln(x) - 44.31$) and Polynomial ($y = -0.098x^2 + 6.155x - 11.13$) models. The activity of *A. florea* depends on maximum temperature up to the extent of 68.9 per cent (Table 3a).

The foraging activity and relative humidity were regressed to know the variability in *A. florea* foraging activity due to relative humidity which could be explained by different models. In the linear model ($y = -9.123x + 125.1$) it could be explained to the extent of 52.1 per cent. The polynomial model ($y = 1.775x^2 - 37.87x + 240.4$) was found to be the best fit, as it could explain variability in *A. florea* foraging activity to the extent of 53.5 per cent due to relative humidity in fennel. Logarithmic model ($y = -73.8\ln(x) + 205.3$) explain variability to the extent of 52.9 per cent. The present studies revealed that the foraging activity of *A. florea* depends on relative humidity to the extent of 53.5 per cent in fennel (Table 3b).

Bee activity was strongly positively and negatively correlated with air temperature and humidity. With an Akaike information criterion (AICc) of 586.17, the most economical minimum model only needed four meteorological variables—air temperature, humidity, rainfall, and wind speed—plus solar elevation. The mean increase in bee activity was 0.046 for every 1°C increase in air temperature.

Although the 95per cent CIs were quite big at high rainfall levels, rainfall was significantly inversely correlated with bee activity. This is mainly because rain falls in heavy, brief summer showers rather than continuously over extended periods of time. Sanderson *et al.* (2015) reported overall bee activity decreased at increasing wind speeds, indicating a lesser but still significant ($P = 0.0007$) detrimental influence of wind speed.

Table 3 a. Different regression models for *Apis florea* foraging activity in relation to maximum temperature

Model	Regression equation	R ²	CD
Linear	$y = 4.569x - 4.773$	0.656	65.6
Logarithmic	$y = 36.68\ln(x) - 44.31$	0.656	65.6
Polynomial	$y = -0.098x^2 + 6.155x - 11.13$	0.656	65.6
Power	$y = 2.96x^{1.139}$	0.689	68.9
Exponential	$y = 10.16e^{0.141x}$	0.684	68.4

R² = Regression Coefficient; CD = Coefficient of Determination

Table 3 b. Different regression models for *Apis florea* foraging activity in relation to relative humidity

Model	Regression equation	R ²	CD
Linear	$y = -9.123x + 125.1$	0.521	52.1
Logarithmic	$y = -73.8\ln(x) + 205.3$	0.529	52.9
Polynomial	$y = 1.775x^2 - 37.87x + 240.4$	0.535	53.5
Power	$y = 992.4x^{-1.43}$	0.489	48.9
Exponential	$y = 211.6e^{-0.17x}$	0.486	48.6

R² = Regression Coefficient; CD = Coefficient of Determination

Regression models for foraging activity of *L. sericata* in relation to weather parameters:

The weather factors in relation to *L. sericata* foraging activity regressed using various linear and nonlinear models to quantify the extent of variability in foraging activity, revealed its impact on the foraging activity.

The *L. sericata* foraging activity and maximum temperature when regressed, the linear model ($y = 4.562x - 0.834$) explained to the extent of 36.2 per cent. The Polynomial model ($y = 3.537x^2 - 50.20x$

Table 3 c. Different Regression models between *Lucilia sericata* foraging activity in relation to maximum temperature

Type of model	Regression equation	R ²	CD
Linear	$y = 4.562x - 0.834$	0.362	36.2
Logarithmic	$y = 33.94\ln(x) - 34.90$	0.339	33.9
Polynomial	$y = 3.537x^2 - 50.20x + 209.4$	0.489	48.9
Power	$y = 5.064x^{0.932}$	0.279	27.9
Exponential	$y = 12.86e^{0.125x}$	0.3	30.0

R² = Regression Coefficient; CD = Coefficient of Determination

+ 209.4) was found to be the best fit, as it could explain variability to the extent of 48.9 per cent due to maximum temperature in fennel. The present studies revealed that the foraging activity of *L. sericata* depends on maximum temperature to the extent of 49 per cent in fennel (Table 3c).

The influence of temperature on foraging likelihood differs significantly among pollinator species, according to logistic regression, which showed that the interaction between species identity and temperature was significant ($X^2 = 13.054$, $df = 3$, $P = 0.005$). *L. sericata*'s foraging likelihood response to temperature, in particular, demonstrated a moderate decline in anticipated probability from high to low temperatures. Devenish (2023) reported that *L. sericata*'s likelihood at high temperatures (0.933) was in the middle of the other three species. Forager activity was positively linked to air temperature and solar elevation, and negatively associated with rainfall, humidity and wind-speed. The effects of increased variability in meteorological conditions are considered by Sanderson *et al.* (2015). From the results, *A. florea* and *L. sericata* were found to be more sensitive to temperature change, resulting in more dramatic changes in foraging behaviour with warming.

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Bio-efficacy of botanical soap formulations fortified with essential oils against *Henosepilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae) and evaluation of its shelf life

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ABSTRACT: Experiments were conducted to evaluate the efficacy of botanical soap formulations against epilachna beetle, *Epilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae), and their repellent effect and the shelf life. In the first experiment, neem oil+ citronella oil soap @2 per cent recorded the highest per cent mortality and was on par with treatments, pongamia+ basil oil soap @ 2 per cent, neem oil+ basil oil soap @ 2 per cent, and pongamia+ citronella oil soap @ 2 per cent after 24 hours after treatment. In the second experiment, neem oil+ citronella oil soap @ 2 per cent recorded maximum repellence (80%) and was on par with pongamia oil+ citronella soap @ 2 per cent (73.33%) and neem oil+ citronella soap @ 1 per cent (65%) after 30 minutes after treatment. In the third experiment, neem oil + citronella oil @ 2 per cent exhibited the highest mortality rates under both refrigerated and room conditions and statistically superior to all other treatments. The lowest per cent mortality was recorded for the control. The soap formulations stored under refrigerated conditions (for a period of three months), showed higher insecticidal efficacy than those stored at room conditions, revealing the effectiveness remained for three months.

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KEY WORDS: Epilachna beetle, bioassay, neem oil, citronella oil, basil oil, pongamia oil, repellent effect, shelf life

INTRODUCTION

The hadda beetle, *Epilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae) is a serious pest of brinjal, *Solanum melongena* L. Both adults and grubs being the damaging stages of the pest, feeds on the epidermal layer of the leaves, reducing the photosynthetic area and cause serious defoliation

(Imura and Ninomiya, 1998). The 3rd and 4th instar grubs were reported to be the most damaging stages. Farmers often rely on harmful chemicals which may prove to be harmful for human health and environment if not used judiciously. Kaur and Singh (2013) reported neem soap @ 1 per cent significantly lowered aphid populations in capsicum followed by pongamia soap @ 2 per cent and

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confidor 17.8 SL @ 0.025 per cent. Pongamia oil soap @2% was found effective in reducing the leaf hopper population, *Amrasca biguttula biguttula* (Ishida) (Thomas and Sreekumar, 2020). Essential oils consist of a mixture of various compounds; Monoterpenes, the primary constituents of essential oils, are lipophilic molecules capable of readily penetrating the insect integument (Tisserand and Young, 2013). Nerio *et al.* (2010) reported that citronella oil possesses repellent effect to insects. Basil oil was reported to contain biologically active constituents that are insecticidal (Chavan and Nikam, 1982), Even though soaps made from plant based insecticides have been proved to be effective in pest management, a combination of botanical soaps fortified with essential oils was lacking. Therefore studies on combined effect of both botanical soaps and essential oils for enhanced pest management were taken up against *E. vigintioctopunctata*.

MATERIALS AND METHODS

The study was carried out at the Department of Entomology, College of Agriculture, Vellayani Kerala, India, during 2021-2023. Botanicals *viz.* neem oil and pongamia oil and essential oils *viz.* basil oil and citronella oil were procured. The saponification value of neem oil, pongamia oil and palm oil estimated were 188, 182, and 192mg KOH/g, respectively. These soft soap formulations were prepared by combining botanicals *viz.*, neem oil and pongamia oil and essential oils *viz.* citronella oil and basil oil. The alkali used for the soap is potassium hydroxide.

Eggs of hadda beetle along with the leaves were collected from infested plants grown in the college campus. The collected egg mass, grubs and adults were placed in separate plastic trays of 28 × 16cm. These trays were covered with white cloth for aeration. Fresh brinjal leaves were provided daily for the grubs and adults. The instars emerged were fed with fresh leaves and placed in new trays. These grubs were reared up to the final instars and the final instar was taken for the laboratory evaluation.

Laboratory bioassay: Leaf dip bioassay was carried out to monitor the toxicity of insecticidal

soaps against the grubs. Laboratory experiments were laid in Completely Randomized Design (CRD) with 19 treatments (Table 1) replicated thrice. Fresh leaves of brinjal were cut into leaf discs of 9 cm diameter and then dipped in different concentrations of botanical soaps. After 10 minutes of exposure, treated leaf discs were kept to dry. After drying, the leaf discs were placed into the Petri plates lined with moist filter paper and 20 grubs representing various treatments were released into each Petri plates. Petri plates were covered with lid to prevent the insect escape. Observations on the mortality were taken after a specific exposure interval of 24 and 48 hours respectively. The corrected mortality was calculated using the following formula (Abbot, 1925). The mortality data were subjected to arcsine transformation and analysis of variance.

$$\begin{aligned} \text{Corrected per cent mortality} = \\ (\% \text{ mortality in treatment} - \% \text{ mortality in control}) \\ \% \text{ mortality in control} \end{aligned}$$

Based on the mortality data, six promising treatments were selected for evaluating repellence effect.

Repellent effect of botanical soap formulations: The repellent effect of different concentrations was determined by modifying the method of Wei *et al.* (2018), with six treatments and three replications in CRD (Table 2). Brinjal leaf discs were cut into two halves (diameter: 3cm) and were placed on the opposite sides of the Petri plates of 9cm diameter. Two halves were separated by gap of 5cm. One half of the brinjal leaves was treated with varying concentration of botanical soaps while the other half was treated with control (water). Following the treatment, the final instars grubs of hadda beetle were released into the centre of the Petri plates and observations were taken at 30 and 60 MAT (Minutes After Treatment). Formula for calculating per cent repellence is given below.

$$PR (\%) = \frac{N_c - N_t}{N_c + N_t} * 100$$

Where PR = per cent repellence, N_c= No. of grubs in control, N_t= No. of grubs in treatment.

Evaluation of shelf life of botanical soap formulations:

The shelf life of best three botanical soap formulations from the bioassay experiments was evaluated under room (27-29°C) and refrigerated conditions (4-7°C) and its effect on epilachna grubs for a period of three months at monthly intervals. The experiment was laid out in the CRD design and data was analysed and values in parentheses were subjected to arc sign transformation. Significant differences between each treatment were compared by using GRAPES (General R-shiny based Analysis Platform Empowered by Statistics) software (Gopinath *et al.*, 2020).

RESULTS AND DISCUSSION

Bioassay: After 24h of exposure, mortality was maximum in the treatment, neem oil+ citronella oil soap @ 2% (54.42%) and was on par with treatments, pongamia+basil oil soap @ 2% (40.89), neem oil+ basil oil soap @ 2% (38.61), and pongamia + citronella oil soap @ 2% (37.56). Palm oil soap @2% (8.60%) and palm oil soap @1% (3.33%) were found to be least effective and were found to be inferior to other treatments (Table 1).

After 48 h of exposure, the treatment neem oil + citronella oil soap @ 2% showed the highest per cent mortality (80.44) followed by neem oil + basil oil soap @2% (70.16). However, both these treatments were found to be on par with each other. Palm oil soap @2% was found to be least effective. The effect of insecticidal effect of neem oil has been proved by many workers. Anam *et al.* (2004) reported that neem oil @ 4% caused highest larval mortality (100%) of *E. dodecastigma*. Similarly, Hanif *et al.* (2021) revealed that neem extract @10 % recorded highest mortality of hadda beetle grubs (73.4%). This might be due to the presence of azadiractin, which alters the feeding habits of insects, and physiological disturbances that lead to the death of insects. Swaminathan *et al.* (2010) reported that pongamia oil @ 5% exhibited a cent percent mortality to epilachna grubs after 7 days of treatment. Lee *et al.* (2004) noticed that the chemical composition of essential oil is lipophilic in nature, which will enter into an insect and cause biochemical dysfunction and mortality.

Table 1. Evaluation of the efficacy of botanical soap formulations on epilachna beetle

Treatments	*Mortality (%)	
	24 HAT	48 HAT
T1: Neem oil + citronella oil soap @1%	20.88 (26.64) ^{bc}	48.07 (43.88) ^{cd}
T2: Neem oil + citronella oil soap @2%	54.42 (47.54) ^a	80.44 (63.83) ^a
T3: Neem oil + basil oil soap @1%	11.93 (19.58) ^{cd}	34.39 (35.82) ^{de}
T4: Neem oil +basil oil soap @2%	38.61 (38.41) ^{ab}	70.18 (57.02) ^{ab}
T5: Pongamia + citronella oil soap @1%	8.51 (16.43) ^{de}	20.53 (26.67) ^{efg}
T6: Pongamia + citronella oil soap@2%	37.56 (37.78) ^{ab}	62.02 (52.00) ^{bc}
T7: Pongamia + basil oil soap @1%	10.35 (18.36) ^d	20.61 (26.59) ^{efg}
T8: Pongamia +basil oil soap @2%	40.89 (39.73) ^{ab}	61.42 (51.66) ^{bc}
T9 : Pongamia oil soap @1%	10.35 (18.36) ^d	17.28 (24.50) ^{fg}
T10- Pongamia oil soap @2%	17.02 (23.69) ^{cd}	24.04 (29.27) ^{efg}
T11: Neem oil soap @1%	10.18 (17.69) ^d	25.79 (30.46) ^{efg}
T12: Neem oil soap @2%	25.53 (30.17) ^{bc}	52.98 (46.81) ^c
T13: Palm oil soap @1%	3.33 (6.14) ^f	5.00 (7.59) ^h
T14: Palm oil soap @2%	8.60 (16.87) ^f	9.33 (10.03) ^h
T15: Palm oil + citronella oil soap@1%	20.88 (26.64) ^{cd}	29.47 (32.63) ^{ef}
T16: Palm oil + citronella oil soap @2%	18.95 (25.45) ^{cd}	34.56 (35.98) ^{de}
T17: Palm oil +basil oil soap @1%	10.35 (18.36) ^{de}	20.61 (26.90) ^{efg}
T18- Palm oil +basil oil soap @2%	12.02 (19.82) ^{cd}	24.04 (29.16) ^{efg}
T19- Control	0.00 (0.00) ^f	0.00 (0.00) ^h

* Mean of three replications

Values in the parenthesis were subjected to arc sine transformation

HAT – Hours after treatment

In a column, the mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Repellent effect of soap formulations: Six most effective treatments from bioassay experiment were further assessed for their repellent effect against the fourth instar grubs (Table 2). Neem oil + citronella oil soap @ 2% recorded maximum repellence (80%) and was on par with pongamia oil + citronella soap @ 2% (73.33%) and neem oil + citronella soap @ 1% (65.00%) after 30 minutes after treatment (MAT). The treatment neem oil + citronella oil soap @ 2% recorded maximum repellence (85%) at 60 MAT and was on par with pongamia oil + citronella oil soap @ 2% (76.67%). Similarly, Ikeura *et al.* (2013) also reported the repellence activity of neem against cabbage armyworms under laboratory conditions and they mentioned that the repellent activity of neem was due to azadiractin content. Manoj (2022) also reported that both basil and citronella oil exhibited 90 to 100 percent repellence against hadda beetle grubs after 30 and 60 minutes of treatment, respectively.

Evaluation of shelf life of botanical soap formulations: The effect of stored botanical soap formulations was evaluated against epilachna grubs on the first, second and third months of storage. After 24 hours of treatment, the freshly prepared soap solutions of neem oil + citronella oil @2% recorded highest mortality (54.42%) and was statistically superior to all other treatments. After

one month of storage, soap formulations stored under refrigerated conditions showed greater mortality compared to those stored at room temperature. Soap formulation of neem oil + citronella oil @ 2% exhibited the highest mortality, under both refrigerated and room conditions (46.71

Table 2. Repellent effect of botanical soap formulations against epilachna beetle grubs

Treatments	*Repellence (%)	
	30 MAT	60 MAT
T1: Neem oil + citronella oil soap @2%	80.00 (63.54) ^a	85.00 (67.40) ^a
T2: Pongamia + citronella oil soap @2%	73.33 (59.32) ^a	76.67 (61.76) ^{ab}
T3: Neem oil + citronella oil soap @1%	65.00 (59.32) ^a	68.33 (55.97) ^{bc}
T4:Neem oil +basil oil soap @2%	55.00 (47.91) ^b	60.00 (50.79) ^{cd}
T5: Pongamia +basil oil soap @2%	55.00 (47.89) ^b	61.67 (51.81) ^{bcd}
T6: Neem oil soap @2%	45.00 (42.09) ^b	50.00 (45.00) ^d

* Mean of three replications; Values in the parenthesis were subjected to arc sine transformation

MAT – Minutes after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Table 3 a. Shelf life efficacy of botanical soap formulations @2% against epilachna beetle after 24 HAT

Treatments	*Mortality (%) – after 1 – 3months of storage						
	Laboratory	First month		Second month		Third month	
		Refrigerated bioassay	Room	Refrigerated	Room	Refrigerated	Room
Neem oil+ citronella oil	54.42 (43.87) ^a	46.71 (43.53) ^a	43.03 (40.91) ^a	41.84 (40.14) ^a	35.33 (34.56) ^a	33.35 (35.07) ^a	31.25 (33.91) ^a
Neem oil + basil oil	38.61 (35.94) ^b	35.39 (36.49) ^b	32.89 (34.79) ^b	35.59 (36.39) ^b	27.76 (30.03) ^b	28.09 (31.77) ^b	26.25 (29.56) ^b
Pongamia oil + citronellaoil	37.56 (34.92) ^b	34.14 (35.64) ^b	31.64 (34.06) ^b	29.08 (32.39) ^b	24.14 (28.25) ^b	26.91 (31.17) ^b	23.75 (27.45) ^b
Control	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c

* Mean of three replications; Values in the parenthesis were subjected to arc sine transformation

HAT – Hours after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Table 3 b. Shelf life of botanical soap formulations @2% against epilachna beetle after 48 HAT

Treatments	*Mortality (%) – after 1 – 3months of storage						
	Laboratory	First month		Second month		Third month	
		Refrigerated bioassay	Room	Refrigerated	Room	Refrigerated	Room
Neem oil+ citronella oil	80.44 (63.83) ^a	65.09 (53.85) ^a	61.25 (51.79) ^a	61.31 (51.82) ^a	53.75 (47.16) ^a	53.43 (46.99) ^a	47.56 (35.07) ^a
Neem oil + basil oil	70.18 (57.02) ^b	58.36 (49.86) ^a	50.99 (45.71) ^a	53.75 (47.23) ^a	47.64 (43.63) ^a	49.13 (44.50) ^a	44.87 (31.77) ^a
Pongamia oil + citronellaoil	62.02 (52.00) ^b	54.28 (47.53) ^a	48.95 (44.38) ^a	52.70 (346.57) ^a	45.14 (42.18) ^a	45.25 (42.26) ^a	42.43 (31.17) ^a
Control	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b

* Mean of three replications; Values in the parentheses were subjected to arc sine transformation; HAT – Hours after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

and 43.03% respectively) and this treatment was found to be superior to all other treatments. The same trend in the mortality of the test insect has been exhibited by soap formulations kept after two and three months of storage respectively (Table 3a).

After 48 hours of treatment, the freshly prepared soap solutions of neem oil + citronella oil @ 2% recorded highest mortality (80.44%) and was statistically superior to all other treatments. After one month of storage, soap formulations kept under refrigerated conditions demonstrated a higher percentage of grub mortality compared to those stored at room temperature. The formulation neem oil + citronella oil @ 2% exhibited the highest mortality, under refrigerated and room conditions (65.01 and 61.25%, respectively) and were found to be superior statistically. The same trend in the mortality of the test insect was showed when the soap formulations were stored after two and three months respectively (Table 3b).

This finding was in line with the findings of Varma (2018), who reported that neem oil garlic soap can be stored for up to three months without reduction in its efficacy. After the storage period, the soap formulations stored under refrigerated conditions showed higher efficacy than those stored at room conditions. This finding was supported by Rowshan *et al.* (2013), who reported that the primary quality

of essential oils was maintained by storing them under refrigerated (4°C) and freezer conditions (-20°C) when compared to room conditions during a period of storage of three months. Farahbakhsh *et al.* (2021) found that the quality of essential oil constituents with low molecular weight decreased over time, especially at room temperature. This quality deterioration may be due to the oxidation, evaporation and other undesired changes in essential oil components during storage. Evaluation of shelf life of these formulations revealed that their effectiveness remained for three months.

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Descriptions of polar gynandromorphic specimens of *Culicoides oxystoma* Kieffer and *Obsoletus* group (Diptera, Ceratopogonidae) from West Bengal, India

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ABSTRACT: Five rare polar gynandromorphic specimens of *Culicoides* (Diptera, Ceratopogonidae), four of *Culicoides oxystoma* Kieffer, and one specimen of *Obsoletus* group were studied. These individuals were collected during pre-monsoon, monsoon and post-monsoon seasons using UV LED light traps from cattle shed and poultry farm located in Purulia, West Bengal, India. The gynandromorphic individuals of *C. oxystoma* revealed a typical one with male genitalia with a slender abdomen. It had the head, antenna, mouthparts, and the associated accessory structures together with the wings were similar to that of a female. On the other hand, three specimens had female genitalia and head, antenna, mouthparts like male. Very interestingly, the gynander specimen of *Obsoletus* group possesses antennal flagellomeres, mouthparts, and wings of females but enlarged pedicel-like male and the presence of basistyle, dististyle but devoid of aedeagus and parameres. © 2025 Association for Advancement of Entomology

KEY WORDS: *Culicoides oxystoma*, *Obsoletus* group, gynandromorph, morphology, India

INTRODUCTION

Gynandromorph individuals are genetically and phenotypically chimeric and can be easily distinguished from others (Goldschmidt, 1934; Laugé, 1985). Gynandromorphism is usually categorized as polar, bilateral, spiral, and oblique (Oke *et al.*, 2020). A polar gynander individual possesses the head of one sex while the abdomen of the other sex. However, a bilateral gynander has the boundary line between the sexes following the longitudinal axis. In contrast, an oblique gynander has the head and one side of the thorax of one sex while the other side of the thorax and the abdomen are of the other. The probable reasons behind gynander formation include loss or damage of a

sex chromosome, polyspermy, binucleate eggs, post-cleavage fertilization, and also due to some symbionts or parasitism (Narita *et al.*, 2010). Mermithid nematodes may produce stunning morphological alterations in their hosts and form an intermediate morph between the sexes (Wülker, 1975).

The occurrence of gynandromorphs has been documented recurrently in various families of the order Diptera (Narita *et al.*, 2010). Gynandromorphs of the nematoceran family Chironomidae, aberrant females copulated with males (Rempel, 1940); in Baltic amber fossils, that deviant female was seen mating with normal females (Boucot and Poinar, 2010). Therefore, a

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gynander can provide essential information about their behavioural and sexual biology (Crosskey, 1990). Mullens *et al.* (2008) state that mermithid parasitism may trigger abnormal host development or morphological features and behaviour.

Beck (1958) recorded the first gynandromorph ceratopogonids parasitized with mermithids. Of the Ceratopogonidae, gynandromorphism was reported in several *Culicoides* spp. (Diptera, Ceratopogonidae). They are incriminated as biological vectors as they transmit various arboviruses, protozoa, and nematodes to livestock and humans (Mellor *et al.*, 2000; Sick *et al.*, 2019). Callot and Kremer (1963) documented mermithids causing intersexes of *Culicoides* spp. Most *Culicoides* intersexes were parasitized by mermithid nematodes with male genitalia and secondary sexual traits of females (McKeever *et al.*, 1997). Intersex male *Culicoides circumscriptus* Kieffer parasitised by *Heleidormis cataloniensis* also documented by Poiner and Monteys (2008).

Although the frequency of this phenomenon is very low in insects, earlier records of gynandromorphism were documented in several biting midges such as *Culicoides varipennis* (Coquillett) (Curtis, 1962), *Culicoides furens* (Poey), *Culicoides leopoldi* Ortiz (Hawkins, 1962), *Culicoides hollensis* (Melandar and Brues) (Khalaf, 1965), *Culicoides crepuscularis* Malloch, *Culicoides haematopotus* Malloch, *Culicoides stellifer* (Coquillett) (Smith and Perry, 1967) and *Culicoides lailae* Khalaf (Navai, 1969). A polar gynandromorph was reported by Oke *et al.* (2020) in *Culicoides subschultzei* Cornet and Brunhes belonging to the subgenus *Remmia* Glukhova (Borkent and Dominiak, 2020) along with observed bi-abdomens in another member of *Schultzei* group, *Culicoides oxystoma* Kieffer in which a distinct abdomen attached to the meta-thorax and interestingly both abdomen of that individual possesses three spermatheca.

The species *C. oxystoma* vectors the following viruses: i) *Orbivirus*: bluetongue virus (BTV),

African horse sickness virus (AHSV), epizootic hemorrhagic disease virus (EHDV), Chuzan virus (CHUV), D'Aguilar virus (DAGV), Ibaraki virus (IBAV); ii) *Orthobunyavirus*: Akabane virus (AKAV), Aino virus (AINOV) (Kurogi *et al.*, 1987; Yanase *et al.*, 2005; Dadawala *et al.*, 2012); nematode: *Onchocerca gibsoni*, the causative agent of filaria of cattle (Buckley, 1938); protozoa: *Leucocytozoon* sp., an intracellular haemosporidian blood parasite and *Leishmania (Mundinia) martiniquensis*, *L. (M.) orientalis*, and *Crithidia* spp. (Sunantaraporn *et al.*, 2022; Songumpai *et al.*, 2022). It is a significant vector species with a wide geographic distribution (Wirth and Hubert 1989). It is one of the seven BTV vectors reported from India (Harrup *et al.*, 2016). It is the predominant species in cattle sheds in the eastern state of India, West Bengal, and in Bluetongue disease (BTD) prone areas of southern India (Chanda *et al.*, 2019; Harsha *et al.*, 2020).

Biting midge species such as *Culicoides obsoletus* Meigen, *Culicoides scoticus* Downes and Kettle, *Culicoides dewulfi* Goetghebuer, and *Culicoides chiopterus* Meigen are members of the subgenus *Avaritia* and belonging to *Obsoletus* group (Campbell and Pelham-Clinton, 1960; Borkent and Dominiak, 2020) and they gained particular importance as they have been implicated as vectors of BTV and SBV in northern Europe (Hoffman *et al.*, 2009; Elbers *et al.*, 2013). Biting midges of the *Obsoletus* species group are increasingly suspected as vectors of the recent emergence of the bluetongue virus in Europe (Augot *et al.*, 2010). In India, bluetongue disease is endemic, and outbreaks have been reported in several states in native and exotic breeds of sheep (Prasad *et al.*, 2009). Morphological anomalies were found in females of *C. obsoletus*. It was concluded that probable causes of these anatomical alterations occur due to some genetic or morphogenetic malformation (Ramilo *et al.*, 2021). This present investigation reports *C. oxystoma* and *Obsoletus* group possessing three types of polar gynandromorphism (anterior-posterior) along with morphometrics firstly from India.

MATERIALS AND METHODS

Study area and collection of wild midges

Adult midges were collected using UV LED light traps from a backyard cattle shed and a poultry farm. The trap was operated overnight from 5.30 pm to 7.00 am. The sampling sites were located in Rajabandh para (23°19' N; 86°22' E) and Kapur Bagan (23°20' N; 86°22' E), respectively, in the district of Purulia, West Bengal, India. Biting midges were collected for three consecutive seasons (pre-monsoon, monsoon, and post-monsoon) from March 2022 to April 2023 from the cattle shed, while sampling was performed for three months (May 2023-July 2023) from a poultry farm.

Mounting and identification of midges

Adult *Culicoides* spp. was sorted out from the rest of the trapped insects and some of them were slide mounted onto glass slides following Wirth and Marston (1968). After that, these specimens were subjected to morphometric measurements and identified based on the key of Wirth and Hubert (1989). Morphometric measurements were noted by following Wirth and Hubert (1989). The characters described by Campbell and Pelham-Clinton (1960), Kluiters *et al.* (2016) and Pagès and Monteys (2005) were applied to morphologically distinguish specimens of the *Obsoletus* group. Morphometric data of the gynanders were recorded in millimeters (mm). Slide-mounted specimens are presently retained with the Entomological collections of Diptera in the Department of Zoology, The University of Burdwan.

