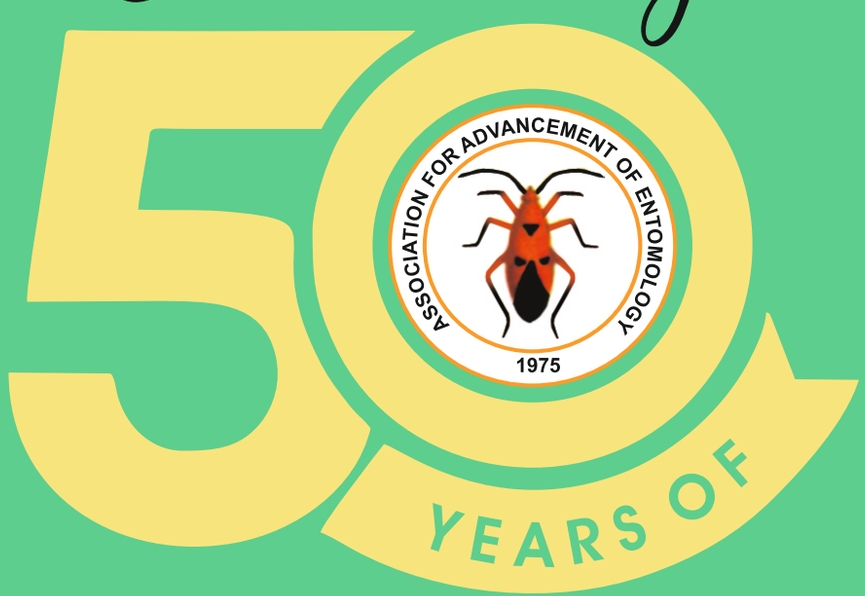
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ENTOMON

Vol. 51 *September 2026* *No. 3*

Contents

Articles Page

https://doi.org/10.33307/entomon.v51i3.1702 275
A taxonomic account of four species of the genus *Palpita* Hübner, 1806 (Lepidoptera, Crambidae) from Jammu and Kashmir, India
Amritpal Singh Kaleka, Sami-Ul-Hassan Tantary and Manmeet Pal Kaur

https://doi.org/10.33307/entomon.v51i3.1703 289
Evaluation of antagonistic *Pseudomonas* and *Bacillus* spp. against *Meloidogyne incognita* causing root-knot disease in okra
Jayanta Kumar Mahalik, Siddhartha Das, Dwity Sundar Rout, Basanta Kumar Samal and Arnab Adhikary

https://doi.org/10.33307/entomon.v51i3.1704 299
Influence of seasonal fluctuations on eyespot variation in *Ypthima asterope* Klug and *Y. inica* Hewiston (Nymphalidae, Satyrinae)
Babita Sharma, Sushmita Prakash and Ashok Kumar

https://doi.org/10.33307/entomon.v51i3.1705 303
Spider (Arachnida, Araneae) diversity and distribution in the Gandhigram Rural Institute campus, Dindigul district, Tamil Nadu, India
S. Jeswin, John T.D. Caleb and M.R. Rajan

https://doi.org/10.33307/entomon.v51i3.1707 321
Investigation of P-glycoprotein (P-gp) ATPase gene variation in temephos resistant *Aedes albopictus* (Skuse, 1894), Chidambaram, India
Soliang Manyu, C. Elanchezhiyan and P. Basker

https://doi.org/10.33307/entomon.v51i3.1708 329
Parasitoids of butterflies: Diversity and host associations in Kolhapur district, Maharashtra, India
Aarati N. Patil, Ankita Gupta and Sunil M. Gaikwad

https://doi.org/10.33307/entomon.v51i3.1709 337
Influence of weather parameters on the seasonal incidence of shoot fly (*Atherigona soccata* Rondani) on sorghum in Gujarat, India
Koosi Sai Thilak, Bindu Panickar, M.K. Chandaragi, Jyotika Purohit, G. Balraj, Gandham Sumathi and Bhanavathu Sai

https://doi.org/10.33307/entomon.v51i3.1711 343
Potential insecticidal effects of *Valeriana jatamansi* and *Isodon rugosus* against *Acanthoscelides obtectus* (Say) (Coleoptera, Chrysomelidae), a pest of *Phaseolus vulgaris* L.
Beena Devi and D.R. Thakur

https://doi.org/10.33307/entomon.v51i3.1712 349
Evaluation of larvicidal efficacy of *Clerodendrum infortunatum* Linn extracts against *Culex quinquefasciatus* Say (Diptera, Culicidae)
A.S. Sheenu, Neethu Karan and V.S. Ajitha

https://doi.org/10.33307/entomon.v51i3.1714 357
Annual life history of okra shoot and fruit borer, *Earias vittella* (Fabricius), under mid-hill conditions of Himachal Pradesh, India
Manjeet Kumar, Vasu Mehta, Mohammad Ilyas Motmayen, Khushwinder Singh and Anjana Thakur

Short Communications

https://doi.org/10.33307/entomon.v51i3.1716 363
Efficacy of horticultural mineral oil and its combinations against leafhopper *Amrasca biguttula biguttula* (Ishida) in brinjal and whitefly *Aleurodicus dispersus* Russell in chilli, Kerala, India
S. Sajay and K.M. Sreekumar

https://doi.org/10.33307/entomon.v51i3.1720 369
Bio-insecticidal potential of methanolic seed extract of *Datura innoxia* (Solanaceae) against housefly *Musca domestica* L. (Diptera, Muscidae)
Deepika Vishwanath Jangam, Sunil Dagadu Patil and Prashant Bhimrao Koli

https://doi.org/10.33307/entomon.v51i3.1721 375
New records of short-horned grasshoppers (Orthoptera, Acrididae) from Nagaland, India
Amlanjyoti Gautam and Rajnish Ranjan

https://doi.org/10.33307/entomon.v51i3.1723 383
Report of *Donda continentalis* Behounek, Han and Kononenko, 2012 (Lepidoptera, Noctuidae) from India
Joslin Treesa Jacob and Abhilash Peter

https://doi.org/10.33307/entomon.v51i3.1724 387
Butterflies of Sasthamkotta Lake –
A Ramsar site in Kerala, India
P. Kiran, Kavya Sukesh and D. Dhanyaraj

https://doi.org/10.33307/entomon.v51i3.1725 393
Record of pleasant fungus beetle genus *Megalodacne* Crotch (Coleoptera, Erotylidae) on oyster mushrooms from Nainital, Uttarakhand, India
Priyanka Bhatt and Jeet Ram

https://doi.org/10.33307/entomon.v51i3.1727 395
Traditional uses of insects in folk medicine by the Bodo tribe in Baksa District, Bodoland Territorial Region, Assam, India
Hrishikesh Das, Dhritismita Talukdar, Ali Ashique Iqbal, Biman kr. Sarma and Alakesh Barman

Author index 399

https://doi.org/10.33307/entomon.v51i3.1702
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A taxonomic account of four species of the genus *Palpita* Hübner, 1806 (Lepidoptera, Crambidae) from Jammu and Kashmir, India

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ABSTRACT: The genus *Palpita* Hübner, 1806 referable to subfamily Spilomelinae of family Crambidae is characterized by distinctive wing maculation and genitalic features. A detailed taxonomic account of four species of *Palpita i.e., P. kiminensis* Kirti & Rose, *P. warrenalis* (Swinhoe), *P. asiaticalis* Inoue and *P. pajnii* Kirti & Rose, from Jammu and Kashmiris presented. The descriptions of adult morphology, wing venation and genitalia are provided along with illustrations. A diagnostic key to the studied species is also presented to facilitate accurate identification. Notably, all four species are recorded for the first time from Jammu and Kashmir, representing significant range extensions within India. The present findings contribute to a better understanding of the taxonomy, distribution and species composition of the genus *Palpita* in the north-western Himalayan region. © 2026 Association for Advancement of Entomology

KEY WORDS: Spilomelinae, adult morphology, wing venation, genitalia, new records

INTRODUCTION

The genus *Palpita* Hübner, 1806 belongs to subfamily Spilomelinae of family Crambidae and is one of the most diverse and extensively distributed genera within this group. The moths of genus *Palpita* are medium-sized and typically characterized by white or pale wings with brown to black spots and a prominent submarginal fascia. The diagnostic features of the genus include obliquely upturned labial palpi, scaled maxillary palpi, filiform antennae, a closed discal cell in both wings with notable distinct features in male and female genitalic structures such as modified fibula and various cornuti in males and paired spinose signa in females. These genitalic features help in species-level identification within the morphologically comparable genus.

The taxonomic history of genus *Palpita* remained notably complex, characterized by repeated reclassifications and extensive synonymization. Several genera, including *Conchia* Hübner, 1821; *Margaronia* Hübner, 1825; *Paradosis* Zeller, 1852; *Margarodes* Guenée, 1854; *Tobata* Walker, 1859; *Sarothronota* Lederer, 1863; *Cryptographis* Lederer, 1863; *Sebunta* Walker, 1863; *Apyrausta* Amsel, 1951 and others, treated as distinct taxa at different times before ultimately being synonymized under genus *Palpita*. The taxonomic stability was achieved after the revisionary work of Munroe (1950), who revived *Palpita* with *Pyralis unionalis* Hübner as its type species. Subsequent regional revisions and systematic studies substantially advanced the understanding of species diversity, diagnostic characters, and interspecific relationships within the genus. The other notable

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contributions are those of Kirti and Rose (1992) and Kirti and Singh (1992) from India, as well as a series of comprehensive studies by Inoue (1996, 1997, 1999) focusing on Southeast Asia diversity. Zhang and Li (2005) discussed 18 species from China. Rathikannu and Chitra (2020) reported three species from Tamilnadu. More recently, Ko *et al.* (2021) further refined the taxonomy of *Palpita* by providing an identification key and documenting 15 species from Laos, thereby contributing valuable data on the regional diversity and distribution of this genus. *Palpita* has around 160 species worldwide (Nuss *et al.,* 2024). In India 25 species have been identified, with the majority coming from north-eastern and Himalayan regions (Inoue, 1999; Anonymous, 2026). However, the fauna of *Palpita* in the north-western Himalaya, particularly Jammu and Kashmir, has received little attention, with scattered data and minimal taxonomic study. The current study intends to address this gap by presenting a detailed taxonomic account of four species of genus *Palpita* collected for the first time from Jammu and Kashmir.

MATERIALS AND METHODS

A detailed taxonomic account of four species of *Palpita i.e., P. kiminensis* Kirti & Rose, *P. warrenalis* (Swinhoe), *P. asiaticalis* Inoue and *P. pajnii* Kirti & Rose, from Jammu and Kashmir is presented. The descriptions of male and female adult morphology, wing venation and genitalia are provided along with illustrations and identification key for the examined species. A diagnostic key to the studied species is also presented to facilitate accurate identification. The new distributional records presented herein considerably broaden the known range of these species within India and highlight the biogeographical importance of the north-western Himalayan region for Spilomelinae moth diversity.

The collected specimens have been deposited in the Lepidoptera Lab., Department of Zoology and Environmental Sciences, Punjabi University, Patiala.