RESULTS AND DISCUSSION

Following *Culicoides* spp. were trapped in a backyard cattle shed, including *C. oxystoma*, *C. peregrinus* Kieffer, *C. fulvus* Sen and Das Gupta, *C. actoni* Smith, *C. imicola* Kieffer, *C. orientalis* Macfie, *C. homotomus* Kieffer, *C. palpifer* Das Gupta and Ghosh, *C. anophelis* Edwards and *C. shortti* Smith and Swaminath. *C. oxystoma*, *C. huffi* Causey, *C. arakawae* (Arakawa), and specimens of *Obsoletus* group were identified from the catch of the poultry farm. It was noted that *C.*

oxystoma was the most predominant species in cattle shed and poultry farm.

Four polar gynandromorph individuals of *C. oxystoma* were reported: one with male genitalia and female mouthparts (Fig. 1) and three other specimens with female genitalia and male mouthparts (Fig. 2). Morphometric data of these specimens showed that the head, associated accessory structures, and wings were typical of a female biting midge (Table 1). In contrast, the posterior part was characteristic of a male biting midge with a slender abdomen and conspicuous terminal male genitalia. The labrum-epipharynx bears well-developed terminal teeth; the lacinia was armed with minute teeth; the well-developed mandibles contain a series of minute, broad teeth, and the maxillary palps include a well-developed palpal sensory pit. The other three specimens had head and accessory parts of males and were devoid of basistyle, dististyle, aedeagus, and parameres. However, two of them had punctured and swollen female-like abdomen, although any formations of spermatheca were not found clearly.

Gynander of *Obsoletus* group exhibited polar sexual dimorphism, mixing male and female phenotypic characteristics in the head and genitalia (Table 2). The specimen contained flagellomeres, mouthparts like females (Fig. 3a) but enlarged pedicel-like a male (Fig. 3b), wings of females (Fig. 3c), and a swollen female-like abdomen (Fig. 3d) with the presence of basistyle, dististyle but devoid of aedeagus, and parameres (Fig. 3e). The sensilla coeloconica pattern in the antenna was 3, 13-15. Therefore, this individual did not demonstrate discrete male and female characters in the head and abdomen.

Occurrences of gynandromorphs are infrequent (Hall, 1988). Ratios of events of gynandromorphs within Simuliid range from 1 in 2,300 to 1 in 50,000 as given by Crosskey (1990). Several researchers proposed reasons behind the generation of gynanders. It was also recorded by Mori and Perondini (1980) that the origin of gynandromorphs in *Sciara ocellaris* (Diptera, Sciaridae) was due to the loss of a chromosome. This type of aberration forms in *Drosophila melanogaster* (Diptera,

Table 1. Morphometrics of polar gynandromorph specimens of *Culicoides oxystoma*

Specimens	Antennal ratio	Palpal ratio	Proboscis/ Head ratio	Hind tibial spines	Wing length (mm)	Costal ratio
Female head/ male abdomen (n=1)	1.01,1.09	2	0.65	4	0.96, 0.86	0.52, 0.54
Male head/female abdomen (n=3)	0.96-0.98	1.4-1.6	-	4	0.66	0.50

Drosophilidae) due to chromosomal elimination (Morgan and Bridges, 1919). The number of X chromosomes relative to the number of autosomes is the initial prime factor for sex determination. According to Narita *et al.* (2010), they were losing one of the X chromosomes during the first mitotic division, resulting in individuals with XX and XO cells possessing 50 per cent male and 50 per cent female tissues. This kind of gynander formation arises as a result of double fertilization, where a polar body is fertilized by one spermatozoon and the female pronucleus by another in the case of *Culex molestus* and *Aedes aegypti* (Diptera, Culicidae) (Gilchrist and Haldane, 1947; Rai and Craig, 1963). The gynandromorph individuals of *Culex molestus* and *Aedes cinereus* possess palpus of a male and female antennae on one side, and the other side harbours palpus of a female and antennae of different sex. It would result from somatic crossing-over, as proposed by Gilchrist and Haldane (1947).

Following general observations, gynandromorphic specimens of *Culicoides* have not shown any sign of parasitism (Curtis, 1962; Navai, 1969). *Mermis* Dujardin, a nematode belonging to the family Mermithidae was recorded in *Culicoides alatus* Das Gupta and Ghosh (synonymised as *C. oxystoma*) by Sen and Das Gupta (1958). Mermithidae or other parasites were not found during microscopic observation, which might cause such abnormalities. However, two of three individuals of *C. oxystoma* with male head and female abdomen had punctured abdomen or holes so the nematodes might have escaped through this. Also, external boundaries between female and male

body parts were obvious. The appearance of the punctured abdomen of this morph could not be ascertained as no mermithids were discernible.

Morphological identification of species belonging to the *Obsoletus* group is often difficult or impossible as the species are quite similar. For example, only males can be discriminated between species based on genitalia characteristics. For females, the measurement of the size of the spermathecae is not sufficient for precise identification (Pages and Monteys, 2005). During the present study, only four females of this group were collected of which one was gynandromorphic whose male genitalia was quite abnormal. But based on morphology it was noted that the female specimens along with gynandromorphic individual possess morphometric characters of this group. However, identification up to the species level leads to further extension of works by studying more specimens or applying DNA-based approaches.

Mechanisms causing the development or production of gynanders are of interest due to the implications for vector control strategies. However, there is no procedure for readily producing many gynanders (Hall, 1987). Gynandromorphs could be helpful in genetic, embryological, physiological, neurological, and parasite interaction studies (Hall, 1987, 1990; Sethuraman and O'Brochta, 2005). A gynander with the abdomen engorged with fully developed eggs was noticed by Craig and Crosby (2008). During this study, despite the gynanders possessing typical female mouthparts, there was no evidence of engorgement with blood. It is unknown whether it

Table 2. Morphometric measurements of polar gynandromorph specimen of *Obsoletus* group

Antennal ratio	Length of pedicel (mm)	Palpal ratio	Proboscis/ Head ratio	Hind tibial spines	Wing length (mm)	Costal ratio
1.03, 1.04	0.088	3, 4	0.90	5	1.16	0.60



Fig. 1 Anterior female and posterior male polar gynandromorph specimen of *Culicoides oxystoma*. a) whole specimen, b) head with antenna and c) male genitalia



Fig 2. Anterior male and posterior female polar gynandromorph specimen of *Culicoides oxystoma*. a) whole specimen and b) head with antenna

would have been possible for this individual to have taken a blood meal due to these modifications or not. However, there could be the possibility of alterations in the feeding habits, but this could not be established since the gynander died after trapping. The insect antennae are the principal sensory organs involved in foraging, mate detection,

and various environmental stimuli so that gynander individuals may possess divergent behavioural aspects of mating, breeding, feeding, or habitat selection due to morphological alteration in their antennae (Lane, 1984; Yuval, 2006; Muñoz-Muñoz *et al.*, 2016). As the number and localization of the different types of sensilla are also influences in their behaviour, this leads to the need for further extension of works to observe their alteration of biological and behavioural aspects. The occurrence of such abnormalities in *C. oxystoma* samples, especially during pre-monsoon (March-April), suggests environmental input on morphogenetic dynamics in this embryo of this species, although this requires further explorations. The probable reason behind such a mosaic gynander formation of the specimen belonging to *Obsoletus* group also cannot be stated.

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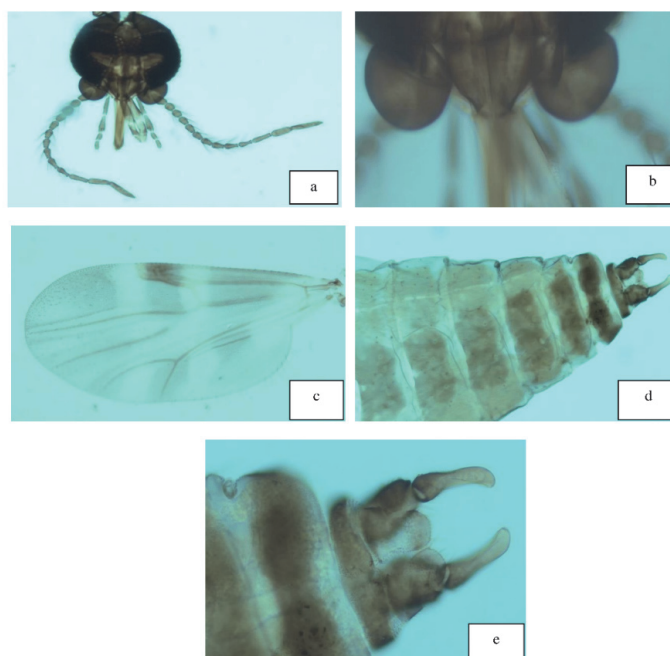


Fig. 3. Dissected parts of polar gynandromorph specimen of *Obsoletus* group. a) head, b) enlarged pedicel-like male, c) wing, d) tapered abdomen, e) male genitalia devoid of aedeagus and parameres

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Odonata diversity of mid-elevation catchments of the Bavali river from Coorg landscape of central Western Ghats, peninsular India

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ABSTRACT: The odonate diversity and distribution of the mid-elevation catchment area of Bavali River in the Coorg landscape of central Western Ghats is discussed. A total of 114 species of odonates including 32 Western Ghat endemic species were recorded from the study areas of Kanichar Gramapanchayath (KGP) which include 69 dragonflies (Anisoptera) and 45 damselflies (Zygoptera). Family Libellulidae dominated the odonate diversity with 39 species followed by Coenagrionidae (18 species) and Gomphidae (15 species). *Cyclogomphus flavoannulatus* Rangnekar *et al.*, 2019 reported in this study is the first report from Coorg landscape of central Western Ghats. The study area harbours 52.53 per cent of the Western Ghats (WG) and 60.64 per cent of the odonate diversity of Kerala. Additionally, it includes 36.78 per cent of WG and 42.67 per cent of endemic odonates of Kerala. About 28.07 per cent of the odonates recorded from the area are endemic to WG. Among the 114 species identified from the area, as per the IUCN Red List of Threatened species, there were 87 'least concern' species, 18 species were 'data deficient', four species belong to 'near threatened' category, three species belong to 'not assessed' category and two species belong to 'vulnerable' category. None of the species listed in the study area is protected under the Indian Wildlife (Protection) Amendment Act, 2022. This area lying outside any designated Protected Area harbours the largest number of odonate species in any region of Kerala known so far and shows high diversity and endemism comparable to that of other protected areas. © 2025 Association for Advancement of Entomology

KEY WORDS: Anisoptera, Zygoptera, Kerala, non-protected area, IUCN Red List, endemic

INTRODUCTION

Biodiversity conservation, in the last few decades, has focused on protecting different vulnerable hot spots but the protected areas (PAs) represent only a very small proportion of the ecosystem they intend

to protect and also majority of the research works are focused only on these PAs (Myers *et al.*, 2000; Oldekop *et al.*, 2016; Avigliano *et al.*, 2019). This strategy leaves the non-protected areas (NPAs) aside which are sometimes more likely to disappear (Western *et al.*, 2009; Avigliano *et al.*, 2019).

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Regarding taxa like Lepidoptera, fishes, birds and many other arthropods, strong reasons support that the study of biodiversity of NPAs is necessary and relevant (Avigliano *et al.*, 2019). Many species, even a considerable percentage of the population of threatened birds at the global scale, are found outside protected areas (Rodrigues *et al.*, 2004; Avigliano *et al.*, 2019).

Odonata in Kerala have been a well-studied group and has been continuing with discovery of many new species (Fraser 1933, 1934, 1936; Peters 1981; Rao and Lahiri 1982; Mathavan and Miller 1989; Radhakrishnan and Emiliyamma 2003; Emiliyamma 2005; Emiliyamma *et al.*, 2007; Emiliyamma 2014; Kiran and Raju 2013; Subramanian *et al.*, 2020; Emiliyamma *et al.*, 2020; Nair *et al.*, 2021; Nair *et al.*, 2022; Sadasivan *et al.*, 2022a; Sadasivan *et al.*, 2022b, Sadasivan *et al.*, 2022c, Vijayakumaran *et al.*, 2022, Payra *et al.*, 2023, Sadasivan *et al.*, 2023, Nair *et al.*, submitted to ENTOMON). As per recent studies, the odonate fauna of Kerala includes 189 species with 75 WG endemics (Nair *et al.*, submitted to ENTOMON). The Western Ghats which runs parallel to the west coast of India with nearly 1600km length, covers six states, Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, and Kerala and is divided into three, the southern, central and northern segments. Section south of Palakkad gap till the spur hills in Kanyakumari is Southern WG, central part is from Palakkad gap to Amboli and northern part ranges in Maharashtra and Gujarat north of the Amboli Ghat (Sadasivan and Sengupta, 2024). The Coorg landscape extends from Northern border of Wayanad plateau to Nethravathi valley.

Bavali River is a major drainage of the western slopes of the Coorg landscape of Central Western Ghats. The river is nearly 84 km long, flows from Wayanad district to eastern hilly regions of Kannur district reaching Kottiyoor and passes through Kelakam, Kanichar and Pala, and then Aralam River joins it and finally near Iritty, Veni River also joins with Bavali which ends in the Valapattanam River finally draining into the Arabian Sea. Kanichar Gramapanchayath (KGP) is situated in the hilly regions of the western slopes of Western Ghats

and is on the eastern side of Kannur District between 11.54° N and 75.47° E with 51.96km² areas. The elevation of the region varies from 100–750 m ASL. This region is one of the main catchment areas of the Bavali River. The nearest PAs in the locality are Kottiyoor Reserve Forest (12 km away) and Aralam Wildlife Sanctuary (7km away). The odonate diversity and distribution of the mid-elevation catchment area of Bavali River in the Coorg landscape of central Western Ghats is discussed.

MATERIALS AND METHODS

The diversity and distribution of odonates were studied in the catchment areas of the Bavali River in Kanichar Gramapanchayath (KGP) of the Coorg landscape. The study was conducted at three stations in the low to mid-elevation range namely–Attamcherry Paddyfields (100m ASL), Poolakkutty (350m ASL), and Elapeedikka (750m ASL). Attamcherry is at the foothills of the Coorg-Kannur Hills of WG, 2 km away from Kanichar Town and is a long stretch of paddy fields with streams running through the borders. Poolakkutty 6km away from Kanichar town is a small town on the slopes of Periya Ghat, with numerous montane streams flowing down from the hills. Elapeedikka is a small hill station located in the mid-elevations about 6 km westwards from Kanichar town, bordering the Periya Ghats and Kottiyoor RF with numerous montane streams.

Field surveys were conducted at the above three sites, Attamcherry, Poolakkutty and Elapeedikka from 2020–2023. At least two visits of each of the stations were conducted every month during the Dry-Winter (Dec–Feb), Dry Summer (March–May), South- West Monsoon (June–August) and North-East monsoon (September–November) during morning hours (09.00am – 01.00pm) (IST: UTC +5.30). A modified line transect method was followed by walking for 1 hr in a transect through the periphery of waterbody and every 100m was divided into 10m distance of 10 numbers and adult odonates were thoroughly sampled (Cezario *et al.*, 2021; Kulkarni and Subramanian, 2013). Odonates were photographed, some were caught using insect

nets, identified and released and difficult-to-identify individuals were retained as voucher specimens in the TORG collection. Identification of species was done using field guides (Subramanian, 2009; Kiran and Raju, 2013; Fraser, 1933, 1934, 1936). Nomenclature and classification follow Kalkman *et al.* (2020) and Nair *et al.* (2021). The IUCN Red List of threatened species was referred from the IUCN site <http://www.iucnredlist.org> (IUCN, 2024).

Abbreviations used: PA—Protected Area, NPA—Non-protected Area, WG—Western Ghats, WLS—Wildlife Sanctuary, ASL—Above Sea Level, TORG—TNHS Odonata Research Group, IUCN—International Union for Conservation of Nature.

RESULTS AND DISCUSSION

A total of 114 species of odonates including 32 Western Ghat endemics were recorded from the study areas which includes 69 dragonflies (Anisoptera) and 45 damselflies (Zygoptera). Family Libellulidae dominated the diversity with 39 species followed by Coenagrionidae (18 species), and Gomphidae (15 species). The study area harbours 52.53 of WG and 60.64 per cent of the odonate diversity of Kerala. Additionally, it includes 32 endemics out of 87 (36.78 %) of WG and 32 out of 75 (42.67%) endemic odonates of Kerala. About 28.07 per cent (32 out of 114) of the odonates recorded from the area endemic to WG. Among the 114 species identified from the region, as per the IUCN Red List of Threatened species, there are 87 least concern species, 18 species were data deficient, four species belonged to near threatened category, three species belong to not assessed category, and two species belonged to vulnerable category. Among the three stations studied, the highest diversity was recorded from Attamcherry (99 species) with 24 WG endemics followed by Elapeedika (66 species) with 14 WG endemics and Poolakkutty (49 species) with 10 WG endemic species (Table 1).

Dragonflies Suborder: Anisoptera

A total of 69 dragonflies (Anisoptera) belonging to six families were found in the study areas. A

total of five species under two genera were found in the study areas under Aeshnidae. *Anax immaculifrons* (Rambur, 1842) and *Gynacantha dravida* Leiftnick, 1960 are present in all three locations where *G. millardi* Fraser, 1920 in two locations and *A. ephippiger* (Burmeister, 1839) at Attamcherry and *A. guttatus* (Burmeister, 1839) at Elapeedika only. Corduliidae was represented by a single member *Hemicordulia asiatica* (Selys, 1878) and was present only at low elevation, paddy fields of Attamcherry.

Family Gomphidae was represented by 13 genera with 15 species. *Gomphidia kodaguensis* Fraser, 1923 and *Heliogomphus kalarensis* Fraser, 1934, WG endemics, were present in all three locations and are very common. *Heliogomphus promelas* (Selys, 1873) were comparatively not common but were found in lower and higher elevations only. *Burmagomphus chaukulensis* Joshi *et al.*, 2022, a recently discovered WG endemic gomphid, was very common in the area, at both low and high elevations (Attamcherry and Elapeedika). *Burmagomphus pyramidalis* Laidlaw, 1922 was very rare and found only once at low elevations (Attamcherry) during the study. A rare WG endemic, *Davidioides martini* Fraser, 1924 was recorded from low elevations. *Cyclogomphus flavoannulatus* Rangnekar *et al.*, 2019 has been recorded once from Attamcherry (wetland - paddy field), a rare record from North Kerala and an endemic from low elevations. It is the first record from the Coorg landscape. Previously it has been reported from Nilgiri-Silent Valley landscape, Lower Periyar, Cardamom Hills, Pandalam Hills and Agastyamalai landscapes (Nair *et al.*, 2021). *Ictinogomphus rapax* (Rambur, 1842) and *Paragomphus lineatus* (Selys, 1850) were common at low elevations. *Nychogomphus striatus* Fraser, 1924 was very rare and was found in low elevations. *Megalogomphus hannyngtoni* (Fraser, 1923) considered to be Near threatened as per IUCN Red List was found during South-West monsoon season at low elevations (Attamcherry). *Melligomphus acinaces* (Laidlaw, 1922), another WG endemic species, was common along mid and lower altitudes during monsoon seasons. *Macrogomphus wynaadicus* Fraser,

1924, *Merogomphus tamaracherriensis* Fraser, 1931 and *Microgomphus souteri* Fraser, 1924 were uncommon in low altitudes (Figs. 1 A, B,C, E,F, G).

Libellulidae was represented by 28 genera with 39 species. Interesting species under Libellulidae were *Epithemis mariae* (Laidlaw, 1915), a WG endemic (Fig. 1D), seen during monsoon and *Lyriothemis acigastra* (Selys, 1878). Macromiidae was represented by two genera (*Epophthalmia* and *Macromia*) with five species. *Macromia* includes *M. falcocolorata* Fraser, 1922, common during monsoon at low elevations, *M. ida* Fraser, 1924, *M. indica* Fraser, 1924 and *M. irata* Fraser, 1924, not uncommon at low elevations during monsoon. Genera Insertae sedis is represented by two genera (*Idionyx* and *Macromidia*) with four species and all were present in low elevation. *Idionyx corona* Fraser, 1921, *I. travancorensis* Fraser, 1931 and *I. saffronata* Fraser, 1924 (Fig. 1H) were not uncommon during monsoon and the first two are new records from the Coorg landscape.

Damselflies suborder: Zygoptera

Calopterygidae is represented by two genera with four species. *Vestalis submontana* Fraser, 1934 is found only at Elapeedika (750m ASL). Family Chlorocyphidae is represented by *Heliocypha bisignata* (Hagen in Selys, 1853) and *Libellago indica* (Fraser, 1928) which were common in all the localities surveyed. Coenagrionidae is represented by six genera with 18 species. Among Coenagrionidae notable species were *Aciagrion approximans krishna* Fraser, 1921, a WG endemic, commonly found at midlands and high elevations. Also, WG endemics like *Ceriagrion chromothorax* Joshi and Sawant, 2019 (Fig. 2B) and *Pseudagrion indicum* Fraser, 1924 (Fig. 2D) were common at low elevations during monsoon. Family Euphaeidae includes *Dysphaea ethela* Fraser, 1924, *Euphaea dispar* (Rambur, 1842) and *E. fraseri* (Laidlaw, 1920) (Fig. 2A, E) where *E. dispar* is present only at high altitudes (Elapeedika, 750m ASL). *Euphaea dispar* and *E. fraseri* are endemic to WG. Lestidae is represented by *Lestes dorothea* Fraser, 1924, *L. elatus* Hagen in Selys, 1862, and *L. praemorsus* Hagen in Selys,

1862 where *L. dorothea* was present in all three locations. Platynemididae includes eight genera with 10 species and the narrowly distributed WG endemic, *Elatoneura souteri* (Fraser, 1924) (Fig. 2F) was found at Attamcherry paddy fields. *Melanoneura bilineata* Fraser, 1922 and *Phylloneura westermanni* (Hagen in Selys, 1860) are near threatened species as per the IUCN Red List and are WG endemic species, recorded from high altitude, Elapeedika.

Platystictidae is represented by two genera and five species and all of them are endemic to WG. *Indosticta* had one species *I. deccanensis* Laidlaw, 1915 (Fig. 2C) which was recorded from Poolakkutty and Elapeedika, thus distributed in midlands and higher elevations. Elapeedika is the type locality of the recently discovered *P. francyi* Sadasivan, Vibhu, Nair & Palot, 2022 (Fig. 2G). The commonest species of the family *P. graveleyi* Laidlaw, 1915 was recorded from all three localities, while *P. mertonii* Fraser, 1924 (Fig. 2H) was commonly encountered in the monsoons from the high elevations. The distribution of *P. sanguinostigma* Fraser, 1922 was similar to that of *P. francyi* and is not uncommon in the monsoons.

From Kerala State, many studies are available on odonate diversity from Protected Areas. From Silent Valley and New Amarambalam Reserve, Rao and Lahiri (1982) recorded 23 species; Emiliyamma and Radhakrishnan (2000, 2014) reported 39 species from Parambikulam; Mathavan and Miller (1989) reported 36 species from Periyar Tiger Reserve; Gnanakumar *et al.* (2012) reported 55 species from Chimmony WLS; Adarsh *et al.* (2015) recorded 48 species from Chinnar; Varghese *et al.* (2014) reported 82 species from Thattekkad Bird sanctuary; Palot and Kiran (2016) listed 93 species from Aralam WLS, and Muneer *et al.* (2023) reported 85 species from Wayanad WLS. Sadasivan *et al.* (2022a) reported 116 species including 33 WG endemics from Shendurney WLS. Sadasivan *et al.* (2024) recently reported 111 species from Silent Valley National Park and its environs.

Regarding Non-protected areas in Kerala, certain

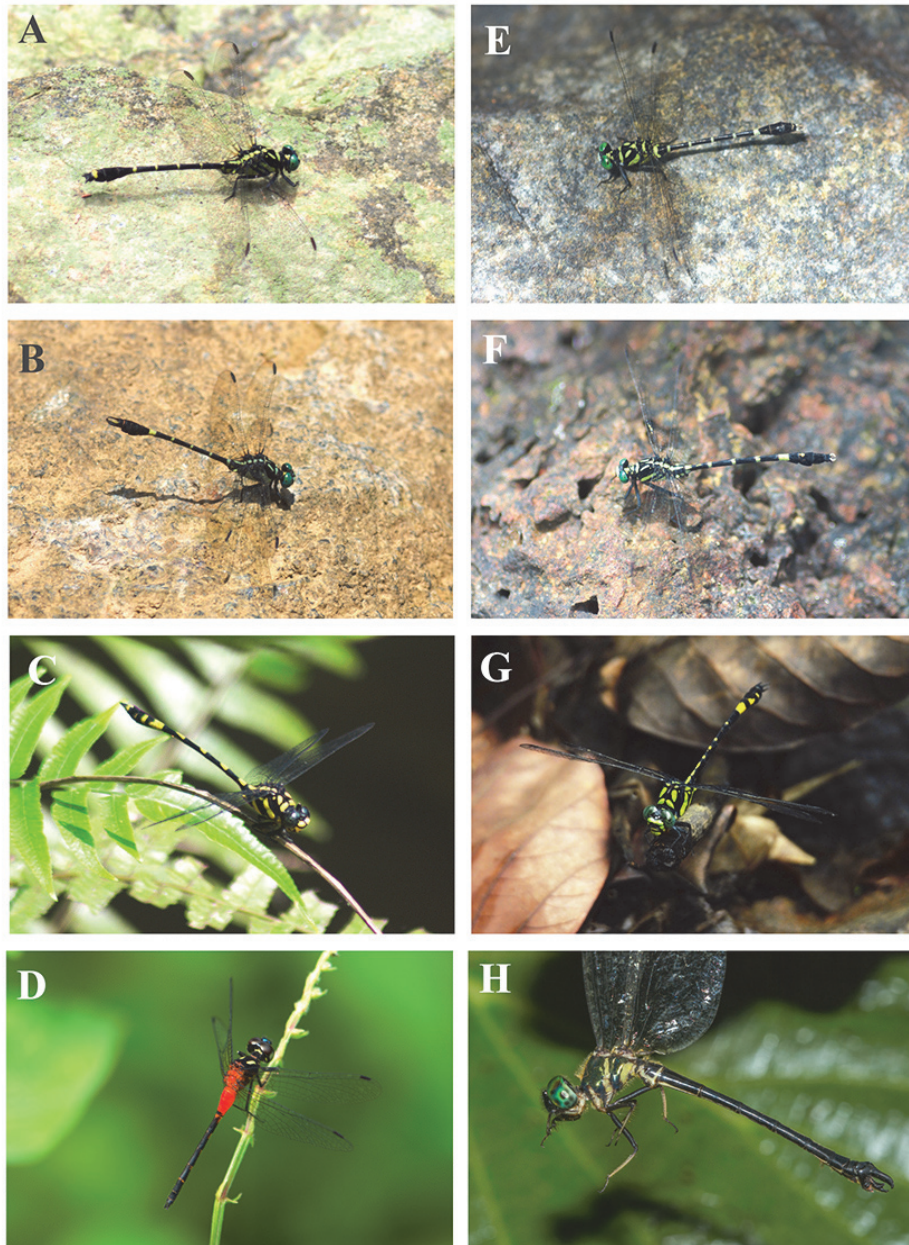


Fig. 1 A. *Burmagomphus chaukulensis* Joshi et al., 2022 © Vibhu V; B. *Melligomphus acinaces* (Laidlaw, 1922) © Vinayan P Nair; C. *Gomphidia kodaguensis* Fraser, 1923 © Vinayan P Nair; D. *Epithemis mariae* (Laidlaw, 1915) © Vinayan P Nair; E. *Microgomphus souteri* Fraser, 1924 © Vinayan P Nair; F. *Merogomphus tamaracherriensis* Fraser, 1931 © Vinayan P Nair; G. *Megalogomphus hanningtoni* (Fraser, 1923) © Vibhu V; H. *Idionyx saffronata* Fraser, 1924 © Kalesh Sadasivan