Abbreviations

1A: First anal vein; 2A: Second anal vein; 3A: Third anal vein; AED: Aedeagus; ANT.APO: Anterior apophyses; CRN: Cornuti; CRP.BU: Corpus bursae; CU_1: First cubital vein; CU_2: Second cubital vein; DU.BU: Ductus bursae ; DU.EJ : Ductus ejaculatorius; FIB: Fibula; JX: Juxta; M_1: First median vein; M_2: Second median vein; M_3: Third median vein; PAP.A: Papilla analis; PO.APO: Posterior apophyses; R_1: First radial vein ; R_2: Second radial vein; R_3: Third radial vein; R_4: Fourth radial vein; R_5: Fifth radial vein ; SA: Saccus; Sc: Subcosta; $Sc+R_1$: Subcosta+ First radial vein; TU.A: Tuba analis; UN: Uncus; VIN: Vinculum VLV: Valva

RESULTS AND DISCUSSION

Genus *Palpita* Hübner, 1808

Palpita Hübner, [1808]: pl. 209 [in Hübner 1806-1832]. TS: *Pyralis unionalis* Hübner, 1796 [in Hübner 1796-1833]; Kirti and Rose, 1992, *J. Ent. Res*., 16(1): 62-77; Kirti and Singh, 1992, *Geob. New. Rep*., 11(2): 113-117; Kendrick, 2002, *Moths Hong Kong*, 2002: 123, 137-138, 437, 550, 654; pl. 16, figs. 1-5, pl. 17, fig. 33; Poltavsky, 2018, *Zootaxa*, 4457(1): 22- 23; Rathikannu and Chitra, 2020, *Plant Archives 20(2): 9007-9010*; Ko *et al*. 2021a, *J. Asia-Pacific Biod.,* 14: 348-354; Ko *et al*., 2021b, *J. Asia Pac. Biod.,*14: 539-543.

Hapalia Hübner, 1818: 19, [35], pl. [17] figures 95-96 [in Hübner 1808-1818]. TS: *Hapalia illibalis* Hübner, 1818.

Conchia Hübner, 1821: 7. TS: *Pyralis unionalis* Hübner, 1796.

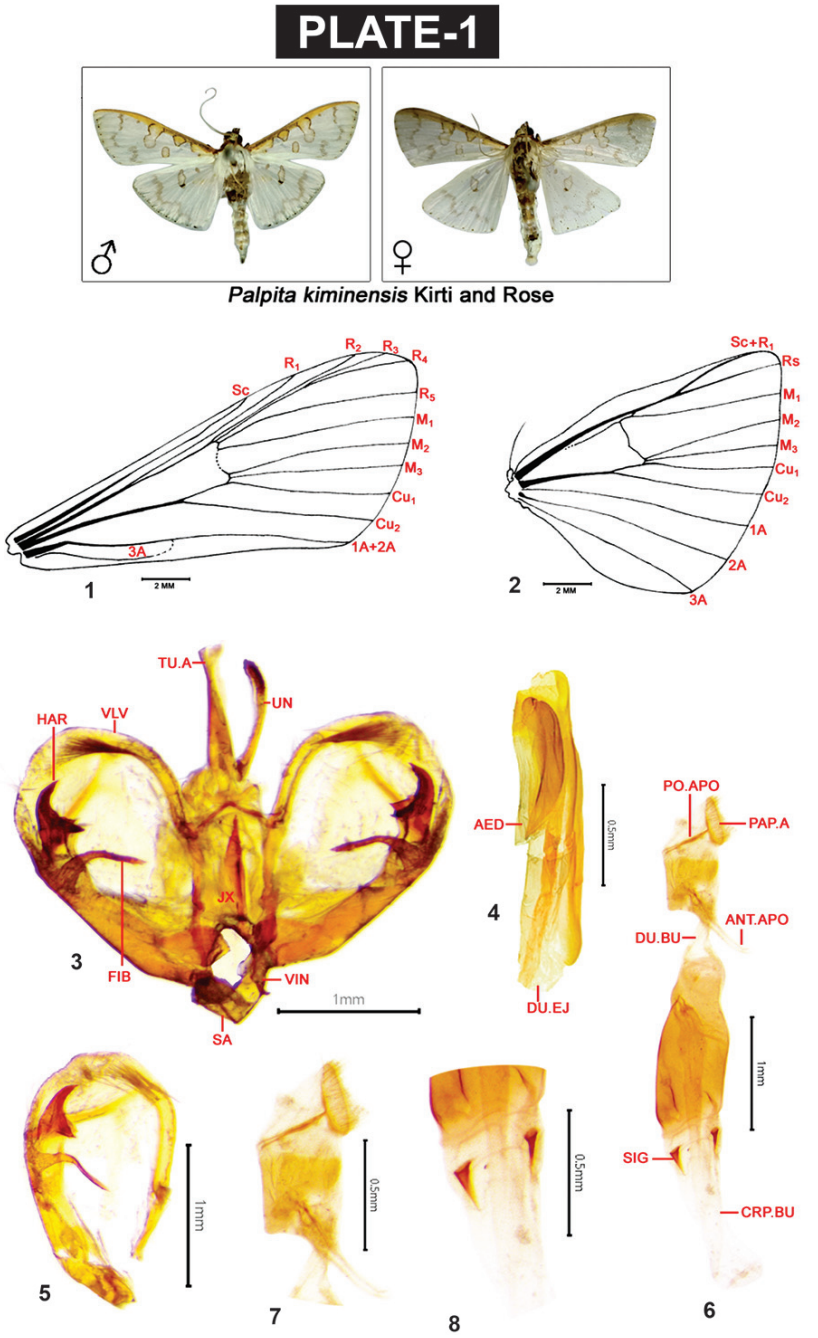
Margaronia Hübner, [1825]: 358 [in Hübner 1816-1826]. TS: *Pyralis unionalis* Hübner, 1796.

Paradosis Zeller, 1852: 58. TS: *Paradosis villosalis* Zeller, 1852.

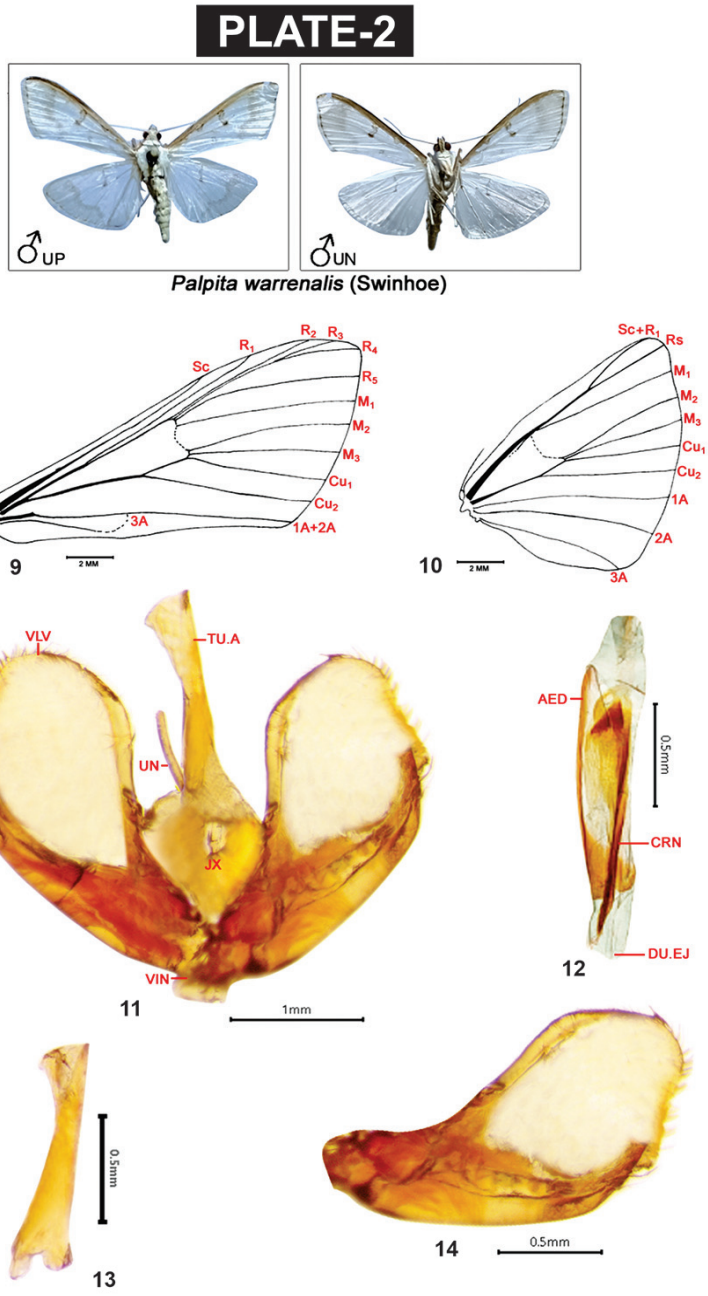
Margarodes Guenée, 1854: 301. TS: *Pyralis unionalis* Hübner, 1796.

Tobata Walker, 1859: 516. TL: *Tobata elealis* Walker, 1859.

Sarothronota Lederer, 1863: 278 (key), 394, pl. 5 figure 7, pl 13 figure 9. TS: *Phalaena flegia* Cramer, 1777.



Figs. 1-8 *Palpita kiminensis* Kirti and Rose, 1992



9. Forewing 10. Hindwing 11. Male genitalia 12. Aedeagus 13. Tuba analis 14. Valva.

Figs. 9-14 *Palpita warrenalis* (Swinhoe, 1894)

Cryptographis Lederer, 1863: 399-400, pl. 5 figure 9. TL: *Cryptographis rogenhoferi* Lederer, 1863.

Sebunta Walker, 1863: 77. TS: *Sebunta guttulosa* Walker, 1863.

Ledereria Marschall, 1873: 299. Objective replacement name for *Margarodes* Guenée.

Hvidodes Swinhoe, 1900: 499. Objective replacement name for *Margarodes* Guenée.

Sylora Swinhoe, 1900: 472. TS: *Sisyrophora cirralis* Swinhoe, 1897.

Apyrausta Amsel, 1951: 552. TS: *Apyrausta persicalis* Amsel, 1951.

Type species: *Pyralis unionalis* Hübner, 1796

Distribution: Cosmopolitan

Diagnosis: Medium sized moths; head with frons rounded, white; labial palpi obliquely upturned, third segment usually concealed in long vestiture; maxillary palpi scaled, brush-like; proboscis well-developed; antennae filiform; Wings with submarginal fascia strongly curved medially or parallel with termen; forewing mostly with basal, antemedial, discal and infracellular spots edged with black; hindwing with discoidal dot on lower angle of cell. Forewing triangular; discal cell closed, less than half the length of wing; discocellulars curved; veins Cu_1, M_3 and M_2 from lower angle of cell; R_5 curved, approximated at base to R_{4+3}; latter two veins well stalked; Sc from base of wing. Hindwing globular; discal cell closed, less than half the length of wing; discocellulars curved; Cu_1, M_3 and M_2 from lower angle of cell; M_3 and M_2 approximated at base; M_1 from upper angle of cell; $Sc+R_1$ anastomosed with Rs after its origin from upper angle of cell. Male genitalia with uncus usually slender, long, rod-like or clubbed; sacculus having variably modified fibula; aedeagus with various shape and length cornuti (modified fibula and various cornuti very important characters for identification); Female genitalia with corpus bursae having a pair of spinose signa; ostium sclerotized in both sides; ductus bursae very short.