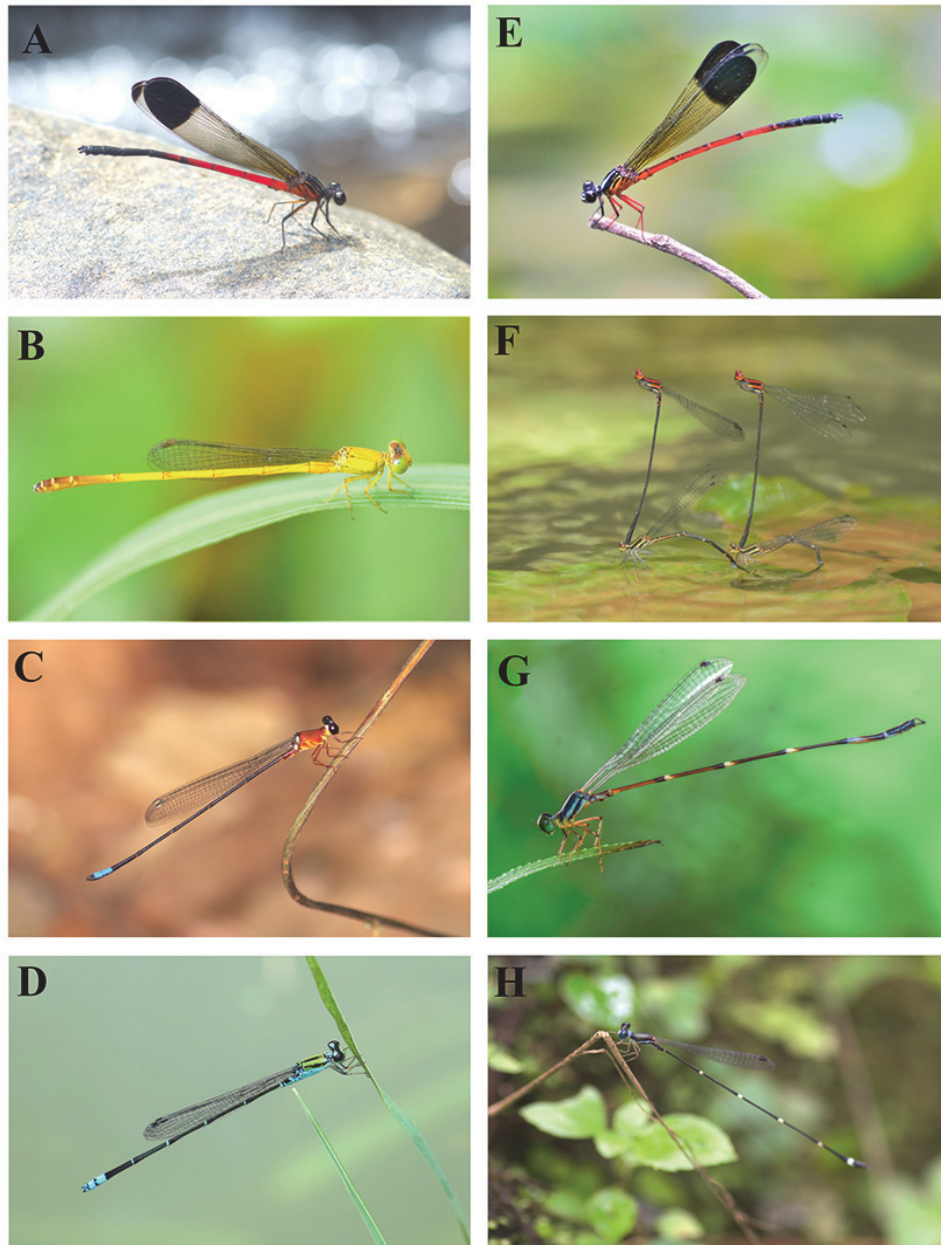


Fig. 2. A. *Euphaea dispar* (Rambur, 1842) © Vinayan P Nair; B. *Ceriagrion chromothorax* Joshi & Sawant, 2019 © Vinayan P Nair; C. *Indosticta deccanensis* Laidlaw, 1915 © Vinayan P Nair; D. *Pseudagrion indicum* Fraser, 1924 © Vinayan P Nair; E. *Euphaea fraseri* (Laidlaw, 1920) © Vinayan P Nair; F. *Elatoneura souteri* (Fraser, 1924) © Vibhu V; G. *Protosticta francyi* Sadasivan et al., 2022 © Vibhu; H. *Prorosticta mertonii* Fraser, 1924 © Vinayan P Nair

Table 1. Checklist of Odonates in locations of Kanichar grama panchayath

No.	Scientific name	IUCN	A [#]	B [#]	C [#]
Dragonflies suborder: Anisoptera					
Family: Aeshnidae					
1.	<i>Anax ephippiger</i> (Burmeister, 1839)	LC	√		
2.	<i>A. guttatus</i> (Burmeister, 1839)	LC			√
3.	<i>A. immaculifrons</i> (Rambur, 1842)	LC	√	√	√
4.	<i>Gynacantha dravida</i> Lieftinck, 1960	DD	√	√	√
5.	<i>G. millardi</i> Fraser, 1920	LC	√		√
Family: Corduliidae					
6.	<i>Hemicordulia asiatica</i> (Selys, 1878)	LC	√		
Family: Gomphidae					
7.	<i>Burmagomphus chaukulensis</i> Joshi, Ogale & Sawant, 2022*	NA	√		√
8.	<i>Burmagomphus pyramidalis</i> Laidlaw, 1922	LC	√		
9.	<i>Cyclogomphus flavoannulatus</i> Rangnekar <i>et al.</i> 2019*	DD	√		
10.	<i>Davidioides martini</i> Fraser, 1924*	DD	√		
11.	<i>Gomphidia kodaguensis</i> Fraser, 1923*	DD	√	√	√
12.	<i>Heliogomphus kalarensis</i> Fraser, 1934*	DD	√	√	√
13.	<i>H. promelas</i> (Selys, 1873)	NT	√		√
14.	<i>Ictinogomphus rapax</i> (Rambur, 1842)	LC	√		
15.	<i>Macrogomphus wynaadicus</i> Fraser, 1924*	DD	√		
16.	<i>Megalogomphus hanningtoni</i> (Fraser, 1923)	NT	√		
17.	<i>Merogomphus tamaracherriensis</i> Fraser, 1931*	NA	√		
18.	<i>Melligomphus acinaces</i> (Laidlaw, 1922)*	DD	√	√	
19.	<i>Microgomphus souteri</i> Fraser, 1924*	LC	√		
20.	<i>Nychogomphus striatus</i> Fraser, 1924	DD	√		
21.	<i>Paragomphus lineatus</i> (Selys, 1850)	LC	√		
Family: Libellulidae					
22.	<i>Acisoma panorpoides</i> Rambur, 1842	LC	√		√
23.	<i>Aethriamanta brevipennis</i> (Rambur, 1842)	LC		√	
24.	<i>Brachydiplax chalybea</i> Brauer, 1868	LC	√		

25.	<i>B. sobrina</i> (Rambur, 1842)	LC	√		
26.	<i>B. contaminata</i> (Fabricius, 1793)	LC	√		
27.	<i>Bradinopyga geminata</i> (Rambur, 1842)	LC	√		√
28.	<i>Cratilla lineata calverti</i> (Forster, 1903)	LC	√		√
29.	<i>Crocothemis servilia</i> (Drury, 1770)	LC	√		√
30.	<i>Diplacodes trivialis</i> (Rambur, 1842)	LC	√	√	√
31.	<i>Epithemis mariae</i> (Laidlaw, 1915)*	LC	√		
32.	<i>Hydrobasileus croceus</i> (Brauer, 1867)	LC	√		
33.	<i>Hylaeothemis apicalis</i> Fraser, 1924	DD	√	√	√
34.	<i>Lathrecista asiatica</i> (Fabricius, 1798)	LC	√	√	√
35.	<i>Lyriothemis acigastra</i> (Selys, 1878)	DD	√		
36.	<i>Neurothemis fulvia</i> (Drury, 1773)	LC	√	√	√
37.	<i>N. intermedia intermedia</i> (Rambur, 1842)	LC	√	√	√
38.	<i>N. tullia</i> (Drury, 1773)	LC	√	√	√
39.	<i>Onychothemis testacea ceylanica</i> Ris, 1912	LC	√	√	√
40.	<i>Orthetrum chrysis</i> (Selys, 1891)	LC	√	√	√
41.	<i>O. glaucum</i> (Brauer, 1865)	LC		√	√
42.	<i>O. luzonicum</i> (Brauer, 1868)	LC	√	√	√
43.	<i>O. pruinosum neglectum</i> (Rambur, 1842)	LC	√	√	√
44.	<i>O. sabina sabina</i> (Drury, 1770)	LC	√	√	√
45.	<i>Paplopleura sexmaculata</i> (Fabricius, 1787)	LC		√	√
46.	<i>Pantala flavescens</i> (Fabricius, 1798)	LC	√	√	√
47.	<i>Potamarcha congener</i> (Rambur, 1842)	LC	√		
48.	<i>Rhodothemis rufa</i> (Rambur, 1842)	LC	√		√
49.	<i>Rhyothemis triangularis</i> Kirby, 1889	LC	√	√	√
50.	<i>R. variegata variegata</i> (Linnaeus, 1763)	LC	√	√	√
51.	<i>Tetrathemis platyptera</i> Selys, 1878	LC	√	√	√
52.	<i>Tholymis tillarga</i> (Fabricius, 1798)	LC	√	√	√
53.	<i>Tramea basilaris</i> (Palisot de Beauvois, 1805)	LC	√		
54.	<i>T. limbata</i> (Desjardins, 1832)	LC	√	√	√
55.	<i>Trithemis aurora</i> (Burmeister, 1839)	LC	√	√	√

56.	<i>T. festiva</i> (Rambur, 1842)	LC	√	√	√
57.	<i>Trithemis pallidinervis</i> (Kirby, 1889)	LC	√		
58.	<i>Urothemis signata</i> (Rambur, 1842)	LC	√		
59.	<i>Zygonyx iris malabarica</i> Fraser, 1926	LC	√	√	√
60.	<i>Zyxomma petiolatum</i> Rambur, 1842	LC	√		
Family: Macromiidae					
61.	<i>Epophthalmia vittata vittata</i> Burmeister, 1839	LC	√		
62.	<i>Macromia flavocolorata</i> Fraser, 1922	LC	√		
63.	<i>M. ida</i> Fraser, 1924*	LC	√		
64.	<i>M. indica</i> Fraser, 1924*	DD	√		
65.	<i>M. irata</i> Fraser, 1924*	LC	√		
Genera insertae sedis					
66.	<i>Idionyx corona</i> Fraser, 1921*	DD	√		
67.	<i>I. saffronata</i> Fraser, 1924*	DD	√		
68.	<i>I. travancorensis</i> Fraser, 1931*	DD	√		
69.	<i>Macromidia donaldi donaldi</i> (Fraser, 1924)*	LC	√		
Damselflies Suborder: Zygoptera					
Family: Calopterygidae					
70.	<i>Neurobasis chinensis</i> (Linnaeus, 1758)	LC	√	√	√
71.	<i>Vestalis apicalis</i> Selys, 1873	LC	√	√	√
72.	<i>V. gracilis</i> (Rambur, 1842)	LC	√	√	√
73.	<i>V. submontana</i> Fraser, 1934	LC			√
Family: Chlorocyphidae					
74.	<i>Helioocypha bisignata</i> (Hagen in Selys, 1853)	LC	√	√	√
75.	<i>Libellago indica</i> (Fraser, 1928)	LC	√	√	√
Family: Coenagrionidae					
76.	<i>Aciaagrion approximans krishna</i> Fraser, 1921*	LC		√	√
77.	<i>Ac. occidentale</i> Laidlaw, 1919	LC	√		
78.	<i>Agriocnemis pieris</i> Laidlaw, 1919	LC	√	√	√
79.	<i>Ag. pygmaea</i> (Rambur, 1842)	LC	√	√	√
80.	<i>Ag. splendidissima</i> Laidlaw, 1919	LC		√	

81.	<i>Archibasis oscillans</i> (Selys, 1877)	LC	√		
82.	<i>Ceriagrion olivaceum aurantiacum</i> Fraser 1924	LC	√		
83.	<i>C. cerinorubellum</i> (Brauer, 1865)	LC	√		
84.	<i>C. chromothorax</i> Joshi & Sawant, 2019*	DD	√		
85.	<i>C. coromandelianum</i> (Fabricius, 1798)	LC	√	√	√
86.	<i>C. rubiae</i> Laidlaw, 1916	LC	√	√	√
87.	<i>Ischnura rubilio</i> Selys, 1876	LC	√		
88.	<i>Pseudagrion australasiae</i> Selys, 1876	LC	√		
89.	<i>P. decorum</i> (Rambur, 1842)	LC	√		
90.	<i>P. indicum</i> Fraser, 1924*	LC	√		
91.	<i>P. malabaricum</i> Fraser, 1924	LC	√		√
92.	<i>P. microcephalum</i> (Rambur, 1872)	LC	√		√
93.	<i>P. rubriceps</i> (Selys, 1876)	LC	√	√	√
Family: Euphaeidae					
94.	<i>Dysphaea ethela</i> Fraser, 1924	DD	√		
95.	<i>Euphaea dispar</i> (Rambur, 1842)*	LC			√
96.	<i>E. fraseri</i> (Laidlaw, 1920)*	LC	√	√	
Family: Lestidae					
97.	<i>Lestes dorothea</i> Fraser, 1924	LC	√	√	√
98.	<i>L. elatus</i> Hagen in Selys, 1862	LC	√		√
99.	<i>L. praemorsus decipiens</i> Kirby, 1893	LC	√		√
Family: Platycnemididae					
100.	<i>Caconeura ramburi</i> (Fraser, 1922)	DD			√
101.	<i>Ca. risi</i> (Fraser, 1931)*	DD	√	√	√
102.	<i>Copera marginipes</i> (Rambur, 1842)	LC	√	√	√
103.	<i>Co. vittata deccanensis</i> Laidlaw, 1917	LC	√	√	√
104.	<i>Elatoneura souteri</i> (Fraser, 1924)*	DD	√		
105.	<i>Esme longistyla</i> Fraser, 1931*	LC			√
106.	<i>Melanoneura bilineata</i> Fraser, 1922*	NT			√
107.	<i>Onychargia atrocyana</i> (Selys, 1865)	LC	√		√
108.	<i>Phylloneura westermanni</i> (Hagen in Selys, 1860)*	NT			√

109.	<i>Prodasineura verticalis annandalei</i> (Fraser, 1921)	LC	√		
Family: Platystictidae					
110.	<i>Indosticta deccanensis</i> Laidlaw, 1915*	VL		√	√
111.	<i>Protosticta francyi</i> Sadasivan, Vibhu, Nair & Palot, 2022*	NA		√	√
112.	<i>P. gravelyi</i> Laidlaw, 1915*	LC	√	√	√
113.	<i>P. mertonii</i> Fraser, 1924*	LC			√
114.	<i>P. sanguinostigma</i> Fraser, 1922*	VL		√	√
Total 114 species			99	49	66

Note: *—Endemic to Western Ghats, IUCN—Red List status, LC—Least Concern, NA—Not Assessed, DD—Data Deficient, VL—Vulnerable, NT—Near Threatened;

#Locations: A- Attamcherry (100m ASL), B- Poolakkutty (350m ASL) and C. Elapeedika (750m ASL)

studies are available. Raju (2007) reported 45 species from Kuttanad Wetlands. Adarsh *et al.* (2014) reported 52 species from Kerala Agricultural University Campus, Thrissur, while Nair (2014, 2017, per. rec.) reported 85 species from Varadoor, Kannur, South India. Further Rodrigues *et al.* (2022) reported 66 species from Kattampally wetland, Kannur. Later Chandran and Chandran (2021) reported 93 species from Aryanad Gramapanchayath with 24 WG endemics.

Considering the high rate of deforestation and the relatively low sampling effort, the NPAs could disappear before being studied, losing potential scientific, environmental and cultural richness. Due to their relatively small area and proximity to human settlements, the NPAs are also prone to be affected by several threats. Anthropogenic environmental pollution proves to be one of the most concerning human effects on the quality of the environment and biodiversity conservation (Avigliano *et al.*, 2009). As per Gray *et al.* (2006) rarefaction-based richness and endemism often do not differ significantly among unprotected and protected areas. The NPAs harbour a good number of species which are ecologically generalist and possess a good amount of ecological plasticity (Büchli and Vuilleumier, 2014; Gaynor *et al.*, 2018). The report of 114 species with 32 Western Ghat endemics from this study forms the highest Odonata diversity reported so far from any non-protected area in

Kerala. The high degree of endemism and presence of rare and narrow endemic species like *Davidioides martini* Fraser, 1924, *Elatoneura souteri* (Fraser, 1924), *Melanoneura bilineata* Fraser, 1922 etc., increase the ecological importance of the area. Moreover, the area is the type locality of *Protosticta francyi* Sadasivan, Vibhu, Nair & Palot, 2022, a recently described endemic damselfly.

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Pollination potential of stingless bee *Tetragonula iridipennis* Smith. (Hymenoptera, Apidae) in pumpkin (*Cucurbita moschata* Duchex Poir)

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ABSTRACT: An experiment was conducted to investigate the pollination potential and effectiveness of the stingless bee (*Tetragonula iridipennis* Smith.) on pumpkin. The abundance and diversity of foragers in male and female pumpkin flowers were assessed by measuring the number of foragers per flower per minute. The pumpkin fruit set and yield were evaluated under three different conditions: open pollination, bee pollination, and pollination exclusion. The foraging rate of bees was higher in male pumpkin flowers (0.22 bees/flower/min) than in female flowers (0.14 bees/flower/min) on the 45th day of flowering. Foraging time was longer on male flowers (40.66 seconds) than on female flowers (35.33 seconds). In terms of yield, the bee-pollinated pumpkin field achieved an average of 3.15 fruits per plant, compared to 3.12 fruits in open-pollinated plots. The bee-pollinated field also produced heavier fruits, with an average fruit weight of 3.45 kg (4.12% increase over open pollination). The total yield in the bee-pollinated plot was 36.1 t ha⁻¹, compared to 34.6 tons in the open-pollination plot. Colony monitoring showed a 1.93 per cent weight gain, from 897 to 924g. Foraging activity at the hive entrance was maximum during the morning hours (07 00–09 00), with on average 10.58 outgoing bees and 8.19 bees at peak nectar forager activity. In contrast, the pollination exclusion condition failed to produce any fruit, emphasizing the positive impact of *T. iridipennis* on pumpkin pollination and yield, particularly during peak flowering periods.

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KEY WORDS: Bee abundance, foraging activity, pollination efficiency, Open pollination, bee pollination, pollination exclusion

INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch. ex Poir.) is an economically and nutritionally significant vegetable cultivated worldwide. It is a rich source of essential nutrients, particularly vitamin A, minerals, and antioxidants, while its seeds contain high levels of protein (40.27%), crude fibre (34.59%), and ash content (44.5%) (Rana, 2014). Archaeological evidence suggests that pumpkins

originated in Mexico and Peru, later spreading to India through South American traders. In India, pumpkins are widely consumed as fresh vegetables, processed products, and livestock feed (Thamburaj and Narendrasingh, 2001). Pollination is crucial for the fruit set and seed development in cucurbits, which largely depend on insect pollinators for successful reproduction (Roubik, 1995). Among pollinators, honey bees (*Apis* spp.), stingless bees (*Meliponini*), and bumble bees (*Bombus* spp.) play

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a vital role in enhancing crop productivity (Free, 1993; Chauhan *et al.*, 2013). Honey bees contribute to approximately 80% of crop pollination, improving yield and quality across various horticultural crops. In tropical regions, stingless bees serve as key pollinators for native flora and cultivated crops (Roubik and Roubik, 1992). Studies highlight their efficiency in pollinating economically valuable crops such as coconut, cotton, mango, strawberries, tomatoes, and sweet peppers (Rindfleisch, 1980; Maeta *et al.*, 1992; Roubik and Roubik 1992; Kakutani *et al.*, 1993; Heard, 1999; Malagodi-Braga and Kleinert, 2004). These bees exhibit flower constancy, ensuring effective pollen transfer and their stingless nature makes them suitable for pollination in managed environments like greenhouses (Robinson and Decker-Walters, 1999; Slaa, 2006).

Despite the well-established role of pollinators in improving agricultural yields, studies specifically evaluating *Tetragonula iridipennis* in pumpkin pollination under open-field conditions remain limited. Most research has primarily focused on *Apis cerana* and *Apis dorsata*, leaving a knowledge gap regarding the effectiveness of stingless bees in pumpkin cultivation. Furthermore, comparative studies on open pollination, bee pollination, and pollinator exclusion in pumpkin fields are scarce. A recent study revealed that *Apis cerana* exhibited the highest foraging rate and pollination efficiency, leading to significant increases in fruit set (77.5%), fruit weight (1.71 kg), and seed count (162), whereas open-pollinated plots had a higher percentage of crooked fruits (Longkumer *et al.*, 2024). This study aims to assess foraging behaviour, and their impact on fruit set and yield under natural field conditions; and to assess the effectiveness of different pollination methods for enhancing pumpkin productivity.

MATERIALS AND METHODS

Evaluation of colony growth parameters: In the pumpkin field at Pollachi, two stingless bees were introduced at the beginning of the flowering stage. Colony growth was evaluated by calculating the initial and final weight of the shifted colony at 15-day intervals. This measurement provides insights

into the changes in the colony's weight and potential growth over time.

Foraging activity at the hive entrance: The foraging activity of *T. iridipennis* at the hive entrance was assessed by counting the number of returning foragers carrying nectar and pollen, as well as the number of outgoing bees, during specific time intervals throughout the day. The observations were conducted for a period of 5 minutes each at the following time slots: 8:00am to 10:00am; 12:00pm to 1:00pm and 4:00pm to 6:00pm. During these time intervals, the number of returning foragers carrying nectar and pollen was recorded. Additionally, the number of outgoing bees from the hive was also counted.

Field experiments: Two field experiments were conducted to evaluate the pollination potential of managed bee pollinators in enhancing the pumpkin yield. It was carried out in a farmer's field in Pollachi (10.757°N; 76.967°E) ensuring that the pumpkin field was located 2 km away from an open-pollination field to avoid interference. The experimental design followed a randomized block structure with three treatments and seven replications for each treatment, with each plot measuring 4m × 10m. The experiments aimed to assess the impact of pollination by managed bee pollinators (stingless bee colonies) compared to pollinator exclusion and open pollination on the yield of pumpkins.

Pollinator exclusion: In this treatment, female flower buds were covered with sleeve net cages before blooming to prevent pollinators from accessing the flowers. Two flowers from five selected plants per replication (70 flowers in total) were covered with cages. After one week of flowering, the cages were removed, and the flowers were tagged for further assessment of fruit set under the caged condition. The crop was grown in an open field without protected conditions, and this treatment ensured that no pollinators, including *T. iridipennis*, contributed to the fruit set, allowing a controlled evaluation of pollination dependency.

Bee pollination: This treatment involved the introduction of managed stingless bee colonies into

the pumpkin fields. Two stingless bee colonies were placed in the field during February 2023. The colonies were introduced when the crop was at (approximately 10%) flowering stage.

Open pollination: This treatment served as a control where the natural process of open pollination by various pollinators was allowed. Observations were recorded on the different pollinators that visited the flowers, and various yield parameters were also recorded.

Number of fruits per plant, weight of each fruit and total yield were recorded in different pollination treatments. The harvested yield in different treatments was scaled up based on the area covered by the plants. ANOVA was used to analyse the data, and AGRES Software are used to carry out all additional calculations.

RESULTS AND DISCUSSION

Abundance of managed bee pollinator *T. iridipennis* in pumpkin flowers:

The study revealed that *T. iridipennis* exhibited higher activity in pumpkin male flowers compared to female flowers. The average number of bees visiting male flowers was recorded at 0.13 bees per flower per minute, while it was slightly lower for female flowers (0.09). Peak bee activity was observed on the 45th day of the flowering period, with an average of 0.22 bees in male flowers and in female flowers it was 0.14 bees per flower per minute. The lowest bee activity was recorded on

the 15th day, with 0.07 bees in male flowers and in female flowers It was 0.04 bees per flower per minute (Table 1)

Regarding foraging behaviour, *T. iridipennis* spent more time collecting rewards from male flowers, with an average of 32.06, compared to 28.92 seconds per female flower. The longest foraging time was recorded on the 45th day, where bees spent 40.66 seconds on male flowers and 35.33 seconds on female flowers (Table 2).

Colony growth parameter of *T. iridipennis*:

Growth of the two *T. iridipennis* colonies introduced into the pumpkin field was monitored, and the colonies showed a slight increase in weight during the study period. The initial weight of the colonies before placement in the field was 897g, which increased to an average of 908g on 15th day (0.78% increase in weight), 915g on 30th day (1.28% increase) and on 45th day 924g with a weight gain of 1.93 per cent, indicating that the colonies experienced growth during their time in the field.

Foraging activity of *T. iridipennis* at the hive entrance:

The foraging activity of *T. iridipennis* was monitored at the hive entrance at various intervals throughout the day. On average, the number of outgoing bees was higher, with a mean of 10.58 bees, and the peak outgoing activity occurred between 07:00 and 09:00 hours. Incoming nectar foragers were more abundant, averaging 6.68 bees,

Table 1. Abundance of *T. iridipennis* in pumpkin flowers. Location: Pollachi, Coimbatore district on 2023

No. of foragers/flower/min $\pm$ S. D						
	15 th day	30 th day	45 th day	60 th day	75 th day	Mean
Male flower	0.07 (0.75)	0.12 (0.78)	0.22 (0.84)	0.16 (0.81)	0.11 (0.78)	0.13(0.79) ^a
Female flower	0.04 (0.73)	0.09 (0.76)	0.14 (0.80)	0.11 (0.78)	0.08(0.75)	0.09(0.76) ^b
Mean*	0.055 (0.74) ^c	0.10 (0.77) ^B	0.18 (0.82) ^A	0.13(0.79) ^A	0.09 (0.76) ^B	
C.D @ 0.05						
Treatment A ^s						0.01
Treatment B ^s						0.01
Treatment A*B ^s						0.03

Note: *Mean of 10 plant observation; figures in parentheses are square root ($X+0.5$) transformed values. In a column, the means followed by a common lower-case alphabet and in a row, the means followed by a common upper-case alphabet are significantly different at $p=0.05$ by LSD.

Table 2. Foraging activity of *T. iridipennis* in pumpkin flowers. Location: Pollachi, Coimbatore district on 2023

Time spent by a forager/flower/min in seconds± S. D						
	15 th day	30 th day	45 th day	60 th day	75 th day	Mean
Male flower	26.33 (5.1)	30.33 (5.5)	40.66 (6.4)	34.66 (5.9)	28.33 (5.3)	32.06 (5.6) ^a
Female flower	24.33 (4.9)	28.66 (5.4)	35.33 (5.9)	30.66 (5.5)	25.66 (5.0)	28.92 (5.4) ^b
Mean*	25.33 (5.1) ^E	29.49 (5.4) ^C	37.99 (6.2) ^A	32.66 (5.7) ^B	26.99 (5.2) ^D	

Note: *Mean of 10 plant observations; figures in parentheses are square root ($X+0.5$) transformed values. In a column, the means followed by a common lower-case alphabet and in a row, the means followed by a common upper-case alphabet are significantly different at $p=0.05$ by LSD

compared to pollen foragers, who averaged 5.43 bees. The highest incoming nectar forager activity was also recorded between 07 00 and 09 00 hours, with an average of 8.19 foragers, followed by 15 00 to 17 00 hours, which saw an average of 7.74 nectar foragers (Table 5). These findings suggest that *T. iridipennis* exhibits higher foraging activity in the morning, particularly for nectar collection, with a second peak in the late afternoon.

Effect of *T. iridipennis* on pollination and yield of pumpkin:

In the field, where two colonies of stingless bees were introduced, the fruit set was recorded at an average of 3.15 fruits per plant, slightly higher than the 3.12 fruits per plant in the open pollination plot. Moreover, the bee-pollinated plot produced heavier fruits, averaging 3.45kg per fruit, compared to 3.37kg fruit in the open pollination plot, representing a 4.12 per cent increase in fruit weight. The yield in the bee-pollinated plot was 36.1 t ha⁻¹, compared to 34.6 t ha⁻¹ in the open pollination plot (Table 3).

No fruit set was observed in the pollinator exclusion treatment, where flowers were sleeve-caged to prevent pollination, confirming the importance of bee pollination in ensuring fruit set and yield. The effect of pollination modes on pumpkin yield was determined through statistical analysis, not just observed data. The results demonstrate that *T. iridipennis* positively influenced both the quantity and quality of pumpkin production compared to open pollination.

Introducing *T. iridipennis* as a managed pollinator in pumpkin fields led to modest improvements in pumpkin yield and colony growth. The fluctuation

in bee activity throughout the flowering period can be attributed to variations in flower availability, as noted in earlier studies (Yogapriya *et al.*, 2019). Similar patterns of abundance were reported by Subhakar *et al.* (2011), where *T. iridipennis* was the most abundant flower visitor in bitter gourd fields. These findings confirm the significance of *T. iridipennis* as an active pollinator, particularly during peak flowering periods.

The effect of *T. iridipennis* on pumpkin pollination and yield was positive but less pronounced compared to other crops. Managed bee pollination resulted in a slight increase in the number of fruits per plant, fruit weight, and overall yield when compared to open-pollinated plots. Specifically, the fruit weight increased (4.12%), and the yield was higher in bee-pollinated plots, indicating that *T. iridipennis* contributed to enhanced productivity. However, studies on other crops such as watermelon and ash gourd have shown greater benefits. Layek *et al.* (2021) reported a 14 per cent increase in watermelon fruit set, with significant improvements in fruit length and yield after the introduction of *T. iridipennis*. Chauhan and Singh, (2021) noted a 27.96 per cent increase in fruit set in ash gourd with *T. iridipennis* pollination. While the present study shows that *T. iridipennis* benefits pumpkin crops, its impact may be more limited than in other cucurbits due to differences in flower structure and pollination requirements. The large, deep, campanulate structure of pumpkin flowers and their high pollen demands limit the foraging efficiency of *T. iridipennis*, as their smaller body size and short proboscis reduce effective pollen transfer, resulting in lower pollination efficiency compared to larger pollinators. The slight increase

Table 3. Effect of different modes of pollination on pumpkin yield

Modes of pollination	No. of female flowers observed	No. of picking /plant	No. of fruits / plants	Fruit weight (kg)	Percent increase in fruit weight	Yield of 10 plants (kg)	Yield (t/ha)	Percent increase in yield (t/ha)
T1 Pollinator exclusion	70	0 (0.71) ^b	0(0.71) ^b	0(0.71) ^b	-	0	0	-
T2 Bee pollination	70	2.63(1.76) ^a	3.15(1.91) ^a	3.45(1.98) ^b	2.31	98.5	36.1	4.12
T3 Open pollinated	70	2.6(1.76) ^a	3.12(1.90) ^a	3.37(1.96) ^b	-	94.9	34.61	-

Note: *Mean of five observations, No. fruit set was observed in pollinator exclusion. Figures in parentheses are the square roots of $(X+0.5)$ transformed values. In columns, means followed by alphabet are highly significant different at 5% level LSD

in colony weight of *T. iridipennis* from 897 to 924 g while foraging on pumpkin flowers suggests that these flowers provide some resources, but they may not be as rich or suitable as those found in other crops, such as mangoes. Walters and Taylor (2006) reported that honey bee pollination significantly enhances fruit size and yield in cucurbits, with honey bees being crucial for maximizing fruit set and seed development. In contrast, studies have shown that different floral resources can lead to varying foraging efficiencies among bee species. For instance, the presence of honey bee hives in pumpkin fields increased their visitation rates, but this did not translate to a proportional increase in native bee visits, indicating a complex interaction between floral resource availability and bee foraging behaviour (Artz *et al.*, 2011). Therefore, while pumpkin flowers do support *T. iridipennis*, the more substantial weight gain observed in mango orchards may reflect a higher quality or quantity of available resources (Saffari *et al.*, 2010).

Finally, foraging activity at the hive entrance revealed a peak in outgoing, nectar, and pollen foragers during the morning hours between 07 00

and 09 00 am, which coincides with pumpkin's peak flowering and anthesis period. This synchronization of foraging activity with floral resources maximizes pollination efficiency, as noted in earlier research (Lintu, 2020). The timing of foraging behaviour aligns with the availability of floral resources, ensuring that *T. iridipennis* can effectively pollinate crops during peak periods. Studies on cucumber crops have shown similar patterns of peak foraging activity, which may vary slightly based on crop type and flowering behaviour. This synchronization plays a critical role in enhancing reproductive success and ensuring efficient resource utilization by stingless bees.

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Table 4. Incoming and outgoing of *T. iridipennis* at hive entrance during peak flowering period

Foraging time	Incoming nectar forager	Incoming pollen forager	Outing bees	Mean
07:00- 09:00 h	8.19 (2.86) ^a	7.16 (2.67) ^a	14.42 (3.79) ^a	9.92
11:00- 13:00 h	4.13 (2.03) ^b	3.58 (1.89) ^b	7.42 (2.72) ^c	5.04
15:00-17:00 h	7.74 (2.78) ^a	5.55 (2.35) ^a	9.91 (3.14) ^b	7.73
Mean*	6.68	5.43	10.58	
C.D. (P=0.05)	0.629	0.687	0.595	

Note: * Mean of five observations, figures in parentheses are square root $(X+0.5)$ transformed values. In column means followed by different alphabets are highly significantly different at 5% level LSD

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Effective sanitation practices mitigate coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera, Curculionidae) infestation in robusta coffee in Indonesia

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ABSTRACT: The coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867), is a major pest responsible for substantial economic losses in global coffee production. This study investigated the impact of field management, particularly sanitation practices, on controlling *H. hampei* infestations in robusta coffee (*Coffea canephora*). Field observations were conducted in both maintained and non-maintained plantations to assess pest populations at different fruit development stages. Results showed that well-maintained fields had significantly lower pest populations across all developmental stages compared to non-maintained fields. Statistical analysis confirmed the effectiveness of sanitation measures in disrupting the pest's life cycle. Mature cherries were found to be highly susceptible to infestation, emphasizing the importance of timely field management. Additionally, healthy coffee plants in managed fields showed greater resilience to pest attacks. The research underscores the vital role of consistent field management in mitigating pest damage and enhancing the sustainability of coffee production systems.

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KEY WORDS: *Coffea canephora*, pest populations, field management, pest's life cycle, sustainability

INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera, Curculionidae), is a devastating pest causing significant economic losses in global coffee production (Johnson *et al.*, 2020; Pereira *et al.*, 2021; Abewoy, 2022; Fotso *et al.*, 2023; Myrie *et al.*, 2023). In Indonesia, a major coffee-producing nation, *H. hampei* inflicts severe damage at all stages of berry development, leading

to reduced yields and quality (Avelino *et al.*, 2015; Hayata, 2016; Aristizábal *et al.*, 2017). Global economic losses due to *H. hampei* are estimated at USD 500 billion annually (Infante, 2018). Indonesia experienced a 30 per cent decline in coffee production in 2013, partly attributed to *H. hampei* infestations (Rosiana *et al.*, 2017). Regions like Modayag suffered from high pest damage due to poor plant maintenance (Langkai *et al.*, 2023). The pest also inflicts damage on stored beans,

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further degrading quality (Novianti *et al.*, 2023; Aristizábal *et al.*, 2017). High infestation levels have been reported in Sulawesi, Sumatra, and the Kerinci Regency of Jambi Province, where up to 64.32 per cent of coffee plants were affected (Arifin *et al.*, 2022; Nadiawati *et al.*, 2023; Langkai *et al.*, 2023).

Environmental factors, such as temperature, altitude, and humidity, significantly influence *H. hampei* populations. The pest thrives at altitudes between 400 and 1,000m, where optimal conditions accelerate its life cycle (Moreira *et al.*, 2020). Warmer temperatures at lower altitudes and cooler temperatures at higher altitudes impact infestation rate (Constantino *et al.*, 2021; Myrie *et al.*, 2023; Hendrival *et al.*, 2024).

Cultural practices, including pruning, sanitation, and removing infested cherries, are essential for managing *H. hampei* (Constantino *et al.*, 2021; Kidanu, 2020). However, smallholder farmers often face challenges in implementing these practices due to limited resources and knowledge (Aristizábal *et al.*, 2023). Integrated pest management (IPM) strategies, which emphasize sanitation, have proven effective, often integrating biological agents like *Beauveria bassiana* (Aristizábal *et al.*, 2017; Rasika, 2021).

Tamiai Village in Kerinci Regency, with its suitable climate for coffee cultivation, also faces *H. hampei* challenges. Limited pest management practices and minimal fertilizer use contribute to high infestation rates (Novianti *et al.*, 2023; BP3K Batang Merangin, 2023).

Field surveys in Tamiai have revealed high levels of *H. hampei* infestation, leading to significant losses for farmers (Sri *et al.*, 2021). This reflects broader trends in Indonesian coffee regions, where *H. hampei* remains a major threat. This research aims to understand *H. hampei* dynamics under varying environmental conditions and develop effective strategies to mitigate infestation risks.

MATERIALS AND METHODS

This research was conducted in a robusta coffee plantation in Desa Tamiai, Kecamatan Batang

Merangin, Kabupaten Kerinci, Jambi Province, Indonesia. Two distinct plots were studied: a non-maintained plot (2°09'57"S; 101°38'15"E) and a maintained plot (2°10'06.0"S; 101°38'31.3"E). The plantation, approximately 300 hectares in size, consists of 5- to 10-year-old coffee plants intercropped with cinnamon trees, spaced 1.5 meters apart. The research was conducted over a three-month period, from May to July 2024.

Two farmer plots were selected based on their maintenance conditions. Within each plot, coffee plants were further categorized based on weed conditions and topography into non-maintained and maintained groups. Non-maintained plots received neither organic nor inorganic fertilizers and were not subjected to weeding. In contrast, maintained plots were regularly fertilized with NPK fertilizers and underwent weed control using herbicides.

The primary variables observed in this study were the level of attack and population density of *H. hampei*. The level of attack was expressed as the percentage of coffee berries infested by *H. hampei*. This included the attack levels on young, unripe, and ripe fruits. Additionally, the population density of *H. hampei* at various developmental stages (egg, larva, pupa, and adult) was assessed within infested berries at different stages of ripeness (young, unripe, and ripe).

A total of 16 sample plants were selected from both plots to represent various field conditions, with eight plants from each plot. Four productive branches from each sample plant, oriented to the four cardinal directions, were destructively sampled for each observation. The study involved three observation periods over three weeks, with different sample branches selected for each observation to ensure data accuracy. At each observation, all fruits from the four sampled branches were harvested, placed in plastic bags, and stored in a cooler box for subsequent analysis. Each fruit was individually examined for a maximum of 48 hours to record the number of young, unripe, and ripe fruits; the number of young, unripe, and ripe fruits infested by *H. hampei*; and the number of *H. hampei* eggs, larvae, and pupae on young, unripe, and ripe fruits.

The level of *H. hampei* attack was calculated by counting all coffee berries on four branches of each sample plant and then determining the number of infested berries based on observed symptoms. The percentage attack rate was calculated using the following formula:

$$\text{Percentage of attack (A)} = \frac{\text{Number of Infested Berries (a)}}{\text{Total Number of Berries Observed (b)}} \times 100$$

The attack percentages were categorized based on the scale (source-Directorate General of Plantations, 2010): scale 1 (d" 25% as – Light; 2 (25-50% as – Currently; 3 (51-90% as – Heavy) and 4 (e" 90% as Very Heavy).

The population densities of *H. hampei* eggs, larvae, pupae, and adults were calculated for every 100 fruits within three predetermined phenological stages (young, unripe, and ripe). These densities were expressed as the number of *H. hampei* individuals per 100 coffee berries. The data on attack levels and *H. hampei* populations were analyzed statistically using a t-test to compare the means between the two study plots. Descriptive analysis was employed to further interpret the results. Supporting environmental data, such as temperature and humidity, were used to contextualize the findings.

RESULTS AND DISCUSSION

Attack Level: Field observations revealed significant differences in *H. hampei* attack rates between maintained and non-maintained coffee fields across various fruit development stages (young, unripe, and ripe). Maintained fields, which received regular management practices, exhibited significantly lower infestation. The average attack rates were 9.51 ± 4.82 , 14.23 ± 6.17 and 11.35 ± 3.13 per cent, for young, unripe and ripe fruits, respectively, categorizing the overall infestation level as "Light." In contrast, non-maintained fields showed higher infestation. The average infestation was 25.55 ± 8.14 , 34.91 ± 15.35 and 38.07 ± 19.36 per cent for young, unripe, and ripe fruits, respectively, categorizing the overall infestation level as "Currently." There were significant differences between maintained and non-maintained coffee

fields across various fruit development stages (*significantly different according to the paired t-test* ($\alpha = 0.05$), emphasizing the effectiveness of regular cultural practices, such as pruning and sanitation, in mitigating *H. hampei* impacts and promoting healthier coffee crops.

Population density: Field observations revealed significant differences in the population density of eggs, larvae, pupae and adults between maintained and non-maintained fields (Table 1, Fig. 1). The data revealed a significant reduction in *H. hampei* populations across all life stages in maintained fields compared to non-maintained fields. Regular management practices, such as pruning and sanitation, implemented in maintained fields effectively suppressed the development and proliferation of *H. hampei* at all stages of the coffee berry borer's life cycle.

A significant difference in egg counts was observed between the two field conditions. Non-maintained fields exhibited higher average egg populations (29.07 ± 7.176 per 100 fruits) compared to maintained fields (10.47 ± 2.54 per 100 fruits). This trend persisted across all fruit development stages: young fruits (22.84 ± 10.66 vs. 9.71 ± 3.68), unripe fruits (30.92 ± 10.661 vs. 11.73 ± 6.73), and ripe fruits (33.45 ± 8.00 vs. 9.96 ± 3.74). These findings demonstrate that effective field maintenance practices can significantly reduce *H. hampei* egg populations and subsequent infestations.

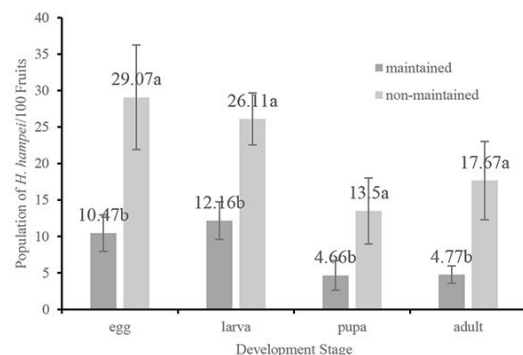


Fig. 1 Comparison of *H. hampei* population dynamics in maintained and non-maintained field conditions. Numbers followed by different letters in the same *H. hampei* development stage are significantly different (paired t-test, $\alpha = 0.05$).

Table 3. Population density at different developmental stages in Maintained and Non-Maintained fields

Field conditions	Young fruit	Unripe fruit	Ripe fruit	Average
Eggs $\pm$ SD/ 100 Fruits				
Maintained	9.71 $\pm$ 3.68b	11.73 $\pm$ 6.73b	9.96 $\pm$ 3.74b	10.47 $\pm$ 2.54b
Non-maintained	22.84 $\pm$ 10.66a	30.92 $\pm$ 10.661a	33.45 $\pm$ 8.00a	29.07 $\pm$ 7.176a
Larvae $\pm$ SD/ 100 Fruits				
Maintained	9.38 $\pm$ 4.24b	16.53 $\pm$ 4.17b	10.56 $\pm$ 2.77b	12.16 $\pm$ 2.58b
Non-maintained	13.57 $\pm$ 6.30a	34.28 $\pm$ 7.45a	30.50 $\pm$ 3.69a	26.11 $\pm$ 3.57a
Pupae $\pm$ SD/ 100 Fruits				
Maintained	2.56 $\pm$ 1.30b	7.39 $\pm$ 5.59b	4.03 $\pm$ 1.82b	4.66 $\pm$ 2.02b
Non-maintained	7.89 $\pm$ 2.00a	15.16 $\pm$ 9.23a	17.01 $\pm$ 4.53a	13.50 $\pm$ 4.52a
Adults $\pm$ SD/ 100 Fruits				
Maintained	4.67 $\pm$ 1.84b	3.68 $\pm$ 1.60b	5.95 $\pm$ 1.90b	4.77 $\pm$ 1.20b
Non-maintained	16.33 $\pm$ 9.58a	14.93 $\pm$ 8.03a	21.75 $\pm$ 3.92a	17.67 $\pm$ 5.37a
Total population				
Maintained	26.35 $\pm$ 8.71b	39.35 $\pm$ 14.76b	30.51 $\pm$ 6.30b	32.07 $\pm$ 7.34b
Non-maintained	60.64 $\pm$ 17.63a	95.75 $\pm$ 22.45a	102.72 $\pm$ 14.33a	86.37 $\pm$ 15.61a

Note: Numbers followed by different letters in the same row are significantly different ($\alpha = 0.05$)

Similar to egg populations, larval populations of *H. hampei* also exhibited significant differences across all fruit stages between maintained and non-maintained fields. The average larval population in non-maintained fields was 26.11 ± 3.57 per 100 fruits, compared to 12.16 ± 2.58 in maintained fields. The highest larval densities were observed in unripe fruits, with 34.28 ± 7.45 larvae per 100 fruits in non-maintained fields and 16.53 ± 4.17 in maintained fields. Even in ripe fruits, non-maintained fields had significantly higher larval counts (30.50 ± 3.69) compared to maintained fields (10.56 ± 2.77). These findings further highlight the importance of maintenance practices in controlling larval populations, which are crucial for limiting subsequent adult emergence and infestation.

A similar trend was observed for pupal populations of *H. hampei*. Non-maintained fields exhibited significantly higher pupal densities compared to maintained fields. The average pupal population in non-maintained fields was 13.50 ± 4.52 per 100 fruits, while in maintained fields, it was significantly lower at 4.66 ± 2.02 . The highest pupal densities were found in ripe fruits (17.01 ± 4.53 in non-

maintained vs. 4.03 ± 1.82 in maintained fields), followed by unripe fruits (15.16 ± 9.23 vs. 7.39 ± 5.59) and young fruits (7.89 ± 2.00 vs. 2.56 ± 1.30). These findings suggest that maintenance practices disrupt the pupal developmental stage, thereby limiting adult emergence and subsequent infestation.

Adult *H. hampei* populations also exhibited significant differences between maintained and non-maintained fields. Non-maintained fields had a higher average adult population of 17.67 ± 5.37 individuals per 100 fruits compared to 4.77 ± 1.20 in maintained fields. The highest adult densities were observed in ripe fruits, with 21.75 ± 3.92 adults per 100 fruits in non-maintained fields and 5.95 ± 1.90 in maintained fields. Unripe and young fruits in non-maintained fields also harbored higher adult populations (14.93 ± 8.03 and 16.33 ± 9.58 , respectively) compared to maintained fields (3.68 ± 1.60 and 4.67 ± 1.84 , respectively). These findings underscore the effectiveness of maintenance practices in limiting adult populations and, consequently, reducing the overall infestation potential of *H. hampei*.

Total population density in maintained fields exhibited significantly lower total *H. hampei* population across all fruit development stages (32.07 ± 7.34 individuals per 100 fruits). While non-maintained fields showed higher total *H. hampei* population (86.37 ± 15.61 individuals per 100 fruits). The population increased from 60.64 ± 17.63 in young fruits to 102.72 ± 14.33 in ripe fruits (Table 1).

This study emphasizes the pivotal role of sanitation practices in controlling *Hypothenemus hampei* infestations in Robusta coffee (*Coffea canephora*). In fields where infested plant debris was consistently removed, pest populations across all life stages were significantly reduced. By disrupting the pest's life cycle, sanitation measures limited the breeding and survival of *H. hampei*, demonstrating their effectiveness as a core pest management strategy.

The findings align with Dufour *et al.* (2021), who reported that mature coffee cherries, due to their higher nutrient content and softer texture, are more vulnerable to *H. hampei*. Similarly, Aristizábal *et al.* (2016, 2017, 2023), Kidanu (2020) and Constantino *et al.* (2021) have underscored sanitation as a cornerstone of Integrated Pest Management (IPM) in coffee production, emphasizing its role in targeting critical stages of the pest's life cycle to mitigate its impact on yield and quality. Regular field maintenance not only curtails pest populations but also promotes overall plant health. Healthier coffee plants are better equipped to synthesize natural defense compounds, which reduce their attractiveness to *H. hampei* (Rasika, 2021).

This study highlights the critical role of effective field management, with a particular emphasis on sanitation practices, in mitigating coffee berry borer (*H. hampei*) infestations in Robusta coffee (*C. canephora*) plantations. The findings underscore the importance of disrupting the pest's life cycle to reduce its population at various developmental stages. Moreover, maintaining plant health through proper cultural practices not only enhances the vigor of coffee plants but also bolsters their natural defenses, rendering them less vulnerable to pest attacks. Future studies should focus on integrating

sanitation practices with biological control agents, such as parasitoids and predators, as well as advanced technological interventions like pheromone traps and remote sensing for early pest detection.

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A new species of *Euphaea* Selys, 1840 (Odonata, Euphaeidae) from Wayanad, Western Ghats, Kerala, India

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ABSTRACT: A new species of *Euphaea* Selys, 1840 (Odonata, Euphaeidae) is described from the Wayanad landscape of Western Ghats of Kerala State based on integrated taxonomy. *Euphaea wayanadensis* **sp. nov.** is differentiated morphologically from the closely similar *E. pseudodispar* Sadasivan & Bhakare, 2021, based on the longer black hind wing patch; broader humeral and antehumeral stripes of males, which are never interrupted and not significantly tapering towards the alar sinus; humeral stripes not terminating in a distinct spot; S10 distal margin wavy at mid-ventrum and apex of the dorsal keel on S10 stepped in lateral view; and the difference in length ratios of male aedeagus and structure of the male genital vesicle. Molecular analysis of the mitochondrial cytochrome oxidase subunit I (mt COI) region showed 12.9 per cent nucleotide divergence between the two species. Phylogenetic analysis with Maximum Likelihood method using MEGA 11 also revealed the distinctiveness of *E. wayanadensis* **sp. nov.** The habitat, distribution and ecology of the new species are discussed, and a key to *Euphaea* species of Western Ghats is also provided. © 2025 Association for Advancement of Entomology

KEY WORDS: Integrated taxonomy, mtCO1, molecular analysis, phylogeny, key to species, distribution, Coorg landscape

INTRODUCTION

The genus *Euphaea* Selys, 1840 (Odonata, Euphaeidae), in Western Ghats of Peninsular India

is characterized by males with hind wing apices broadly opaque black with metallic iridescence, rounded and shorter than the forewing; discoidal cell traversed; distinct keel on 10th abdominal

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segment, and the anal appendages with forcipate cerci and tiny paraprocts (Fraser, 1934). The genus ranges from Sri Lanka, Peninsular and Northeast India, Thailand, Malaysia, Borneo to Indonesia. Currently, five species of *Euphaea* are known from Western Ghats of Peninsular India, namely *E. cardinalis* (Fraser, 1924), *E. dispar* (Rambur, 1842), *E. fraseri* (Laidlaw, 1920), *E. pseudodispar* Sadasivan & Bhakare, 2021 and *E. thosegharensis* Sadasivan & Bhakare, 2021 (Subramanian and Babu., 2017; Bhakare *et al.*, 2021; Nair *et al.*, 2021). The genus *Euphaea* can be a good model for studying the allopatric speciation mediated by the biogeographical gaps of the Western Ghats. Of the three species of *Euphaea* occurring in the Southern and Central Western Ghats, *E. fraseri* has a wide distribution throughout the Western Ghats from Kanyakumari to Maharashtra at 100–1200m (Subramanian *et al.*, 2018; Fraser, 1934), whereas, *E. cardinalis* is restricted to the mountains south of Palghat Gap (Southern Western Ghats), and *E. dispar* is restricted to the north of the Palghat Gap, mainly in the mountains between the Palghat Gap and Coorg (Central WG) (Fraser, 1934). During our fieldwork we observed a morphologically distinct population of *Euphaea* from the Wayanad–Coorg landscape of Western Ghats, which is described here as new to science based on morphological and molecular differences from all other congeners.

MATERIALS AND METHODS

The authors (SSA & CS) came across the distinct morphotype at Kalindi River, Thirunelli, Wayanad district Kerala, during a survey in 2013, followed by observations along the same stretch till 2019. Later field surveys by KS & VPN from 2019 to 2023 revealed similar morphotypes from Thirunelli in Wayanad, Aaralam in Kannur, and the western slopes of Coorg in Karnataka. The specimens of new morphotypes were photographed in the field, collected using an insect net, and preserved in absolute ethanol. Morphometric studies were done using a digital vernier caliper (Mitutoyo CD-6" CS). The field photographs were captured using Canon 600D fitted with Canon EF 100 mm f/2.8L USM Macro lens. Specimen photographs captured in the laboratory were done using a Nikon Z 50 mirrorless camera fitted with a Nikkor Z DX 16-15 f/3.5-6.3

VR lens. Microphotography of specimens were carried out using a camera-mounted Leica M80 stereo zoom microscope and processed using Leica Application Suit V 4.8 Software. Taxonomic description follows Fraser (1934) and Bhakare *et al.* (2021). The terminology for morphological description follows Garrison *et al.* (2006). The distribution map was prepared using QGIS3 software and edited in Adobe Photoshop CS5.

DNA extraction and PCR amplification:

Genomic DNA was isolated from the muscle tissues of the thorax using a DNeasy animal tissue kit following the manufacturer's protocol (Qiagen, Valencia, CA) and was visualized using 0.8% agarose gel. The universal barcode primer described by Folmer *et al.* (1994) (LCO-50-GGT CAA CAA ATC ATAAAG ATA TTG G-30; HCO-50-TAA ACT TCA GGG TGA CCA AAA AAT CA-30) specific to mitochondrial cytochrome oxidase I (COI) was used.

The polymerase chain reaction (PCR) steps were carried out using sterile PCR tubes. The primers were diluted to 10µM using DNAase and RNAase-free molecular water. The PCR master mix used was the Emerald Amp GT PCR Master mix (TAKARA). The master mix included an optimised buffer, PCR enzyme, dNTP mixture, gel loading dye (emerald green), and a density reagent in a 2x premix format Gradient PCR was carried out for standardising the annealing temperature. The best annealing temperature for this reaction was recorded as 51°C. For PCR amplification of mitochondrial DNA, Cytochrome oxidase subunit I locus, a reaction mixture of 40µL was prepared. The composition of the reaction mixture was as follows: 20 µL PCR master mix, 2 pl template DNA, 1µL forward, 1 µL reverse primer, and 16 µl molecular water.

Thermal conditions for PCR amplification were as follows; A. initial denaturation: 94°C for 4 minutes, B. denaturation: 94°C for 30 seconds, C. annealing: 51°C for 1 minute, D. extension: 72°C for 2 minutes, steps B-D were repeated for 30 cycles, E. Final extension: 72°C for 10 minutes. The PCR product obtained was stored at 4°C. For electrophoresis, 1.2 per cent agarose gel was prepared with 100ml

of 1x TAE buffer, and 3 µL of Ethidium bromide was added. The prepared gel was cast in a horizontal gel casting tray. After solidifying, the comb was removed, and the tray was placed in the electrophoresis chamber and filled with 1x TAE buffer. The PCR product (6 µL) was directly loaded into the wells since the dye was already present in the PCR master mix. Three µL of 1kb DNA ladder (Invitrogen TrackIt) was also loaded in a separate well. 1x TAE buffer was used as the electrophoresis buffer, and the run was carried out at 70 V cm. The electrophorised gels were visualised, and images were documented with UV light using the Bio-Rad gel documentation system.

DNA sequencing: Sanger sequencing of PCR product was done by sending PCR product to Eurofins India Pvt. Ltd., Bangalore. For each sample, bidirectional sequencing was done.

Bioinformatic analysis: The forward and reverse sequences obtained were trimmed and aligned using Bioedit software. Multiple sequence alignment was done using MUSCLE algorithm in MEGA 11 software. For phylogenetic classification of the identified species by DNA barcoding, sequences belonging to the two *Euphaea* species which were generated in this study and the relevant mtCOI-5P sequences available in the NCBI database were retrieved and analysed by constructing a phylogenetic tree in MEGA11 software (Tamura *et al.*, 2021). The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. All positions containing gaps and missing data were eliminated. The ML Heuristic method applied was the Nearest-Neighbour-Interchange method (Robinson, 1971). A total of 1000 bootstrap replications (Felsenstein, 1985) were followed in the construction of the phylogeny tree. Nucleotide sequence similarity count matrix, was prepared to confirm the species distinctiveness using Ape package in R studio software and a heat map showing percentage similarity was plotted using ggplot2 package.

The following abbreviations/notations are used in the description — Fw: Forewing, Hw: Hind wing, TL: Total length including appendages, AL: abdominal length, FL: forewing length, HL:

hindwing length, BHL: length of black apices of the hindwing, Ax: antenodal veins, Px: postnodal veins, Pt: pterothorax, S1-S10: corresponding abdominal segments, SSA: S S Anooj, CS: C Susanth, KS: Kalesh Sadasivan, MB: Milind Bhakare, VPN: Vinayan P Nair.

RESULTS

Euphaea wayanadensis Anooj, Susanth, & Sadasivan sp. nov. (Figs. 1, 2, 3, 4)

LSIDurn:lsid:zoobank.org:act:3CD9CAF7-EFAD-4A2E-B7F9-CF6CE312E301

Holotype: Male, Thirunelli, Wayanad district, Kerala, India, (910 m ASL), private land on banks of River Kalindi, Thirunelli, coll. S.S. Anooj on 28.ix.2019, wet specimen in ethanol, to be deposited in ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru.

Paratypes: Four males and three females (including one allotype in ethanol), the same information as the holotype, TORG 1035 male and TORG 1036 male, Aaralam, Kannur, Kerala, wet specimens in ethanol, coll. Kalesh Sadasivan on 19.ix.2022; TORG 1037 male and TORG 1038 male, Thirunelli, Wayanad, coll. Vinayan P.Nair on 18.ix.2022, TORG 1035 and TORG 1037 Paratype specimens to be deposited in ZSI, Calicut, Kerala.

Description of the holotype – Male (Fig. 2): Measurements (in mm): TL (including appendages) 52.2, AL 42.0, FL 36.6, HL 34.1 and BHL 10.4.

Head: Labium pale brown medially and darker laterally; labrum pale bluish with a median black ‘tongue’ shaped mark; mandibles concolourous with labrum with an upper transverse black streak; anteclypeus shiny dark amber-brown to black with an upper azure blue pruinose stripe visible in live specimens; postclypeus matt black; antefrons and postfrons black; genae pale bluish-white; eyes black superiorly, medially and laterally dark amber to brownish-black, inferolateral area brown; antennae shiny black; vertex, occiput, occipital bar, post-ocular area, all matt black (Figs. 2B, E).

Prothorax: Ground colour matt black; anterior lobe completely black; middle lobe black with triangular

yellowish spots laterally; notopleural suture black; propleuron matt black with a pair of indistinct reddish spots; posterior lobe black without any ornamentations or spines (Figs. 2 B, D, F).