Key to the species groups of genus *Palpita* Hübner:

1. Wings with submarginal fascia strongly curved outward between M_1 and M_3, and inward between M_3 and Cu_1; large conspicuous spots on both wings. Male genitalia with uncus long, slender, rod-shaped and weakly expanded at apex..... Species-group A (*P. kiminensis* Kirti and Rose)

- Wings with submarginal fascia parallel with termen, weakly curved or indistinct; conspicuous spots absent on both wings. Male genitalia with uncus usually clubbed..... .....Species-group B

Key to the species of species-group B of genus *Palpita* Hübner:

1. Wings with submarginal fascia slightly excurved medially. Forewing with diffused bean-shaped marking from costa to vein Cu_2 covering discocellulars and distinct lunule at lower angle. Male genitalia with sacculus having straight and broad saccular process .....*P. warrenalis* (Swinhoe)

- Wings with submarginal fascia parallel to termen or indistinct. Forewing without bean-shaped marking and distinct lunule at lower angle. Male genitalia with sacculus having projected saccular process.....2

2. Wings with black specks at terminal ends of the veins. Male genitalia with short rod-like uncus; saccular projection with two processes; fibula absent. Female genitalia with a pair of short thorn like signa ....*P. asiaticalis* Inoue

- Wings without black specks at terminal ends of the veins. Male genitalia with drooping long uncus; saccular projection with single process; fibula present. Female genitalia with a pair of long spine like signa ....*P. pajnii* Kirti and Rose

Species group - *annulifer* group

***Palpita kiminensis* Kirti and Rose, 1992 (Plate 1, Figs. 1-8)**

Palpita kiminensis Kirti and Rose, 1992, *J. Ent. Res.,* 64(1): 70-72; Ko *et al*., 2021a, *J. Asia Pacific Biod.,* 14(2021): 349-352.

Type locality: **Kimin, Arunachal Pradesh**

Diagnosis: Head with frons rounded; vertex and frons dressed with white scales; labial palpi covered with fulvous-brown scales, underside with white scales, third segment short; proboscis well developed, basally clothed with white scales; antennae white. Collar with a very narrow band of ochreous scales followed by a larger band of white scales behind eyes; tegula and thorax densely covered with long, shiny white scales. Legs long, white; foreleg with femur and tibia suffused with fuscous, distally tinged with golden; tarsi banded with white and fuscous; midleg having a pair of tibial spurs with inner one three the length of outer one; hindleg having two pairs of tibial spurs with upper pair having inner one quite longer, almost four times longer than outer one and second pair with inner one more than double the length of outer one. Abdomen white.

Wing maculation: Forewing pure white; costal margin golden-brown upto apex; sub basal spot conjoined with costal margin, followed by another oblong spot representing antemedial line; large bean-shaped marking from costa to vein Cu_2 covering discocellulars; submarginal fascia strongly curved outward between M_1 and M_3, and inward between M_3 and Cu_1; marginal black specks on each vein; fringe white; underside similar. Hindwing pure white; costal margin straight, delicately curved near apex; a pale-brown antemedial spot in cell; an irregular, oblong spot at discocellulars ending into dark marking at lower angle; submarginal fascia medially excurved, extending upto anal vein 1A; marginal black specks on each vein; fringe white; underside almost similar; hindwing less patterned than forewing.