Pterothorax: Ground colour orange-red with yellow dorsally, orange hue inferiorly; triangular mesothoracic acrotergite black, dorsal carina black; mesepisternum matt black; humeral and antehumeral stripes broad, never interrupted, does not taper conspicuously towards alar sinus; mesostigmal plate matt black. Orange posterior lateral and black antero-superior border region of mesinfraepisternum distinctly separated diagonally; mesepimeron superiorly yellow and orange inferiorly, centrally black; a reddish-black patch of interpleural suture extends to about middle of mesepimeron; dorsal third of second lateral suture marked black; metepisternum, metepimeron yellowish-orange; metinfraepisternum brownish-orange; metathoracic spiracle pale yellowish-white (Figs. 2 B, D, F).

Legs: Fore-coxae anteriorly brown, posteriorly grey; trochanter of similarly coloured as former; mid and hind coxae orangish to brownish red; femur, tibia, tarsus red with cyan hue; superior 1/3rd of extensor surface of forelegs black; knees and claws black (Figs. 2A, B, D).

Wings: Fw hyaline. Nodal formula: left Fw Ax-22 & Px-35, right Fw Ax 22 & Px 34, left Hw Ax 20 & Px 35, right Hw Ax 21 & Px 35; Pt black, 10 cells wide in Fw, 11 cells wide in Hw. Hw hyaline, distal 1/3 black with a metallic coppery-lilac shine when viewed in some angles (Figs. 2A, C); anterior margin of this black patch convex, extends to 11 cells proximal to Pt (Fig. 2M); cubital space with three cross veins in all wings.

Abdomen: Proximal segments reddish-orange, distal segments black; S6, dorsally red, black ventrally; distal 1/4th black (Figs. 2A, C); segmental joints black; proximal margin of segments lustrous black; S1 yellowish with posterior part having orange tint; S2 orange anteriorly, reddish-orange distally; pseudoauricles on S2 not flanged, with four teeth interspaced with few erect hairs (Fig. 2H); hamule distal end hooked, blunt, black at distal tip

(Fig. 2I); S3, S4 reddish orange with indistinct orange lateral patch; S5 reddish orange with distal 1/5th brownish-black which merges with ground colour; proximal part of S6 orange red which gradually transits to black at its distal 1/3rd; S7–10 fully black; broad dorsal carina, distal dorsal keel on S10; ventrally base of S8 sternite and base of lateral border of S8 tergum with tuft of black hairs; ventral side of S9 with tuft of long hairs on proximal part of its lateral tergite, small tuft on distal ventral border of S9 tergite, and a small tuft of hairs on the S9 sternite proximal to its ventral beak before gonopore on either side (Figs. 2L, N, O); S9 sternite ventral beak bordered on either side with a prominent node; gonopore margin oval in ventral view; gonocoxae with blunt apices; S10 distal margin wavy at mid-ventrum, apex of dorsal keel on S10 stepped on lateral view (Fig. 2L); medial part of distal margin of S9 extending ventrally, its tip extends over gonopore as a short tooth (Fig. 2L); length of S9 1.5 times that of S10. In lateral view, S10 shows comparatively lesser curvature i.e., comparatively broader on ventrolateral area.

Genitalia: S2 with lustrous brownish black, rhomboidal genital vesicle with distal end angulated, transverse surface rugosities absent, vesicle longer than broad and its distal margin not oval (Figs. 2G, J); genital ligula illustrated; penis with a single seta on either side; length of penile structure after setae comparatively more in dorsal view (Fig. 2K).

Anal appendages: Cerci forcipate, black; paraprocts black, in ventral view with tips medially directed and hence converge at rest. Paraprocts with tip hooked, directed dorsally. Paraproct longer than gonocoxae, of similar size as that of dorsal prominence of S10 (Fig. 2L, N, O).

Description of Female (Fig. 3): Measurements (in mm): TL (including appendages) 45.9, AL 35.6, FL 35.2 and HL 33.2.

Head: labium black, labrum pale bluish-white with a median black 'tongue' shaped mark; mandibles pale bluish-white having an upper transverse black streak similar to male; anteclypeus, postclypeus, antefrons, postfrons, vertex and occiput matt black; genae pale bluish-white; antennae black; eyes as



Fig 1. *Euphaea wayanadensis* **sp.nov** adult male in field © Vinayan P Nair



Fig. 2 *Euphaea wayanadensis* **sp. nov.** A, B: field photo, C: holotype, D: thorax lateral view, E: facial view, F: thorax dorsal view, G: Secondary genitalia and vesicles lateral view, H: Pseudoauricles lateral view showing teeth, I: hamule lateral view, J: secondary genitalia and vesicles ventral view, K: penile structure dorsal view, L: terminal abdominal segments and appendages lateral view, M: hind wing portion showing the extension of black patch with respect to Pt, N: terminal abdominal segments and appendages ventral view, O: terminal abdominal segments and appendages dorsal view © S.S. Anooj and C. Susanth

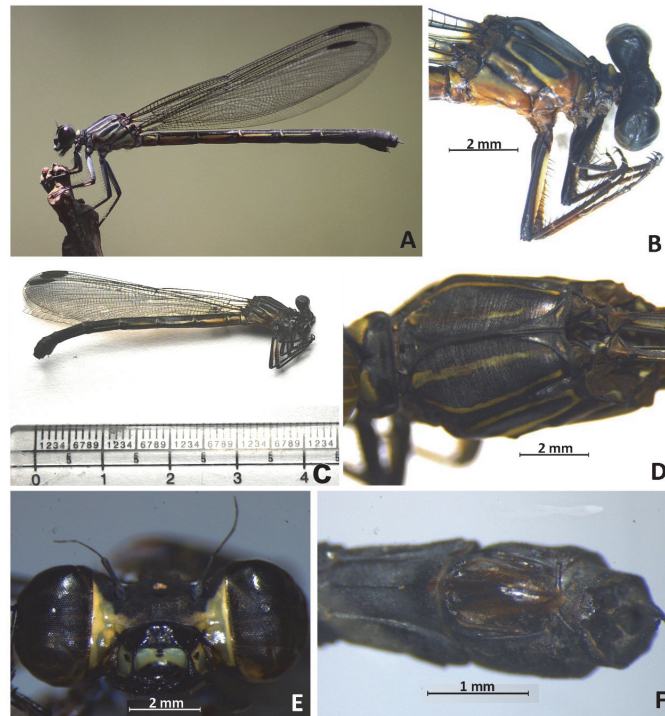


Fig. 3 *Euphaea wayanadensis* **sp. nov.** female, A: field photo, B: thorax lateral view, C: allotype, D: thorax dorsal view, E: facial view, F: terminal abdominal segment dorsal view showing vulvar scale
© S.S. Anooj and C. Susanth



Fig. 4 *Euphaea wayanadensis* **sp. nov.** paratypes, A: paratype males, B: paratype females, C-D: habitat
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in males (Fig. 3E).

Prothorax: as in males; spots on second lobe pale yellowish-white (Fig. 3B, D).

Pterothorax: Pale greenish-white replaces the red colour in males; humeral and antehumeral stripes pale yellowish blue, latter not interrupted; reddish markings of mesepisternum, mesepimeron and metepisternum in males replaced with pale bluish-yellow; mesepisternum and metepisternum with central black area (Figs. 3B, D).

Legs: Coxae posterolaterally pale dirty white and pruinose; trochanters grey; femur, tibia, tarsus, claws black; basal 1/4th of flexor region of forelegs, whole of mid and hind tibia creamy white (Figs. 3A, B).

Wings: Hyaline. Left Fw Ax-24 & Px-31; right Fw Ax21 & Px28; left Hw Ax 7 & Px27; right Hw Ax 18 & Px28. Pt black, 10 cells wide in Fw and Hw (Figs. 3A, C).

Abdomen: All segments black with a dorsal yellow carina that disappears at S6; laterally inferior half of segments with yellow transverse streaks narrowing distally and disappearing on S7; remaining areas, including whole of S8 to S10, black (Fig. 3A, C); vulvar scales longer compared to that

of *E. pseudodispar* and without central sulcus, postero-lateral angle forms blunt tooth (Fig. 3F); appendages and ovipositor black.

Variation: *Males:* In the paratype males (n= 4); TL, AL, FL, HL and BHL (in mm) ranged from 50.3–52.2, 39.2–42.0, 35.5–36.6, 32.1–34.1 and 9.9–10.7 respectively; humeral stripes appeared to be more or less stable (Table 1).

Females: The paratype females (n= 2); TL, AL, FL, HL and BHL (in mm) ranged from 45.1–45.3, 34.6–35.1, 34.2–35.5 and 32.2–33.1 respectively (Table 1).

Etymology: The species name '*wayanadensis*' is derived from the name of the district Wayanad, in which the type locality Thirunelli is located.

Distribution: As far as known, the species is distributed in the central WG from River Kalindi, Thirunelli, Wayanad district, in Kerala, India extending further north in Aaralam WLS, and foothills on western slopes of central Western Ghats south of Someshwara (Fig. 7).

Habitat and Ecology: Fast-flowing streams with rocks/boulders and aquatic vegetation with luxuriant evergreen and Semi-evergreen tree growth on the

Table 1. Morphometrics of *Euphaea wayanadensis* sp. nov.

No	Specimen	Total length (mm)	Abdominal length (mm)	Forewing length (mm)	Hindwing length (mm)	Length of black hindwing patch (mm)	Ratio of the hind wing length to the length of black hindwing patch
1	Holotype ♂	52.2	42.0	36.6	34.1	10.4	3.3
2	Paratype ♂	52.6	41.5	35.7	33.7	9.9	3.4
3	Paratype ♂	52.9	42.6	35.5	33.5	10.1	3.3
4	Paratype ♂	51.0	41.2	36.6	33.4	10.7	3.1
5	Paratype ♂	50.3	39.2	36.0	32.1	9.9	3.2
6	Paratype ♀	45.9	35.6	35.2	33.2	—	—
7	Paratype ♀	45.3	34.6	34.2	32.2	—	—
8	Paratype ♀	45.1	35.1	35.5	33.1	—	—

*Black hind wing patch absent in females

banks, were the most preferred by the species (Fig. 4C–D). The shrubs and rocks on the riverine habitats were preferred by the adults for perching. Males prefer to perch on branches at a height of 1–2m while females prefer higher perches. The emergent rocks and boulders are preferred by males for display. The elevational distribution is from 100–1000m ASL. The species is observed throughout the year, except during the dry seasons of March–April. Monsoon and post-monsoon season from May to October is the flight season based on the field observations.

Differential diagnosis

The species is close to *E. pseudodispar*; however, can be differentiated by the black patch on hind wing apices in males which covers up to 1/3 of the hind wing, whereas in *E. pseudodispar* it is only 1/5th the length of the hind wing; also, the anterior margin of this black hind wing patch extends to 11 cells proximal to the Pt in *E. wayanadensis* **sp. nov.** whereas in *E. pseudodispar* it extends to only 4 cells proximal to the Pt. In males, unlike in *E. pseudodispar*, the humeral and antehumeral stripes are broader, never interrupted and not significantly taper towards the alar sinus. Unlike its congener, the humeral stripes are not terminating in a distinct spot. Also, S9 of *E. wayanadensis* **sp. nov.** in lateral view shows lesser curvature; broader on the lower area in comparison with *E. pseudodispar*. S10 distal margin wavy at mid-ventrum and apex of the dorsal keel on S10 stepped in lateral view. When compared to *E. pseudodispar*, the length of penile structure of *E. wayanadensis* **sp. nov.** after the setae was more, in dorsal view. Male genital vesicle longer than that of *E. pseudodispar* and its distal margin not oval in *E. wayanadensis* **sp. nov.** The *E. wayanadensis* **sp. nov.** is comparatively larger. The TL, AL, FL, & HL of the males are more in *E. wayanadensis* **sp. nov.** i.e., average (n=5), TL, AL, FL, HL of males is 51.8, 41.3, 36.1 and 33.4. In male *E. wayanadensis* **sp. nov.**, there is a pale azure blue pruinose stripe on anteclypeus in fresh specimens. In the case of females, the vulvar scale is longer when compared to *E. pseudodispar* and the TL, AL, FL and HL of the female is more

compared to *E. pseudodispar* i.e., average (n=3) TL, AL, FL and HL of female is 45.4, 35.1, 35.0 and 32.8.

In the field, the new taxon can be easily confused with the rarely co-occurring *E. dispar*; however, can be differentiated by the extensor surface of the fore-femora which is black and red, hind and mid femora red as in *E. fraseri*, however, with no yellow patch on legs as in *E. dispar* (in males). The most commonly co-occurring species *E. fraseri* males can be very easily differentiated in the field by the pale sky blue humeral and antehumeral stripes (yellowish-orange in *E. wayanadensis* **sp. nov.**) and the azure blue labrum (pale blue with a median black knob like marking in *E. wayanadensis* **sp. nov.**). *Euphaea wayanadensis* **sp. nov.** can be easily differentiated from *E. cardinalis* males, seen south of Palghat Gap, which also has an extended black hind wing patch (more extended than *E. wayanadensis* **sp. nov.**) by its pale blue labrum (reddish-ochreous in *E. cardinalis*) and shorter S9 in the former.

Revised key to males of the genus *Euphaea* Selys, 1840 of Western Ghats, Peninsular India

1. Ventral hair tufts present on male terminal abdominal segments; antehumeral stripes on thorax not azure blue; lateral aspect of middle lobe of prothorax with orange spots.....2
 - Ventral hair tufts absent on male terminal abdominal segments; antehumeral stripes bright azure blue and lateral aspect of the middle lobe of prothorax with an azure blue or pale blue spot (male genital vesicle red, longitudinally elongated, apple-shaped. TL 45.7± 2.10 mm).....*E. fraseri* (Laidlaw, 1920)
2. Labrum pale turquoise blue; antehumeral stripe yellowish.....3
 - Labrum orange-ochre to vermilion red; antehumeral stripe reddish-orange.....4
3. Extensor surface of all femora black, flexor surface of proximal 2/3rd, all femora with bright yellow patch; Fw tipped blackish-brown, apical fourth of Hw black; genae black. Male genital

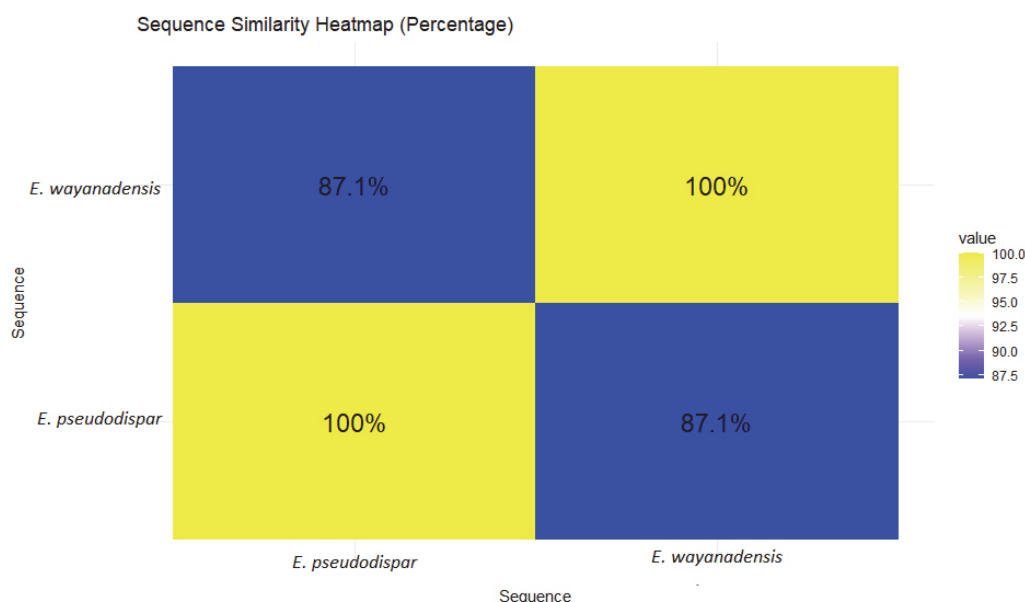


Fig. 5 Nucleotide sequence similarity heat map (percentage) for *Euphaea wayanadensis* sp. nov. and *E. pseudodispar* prepared using Ape package in R studio software and plotted using ggplot 2 package

vesicle black with distal border rounded and with fine transverse rugosities; penis with three pairs of setae; sternite of S9 not prominently extending ventrally. TL 53.25 ± 1.06 mm.....

.....*E. dispar* (Rambur, 1842)

– Extensor surface of all femora black, no yellow patch on legs.....5

4. Extensor and flexor surface of all femora bright red; apical half of Hw black; S8 and S9 with tufts of long ventral hairs; male genital vesicle black, hexagonal with rounded angles; S9 and S10 of equal length. TL 52.5 ± 0.70 mm.....

.....*E. cardinalis* (Fraser, 1924)

– Extensor and flexor surface of all femora black; apical fourth of Hw black; a tuft of sparse stub black hair on either side of tergite of S9; male genital vesicle matt black, with distal border rounded angles; S9 twice the length of S10. TL 50.6 ± 2.10 mm.....

.....*E. thosegharensis* Sadasivan & Bhakare, 2021

5. Humeral stripes narrow, starting well beyond the suture between the episternum-2 and infraepisternum-2 and thus close to the origin of

antehumeral stripe; antehumeral stripes narrow and significantly tapering towards the alar sinus; upper part of infraepisternum black with a small pale yellow spot, just below the origin of the humeral stripes in the episternum 2; HW black patch extends to 4–5 cells proximal to the Pt; node on either side of the midline keel on ventral beak of S9 sternite inconspicuous; apex of the dorsal keel on S10 not stepped in lateral view; penile structure short after the setae; male genital vesicle shorter with oval distal margin. TL 47.0 ± 0.70 mm

.....*E. pseudodispar* Sadasivan & Bhakare, 2021

– Humeral stripes broader starting at the suture between the episternum-2 and infraepisternum-2, well away from the origin of antehumeral stripe; antehumeral stripes broader and not tapering towards the alar sinus; upper part of infraepisternum-2 fully black and no pale yellow spot; HW black patch which extends to 10–11 cells proximal to the Pt; ventral beak of S9 sternite with a prominent node on either side of the midline keel; apex of the dorsal keel on S10 stepped in lateral view; penile structure longer after the setae; male genital vesicle longer with its distal margin not oval but nearly angular. TL 51.0 ± 0.70 mm.....

.....*E. wayanadensis* sp. nov.

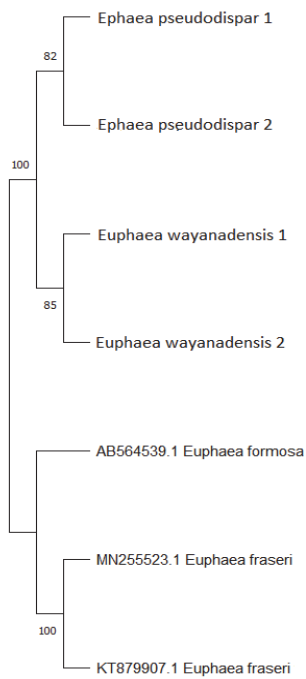


Fig. 6 Phylogenetic tree constructed using Maximum Likelihood method (MEGA 11) with 1000 bootstrap value

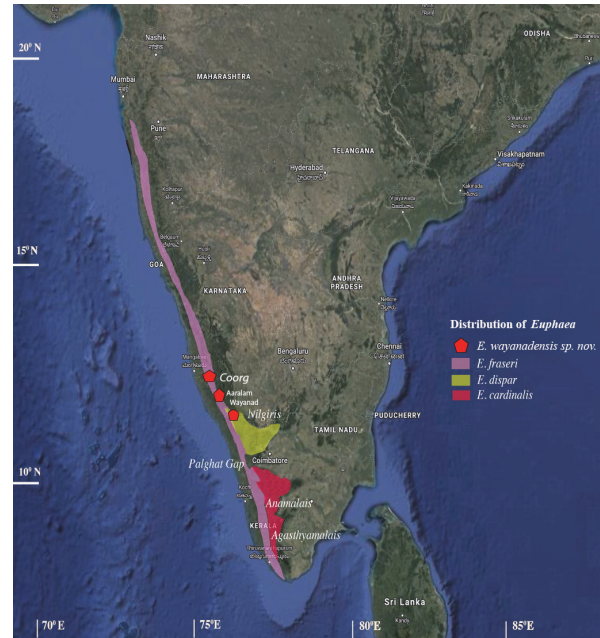


Fig. 7 Distribution map of *E. wayanadensis* sp. nov., *E. dispar*, *E. cardinalis* and *E. fraseri* in the Western Ghats

Molecular analysis

The sequences generated for *Euphaea wayanadensis* sp. nov. and *Euphaea*

pseudodispar and used in the nucleotide similarity study are presented below —

Euphaea wayanadensis sp. nov.:

TCTCCACCTCCTGCAGGGTCTGAAGAATGAAGTGTTAATGTTTCGGTCTGTTAATAGTATT
GTAATTGCACCTGCTAATACAGGTAGTGATAAGAGTAAGAGTAGTGCTGTGATTACTACT
GCTCATACAAATAATGGTATTTGATCTATTTTTATTCTGCGCTTTTTATATTAATTGTT
GTTCTAATAAAGTTAATTGCTCCTAAAATTGACGATACACCTGCTAGGTGTAGTGAAAAA
ATAGTTAGATCTACTGATCCCCCGCATGTGCAATAGCTCCTGCTAATGGGGGGTATACT
GTTTCATCCTGTTCCCTGCTCCTCTTTCTACAAGTCTTCTTGTTAGGAGAAGTGTAATGGAA
GGGGGTAGTAATCAAAATCTTATATTATTAGTCGTGGGAATGCCATATCTGGGGCTCCT
AGTATTAATGGTACTAATCAGTTTCCGAATCCTCCAATTATAATAGGTATAACTATAAAG
AAAATCATTACGAATGCGTGTGCTGTGACAATCACATTATAAATTTGGTCATCTCCAATT
AATGATCCTGGTTGACCAATTCAATTCTGAATTAATATTCTTAGGGCTGTTCTACTATTCTCTG

Euphaea pseudodispar:

TGAAGTGTTAATGTTTCGGTCTGTTAATAGTATTGTAATTGCACCTGCTAATACAGGTAG
TGATAAGAGTAAGAGTAGTGCTGTGATTACTACTGCTCATACAAATAATGGTATTTGATC
TATTTTTATTCTGCGCTTTTTATATTAATTGTTGTTCTAATAAAGTTAATTGCTCCTAA
AATTGACGATACACCTGCTAGGTGTAGTGAAAAAATAGTTAGATCTACTGACCCCCCAGC
ATGTGCAATAGCTCCTGCTAGTGGGGGGTATACTGTTTCATCCTGTTCCCTGCTCCTCTTTC
TACAAGTCTTCTTGTTAGGAGAAGTGTAATGGAAGGGGGTAATAATCAAAATCTTATATT
ATTTAGTCGTGGGAATGCCATATCTGGGGCCCCCTAGTATTAATGGTACTAATCAGTTTCC
GAATCCTCCAATTATAATAGGTATAACTATAAAGAAAATCATTACGAAGGCGTGTGCTGT
GACAATCACATTATAAATTTGGTCATCTCCAATTAATGATCCTGGTTGACCC

Sequences compared using Ape package in R studio software showed 12.9 per cent difference between the two closely resembling species (Fig. 5). The phylogenetic analysis using sequence-based DNA barcode showed a significant correlation between morphological identification and molecular analysis. The phylogenetic analysis revealed that *E. wayanensis* **sp. nov.** formed a separate clade (Fig. 6). *Euphaea pseudodispar* was found to be the nearest associated species. *Euphaea fraseri* formed a separate clade, thus validating the distinct identity of the new species described here.

DISCUSSION

The new species *E. wayanadensis* **sp. nov.**, described here, superficially resembles *E. pseudodispar*, however, they are not sympatric, as per the observations. In the field, *E. wayanadensis* **sp. nov.** can be confused with the occasionally co-occurring *E. dispar*, but the former can be differentiated by the extensor surface of fore femora which is black and red hind and mid femora red as in *E. fraseri*, but with no yellow patch on legs as in *E. dispar* (in case of males). The commonly co-occurring *E. fraseri* males can be very easily differentiated in the field by the pale sky blue humeral and antehumeral stripes (yellowish-orange in *E. wayanadensis* **sp. nov.**) and the azure blue labrum (pale blue with median black knob like marking in *E. wayanadensis* **sp. nov.**). *Euphaea wayanadensis* **sp. nov.** can be easily differentiated from *E. cardinalis* males (seen south of Palghat Gap) which also has an extended black hind wing patch (more extended than *E. wayanadensis* **sp. nov.**), by its pale blue labrum (reddish-ochreous in *E. cardinalis*) and shorter S9 in the former.

Euphaea wayanadensis **sp. nov.** can be differentiated by the black patch on hind wing apices in males which covers up to 1/3rd of the hind wing, whereas in *E. pseudodispar* it is only 1/5th the length of the hind wing; also, the anterior margin of this black hind wing patch extends to 11 cells proximal to Pt in *E. wayanadensis* **sp. nov.** whereas in *E. pseudodispar*, it extends to only 4 cells proximal to the Pt. In males, unlike *E. pseudodispar*, the humeral and antihumeral stripes

are broader, never interrupted and do not significantly taper towards the alar sinus. Unlike its congener, the humeral stripes do not terminate within a distinct spot. Males of *E. wayanadensis* **sp. nov.** have a tuft of hairs on the base of the lateral border of the tergum of S8 and the proximal part of S9 unlike in *E. pseudodispar*. Also, S9 of *E. wayanadensis* **sp. nov.** in lateral view shows lesser curvature; broader on the lower area in comparison with *E. pseudodispar*. When compared to *E. pseudodispar*, the length of penile structure of *E. wayanadensis* **sp. nov.** after the setae is more in dorsal view. Male genital vesicle is longer than that of *E. pseudodispar* and its distal margin is not oval in *E. wayanadensis* **sp. nov.** and is comparatively a large species. In male *E. wayanadensis* **sp. nov.**, there is a pale azure blue pruinose stripe on anteclypeus in fresh specimens. In the case of females, the vulvar scale is longer when compared to *E. pseudodispar*. The molecular analysis reinforced the distinctiveness of *E. wayanadensis* **sp. nov.**

Muneer *et al.* (2022) reported the occurrence of *E. pseudodispar* at the banks of Kalindi River, from Thirunelli, Wayanad. The authors (CS, SSA, KS and VPN) had been studying odonates of Thirunelli and Wayanad since 2013 and had not come across *E. pseudodispar*, and all the '*pseudodispar*' morphotypes caught and examined turned out to be this new species, hence this is a case of misidentification. The mt CO1 gene had been used as a very reliable marker in DNA barcoding of Odonates. The analysis of nucleotide similarity and the phylogenetic tree clearly supports the distinctiveness of the new species discovered. *E. wayanadensis* **sp. nov.** has a very restricted distribution in the Wayanad region, though not uncommon further north on west-flowing rivers of Coorg landscape. Observations at other locations in Wayanad, namely Kalpetta (780m ASL), Kuruva Island (700m ASL) from 2013 to 2017 as a part of a different study by the same authors (Susanth and Anooj, 2020), failed to find this taxon. This could be attributed to the stream ecology and the riparian forest habitat of the new species. The closely resembling *E. pseudodispar* is reported to have similar habitat requirements as for the new species,

except for the slower speed of flow of the streams. The highly restricted distribution and habitat requirement of the new species highlights the need for targeted surveys and prioritizing the conservation of aquatic invertebrates of ecologically vulnerable landscapes like Wayanad.

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Partial characterization of lipase in male moth of *Maruca vitrata* (Fabricius) (Lepidoptera, Pyralidae)

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ABSTRACT: Partial characterization of lipase in male insect moth of *Maruca vitrata* revealed pH 7.6, temperature 37 °C, incubation time 25 minutes, substrate concentration (5%), enzyme concentration (1%) and K_m value 0.0993×10^{-2} mM. Maximum lipase activity noted in 4th day insect moth of *M. vitrata*. The adult longevity of insect moth was found to be seven days. The physiological significance of lipase in male insect moth of *M. vitrata* has discussed. © 2025 Association for Advancement of Entomology

KEY WORDS: Enzyme kinetics, hydrolases, lepidoptera adult, spotted pod borer

The bean pod borer, *Maruca vitrata* (F.) (Lepidoptera, Pyralidae), is one of the most serious pests of cowpea, *Vigna unguiculata* (Taylor, 1967). It is one of the serious pests and occurs during flowering and pod formation stages causing huge losses (Pappu *et al.*, 2010). In insects, lipids are involved in several physiological functions like molting during larval and adult development (Kawooya and Law, 1988). Triacylglycerol lipase hydrolyzes certain positions of triacyl glyceride while different phospholipases act on different ester bonds within phospholipids (Zibae *et al.*, 2008). However, the information about the partial characterization of lipase in male insect moth of *M. vitrata* is rather scanty. In the present work, partial characterization of lipase in male insect moth of *M. vitrata* has been discussed.