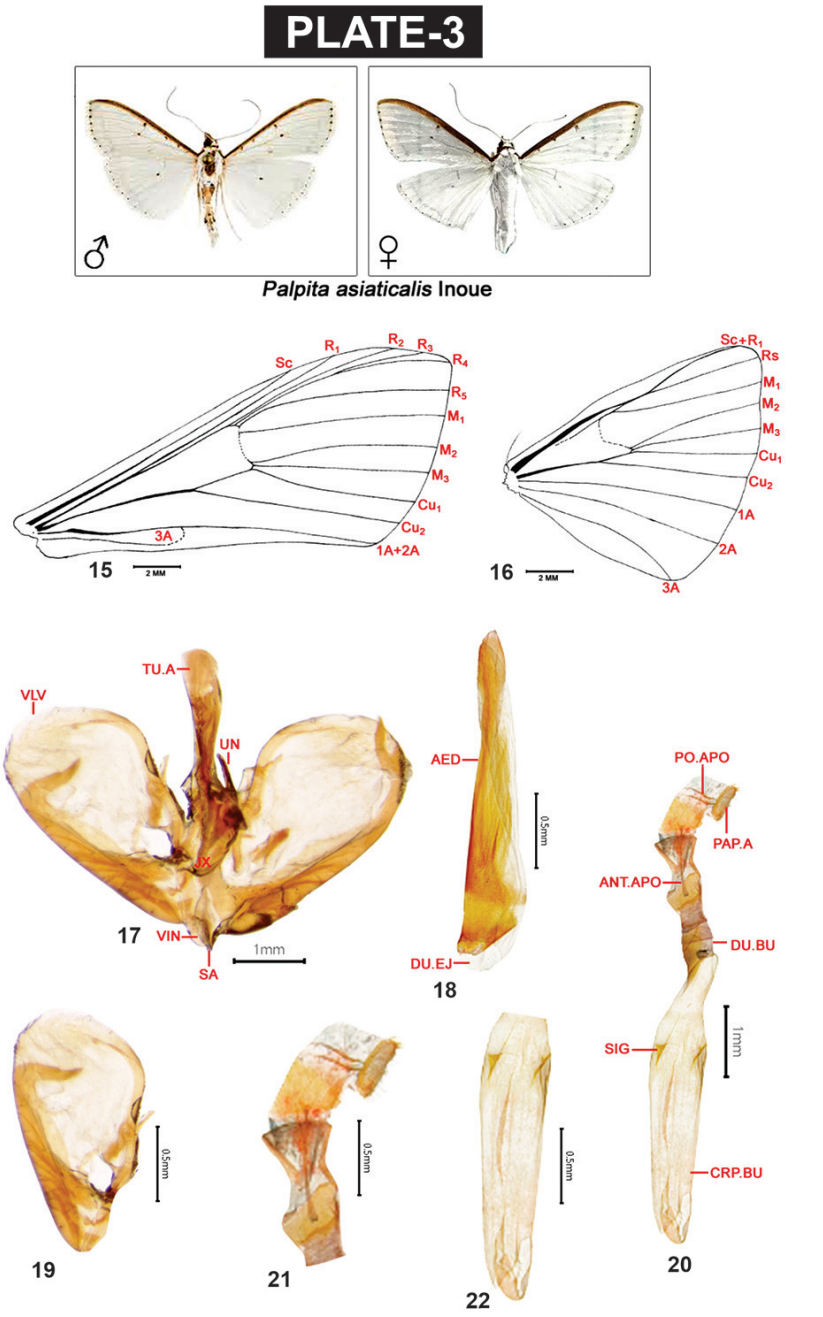
Wing venation: Forewing triangular; discal cell closed, more than half the length of wing; vein 3A curved forward forming a loop and joining with 2A+1A; latter two veins fused from base of wing, reaching margin; Cu_2 from just beyond three-fourth of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from above lower angle of cell; M_1 from upper angle of cell; R_5 free, curved toward R_{4+3} near base; latter two veins well stalked;R_2 from before common stalk of R_{4+3}; R_1 well beyond half of cell; Sc from base of wing, not reaching apex. Hindwing globular; discal cell closed, less than half the length of wing; veins 3A and 2A from base of wing, reaching tornus; 1A from base, reaching margin; Cu_2 from just beyond two-third of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 and Rs stalked from upper angle of cell; $Sc+R_1$ anastomosing with Rs just after its bifurcation from $M_1$and ends at costa near apex.

Wing expanse: **Male: 25-28mm; Female: 26-29mm**

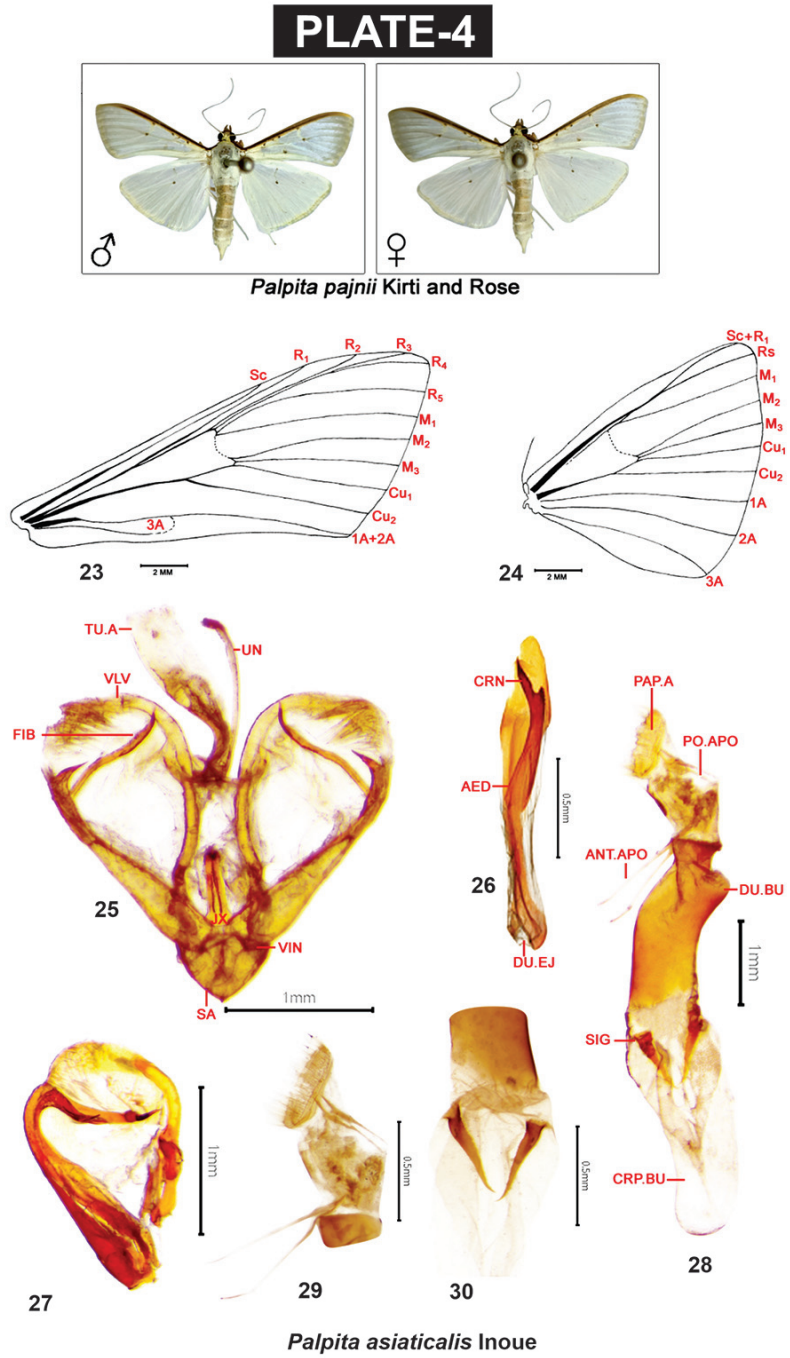
Body length: **Male: 15mm; Female: 14mm**