The larvae from infested pods were kept in cowpea pods in insect rearing cage. Tiny larvae were transferred to cowpea pods in glass tubes and covered with muslin cloth. A full-grown larva bore large exit holes, leave the pea pods and pupates underneath the pods. The pupae were collected immediately after pupation and were kept in glass jar having 15cm in height and 7cm in diameter covered with muslin cloth until the immergence of the adults. The pupation period of *M. vitrata* was noted as 8 days. Newly emerged male and female moths of *M. vitrata* were kept as 10 pairs in round glass jar. Adults were placed in rounded glass jar, having 12cm height and 30cm in diameter and covered with muslin cloth until the deposition of eggs. Adults were fed with sucrose (10%) solution. Pea pods were placed in rounded glass jar as an

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oviposition site and removed daily (Sharma, 1998). Ammonium sulphate precipitation method was used for purification of lipase (Dawson *et al.*, 1969). For uniform and rapid dissolution, the desiccated ammonium sulphate is used. Ammonium sulphate was placed over night in oven at 120°C in a glass beaker. For 70 per cent saturation required ammonium sulphate was 43.6g 100⁻¹ ml. Homogenate was allowed to precipitate for 30 minutes at 4°C with stirring. Centrifugation used for recovered precipitation about 30 minutes and pellet was separated. Lipase assay included 0.25mL enzyme, 0.25mL substrate (olive oil) and one mL phosphate buffer. Reaction was stopped by two mL Cu-TEA reagent; 10mL of chloroform was used as solvent. Then 2mL of chloroform phase was taken in stoppered test tube and 1mL of colour reagent was added (Hayase and Tappel, 1970). Liberated fatty acids were measured by using calorimeter. The absorbance was read at 540nm (Itaya, 1977).

The survival of male moth of *M. vitrata* was found to be 7 days. Partial characterization of lipase in male insect moth of *M. vitrata* revealed pH 7.6, temperature 37°C, incubation time 25 minutes, substrate concentration (5%), enzyme concentration (1%) and K_m value 0.0993×10^{-2} mM. Maximum lipase activity noted in 4th day insect moth of *M. vitrata*.

Effect of pH on lipase activity: In present work, maximum lipase activity in male insect moth at pH 7.6 indicated the presence of alkaline lipase in the male insect moth of *M. vitrata* (Fig. 1). Highest lipase activity was observed at optimum pH 9.5 in male of *Helicoverpa armigera* (Chamani *et al.*, 2016). Lipase activity in *Callosobruchus maculatus* studied at pH 8 (Silva *et al.*, 2017). Lipase activity in host specific *Bemisia tabaci* G., studied at pH 8 (Anokhe *et al.*, 2018). Effect of pH on lipase activity in butterfly, *Junonia atlites* and *Junonia almanac* studied at pH 6.8 (Saptarini *et al.*, 2021).

Effect of enzyme concentration on lipase activity: In present study, maximum lipase activity in male insect moth at one per cent enzyme concentration suggest rate of release of fatty acid

was optimum at one per cent enzyme concentration. Increased enzyme concentration showed more rate of reaction after certain concentration further increase in enzyme concentration never increased the reaction rate (Fig. 2). The maximum lipase activity of the enzyme noted at one per cent enzyme concentration in male of *M. separata* (Pol and Salunkhe, 2001). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera, Chrysomelidae) determined at enzyme concentration (2 %) (Silva *et al.*, 2017).

Effect of temperature on lipase activity: In the study, maximum lipase activity was at 37°C, indicating the optimum temperature for enzymatic reaction (Fig. 3). This result suggest the rate of enzyme reaction increased with increase in temperature, within the temperature range in which the enzyme was stable and retained full activity and this happen up to a certain temperature called as optimum temperature. Highest lipase activity studies on male *H. armigera* showed 40°C as its optimum temperature (Chamani *et al.*, 2016). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) determined 37 °C as its optimum temperature (Silva *et al.*, 2017). Lipase activity of butterfly, *Junonia atlites* higher than *J. almanac* 37°C as its optimum temperature (Saptarini *et al.*, 2021).

Effect of time on lipase activity: The maximum lipase at 25 minutes of incubation time suggested enzyme required 25 minutes for optimum activity (Fig. 4). Lipase activity of *Tribolium confusum* was noted at incubation time 30 minutes (Barra *et al.*, 2015). Digestive lipase of dung beetle, *Chironitis arrowi* was reported 10 minutes as its incubation time (Gaikwad and Bhawane, 2015). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera, Chrysomelidae) studied indicated 30 minutes as its incubation time (Silva *et al.*, 2017).

Effect of substrate concentration on lipase activity: In the present work, maximum lipase activity was at 5 per cent substrate concentration, suggesting it as the optimum substrate concentration. The K_m value 0.0993×10^{-2} mM indicated more affinity of enzyme with the substrate (Fig. 4).

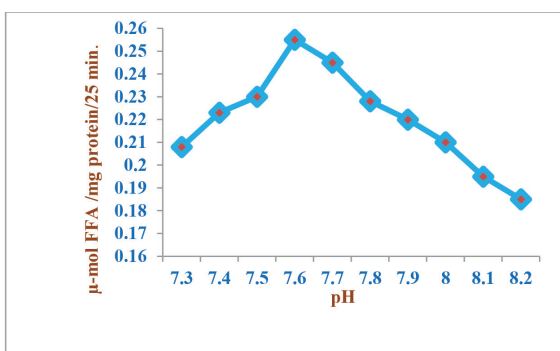


Fig. 1 Effect of pH on lipase activity in male moth in *M. vitrata*

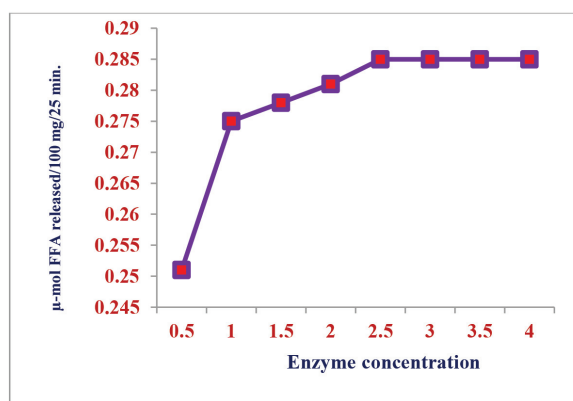


Fig. 2 Effect of enzyme concentration on lipase in male moth of *M. vitrata*

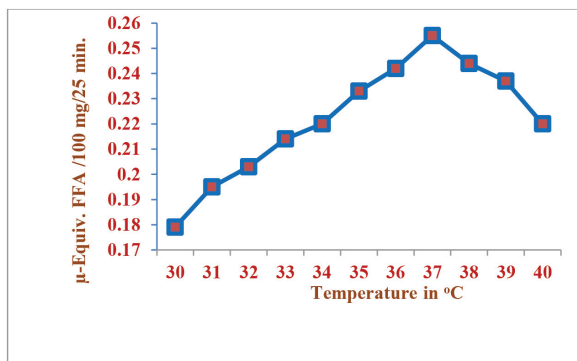


Fig. 3 Effect of temperature on lipase activity in male moth of *M. vitrata*

Maximum lipase activity of phospholipase B in male of *Culex pipiens fatigans* showed $0.8 \mu\text{mol mL}^{-1}$, as the optimum substrate concentration (Rao and Subrahmanyam, 1969). In male of *M. separata* the maximum lipase activity was noted at 5 per cent substrate concentration (Pol and Salunkhe, 2001).

In present study, the increased in lipase activity in male insect moth of *M. vitrata* suggested utilization

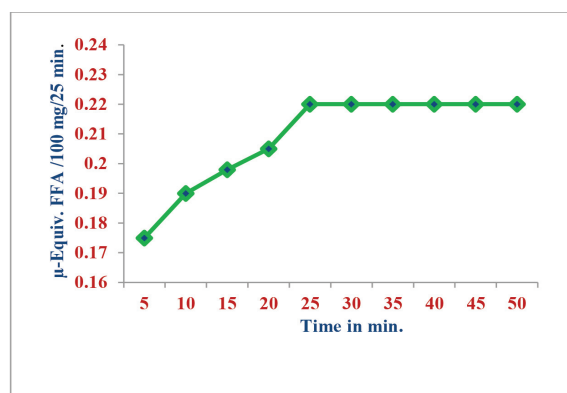


Fig. 4 Effect of incubation time on lipase activity in male moth of *M. vitrata*

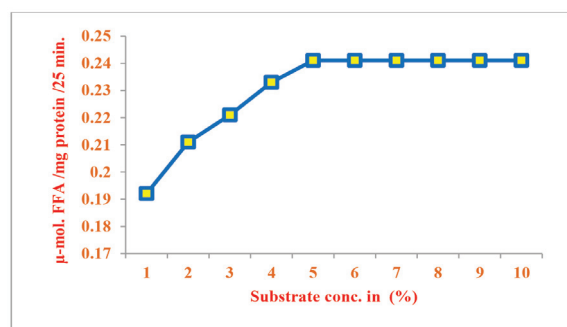


Fig. 5 Effect of substrate concentration on lipase activity in male moth of *M. vitrata*

lipid during the active flight in search of female moth for mating. This increased lipase activity may be concerned with structural components and sperms production. In insects, including male adults, lipase activity can indeed vary and may be higher compared to other developmental stages or female insects. Lipase enzyme in insect is involved in lipid digestion and metabolism. The variation in lipase activity among different developmental stages of male moths can be attributed to differences in nutritional requirements, reproductive physiology, and overall metabolic demands. The maximum lipase activity in 4-day adult male of *M. vitrata* indicates most active stage of moth and energy derived from catabolism of triglycerides. The main source of energy during the male adult development is lipid and lipolytic activity.

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New host and distribution records of *Hemiptarsenus orizae* Narendran (Hymenoptera, Eulophidae) from Kerala, India

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ABSTRACT: Rice leaf folder, *Helcystogramma arotraeum* (Meyrick, 1894) a major defoliator in Kerala was found parasitoid by a solitary endoparasitoid, *Hemiptarsenus orizae* Narendran (Hymenoptera, Eulophidae). New host-parasitoid association with distribution records of *H. orizae* is reported.

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KEY WORDS: Rice leaf folder, Chalcidoidea, endoparasitoid, *Helcystogramma arotraeum*

Hemiptarsenus Westwood, 1833, a moderate-sized parasitic wasp in Eulophinae, was erected for *Hemiptarsenus fulvicollis* Westwood, 1833. Majority of the parasitoids in the genus *Hemiptarsenus* attack larval or nymphal stages of various species of *Agromyza*, *Butornornyza*, *Cerodontha*, *Chromatornyia*, *Lirionyza*, *Ophiomyia*, *Phytolirionyza*, *Phytornyza*, and *Pseudonafirnyza* (*Agromyzidae*); *Merornyza* (*Chloropidae*, Diptera); *Eriopeltis* (*Coccidae*); *Bucculatrix* (*Bucculatricidae*); *Stugnatophora* (*Cosmopterigidae*); *Elachista* (*Elachistidae*); *Hydrellia* (*Ephydriidae*); *Phyllonorycter* (*Gracillariidae*); *Leucoptera* (*Lyonetiidae*); *Mornpha* (*Mmiphidae*); *Ectoedernia*, *Parafororia*, *Stigmella*, *Trifurcula* (*Nepticulidae*, Homoptera); *Gortynu* (*Noctuidae*); *Ostrinia* (*Pyralidae*); *Prays* (*Yponomeutidae*, Lepidoptera);

Blastophagus, *Ceutorhynchus*, *Hypurus*, *Rharnphus* (*Curculionidae*, Coleoptera); and *Hpteralhrus* (*Tenthredinidae*, Hymenoptera) (Chac-dong *et al.*, 2000). *Hemiptarsenus orizae* was discovered in the rice ecosystem and is reported only from Tamil Nadu and West Bengal (Narendran, 2011).

Parasitoids are the essential components of agroecosystems, particularly those from the Hymenoptera (Godfray, 1994). The primary foundational research on insect pest management for subsequent applicative investigations is based on faunistic parasitoid surveys (Tomanoviæ *et al.*, 2014; Petroviæ *et al.*, 2019). If a favourable environment causes considerable stress in agriculture, several pest species, particularly lepidopterans, are likely to become more prevalent (Elkinton and Liebhold, 1990; Devetak *et al.*, 2014).

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The abundance of hosts affects the population dynamics of parasitoids since many of them may prey on many host species or specialise in one. As a result, parasitoids play a crucial role in pest management (Hassell, 1980; Hassell and May, 1986; Barbosa, 1998; Gagiæ *et al.*, 2016).

Rice defoliator, *Helcystogramma arotraeum* Meyrick 1894 (Lepidoptera, Gelechiidae) is a pest of rice in Japan, Taiwan, China (Hainan, Jiangxi, and Yunnan), Myanmar, Thailand, India, Sri Lanka, Malaysia, and Indonesia (Ueda, 1995; Ponomarenko, 1997). *H. arotraeum* (= *Brachmia arotrae* Meyr.) larvae were seen folding and feeding on rice leaves. The caterpillars of this pest folds the leaves and feed with in and is wide spread in late-planted rice, (30–40% of the population of leaf-folders during the first week of December), and was seen parasitized by *Apanteles* sp. and *Brachymeria* sp. Little focus has been given to this rice pest despite its startling resemblance to *Cnaphalocrocis medinalis* Guenée (Natarajan *et al.*, 1978). *Oryza sativa* L. and *Zizania latifolia* Turcz (Poaceae) were the known hosts of this pest (Ponomarenko, 1997). The study aimed to shed light on the new host association of *H. orizae* in the rice fields of North Kerala.

Caterpillars of *H. arotraeum* were collected from the rice fields of Muiyyorath (11°52'30.8"N; 75°38'32.3"E), Kannur, Kerala, India, in the wet season (June–September). and brought to the laboratory for the emergence of parasitoids (at 28 ± 2°C and 70–80% relative humidity). Parasitoids preserved (in 70% ethanol) were processed using hexamethyldisilazane (HMDS) and later card-mounted. The parasitoids were identified using the available taxonomic keys (Narendran, 2011). Images of parasitoids were taken with a Leica DFC 295 camera attached to a Leica S8 APO stereozoom trinocular microscope with an 8x objective. Figures were produced from stacked images and combined automatically into a single image using Leica Automontage Software® V4.2. All the specimens were deposited at the Department of Zoology, Malabar Christian College, Kozhikode, Kerala, India. Moreover, the developmental stages of the rice insect pest, *H.*

arotraeum, were photographed using a Canon EOS 1500D 24.1-megapixel digital SLR camera.

The parasitoid *H. orizae* was found attacking the larvae of *H. arotraeum* in the rice fields, similar to the rice leaf folder *C. medinalis*. Larvae of *H. arotraeum* fold the leaves by sticking leaf margins, scrape the green tissues of leaves, leaving the blade with long, transparent white streaks (Fig. 1A-F). In mid-July, *Hemiptarsenus orizae* parasitized 3.6 per cent of *H. arotraeum* larvae, emerging from 2 of the 55 collected during the booting stage of paddy.

***Hemiptarsenus* Westwood, 1833**

Hemiptarsenus Westwood, 1833: 122-123. Type species: *H. fulvicollis* Westwood, designated by Westwood, 1839.

Notanisomorpha Ashmead, 1904: 356. Type species: *N. collaris* Ashmead. by original designation (synonymized by Schauff and La Salle, 1993: 494).

Eriglyptoideus Girault, 1913: [167]: 154. Type species: *E. varicornis* Girault. by original designation (synonymized by Bouček, 1988: 626).

Hemiptarsenoideus Girault, 1916: [274]: 220. Type species: *H. semialbiclava* Girault, designated by Gahan and Fagan, 1923 (Synonymized by Girault 1924: 376).

Neodimmockia Dodd, 1917: 361-362. Type species: *N. agromyzae* Dodd, by monotypy (Synonymized by Girault, 1924: 376).

Cleolophus Mercet, 1924: [24]: 461. Type species: *C. autonomus* Mercet, original designation and monotypy (Synonymized by Burks, 2012: 28-31).

Parpholema Szelenyi, 1981: [1]: 276. Type species: *P. collaris* Szelenyi, original designation and monotypy (Synonymized by Burks, 2012: 28-31).

Diagnosis: Scape exceeding level of vertex; funicle 4 segmented in female and with 3 branches in male; notauli incomplete; axillae usually not angulately advanced; scutellum without sublateral grooves; propodeum with median carina and plicae indistinct or absent in majority of species; forewing

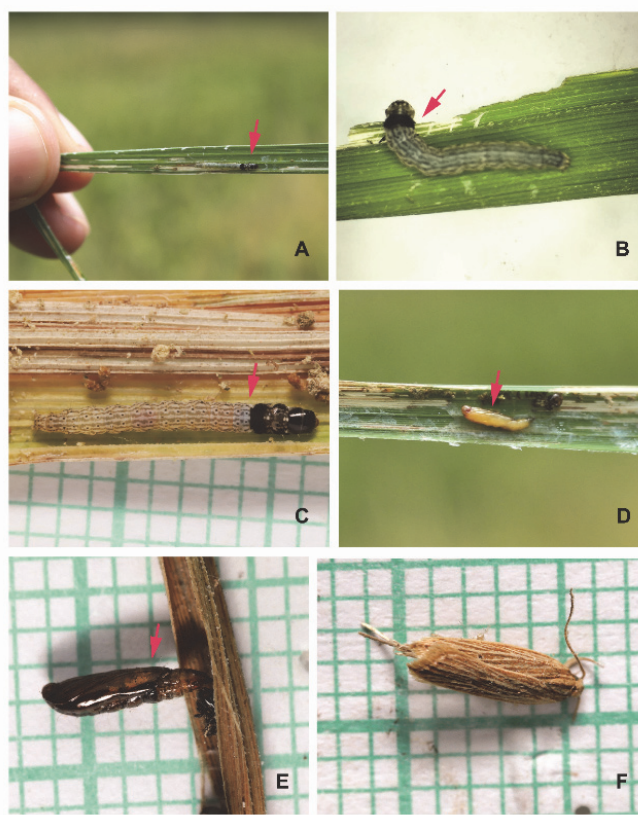


Fig. 1 Biology of *Helcystogramma arotraeum* (Meyrick): A-B. Larva feeding the rice leaf; C. Late instar larva; D-E. Pupal stage; F. Adult

and costal cell long and narrow; forewing at least 2.6x as long as wide and costal cell 10- 15x as long as wide.

Hemiptarsenus orizae (Narendran, 2011)

Type material: **Paratypes Examined**

Material examined: 1 ♀, INDIA: Kerala, Kannur, Muyyorath, 16.vii.2019, coll. S.K. Sreejina.

Diagnosis: Female: Length 2.31 mm. Head dark metallic green with bronze refringence; eye gray; ocelli pale brownish yellow; antenna brown with pedicel and scape pale yellowish brown (Fig. 2); pronotum orange yellow with a transverse area adjacent to posterior border medially metallic green; mesoscutum, scapula, axilla, scutellum and meso and metapleuron slightly dark with metallic green and bronze refringence; petiole pale brown; gaster with yellow and black patches; legs completely pale yellow except fourth tarsal segment and pretarsus

of forelegs also brown; wings hyaline with veins brown.

Variation: As reported by Narendran (2011), the paratypes from West Bengal are more brightly metallic green without bronze refringence and antenna pale brownish; length of female varies from 2.4-2.5mm.

Distribution: India: Tamil Nadu, West Bengal (Narendran, 2011) and Kerala.

Host: *Helcystogramma arotraeum* (Meyrick) (Lepidoptera, Gelechiidae) is the only known host. According to Chac-dong *et al.* (2000), two lepidopteran families, Noctuidae and Pyralidae, are hosts to the genus *Hemiptarsenus*.

Biology: *H. orizae* (Fig. 2) was reared from the larvae of *H. arotraeum*, inhabiting the foliage of *O. sativa* (Fig. 1B). Females of *H. orizae* probably oviposit into the early larval instars of *H.*

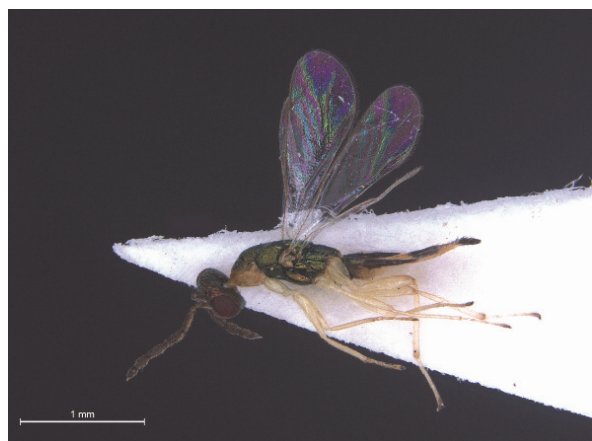


Fig. 2 Adult *Hemiptarsenus orizae* (Narendran)

arotraeum (Fig. 1A) as the parasitoids were reared from the late larvae (Fig. 1C) of the host. Hence, it is a larval parasitoid and exhibits solitary endoparasitism through koinobiosis. The parasitoid *H. orizae* was reported from the rice fields by Narendran (2011). However, no hosts have been reported for the parasitic wasp, and this study reports the first host-parasitoid association and distribution record of *H. orizae*.

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Rediscovery of *Trochoideus desjardinsi* Guérin–Méneville, 1838 (Coleoptera, Endomychidae, Pleganophorinae) with a new record from Mumbai, Maharashtra, India

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ABSTRACT: After nearly a century, *Trochoideus desjardinsi* Guérin-Méneville, 1838 was rediscovered from India. Earlier records from India are by Arrow (1925) from Kerala and Andaman Islands. This species is reported for the first time from Maharashtra. Additionally, a brief redescription of the species is provided with habitus image. © 2025 Association for Advancement of Entomology

KEY WORDS: Range, extension, beetle fauna, light trap, Western Ghats

The “Handsome fungus beetle” is a common name for mycophagous beetles of the family Endomychidae, which are vibrant in colour. Global Endomychidae fauna consists of 1782 species in 130 genera and 12 subfamilies (Shockley *et al.*, 2009a). However, in India, this family of beetles is only represented by 116 species in 35 genera and six subfamilies (Shockley *et al.*, 2009a). One of the subfamilies is Pleganophorinae, which according to Strohecker (1953), Tomaszewska (2005) and Shockley *et al.* (2009a), has only three genera globally: *Pleganophorus* (Palearctic Region), *Dadocerus* (Oriental Region) and *Trochoideus* (Neotropical, Afrotropical and Oriental Regions).

Trochoideus Westwood, 1833 contains 19 species distributed worldwide, one of which, *Trochoideus desjardinsi* Guérin–Méneville, 1838 (Coleoptera, Endomychidae, Pleganophorinae) has been reported

from Borneo, Cuba, Fiji Islands, India, Indonesia, Japan, Java, Kenya, Madagascar, Malaysia, Mauritius, Micronesia, Myanmar, Papua–New Guinea, Philippines, Samoa, Seychelles, Sri Lanka, Taiwan, Tanzania, Uganda, and the United States (Shockley *et al.*, 2009a; Shockley and Kovalenko, 2018). Thus, the species is now considered cosmopolitan across the tropical regions of the world. Arrow (1925) revised the *Trochoideus* fauna from British India and reported *T. desjardinsi* from Indian regions like Quilon, Travancore (currently Kollam), and Port Blair, Andaman Islands (currently Sri Vijaya Puram). Therefore, this species in India has, so far, only been reported from the state of Kerala (Kollam district) and a union territory, Andaman and Nicobar Islands (South Andaman district). There are no new records of this species from India in any recent literature except the mention in Skelley and Burgess (1995) and a

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checklist by Shockley *et al.* (2009a), both with just a country name (“India”), likely referring to the specimen localities reported by Arrow collectively. Now the rediscovery of *T. desjardinsi* (Fig. 1) from India after nearly a century, with a record new for Maharashtra State in Sanjay Gandhi National Park, Mumbai is reported.

Sanjay Gandhi National Park (SGNP) is located in the metropolitan area of Mumbai. Yazdani *et al.* (1992) described a wide range of habitats present in this national park, such as: coastal, dry and moist deciduous, mixed deciduous to semi evergreen and open scrubland. These habitats are typical of the Western Ghats. The elevation, in SGNP, ranges from 30 meters to more than 500 meters above sea level, and vegetation is also highly diverse due to the area’s proximity to the sea, numerous streams, and mountainous topography (Yazdani *et al.*, 1992). An on-going field study to understand the diversity of beetle fauna from SGNP revealed a female specimen (Reg. No. KN210; deposited in the collection of the Department of Zoology, K.J. Somaiya College of Science and Commerce, Mumbai) on a light trap, during mid-monsoon on 23.VIII.2022 at Kashi, SGNP. The light trap (Fig. 2) was set at 51 m above sea level in the Dharavi Mandir under the canopy of a huge mango tree, facing downhill to a seasonal stream.

The collected female specimen was identified as *T. desjardinsi* using Arrow (1925), Strohecker (1953), and Tomaszewska (2000). The species is characterized by having modified antennae with probable association with ants and termites (Arrow, 1925; Strohecker, 1978). The female possesses antennae similar to that of the male but with a diameter of antennomere IV one-fourth of the length, while in male antennomere IV is swollen considerably (Arrow, 1925; Skelley and Burgess, 1995- images on p.290). According to Arrow (1925), the species is also found in heaps of refuse coconut husks. Additionally, this species also inhabits under the bark, can survive on stored products, and has a potential to be an economically important insect (Beeson, 1941; Shockley *et al.*, 2009b; Shockley and Kovalenko, 2018). The species has been redescribed in Arrow (1925) and a recent detailed

diagnosis of the genus has been given by Tomaszewska (2000).

Brief Redescription:

Female (Fig. 1). Body elongate-oval, almost twice as long as wide (3mm long, 1.5mm wide). Brown red integument with small and numerous punctures. Pubescence yellow, short, thin and moderately dense over the body.

Head width including eyes wider than the half of the pronotal width. Eyes small, widely placed with distinct facets. Antenna modified with four joints; antennal insertions visible from above. Antennomere I somewhat cylindrical and curved, broadening apically. Antennomere II smallest, narrower than III. Antennomere IV longest, largest, widening apically to somewhat rounded apex. Vestiture on antennomere IV. Frons convex, without frontal carina, carinate over antennal insertions. Clypeus punctate, light brown, anterior margin straight. Labrum rectangular, setose, as broad as clypeus, its anterior margin broadly emarginate. Mandibles slender. Maxillary palpi three segmented, terminal segments cylindrical with pointed apex. Labial palp with the terminal segment distinctly cup-shaped.

Thorax with pronotum transverse, widest near middle, its surface convex with shallow longitudinal depression in the middle. Lateral edges carinate, convex, slightly constricted submedially; posterior angles right angled. Scutellum small, flat with sides broadly rounded converging posteriorly to apex. Elytra only slightly broader than pronotum, with numerous punctures and without distinct striae, elytral humeral angle rounded. Elytral sides convex, uniformly sloping posteriad to almost rounded apex. Elytral epiplura broad, almost up to the middle of first abdominal ventrite. Prosternal process very narrow. Mesosternum convex, projecting between mesocoxae. Metasternum convex with distinct metaventral discrien along the entire length; broad and truncate between metacoxae. Metepimeron long and parallel sided.

Legs with procoxae broad anteriorly, narrowing posteriorly, placed very close to each other.



Fig. 1 Female of *Trochoideus desjardinsi* Guérin-Méneville, 1838 collected from SGNP, Mumbai

Mesocoxae parallel to each other. Metacoxae transverse, widely separated. Protochantin visible. Trochanter length in increasing order from anterior to posterior: protochanter < mesotrochanter < metatrochanter. Femora twice as broad as tibia; tibia slender, length subequal to femoral length. Tibial

spur absent. Tarsal formula 4-4-4; tarsomeres densely pubescent. Tarsomere IV long. Tarsal claws slender, blades uniformly curved to pointed apex. Abdomen with six visible ventrites. Ventrite I long almost equal to combined length of II–IV. Ventrite VI small, broadly rounded. All ventrites finely, uniformly punctate.