Male genitalia: Uncus long, narrow with spatulate distal end, dorsally setosed with backwardly directed short hair; tuba analis straight, well sclerotized, slightly broader and longer than uncus; tegumen dome shaped; juxta long, triangular, spine-like, well sclerotized; vinculum narrow, ending into broad saccus. Valva ovoid distally, extending beyond half the level of uncus; costa well differentiated; sacculus broad, well sclerotized, saccular process broad, lunate; harpe long and narrow, with pointed tip. Aedeagus cylindrical, of moderate size, well sclerotized; vesica armed with a long and broad cornutus.

Female genitalia: Corpus bursae narrow, membranous; a pair of triangular signa on lateral sides of corpus bursae; ductus bursae almost of same size, decorated with numerous minute denticles; anterior apophyses longer than posterior ones with dilated apices; posterior ones narrower with narrow apices; papilla analis obliquely oblong, decorated with long setae.



Figs. 15-22 *Palpita asiaticalis* Inoue, 1994



23. Forewing 24. Hindwing 25. Male genitalia 26. Aedeagus 27. Valva
28. Female genitalia 29. Papilla Analis (Enlarged) 30. Corpus Bursae (Enlarged).

Figs. 23-30 *Palpita pajnii* Kirti and Rose, 1992

Material examined: Jammu and Kashmir: Aru, 06.vi.2018, 5♂, 3♀; Pahalgam, 13.vi.2018, 7♂, 5♀; Phraslun, 19.vi.2018, 6♂, 5♀.

Distribution: India: Arunachal Pradesh (Kimin); Australia; Borneo; China; Indonesia; Laos; Malaysia; Nepal; Thailand.

Remarks: Kirti and Rose (1992) reported this species as a new species from Arunachal Pradesh and named it after the name of its type locality i.e., Kimin. Ko *et al*. (2021a) described this species for the first time from Laos. The collection of this species from Aru, Pahalgam and Phraslun is its first record from North India particularly Jammu and Kashmir. The forewing wing expense is 13 to 14 mm in both sexes. This species is quite similar to *P. annulifer* Inoue, but can be distinguished by on the basis of wing maculation and genitalic characters. The infracellular spots on forewings are ovoid and brighter than *P. annulifer* Inoue and submarginal fascia of fore and hindwing brighter than *P. annulifer* Inoue. In male genitalia, sacculus is broader than *P. annulifer* Inoue, with lunate process at tip and fibula spine-shaped and waved.

Speices group-*unionalis* group

***Palpita warrenalis* (Swinhoe, 1894) (Plate 2, Figs. 9-14)**

Margaronia warrenalis Swinhoe, 1894b, *Ann. Mag. Nat. Hist*., 14(6): 148.

Palpita warrenalis Swinhoe: Kirti and Singh, 1992, *Geob. New. Rep*., 11: 114; Kirti and Rose, 1992, *J. Ent. Res*., 16(1): 65; Kendrick, 2002, *Moths Hong Kong*, 2002: 138; Irungbam *et al*., 2016, *J. Asia-Pacific Biod*., 9: 374.

Type locality: **Meghalaya, India**

Diagnosis: Head with vertex and frons clothed with white scales; labial palpi porrect, basal segment clothed with white, second segment broad, covered with dark-brown scales, third segment blunt; proboscis well developed; antennae white. Collar, tegula and thorax densely covered with shiny-white scales; collar and tegula having a golden-brown band showing continuity with costal margin of forewing. Legs long, narrow; pure white; foreleg tinged with fuscous; midleg having a pair of tibial spurs with inner one four times the length of outer one; hindleg with two pairs of tibial spurs with upper pair having inner one more than twice the length of outer one and lower ones with inner one approximately twice the length of outer one. Abdomen white.

Wing maculation: Forewing pure white; costal margin golden brown; a antemedial narrow patch along costal margin and one below vein Cu_2; brown lunule on discocellulars with specks at both angles; submarginal fascia medially excurved ; margins specked with fuscous; fringe white. Hindwing pure white, weakly marked at discocellulars; submarginal fascia medially excurved, extending upto anal vein 2A; margins weakly specked with fuscous; fringe white; dorsum ciliated with white scales; underside similar.

Wing venation: Forewing triangular; discal cell closed, equal to half the length of wing; vein 3A curved forward forming a loop and with joining 2A+1A, latter two veins fused from base of wing, reaching margin; Cu_2 from three-fourth of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 from upper angle of cell; R_5 free, curved toward R_{4+3} near base; latter two veins well stalked; $R_2$from well before common stalk of R_{4+3}, running in its close proximity; R_1 from half of cell; Sc from base of wing, not reaching apex. Hindwing globular; discal cell closed, less than half the length of wing; veins 3A and 2A from base of the wing reaching tornus; 1A from base reaching margin; Cu_2 from beyond two-third of the cell; Cu_1 just before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 and Rs stalked from upper angle of cell; $Sc+R_1$ anastomosing with Rs just after its bifurcation from M_1 and ends at costa near apex.