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Fig. 2 Light trap and a part of the habitat at Kashi

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Life cycle and morphometrics of leaf roller, *Haritalodes derogata* (Fabricius) on cotton in Surat, Gujarat, India

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ABSTRACT: The life cycle and morphometrics of cotton leaf roller, *Haritalodes derogata* (F.) (Lepidoptera, Pyralidae) study revealed that the average duration of eggs, larvae, male pupae, female pupae, male adult and female adult moths varied from 3.84 ± 0.99 , 15.40 ± 1.63 , 7.32 ± 0.69 , 7.88 ± 0.97 , 9.32 ± 0.75 and 10.40 ± 0.82 days, respectively. Pre-oviposition and post-oviposition periods were 3.36 ± 0.70 and 2.40 ± 0.65 days, respectively and the fecundity was 265.40 ± 42.55 eggs, within oviposition periods of 3.56 ± 0.58 days. The total life span for male and female was 37.40 ± 2.06 and 39.68 ± 2.36 days, respectively. The length and breadth of egg measured 0.54 ± 0.03 and 0.47 ± 0.02 mm, of full-grown larvae was 27.78 ± 1.18 mm and of male and female pupae were 11.95 ± 0.78 and 4.19 ± 0.14 and 13.34 ± 0.49 mm and 4.38 ± 0.11 mm, respectively. The length and breadth with wings expanded of male was 11.48 ± 0.70 and 22.27 ± 0.67 mm, while that of female moth was 13.79 ± 0.53 and 28.12 ± 1.42 mm, respectively. © 2025 Association for Advancement of Entomology

KEY WORDS: Biology, life span, measurements, stages, wings

Cotton (*Gossypium hirsutum* L.), is grown worldwide for its natural fiber and oil. Cotton leaf roller, *Haritalodes derogata* (F.), (Lepidoptera, Pyralidae) nowadays appeared regularly especially in non-Bt cotton varieties/hybrids. The pest occurs in India, Pakistan, Bangladesh, Burma, Australia, Africa, China, Japan and Sri Lanka. The pest is active from the month of September to November. The leaf roller is a polyphagous that feeds on malvaceous plants such as *G. hirsutum*, *Abelmoschus esculentus*, *Hibiscus rosasinensis*, *Urena lobata*, *Althaca rosea*, *Sida cordifolia* and *Malvastrum tricuspidatum*. The leaves rolled in the trumpets fastened by silken threads, feed on the green tissue in early stage, and eat up a large portion of the leaf as it grows. Caterpillar infestation can vary with some leaves having as few as 5 to 6,

while others may have as many as 32 young caterpillars on a single damaged leaf (Mariselvi and Manimegalai, 2016). In case of heavy infestation, there was complete defoliation. Larvae's destructive feeding activity limits photosynthetic area, as well as the quantity and quality of minerals and water uptake by the plant. If the above procedure continues, the plant will dry totally (Gahramanova *et al.*, 2020). Yield loss due to leaf roller in cotton up to 50 per cent has been reported in the northwest ecological zone of Nigeria (Yahaya, 2008). On the underside of leaves, the female moth lays 200-300 eggs, each of which takes 2-6 days to hatch. The resulting larvae undergo a developmental stage lasting 15 to 35 days, during which they can pupate on the plant, within curled leaves or among plant remains in the soil. The pupal

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stage lasts from 6 to 12 days and the entire life cycle lasts between 23 to 53 days (Mansion *et al.*, 2022). Owing to its regular occurrence and potential to be key pests, there is dire need to relook the life cycle parameters of the pest before formulating effective management tactics. A study was carried out to study the life cycle and morphometrics of different life stages on cotton in the laboratory of Department of Entomology, N. M. College of Agriculture, Navsari Agricultural University (NAU), Navsari, Surat, Gujarat, India.

Initial culture of larvae of *H. derogata* were obtained from the unsprayed plot of non-Bt cotton hybrid (RCH 659 NBt) grown on Research farm, Main Cotton Research Station, NAU, Surat. The culture reared in the laboratory during *Kharif*, 2022-23. The larvae were reared in round plastic jar (15cm diameter × 10cm height) having fine netting cover as lid for aeration. Such five cages were prepared for mass rearing. Fresh cotton leaves were supplied daily after cleaning to the larvae as food. The newly formed pre-pupae with folded cotton leaves were gently collected and placed in another rearing jar covered with a fine netting lid. The pupae thus formed were separated as male and female pupae based on distance of genital and anal aperture and shape by critically examining under trinocular microscope and placed in separate acrylic rearing cage (30 × 30 × 30cm) for emergence of adults. The pair of male and female adults emerged as same dates were released into an oviposition cage for mating. A tender shoot of cotton with three to four leaves, cut and wrapped with absorbent cotton to maintain its turgidity was placed inside the oviposition cage as a place for egg laying. A swab of absorbent cotton dipped in a 5 per cent honey solution was hanged inside the cage as food for the adults (Sontakke *et al.*, 2015). The leaves with freshly laid eggs were detached and kept on wet blotting paper affixed to petridish (9cm diameter × 3cm height). These eggs were used for further study of life cycle and morphometrics. The five sets/replications each of twenty-five eggs of *H. derogata* was observed for hatching percentage. The different stages of *H. derogata* were critically observed under a dissecting stereo-trinocular microscope, using a very fine needle and moistened brush to determine the

shape and duration of each stage. Further, the measurement of length and breadth of various stages were also carried out under trinocular microscope (Make: Olympus-SZ 61) fitted with a Brand Catcam-130 camera and software power Scopephoto (Version 3.1). Average temperature and relative humidity were recorded as $27.77 \pm 1.66^{\circ}\text{C}$ and 65.11 ± 6.97 per cent, respectively during the study.

The life cycle of *Haritalodes derogata* consisted of egg, larvae (six instars), pupae and adult stages. The female moths laid eggs mostly singly during the night. The eggs were typically deposited on the lower surface of leaves, on leaf petioles, stems and squares of cotton plant. The freshly laid eggs were found small, whitish, translucent and spherical in shape. Within a day, the colour of the eggs becomes light yellow before hatching. The length of the eggs ranged from 0.50 to 0.62mm with an average of $0.54 \pm 0.03\text{mm}$, while the breadth varied between 0.45 to 0.55 mm, with an average of $0.47 \pm 0.02\text{mm}$. The incubation period varied from 2 to 6 days with an average of 3.84 ± 0.99 days. In first, second, third, fourth and fifth sets of hatching percentage were 90, 84, 96, 84 and 80, respectively. The minimum and maximum hatching percentage was 80 to 96, respectively with an average of 86.00 ± 6.26 per cent (Table 1, 2).

The larva was found to pass through six instars on cotton leaves under the laboratory condition. The freshly hatched larva had yellowish and transparent body colour, which later change to a light green colour as it starts feeding on cotton leaf. The body of the larva was slender and slimy with a comparatively transparent broad head. This stage of the larva was notably more active and sensitive compared to subsequent instars. The first instar larva had small head and enlarged prothorax. Each thoracic segment bears a pair of legs. Four abdominal prolegs are located on the 3rd to 6th segments. The last abdominal segment bears the 5th abdominal prolegs or claspers. The body was covered with numerous small setae. The length and breadth of first instar larva varied from 1.80 to 2.30mm with an average of 2.04 ± 0.16 mm and 0.24 to 0.31 mm with an average of $0.28 \pm 0.02\text{mm}$, respectively (Table 2). The width of head capsule

varied from 0.21 to 0.25 mm with an average of 0.23 ± 0.01 mm (Table 3). The duration of first instar larva varied from 1 to 3 days with an average of 2.20 ± 0.50 days (Table 1).

The second instar larva had yellowish green body, light brown head and slightly larger than first instar. It was more actively feeding on leaves than first instar. It possessed clearly visible setae on the body. The presence of two small brown spots on dorsal region of prothorax was distinctive feature of second instar larva. The length of second instar larva ranged from 3.28 to 4.00 mm with an average of 3.51 ± 0.18 mm, while the breadth ranged from 0.40 to 0.47 mm with an average of 0.43 ± 0.02 mm (Table 2). The width of head capsule varied from 0.37 to 0.43 mm with an average of 0.40 ± 0.02 mm (Table 3). The duration of second instar larvae varied from 2 to 3 days with an average of 2.44 ± 0.51 days (Table 1).

The third instar larva had dark green coloured body with brown head. The two brown spots on the prothorax start to develop into thoracic shields. The typical behaviour of feeding on the inner tissue of the rolled leaves was observed in third instar, which was not the case in first and second instars. The length and breadth of third instar larva ranged from 7.00 to 8.00 mm with an average of 7.70 ± 0.23 mm, 1.11 to 1.20 mm with an average of 1.15 ± 0.03 mm, respectively (Table 2). The width of head capsule varied from 0.89 to 0.95 mm with an average of 0.93 ± 0.02 mm (Table 3). The duration of third instar larva varied from 2 to 3 days with an average of 2.48 ± 0.51 days (Table 1).

Fourth instar larva had thick and strong body and was deep green colour with dark brown head and black thoracic shield. Larval prothoracic segment becomes slightly flattened on the ventral surface of the body. The thoracic legs were black with white claws and the abdominal prolegs were dirty white. The length and breadth of fourth instar larva ranged from 11.00 to 12.14 mm with an average of 11.91 ± 0.40 mm, 1.50 to 1.60 mm with an average of 1.54 ± 0.03 mm, respectively (Table 2). The width of head capsule varied from 1.10 to 1.25 mm with an average of 1.19 ± 0.04 mm (Table 3). The duration of fourth instar larvae varied from 2 to 4

Table 1. Duration (days) of different life stages of *H. derogata*

Life stages	Duration (days)	
	Range	Mean $\pm$ SD
Egg	2-6	3.84 ± 0.99
Hatching (%)	80-96	86.80 ± 6.26
Larva		
I instar	1-3	2.20 ± 0.50
II instar	2-3	2.44 ± 0.51
III instar	2-3	2.48 ± 0.51
IV instar	2-4	2.56 ± 0.58
V instar	2-4	2.68 ± 0.75
VI instar	2-4	3.04 ± 0.79
Total	13-18	15.40 ± 1.63
Pre-pupa	1-2	1.80 ± 0.41
Pupa		
Male	6-8	7.32 ± 0.69
Female	7-10	7.88 ± 0.97
Adult		
Pre-oviposition	2-4	3.36 ± 0.70
Oviposition	2-4	3.56 ± 0.58
Post-oviposition	1-3	2.40 ± 0.65
Longevity		
Male	8-10	9.32 ± 0.75
Female	9-11	10.40 ± 0.82
Fecundity	200-305	265.40 ± 42.55
Sex ratio		
Field	1:1-1.50	1:1.36
Laboratory	1:1-1.27	1:1.15
Total life span		
Male	30-39	37.40 ± 2.06
Female	35-43	39.68 ± 2.36

*Five sets of twenty five numbers each

days with an average of 2.56 ± 0.58 days (Table 2).

The fifth instar larva had a stout body with dark brown head. It was dorsally dark green and ventrally light green. The black thoracic shield started to degenerate from the posterior portion. The length and breadth of fifth instar larva ranged from 15.40 to 18.00 mm with an average of 16.60 ± 0.75 mm and 1.73 to 1.99 mm with an average of 1.84 ± 0.09 mm, respectively (Table 2). The width of head capsule varied from 1.40 to 1.64 mm with an average of 1.55 ± 0.06 mm (Table 3). The duration of fifth instar larvae varied from 2 to 4 days with an average of 2.68 ± 0.75 days (Table 1).

The fully grown sixth instar larva having a stout body with dark brown head and green in colour but towards pupation turns light green. The black thoracic shield continued to degenerate, so exposing the greater part of the prothoracic segment. The length and breadth of sixth instar larva ranged from 25.50 to 29.00mm with an average of 27.78 ± 1.18 mm and 2.40 to 3.00mm with an average of 2.74 ± 0.24 mm (Table 2). The width of head capsule varied from 1.83 to 2.20mm with an average of 2.08 ± 0.11 mm (Table 3). The duration of sixth instar larvae varied from 2 to 4 days with an average of 3.04 ± 0.79 days. In present study the total larval period varied from 13 to 18 days with an average of 15.40 ± 1.63 days and comprised of six larval instars as confirmed through the Dyar's law of ratio of width of head capsules (Table 1-3).

Pre pupa: The mature larva stopped feeding and began searching for a suitable location to spin its cocoon. The larva spun a delicate silken cocoon in various locations such as the rolled edge of a leaf, between two leaves that are glued together. During this period, the larva contracts its body length and appendages, turning pinkish in colour. After these transformations, the larva enters the pre-pupal stage and becomes sluggish. The length of the pre-pupae ranged from 13.60 to 18.00mm with an average of

15.90 ± 1.50 mm, while the breadth ranged from 2.00 to 3.20mm with an average of 2.24 ± 0.32 mm (Table 2). The duration of pre-pupal stage varied from 1 to 2 days with an average of 1.80 ± 0.41 days (Table 1).

Pupa: The larvae of *H. derogata* preferred to pupate in rolled edge of cotton leaf or between two leaves. The newly formed pupae of *H. derogata* was reddish brown in colour but changed to dark brown colour after one day, long and cylindrical in shape. The length of male pupae ranged 10.10 to 12.90 with an average of 11.95 ± 0.78 mm. The length of female pupae varied from 12.50 to 13.85 with an average of 13.34 ± 0.49 mm, which was slightly more than male pupa. The breadth of male pupae ranged from 3.88 to 4.32 with an average of 4.19 ± 0.14 mm. Similarly, it was 4.00 to 4.43 with an average of 4.38 ± 0.11 mm in case of female pupa, which was slightly more than male pupa (Table 2). The duration of male pupae varied from 6 to 8 days with an average of 7.32 ± 0.69 days while, duration of female pupae varied from 7 to 10 days with an average of 7.88 ± 0.97 days, which was slightly higher than male pupal duration (Table 1).

Morphometrics of male and female pupae: The distance between genital pore and anal pore was

Table 2. Morphometrics (mm) of different life stages of *H. derogata*

Life stages	Length (mm)		Breadth (mm)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Egg	0.50-0.62	0.54 ± 0.03	0.45-0.55	0.47 ± 0.02
Larval instars				
I instar	1.80-2.30	2.04 ± 0.16	0.24-0.31	0.28 ± 0.02
II instar	3.28-4.00	3.51 ± 0.18	0.40-0.47	0.43 ± 0.02
III instar	7.00-8.00	7.70 ± 0.23	1.11-1.20	1.15 ± 0.03
IV instar	11.00-12.14	11.91 ± 0.40	1.50-1.60	1.54 ± 0.03
V instar	15.40-18.00	16.60 ± 0.75	1.73-1.99	1.84 ± 0.09
VI instar	25.50-29.00	27.78 ± 1.18	2.40-3.00	2.74 ± 0.24
Pre-pupa	13.60-18.00	15.90 ± 1.50	2.00-3.20	2.24 ± 0.32
Pupa				
Male	10.10-12.90	11.95 ± 0.78	3.88-4.32	4.19 ± 0.14
Female	12.50-13.85	13.34 ± 0.49	4.00-4.43	4.38 ± 0.11
Adult				
Male	9.50-12.40	11.48 ± 0.70	20.80-23.30	22.27 ± 0.67
Female	13.20-14.80	13.79 ± 0.53	26.20-30.00	28.12 ± 1.42

*Five sets of twenty five numbers each

more in case of female (0.80 to 0.90mm with an average of 0.85 ± 0.03 mm) than the male (0.36 to 0.50mm with an average of 0.39 ± 0.03 mm). Male pupa had genital aperture on ninth abdominal segment while, in case of female, it was on eighth abdominal segment (Table 1).

The moths of *H. derogata* displayed characteristics such as medium size and an attractive colour pattern. The colour ranged from white to creamy, with a network of brown markings on both the forewings and hind wings. These moths had filiform antennae and their thorax and abdomen were whitish yellow with yellow-brown bands and covered with white hairs. In terms of size, the males were smaller than the females. The male abdomen was slender and gently tapering at the tip, while the female abdomen was stouter and blunt at the tip. The length of male moths ranged from 9.50 to 12.40 with an average of 11.48 ± 0.70 mm, while the breadth with wing expanded varied from 20.80 to 23.30 with an average of 22.27 ± 0.67 mm. The length of the female moths ranged from 13.20 to 14.80 with an average of 13.79 ± 0.53 mm, while the breadth with wing expanded varied from 26.20 to 30.00 with an average of 28.12 ± 1.42 mm. The female moths were slightly bigger in size than the male moths (Table 2).

Pre-oviposition, oviposition, post-oviposition, longevity and fecundity: The pre-oviposition period of female moth of *H. derogata* varied from 2 to 4 with an average of 3.36 ± 0.70 days. The oviposition period of female moth ranged from 2 to 4 with an average of 3.56 ± 0.58 days (Table 1). After completion of egg laying the female moths lived for 1 to 3 days with on average post-oviposition period of 2.40 ± 0.65 days. The longevity of female moths ranged from 9 to 11 with an average of 10.40 ± 0.82 days, while the longevity of male moths ranged from 8 to 10 with an average of 9.32 ± 0.75 days (Table 2). The egg laying capacity of female varied from 200 to 305 with an average of 265.40 ± 42.55 eggs. The sex ratio of male: female was found to be 1:1.36 and 1:1.15 under field and laboratory condition, respectively indicating preponderance of female over male in field as well as in laboratory (Table 2). The total life cycle of *H. derogata* occupied on an average of 37.40 ± 2.06

days ranging from 30 to 39 days in case of male, while 39.68 ± 2.36 days ranging from 35 to 43 days in case of female (Table 1).

Bapodara (1973), Anioke (1989) and Bhatnagar *et al.* (1993) noticed that eggs were laid on upper or both side of the leaves. Bapodara (1973), Anioke (1989), Bhatnagar *et al.* (1993), Darji (1995), Shrivastava and Dhaliwal (2013) and Shakya and Saxena (2016) described that eggs were whitish in colour, spherical shape. However, Dhawan *et al.* (1981), Kumar and Nigam (1989) and Karpun *et al.* (2022) reported the eggs were greenish in colour. As per the report of Anioke (1989), who documented larger-sized eggs of *H. derogata* on okra and the eggs had length and breadth measurements of 0.76 ± 0.03 mm and 0.51 ± 0.01 mm, respectively which differ from the present study. The incubation period was 2.50, 3.00 and 3.50 days during July and August, September, October, respectively (Lal and Singh, 1951), 2.50 to 3.50 days (Khan and Rao, 1960), 5 to 7 and 3 to 5 days with different temperature and relative humidity (Bapodara, 1973), 2 to 4 days (Dhawan *et al.*, 1981), 3.10 ± 0.05 days (Anioke, 1989), 3 to 4 days (Kumar and Nigam, 1989), 2.40 ± 0.13 days (Bhatnagar *et al.*, 1993), 2 to 3 days (Darji, 1995) and 2 to 9 days (Shakya and Saxena, 2016). For the larval instar, six or occasionally seven instars in cotton (Bapodara, 1973), five or six instars on cotton and okra (Ahsan and Khalequzzaman, 1982; Anioke, 1989), five instars in cotton (Bhatnagar *et al.*, 1993; Darji, 1995), five instars in okra (Iyamu, 2012), seven larval instars in different hosts (Shrivastava and Dhaliwal, 2013; Atwal and Dhaliwal, 2013 and Sontakke *et al.*, 2015). In present study, *H. derogata* passed through six larval instars which were confirmed through application of Dyar's law. In case of sex ratio, Iyamu (2012) showed 1.5:1 in okra. These contrasts might be due to the difference in host crops and climatic condition. Further in the fields, no overlapping generations were observed and the pest easily monitored through the cut and half rolled leaves inside which larvae stayed and feed. The life cycle of leaf roller revealed the active period of 13-18 days mostly living their lives within the rolled leaves and that required managing through simply plucking the rolled leaves in September-

Table 3. Head capsule width of *H. derogata*

Larva	Head capsule width (mm)*	
	Range	Mean $\pm$ SD
I instar	0.21-0.25	0.23 $\pm$ 0.01
II instar	0.37-0.43	0.40 $\pm$ 0.02
III instar	0.89-0.95	0.93 $\pm$ 0.02
IV instar	1.10-1.25	1.19 $\pm$ 0.04
V instar	1.40-1.64	1.55 $\pm$ 0.06
VI instar	1.83-2.20	2.08 $\pm$ 0.11

S.D. = Standard deviation; *Application of Dyar's law on head capsule's width ratio of subsequent instars revealed uniform/constant variation

November and can be effectively managed by application of systemic insecticide in severe cases.

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Screening of rice cultivars against Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera, Gelechiidae)

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ABSTRACT: A study was conducted to screen twenty-five different rice cultivars against Angoumois grain moth, *Sitotroga cerealella* (Olivier) under laboratory conditions during 2020 - 2021 and 2021-2022 at Navsari Agricultural University, Navsari. From the pooled data of two year, it is observed that the cultivar IET-25470 recorded lowest number of adult emergence (72.83 adults) whereas the cultivar GNR -4 recorded highest number of adult emergence (119.33 adults) followed by the cultivar Ratnagiri-4 (110.50 adults). Regarding susceptibility index, the cultivar GNR 4 recorded highest susceptibility index (15.36) whereas cultivar IET- 25470, IET-25453, IET-24336, and IET-28713 recorded the lowest susceptibility index. The damage caused by *S. cerealella* to the grains of different rice cultivars revealed that the percentage of loss in grain weight was ranging between 4.08 to 8.19 per cent in different paddy cultivars. Further, Ratnagiri-5 recorded the lowest weight loss (4.08%) while the maximum weight loss was recorded in GNR 4 (8.19%).
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KEY WORDS: Resistance, cultivars, adult emergence, susceptibility index, grain weight loss

Rice (*Oryza sativa* L.) is the most widely consumed staple food in the world. It accounts for about of 60 to 70 per cent of the body calorie intake of the consumers (Das *et al.*, 2018). It is commonly known as ‘the Global grain’. About 90 per cent of the world’s rice is grown and consumed in Asia. Among Asia, India has the largest area under rice of 46.25 m ha (Kamboj *et al.*, 2023). The common biological agent for the loss and deterioration of stored rice are beetles and moths. Among the moths, the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera, Gelechiidae) is one of the principal causes of loss in storage (Hall, 1970). It is carried over from field to the storage through infested grains. In a field level survey Zote and Shukla (2023) reported 62.26 to 70.83 per cent grain

infestation. It is cosmopolitan in distribution. The moths are generally able to infest the surface layer of bulk-stored grain, as adults are unable to penetrate deeply. Only larvae are able to damage the commodities by boring into the grains and feeds about 30-50 per cent contents of the grain which ultimately gives unpleasant smell and unhealthy appearance (Bushra *et al.*, 2013). The emerging adult pushes the flap, which was already made by the final instar larva and forms the typical ‘circular-hole symptom’. The use of chemical insecticides has toxic effects on the environment and humans. It is very important to find an alternative against the chemical method of control. The innate capacity to resist the attack and damage by this pest on different rice genotypes will provide valuable

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information to the plant breeder for developing new cultivars, hence the studies were undertaken.

Experiments were conducted under laboratory conditions during 2020 - 2021 and 2021-2022 at Post Graduate Research Laboratory, Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat. Twenty-five different rice cultivars were screened for their susceptibility against *S. cerealella*. Twenty-five grams of apparently sound paddy grains of each cultivar were kept separately in plastic jars (6.0cm x 8.0cm). The paddy cultivars were obtained from Main Rice Research Station, Navsari Agricultural University, Navsari. Five pairs of newly emerged male and female moths were released for oviposition in each jar for three days. The top of the jar was covered with muslin cloth which was secured firmly with a rubber band. The jars were kept under laboratory conditions at $27 \pm 5^\circ\text{C}$ temperature and 70 ± 5 per cent relative humidity for the fresh moth emergence. From the day the new moth emergence started, moths were counted daily and removed from the jars to prevent further oviposition. The observations were recorded until the emergence stopped. When emergence of adults stopped, number and weight of healthy and damaged grains in each jar were recorded. The experiment was repeated thrice with complete randomized block design. The cultivars were screened on the basis of susceptibility index and damage.

The susceptibility index of each cultivar was calculated by applying formula suggested by Dobie (1977) based on total number of adults emerged and mean developmental period.

$$\text{Susceptibility Index} = \frac{\text{Natural log F}}{D} \times 100$$

Where, F= Number of adults emerged,
D=Mean developmental period

For the determination of damage, healthy and damaged grains in each jar were counted and weighed separately. The per cent weight loss was calculated using formula suggested by Adams and Schulten (1978) as below -

$$\text{Weight loss(\%)} = \frac{(UNd) - (DNU)}{U (Nd + Nu)} \times 100$$

Where, U = Weight of undamaged grains, Nu = Number of undamaged grains, D= Weight of damaged grains and Nd = Number of damaged grains.

The pooled data of two years showed that the cultivar IET -25470 recorded lowest number of adult emergence (72.83 adults) which was significantly superior over rest of the cultivar. The cultivar GNR-4 recorded highest number of adult emergence (119.33 adults) followed by the cultivar Ratnagiri-4 (110.50 adults). Regarding susceptibility index, the cultivar GNR -4 recorded highest susceptibility index (15.36) followed by the cultivar GNR-7(14.98) and Panvel-2 (13.98) and they are at par with each other. The cultivar IET-25470, IET-25453, IET-24336 and IET-28713 recorded the lowest susceptibility index *i.e.* 11.15, 11.18, 11.46, and 11.87, respectively and they were at par with each other (Table 1).

The damage caused by *S. cerealella* to the grains of different rice cultivars on the basis of weight and number, (in the pooled data of two year), showed grain weight loss in the range of 4.08 to 8.19 per cent (Table 1). Among cultivars, Ratnagiri-5 recorded the lowest weight loss (4.08%) where as it was at par with rice cultivars IET-25477, IET-25453, IET-26376 and IET-28005. The maximum weight loss was noticed in cultivar GNR-4 (8.19%), it was at par with the cultivars like GNR-3, GR-17, IET-25453, IET-24336 and IET-26376. The per cent damaged grains (number basis), the cultivar GR-19 recorded lowest number of grain loss (3.15). The maximum grain weight loss was observed in Panvel-1 (10.15).

In present investigation the results revealed that none of the cultivars was completely immune to the attack of *S. cerealella* in storage only the degree of infestation varies. Pandey *et al.* (1980), Khattak and Shafique (1981) and Khattak and Shafique (1986) reported similar results. Shifque and Chaudry (2007) suggested that the low insect population and low weight loss of grain can be used as one of the attributes of the grain resistance to insect. Singh *et al.* (2007) screened 10 rice varieties against

Table 1. Mean number of adults emerged, susceptibility index and grain damage (on weight and number basis) of different paddy cultivars due to *Sitotroga cerealella* (Two year pooled data)

Cultivar	AE	SI	Damage (%) on	
			Weight	No.
GNR-4	119.33	15.36	8.19	9.57
GNR-3	97.67	13.00	7.04	7.05
GNR-7	98.17	14.98	5.73	5.62
GNR-8	73.83	12.64	5.10	5.81
GR-15	90.50	12.72	6.27	5.84
GR-17	84.83	12.12	5.41	7.58
GR-19	95.50	12.67	7.00	3.15
IET-25453	94.00	11.18	5.15	6.23
IET-25470	72.83	11.15	5.64	6.18
IET-25477	77.67	11.86	5.38	5.25
IET-26376	98.83	12.33	6.46	5.05
IET-28713	87.67	11.87	5.75	6.04
IET-24336	82.17	11.46	6.14	5.45
IET-28005	91.67	12.02	6.61	4.03
IET-28705	95.33	12.58	4.66	7.31
NAUR-1	95.50	13.67	5.34	10.05
Ratnagiri-1	107.17	13.86	5.31	8.37
Ratnagiri-4	110.50	12.99	5.58	5.63
Ratnagiri-5	103.00	12.73	4.08	6.75
Ratnagiri-7	98.17	12.78	5.04	7.81
Ratnagiri-8	97.67	12.04	6.80	6.59
Ratnagiri-24	109.00	12.01	5.80	8.31
Panvel-1	103.00	13.02	4.31	10.15
Panvel-2	98.00	13.98	5.20	7.15
Panvel-3	99.00	13.29	4.07	5.18
SEm+	7.52	0.49	0.87	1.29
CD at 5 %	21.96	1.44	2.54	3.76
CV(%)	4.02	3.15	11.21	7.02

AE = Adult emergence; SI = Susceptibility Index

S. cerealella up to 3, 6 and 9 months of storage and reported that Kalanamak appeared with 23.5 per cent grain damage (greater susceptibility) followed by Pankaj, Sona and Basmati during 9 months of storage. However, IR-8 and Jaya showed the least infestation with 7.9 and 9.8 per cent grain damage, respectively. The results of this study closely matched those of Saadia *et al.* (2011), who found that no cultivar was totally resistant to *S. cerealella* infestation. In a study, Kumar *et al.* (2018) also reported that the rice varieties with the most *S. cerealella* grain damage showed the greatest weight loss. Further, Mathew *et al.* (2019) observed that none of the fourteen varieties and sixty-six genotypes of paddy were entirely resistant to *S. cerealella*. These past works mostly support the current findings. From the study it can be concluded that out of 25 rice cultivars, none of the cultivar found resistant against grain moth only the degree of infestation varied. Cultivars IET-25470 and IET-25453 were relatively less susceptible.

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Exploring plant seed extracts as potential sources of trypsin inhibitors for insect pest management: screening and characterization of inhibitory activity

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ABSTRACT: This study aimed to identify seeds containing trypsin inhibitors by screening 17 plant species. Plant seed extracts were prepared by homogenizing soaked seeds in a bicarbonate buffer (pH 9.0), followed by centrifugation. The resulting supernatants, rich in soluble proteins, were subjected to a protease inhibition assay. Among the seventeen seed extracts examined, ten exhibited significant trypsin inhibition, with notable examples including *Pterocarpus marsupium* ($98.20 \pm 0.5\%$), *Barleria repens* ($97.75 \pm 0.25\%$), *Ipomoea sepiaria* ($93.80 \pm 0.15\%$) and *Distimake vitifolius* ($91.79 \pm 0.71\%$) have not been previously reported as sources of trypsin inhibitors. © 2025 Association for Advancement of Entomology

KEY WORDS: Inhibitor, protease inhibition assay, serine protease, pest management

Insect pests intensively feed on host plants to obtain sufficient nutrients for their growth and development. This process involves a complex interplay of proteases, including trypsin, chymotrypsin, aminopeptidase, elastase, and carboxypeptidase, which aid in the digestion of proteins within the caterpillar's digestive system. These proteases are categorized into exopeptidases, which cleave proteins from the N or C-terminus, and endopeptidases, which cleave internal peptide bonds (Fan and Wu, 2005; Rawlings *et al.*, 2010). Lepidopteran insects are some of the most significant pests threatening various economically valuable crops. During their larval stage, these insects predominantly utilize serine proteases for digestion, with trypsin-like enzymes constituting

around 90% of their digestive protease profile, while chymotrypsin-like enzymes represent about 5% (Srinivasan *et al.*, 2006).

Plant protease inhibitors (PIs) are natural, biodegradable defense molecules that can be utilized as biochemical markers to identify crop plants with varying resistance levels against insect pests (Swathi *et al.*, 2021). Seeds harbour relatively high levels of proteinase inhibitors, most of which exhibit activity against serine endopeptidases (Chougule *et al.*, 2008). PIs, particularly serine group inhibitors like Bowman-Birk inhibitors (BBIs) and Kunitz inhibitors (KIs), have shown promise in managing lepidopteran pest populations. These plant PIs often bind to the active site of enzymes, forming enzyme-

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inhibitor complexes that block their activity (Norton, 1991; Nanasahe *et al.*, 2008). The inhibition of protease enzymes leads to a depletion of essential amino acids crucial for insect growth and development (Leo *et al.*, 2002). Specifically, the unavailability of essential amino acids in the insect body due to the inhibition of trypsin-like enzymes can result in insect pest mortality (Samiksha *et al.*, 2019). As evidenced by reports from diverse plant species, plants containing serine protease inhibitors can resist various insects and pathogens (Leung *et al.*, 2000; Oliva and Sampaio, 2009; Ramos *et al.*, 2009). Leguminous plant seeds, in particular, are abundant in PIs, which have been shown to inhibit insect gut proteolytic activity both in vivo and in vitro (Richardson, 1991). Therefore, employing PIs in resistance breeding programs could facilitate the development of innovative defense strategies in domestic crop varieties. Additionally, a gene stacking approach with different mechanistic classes of PIs can effectively target the dynamic expression of digestive proteases in highly polyphagous pests, such as *Helicoverpa* and *Spodoptera* (Swathi *et al.*, 2021). Hence, the study aimed to screen plant seed extracts to identify seeds containing the protease enzyme (trypsin) inhibitors, thereby contributing to our understanding of plant defenses against insect herbivory.

Reagents: N- α -Benzoyl-DL Arginine-P-NitroAnilide (BAPNA) was purchased from Sigma-Aldrich Chemicals, and trypsin was purchased from HIMEDIA, India. All other chemicals were of analytical grade. The reagents used were 200 mM Tris Buffer with 20 mM Calcium Chloride, pH 7.8, 0.1 per cent (w/v) N- α -Benzoyl-DLArginine-P-NitroAnilide (BAPNA). Hydrochloric Acid Solution, 1 mM. Trypsin solution (Dissolved 2.5mg of Bovine trypsin in 20ml of cold Reagent C (7mM Na-K tartrate, 0.81M sodium carbonate, 0.5N NaOH final concentration)), 0.9 per cent (w/v) Sodium chloride solution.

Protease assay: Protease assay was carried out in a total volume of 1ml containing 536 μ l Tris Buffer (200 mM Tris; 20 mM Calcium chloride, pH 7.8), 67 μ l bovine trypsin (0.125 mg/ml; dissolved in 1mM HCl), 67 μ l NaCl (0.9%) and 330 μ l N- α -

Benzoyl-DLArginine-P-NitroAnilide (BAPNA) (1mg/1ml) as substrate. A blank was also done along with it by adding 67 μ l 1mM HCl, instead of trypsin (Divya *et al.*, 2014).

Protease inhibition assay: Seventeen plant species were selected for this study based on the availability of the seeds and traditional knowledge. Fully matured dry seeds of *Albizia lebbbeck*, *Barleria repens*, *Bixa orellana*, *Calliandra haematocephala*, *Cleome viscosa*, *Crotalaria pallida*, *Cucumis sativus*, *Fioria vitifolia*, *Hibiscus surattensis*, *Ipomoea sepiaria*, *Leucaena leucocephala*, *Distimake vitifolius*, *Passiflora foetida*, *Pterocarpus marsupium*, *Swietenia mahagoni*, *Trichosanthes cucumerina*, and *Vigna trilobata* were collected from Calicut University Botanical Garden (11°8'1.57"N; 75°53'21.74"E), Thodeekkalam (11°51'12.51"N; 75°39'18.96"E), and Srirampura (13°3'51.15"N; 77°37'12.76"E). These were harvested at their peak ripeness and then dried under shade to ensure consistency in our extraction process. The seeds were washed and soaked in bicarbonate buffer (pH 9.0; 1 ml/g tissue) overnight, then homogenized. The homogenates were centrifuged at 10,000 $\times$ g in a Rota 4R-V/FA, Plasto Crafts centrifuge for 10 minutes at 4°C, and the supernatant containing soluble proteins was used for the protease inhibition assay.

In protease inhibition assay the extract was pre-incubated with the enzyme (25 μ l of plant extract with 67 μ l of trypsin (0.125 mg/ml⁻¹) for 10 minutes, followed by addition of 67 μ l NaCl (0.9%) and 511 μ l Tris buffer. The reaction was started by adding 330 μ l BAPNA (1mg ml⁻¹). Proteolytic activity was measured by continuous spectrophotometric rate determination method using UV Spectrophotometer, by recording the increase in absorbance at 405nm for 5minutes. All assays were done three times in duplicate and percentage of inhibition calculated by taking the activity of the enzyme alone as 100% (Divya *et al.*, 2014). We took meticulous care to ensure that the pH of the assay medium was consistently maintained at 7.8 across all experiments.

Calculations: The absorbance of the control was subtracted from the absorbance of the inhibitor alone to quantify the protease activity attributed to the plant extract. This calculated value was then subtracted from the absorbance measured in the presence of both the inhibitor and the enzyme, allowing for the determination of the actual absorbance devoid of protease activity from the plant extract. The absorbance of the enzyme alone was designated as representing 100 per cent enzyme activity. Control Activity ($\Delta A_{405\text{nm}}/\text{min}$) represents the enzyme's absorbance rate without any plant extract, and taken as 100 per cent activity. With this reference point, the absorbance recorded in the presence of the inhibitor was translated into percentage activity. Finally, this percentage was subtracted from 100 to derive the percentage inhibition.

Percentage inhibition is calculated as follows:

$$= (\Delta A_{405\text{nm}}/\text{minute uninhibited} - \Delta A_{405\text{nm}}/\text{minute inhibited}) / (\Delta A_{405\text{nm}}/\text{minute uninhibited} - \Delta A_{405\text{nm}}/\text{minute blank}) \times 100$$

Seventeen plant seeds were screened to identify protease inhibitors targeting trypsin. Among these, ten seed extracts exhibited significant trypsin inhibition, with inhibition levels exceeding 40 per cent (Table 1). In this study, *Pterocarpus marsupium* showed the highest trypsin inhibition at 98.2 per cent $\pm$ 0.5 per cent, followed closely by *Barleria repens* (97.75% $\pm$ 0.25%), *Ipomoea sepiaria* (93.80 per cent $\pm$ 0.15%), *Distimake vitifolius* (91.79% $\pm$ 0.71%), and *Calliandra haematocephala* (88.74 per cent $\pm$ 0.26%). There are no prior reports of trypsin inhibitors from these seeds, highlighting their potential as novel sources. In contrast, previous research has documented trypsin inhibitors in other species of the *Calliandra*. For example, *C. selloi* Macbride demonstrated a trypsin inhibitor with a molecular weight of approximately 20,000 Da (Yoshizaki *et al.*, 2007), supporting the relevance of *Calliandra* species as potential sources of protease inhibitors.

In agreement with earlier studies, *Albizia lebbbeck*

exhibited substantial trypsin inhibition, showing 80.8 per cent inhibition in our study. Prior study identified a Bowman-Birk inhibitor from *A. lebbbeck* that was effective against gut proteases of *Helicoverpa armigera*, leading to larval mortality and growth suppression in *Pieris brassicae* and *Spodoptera littoralis* (Hivrale *et al.*, 2013). This consistency across studies reinforces the potential of *A. lebbbeck* for pest management.

Similarly, *Vigna trilobata* demonstrated 76.7 per cent trypsin inhibition, despite a lack of prior reports documenting protease inhibitors from this species. Nonetheless, study on *Vigna unguiculata* has identified inhibitors belonging to the Bowman-Birk family (Parveen *et al.*, 2018), indicating a possible commonality within the genus. *Fioria vitifolia* and *Cleome viscosa* showed trypsin inhibition at 49.43 and 40.15 per cent, respectively. The observed inhibition might be linked to phytochemicals known for anti-proteinase activity (Pillai and Nair, 2014). *Crotalaria pallida* demonstrated 46.33 per cent inhibition, which aligns with previous findings of proteinaceous trypsin inhibitors effective against various pests, including *Callosobruchus maculatus* and *Spodoptera mauritia* (Gomes *et al.*, 2005; Remya and Kannan, 2019). For the first time, this study reports seven plant seeds such as *P. marsupium*, *B. repens*, *I. sepiaria*, *D. vitifolius*, *C. haematocephala*, *V. trilobata* and *F. vitifolia* as sources of PIs targeting trypsin. These findings suggest broader applications in pest management, particularly in targeting lepidopteran pests.

Plant-derived protease inhibitors (PIs) play a key role in plant defense, both in natural ecosystems and agricultural systems. The effectiveness of PIs in various plants is well-documented. In this study, among the 17 plant species screened, five species from the Fabaceae family demonstrated significant trypsin inhibition, each exhibiting more than 40% inhibitory activity. This notable trend highlights the potential of Fabaceae species as promising sources of protease inhibitors, warranting further investigation into their biochemical properties. For instance, soybean-derived inhibitors like the Kunitz soybean trypsin inhibitor (STI) and the Bowman-Birk inhibitor (BBI) exhibit significant anti-

Table 1 List of plant seed extracts exhibiting trypsin inhibition, with inhibition values presented as the mean of six experiments along with standard error (SE)

No.	Plant species	Family	Common name	Mean $\pm$ SE % inhibition
1	<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	Indian kino tree	98.20 $\pm$ 0.50
2	<i>Barleria repens</i> Nees.	Acanthaceae	Small bush violet	97.75 $\pm$ 0.25
3	<i>Ipomoea sepiaria</i> Roxb.	Convolvulaceae	Purple heart glory	93.80 $\pm$ 0.15
4	<i>Distimake vitifolius</i> Burm. f.	Convolvulaceae	Grape leaf wood rose	91.79 $\pm$ 0.71
5	<i>Calliandra haematocephala</i> Hassk.	Fabaceae	Red powder puff	88.74 $\pm$ 0.26
6	<i>Albizia lebbeck</i> (L.) Benth.	Fabaceae	Indian siris tree	80.80 $\pm$ 0.06
7	<i>Vigna trilobata</i> (L.) Verdc.	Fabaceae	Wild gram	76.7 $\pm$ 1.90
8	<i>Fioria vitifolia</i> (L.) Mattei	Malvaceae	Wild okra	49.43 $\pm$ 0.57
9	<i>Crotalaria pallida</i> Ait.	Fabaceae	Streaked rattle pod	46.33 $\pm$ 1.66
10	<i>Cleome viscosa</i> Linn.	Cleomaceae	Asian spider flower	40.15 $\pm$ 0.85
11	<i>Bixa orellana</i> Linn.	Bixaceae	Lipstick plant	36.00 $\pm$ 1.10
12	<i>Cucumis sativus</i> Linn.	Cucurbitaceae	Cucumber	30.50 $\pm$ 0.40
13	<i>Hibiscus surattensis</i> Linn.	Malvaceae	Bush sorrel	28.10 $\pm$ 0.80
14	<i>Leucaena leucocephala</i> (Lam.) de Wit	Fabaceae	Lead tree	24.04 $\pm$ 0.75
15	<i>Passiflora foetida</i> Linn.	Passifloraceae	Stinking passion flower	22.23 $\pm$ 1.00
16	<i>Swietenia mahagoni</i> Linn.	Meliaceae	Mahogany	19.50 $\pm$ 1.50
17	<i>Trichosanthes cucumerina</i> Linn.	Cucurbitaceae	Snake gourd	8.79 $\pm$ 1.50

proteolytic activities and are widely distributed across plant families like *Gramineae* (Poaceae), *Leguminosae* (Fabaceae), and *Solanaceae* (Haq *et al.*, 2004; Bijina *et al.*, 2011; Marathe *et al.*, 2019). Bowman-Birk inhibitors, such as those from *Dolichos biflorus*, have demonstrated effectiveness in deterring herbivores and fungal pathogens, highlighting the multifunctionality of PIs (Kuhar *et al.*, 2013). These inhibitors often exist as zymogens, inactive forms activated under stress, enhancing their defensive capabilities (Graham *et al.*, 1985; Koiwa *et al.*, 1997).

In agricultural contexts, PIs are valuable as natural insecticides. These proteins specifically target digestive enzymes in herbivorous insects' midguts, impairing digestion and leading to slowed growth and mortality (De Leo *et al.*, 2001; Ribeiro *et al.*, 2006). PIs offer a sustainable alternative to chemical pesticides by targeting pests with minimal

impact on beneficial organisms, thereby preserving ecological balance (Gatehouse *et al.*, 1997; Schneider *et al.*, 2017; Marathe *et al.*, 2019). Studies have consistently demonstrated the efficacy of PIs in pest control, making them suitable for integrated pest management (IPM) strategies (Dunse *et al.*, 2010; Pawar *et al.*, 2024). Although transgenic crops expressing insecticidal genes have gained popularity, concerns about pest resistance and consumer acceptance remain, particularly regarding Bt crops. PIs, being naturally occurring, offer a safer alternative for pest management with fewer health risks and greater public acceptance (Kumar, 2024). Hence, this study underscores the significance of plant-derived protease inhibitors in natural pest management and sustainable agriculture. The newly identified inhibitors from plant seeds suggest the possibility of developing effective, eco-friendly strategies for IPM.

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Strategy on the predation of arboreal weaver ant *Oecophylla smaragdina* (Fabricius) on a ground nesting ant *Camponotus compressus* (Fabricius)

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ABSTRACT: The weaver ant *Oecophylla smaragdina* (Fabricius) occurring in Jhalda, Purulia of West Bengal, India feeds on different kinds of prey animals. These ants attack the ant *Camponotus compressus* in their natural habitats to feed on them. *C. compressus* are larger in size in respect to the predator ant *O. smaragdina*. Therefore, they have developed the art of group attack to capture the prey individual. In this article, described how *O. smaragdina* targeted and immobilized its prey on way of biting the body parts to ensure stretching the trapped individual prior to carry the same cooperatively to the canopy nest.
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KEY WORDS: Weaver ant, prey, predator behaviour, art of group attack

The widely distributed (Wetterer, 2017) Asian weaver ants *Oecophylla smaragdina* (Fabricius) are predacious in habit as they prey upon various types of arthropods, arachnids and other invertebrates (Belcher and Kuster, 2004; Gathalkar and Barsagade, 2016; Babu and Patil, 2021). Thus, in several cases these ants have been proved effective to control the pest population (Peng *et al.*, 2012; Offenberger *et al.*, 2013; Pierre and Idris 2013). In their natural habitat they actively seek out for honeydew (Blüthgen and Fiedler 2002), while in experimental conditions they never spared jaggery, sugar cubes, honey, fragments of biscuits, fish and meat (Selvam and Nalini, 2021). Also the role of *O. smaragdina* in shaping the plant-pollinator interactions did not escape the sight of

Rodríguez Gironés and coworkers (2013). Reports on the predatory behaviour of *O. smaragdina* though available on various prey animals; information on their predation of other ant species is not documented. However, Gotwald (1972) described the predatory behaviour of *O. longinoda* (Latreille) on *Anomma* (Savage) driver ants. During the course of studies on the bioecology of some ant species noted the predation behaviour of *O. smaragdina* on *Camponotus compressus* (F.) (Hymenoptera, Formicidae), ant in its natural habitat. The arboreal ants *O. smaragdina* are very common in different trees of Jhalda (Latitude- 23° 22' N; Longitude- 85° 59' E) area of Purulia district, West Bengal, India. They regularly descend from their arboreal nests to forage on the ground. They exhibit

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opportunistic foraging behaviour, searching for and capturing a variety of arthropod prey items, including adults, eggs, and larvae. Though, customarily ants prey upon many invertebrates it is not clearly known how many of these ant predators prey upon other ant species.

In the course of experimental studies on the food preference of ants, noted the capturing behaviour of *O. smaragdina* four times to the prey ant *Camponotus compressus*, of course, in a group as *C. compressus* is quiet larger in size in respect to the size of an *O. smaragdina* worker. The nests of *O. smaragdina* were located high in the canopy of a large mango tree (*Mangifera indica* L.) while foraging *C. compressus* were observed emerging from their nest at the base of a moringa tree (*Moringa oleifera* Lam.) locating 2.5 meters away. Documented the observations by recording data on sequential events on the capturing process of the powerful prey and by photographs. Such incidents were seen on sunny days between 10.30 am and 3.50 pm during September-October (dew season) period in the study site.

There were 12 instances where *O. smaragdina* ant isolated one *C. compressus* individual from the row of *C. compressus*, and 8 events where *O. smaragdina* trapped *C. compressus* individual moving alone in the foraging ground. In cases of snatching a *C. compressus* individual from the row an *O. smaragdina* was seen to give a sudden bite to a leg. The said *C. compressus* perhaps, because of biting pain moved out of the row where she had to encounter with many other *O. smaragdina* but finally trapped by the predators' group attacking strategy. In three instances, a solitary *C. compressus* was observed being attacked by a pair of conspecifics, which bit its legs. In the remaining five cases, group attacks were initiated after a single *O. smaragdina* worker bit the leg of a *C. compressus* individual. Surprisingly, in all cases within few seconds the attacked *C. compressus* was encircled by many *O. smaragdina* individuals (Fig. 1A). Of the assembled *O. smaragdina* some were seen in direct attacking state, i.e. each leg of the prey was deactivated due to bite of either one or more individuals. Also the movement of

appendages was seized through the strong biting at the terminal point of the same by the predator ant. Then, each of these biting act, *O. smaragdina* individual was seen to stretch the prey's body for a period of 12-18 minutes so as to stop any kind of movement and/or counter blow of the trapped prey individuals. But, in some cases when the prey individual, even after adoption of such strategy to immobilize the same, tried to escape the predator's trap the predator ants were seen to ride over the body of the prey individual to give a bite at the cervix as well as moved close to the body of the prey to bite the abdominal part. Also, in some cases one predatory individual was seen to find access to bite the petiole even ventrally to induce paralysis (Fig. 1B). That is, grasped the prey biting the legs, antenna, petiole and gaster. Moreover, following grasping in respect to prey individual's repeated attempt to get rid of the danger, on way of undulation of her body, some *O. smaragdina* workers triggered to bite the wall of the abdomen, thorax and cephalic portion of and on. Finally, the prey became silent. But, *O. smaragdina* did not move at least for a period of 3-7 minutes from that spot of the foraging ground. Thereafter, they were seen to move in a coordinated way; forward by applying pulling and pushing strategies by holding the prey in a stretched condition.

It is evident that *C. compressus* being much larger in size are victimized by the much smaller sized *O. smaragdina* but in a cooperative manner through group activities. It seems that the biting of *O. smaragdina* is painful to *C. compressus* and thus only one bite to a leg is enough to detract the prey individual from the trail of the same. It is most likely that *O. smaragdina* releases certain chemicals during such an operation so as to ensure the arrival of many more foragers moving around.

However, the recruitment of additional workers is a time-consuming process. When a large number of workers gather around a *C. compressus* individual, it cannot be excluded that they release chemical signals. In this context information provided by Vidhu and Evans (2011a, b; 2014, 2015a, b) and Gathalkar and Barsagade (2016) that (i) the major and/or intermediate worker castes of



Figs. 1A-B Chronological behavioural sequences during capturing the ant prey *C. compressus* by the weaver ants *O. smaragdina*

- A — On the right side of the photograph: targeted *C. compressus* is snatched away from the row; in the middle of the photograph: the strategy the weaver ants applied to seize her activity
- B — An *O. smaragdina* is trying to bite the cervix of the prey individual, another one is trying to bite the petiole of the prey ventrally, still another one is trying to bite the abdominal wall

O. smaragdina during the course of bite to the prey are habituated to spray formic acid instantly together with a group of volatile compounds by lifting and their gaster towards the site of bite; (ii) the formic acid then enters into the victim's body through the puncture made by mandibles while volatile compounds act as a pheromone and direct the other ants to join the act of predation, and (iii) they are brutally aggressive, are strategic devices applied by *O. smaragdina* during predation. Thus, it could safely be concluded that the behavioural events exhibited by *O. smaragdina* ants during the present study are very much influenced by the factors.

From the photographs it is clear that the weaver ants positioned themselves at all possible points encircling the body of the prey individual where workers found the body part of the prey to bite. Such type of group attack have also been noted in *Oecophylla longinoda* while capturing the *Anomma* ant prey (Gotwald, 1972). Moreover, immobilization of prey individual through prolonged stretching by *Oecophylla* ants is a common device as is evident from the studies of Weber (1949), Ledoux (1950), Way (1954), Gressitt (1956) and Vanderplank (1960). Elongation of doriliane ants is also in practice by the predacious ants *Eciton burchelli* (Westwood) and *Labidus coecus* (Latreille) (Schneirla, 1971). Predators typically launch surprise attacks to immobilize their prey. Immobilization may be achieved by inflicting physical damage, such as biting and creating wounds, or by injecting toxic substances. Since, *O. longinoda*, *Eciton burchelli*, *Labidus coecus* are devoid of such prey capturing weapons they have developed the art of stretching the prey individual for a considerable length of time to kill the same and to torn the body of the prey individual into pieces to facilitate transporting the same to the nest (Schneirla, 1971; Gotwald, 1972).

In the present study it is clear that *O. smaragdina* stretched the prey ant *C. compressus* to kill the same but the body of the prey was never separated into pieces. Rather, the weaver ants transported the prey individual as such to the destination. Though previous workers are in opinion that the

aim of stretching of prey is to torn the body into pieces it is very hard to accept the idea. Removing a leg from the prey's body would not simplify the transportation process. In fact, it would complicate the task of carrying the prey. Because cooperative transportation (Czaczkes and Ratnieks, 2013; McCreery and Breed, 2014; Buffin and Pratt, 2016; Naskar and Raut, 2018; Naskar *et al.*, 2023; Burchill *et al.*, 2023), especially vertically would prove effective and faster if the body of the prey could be kept high up from the surface to avoid any kind of hurdle or obstructions. This could be substantiated from the facts of association of a good number of weaver ant workers encircling the workers engaged in stretching the prey ant (Gotwald, 1972). Food value of *O. smaragdina* is very high and accordingly they are consumed by the tribal people by different means (Vidhu and Evans, 2015a; Mitra *et al.*, 2020).

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OBITUARY



Prof. V.K.K. Prabhu 1935–2025

Born on 17th March 1935 to Mr. Krishna Prabhu and Mrs. Sundari Bhai at Kottayam in Kerala state, Kesav Prabhu has had his early education in the town itself. He passed ESLC examination with first class in 1951. After completing his intermediate programme in CMS college Kottayam with first class and distinction, Keshav Prabhu continued his under graduate education in SB college Changanassery, which also he passed with first rank and first class. His main option was zoology with chemistry as subsidiary, for which he won the C. John Memorial gold medal. Young Prabhu continued his college education and joined for Master's degree in Zoology in the University college, Trivandrum and passed out with colours. He continued for doctoral studies on nerve anatomy and neuro secretion in *Jonespeltis splendius*, an arthropod. All his degrees were from the University of Travancore/ University of Kerala; subsequently he worked as a Post-Doctoral fellow and a Research Associate, mentored by Prof K.K. Nayar at the Department of Zoology, University of Kerala. He started his career as a Lecturer in Zoology in the University of Kerala at Calicut centre from 1964, continued in that capacity or on deputation till he became a Reader in 1975. He was appointed as a Professor in the Department of Zoology, University of Kerala in 1978. In the early 1970s, Prof. Prabhu engaged classes in physiology to Biochemistry students in the Kerala University Senate campus as testified by Prof. P.R. Sudhakaran, who later became the Head of that department. Prof. Sudhakaran remembers him as a very disciplined scientist and as an efficient teacher.

Prof. Prabhu was on deputation in U.S.A as a Fellow at Worcester foundation on experimental biology at Massachusetts under the Ford foundation training program on Reproductive Biology. He also was on deputation for a training in electron microscopy in Germany in early 1970s. Prof. Prabhu participated and presented papers in several international symposia held in Czech Republic, New Delhi, Japan, Hong Kong etc, with Commonwealth foundations grant. He also participated, presented papers and chaired sessions in several National Symposia. He mentored around 8 people for Doctoral Research and several students for their MPhil and Master degrees. Prof. Prabhu was a well-known insect physiologist after his mentor K.K. Nayar, who was a well-known insect neuroendocrinologist and physiologist internationally. In fact, Prof. K.K. Nayar brought in grants to the University of Kerala from Ford foundation and PL480 scheme from U.S. that helped Prof. Prabhu also to continue that legacy. Well known Comparative

Endocrinologists like Prof A. Gorbman and Prof H.A. Bern visited the Department of Zoology several times in the 1970s on his invitation. He has been a member of several scientific societies including the New York Academy of Sciences.

Prof V.K.K. was closely associated with the establishment of Association for the Advancement of Entomology, back in 1975. When Prof. Nayar passed away in 1975, Prof V.K.K. took the leadership in starting the Journal ENTOMON, as Managing Editor and the first issue was brought out in 1976 dedicating to Prof. Nayar. Prof. V.K.K. Prabhu had been Secretary-Treasurer of the Association for the Advancement of Entomology and the Managing Editor of the prestigious journal ENTOMON for several years from 1975. His tenure as the Managing Editor of ENTOMON for many years and brought name and fame to the Journal and still ENTOMON stands out as a prestigious Journal in our Country. It is his hard work during the formative years that maintained the periodicity and quality of the Journal. As part of the Silver Jubilee Celebrations of AAE, Entomocongress - an International Congress was organised in Technopark, Trivandrum organised under the able guidance and leadership of Prof V.K.K.

I am very happy that Prof Prabhu had completed four research projects funded by national funding agencies including UGC and INSA. His contribution to teaching and research in Zoology, particularly in the area of Entomology in our state are enormous. Prof. Prabhu had been the member of Board of Studies of the Universities of Kerala and Calicut, was the member of the Faculty of Sciences in the University of Kerala, Scientific panel of the Indian Council of Agricultural Research for Entomology and Nematology. He was on the panel of Referees of several agencies in India and abroad. He had evaluated several research projects submitted to CSIR, ICAR, DST, and BARC. He was on the board of examiners to evaluate PhD theses of several Universities in India and abroad.

I have had the privilege of working with him for over 20 years as a faculty in the Department and enjoyed his suggestions in the running of Department of Zoology for several years. His contributions to the Department will be remembered by his Doctoral students who later occupied several key positions in India and abroad and all his Masters and M Phil students who also entered into the scientific arena of our state and elsewhere. His doctoral students included Dr. R.V. Varma, the late Dr. V.K. Krishnan Nair, Dr. D. Muraleedharan, Dr. Ambika Varma, Dr. Geetha Bhaskar, Dr. Mariamma Mathew, to name a few. Prof. Prabhu's contributions to science in general and insect physiology, in particular will be remembered by scientists working in those areas through his publications in both well-known national and international journals. His contributions include Hormonal regulation of feeding digestion and reproductive aspects in insects with insight into insect endocrinology as well. His studies on *Dysdercus cingulatus*, *Hyblea puera* etc., demonstrated the endocrine mechanisms controlling insect growth and metabolism. His researches on the effects of plant-derived compounds on ovarian development in juvenile insects paved the way for developing natural alternatives to synthetic pesticides. His works extended beyond physiology and led to environmentally safe pest control strategies.

While we mourn his loss, we also celebrate his enduring legacy-something which will continue to sustain through the knowledge he imparted, the research he pioneered and the students he mentored.

He is survived by his wife, Dr. Nirmala Prabhu, sons, Dr. Nirmalkumar Prabhu and Dr. Pradeep Prabhu, all medical professionals and their families.

May his soul Rest in Peace.

Prof. Dr. Oommen V. Oommen FNASc., FRE, FAS

Former Professor and Head,
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