Wing expanse: Male: 28mm; Female: Not examined

Body length: Male: 14mm; Female: Not examined

Male genitalia: Uncus narrow, slender, slightly curved in middle, reaching half the level of tuba analis; tuba analis straight, well sclerotized, broader and longer than uncus; tegumen dome shaped; juxta short, well sclerotized; vinculum "V" shaped, ending into a hood-like saccus. Valva simple, extending well above the level of uncus; costa well differentiated; sacculus broad, well sclerotized, saccular process simple, narrowing distally; fibula absent. Aedeagus cylindrical, of moderate size; vesica armed with a long narrow and well sclerotized cornutus reaching almost four-fifth of its length.

Female genitalia: Not examined

Material examined: Jammu and Kashmir: Aru, 06.vi.2018, 9♂; Pahalgam, 13.vi.2018, 7♂; Phraslun, 19.vi.2018, 6♂.

Distribution: India: Arunachal Pradesh, Kerala, Meghalaya; Nagaland; Bhutan; Borneo; China; Hong Kong; Indonesia; Nepal; Papua New Guinea; Philippines; Sikkim; Sumatra; Vietnam.

Remarks: Swinhoe (1894) originally described this species under genus *Margaronia* Hübner. Kirti and Singh (1992) placed it under genus *Palpita* Hübner and provided notes and illustrations of its male and female genitalia. Kirti and Rose (1992) studied this species from Meghalaya. Irungbam *et al.* (2016) followed its placement in the present genus. The collection of this species from Aru, Pahalgam and Phraslun is its first record from North India.

***Palpita asiaticalis* Inoue, 1994 (Plate 3, Figs. 15-22)**

Palpita asiaticalis Inoue, 1994, Tyo to Ga, 45(2): 98; Kendrick, 2002, *Moths Hong Kong*, 2002: 137; Irungbam *et al*., 2016, *J. Asia. Pacific Biod*., 9: 374; Sanyal *et al*., 2018, *Faunal Diversity of Indian Himalaya*, Zoological Survey of India, Kolkata, 682 pp.

Type locality: Wushe, Taiwan

Diagnosis: Head with frons rounded, vertex and frons dressed with white scales; labial palpi basal segment clothed with white scales, second segment with fuscous-brown scales, third segment short and pointed; proboscis well developed; antennae pale-white. Collar dressed with white scales behind eyes; tegula and thorax clothed with long, shiny-white scales. Legs long, pure white; foreleg basally, tibia and tarsi edged with ochreous scales; midleg having a pair of tibial spurs with inner one almost double the length of outer one; hindleg having two pairs of tibial spurs with upper pair having inner one almost three times the length of outer one and lower pair with inner one more than twice the length of outer one.

Wing maculation: Forewing pure white; costal band dark-brown; basal spot, followed by antemedial spot in the cell, second spot below Cu_2, dark-brown discoidal spots on upper and lower angle of cell; submarginal fascia parallel with termen, not excurved medially; specks on terminal end of veins; fringe white; underside similar. Hindwing pure white; indistinct speck at lower angle of cell; submarginal fascia parallel with termen, not excurved medially; specks on terminal end of veins; fringe white; dorsum ciliated; underside similar.

Wing venation: Forewing with costal margin almost straight, delicately curved near apex; discal cell closed, more than half the length of wing; vein 3A curved forward forming a loop and joining with 2A+1A; latter two veins fused from base of wing, reaching margin; Cu_2 from well before three-fourth of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 from upper angle of cell; R_5 free, curved toward R_{4+3} near base; latter two veins well stalked; R_2 from well before common stalk of R_{4+3}; R_1 from just beyond middle of cell; Sc from base of wing, not reaching apex. Hindwing with termen straight; discal cell closed, less than half the length of wing; veins 3A and 2A from base of wing, reaching tornus; 1A from base, reaching margin; Cu_2 from well beyond two-third of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 and Rs stalked from upper angle of cell; $Sc+R_1$ anastomosing with Rs after its bifurcation from M_1 and ends at costa near apex.

Wing expanse: Male: 30mm; Female: 30-33mm

Body length: Male: 16mm; Female: 15mm

Male genitalia: Uncus short, narrow, rod-like, well sclerotized; tuba analis well sclerotized, longer and broader than uncus; tegumen arched; juxta finger-like, moderately sclerotized; vinculum narrow ending into "V" shaped saccus. Valva lingulate, extending well above the level of uncus; costa weakly differentiated; sacculus broad, well sclerotized; saccular projection with two sclerotized processes, one shorter and other one longer. Aedeagus cylindrical, of moderate size, well sclerotized; vesica armed with minute denticles.

Female genitalia: Corpus bursae narrow, long, membranous; a pair of short thorn-like signa on lateral sides of corpus bursae; ductus bursae well sclerotized, half the length of corpus bursae; anterior apophyses broader, twice the length of posterior ones; posterior apophyses narrow; both pairs with rounded apices; papilla analis rounded, setosed with short setae.

Material examined: Jammu and Kashmir: Aru, 06.vi.2018, 7♂, 4♀; Ashmuqam, 25.vi.2018, 8♂, 6♀.

Distribution: India: Himalaya; Bhutan; China; Hong Kong; Nepal; Taiwan; Thailand; Vietnam.

Remarks: Inoue (1994) described this species under genus *Palpita* Hübner from Taiwan and provided detailed notes on its male and female genitalic features. Kendrick (2002) and Irungbam *et al.* (2016) reported this species from Hong Kong and Bhutan respectively. Its collection from Aru and Ashmuqam is its first record from Jammu and Kashmir. This species belongs to *nigropunctalis* subgroup of species-group B.

***Palpita pajnii* Kirti and Rose, 1992 (Plate 4, Figs. 23-30)**

Palpita pajnii Kirti and Rose, 1992, *J. Ent. Res.,* 64(1): 72-73; Ko *et al*., 2021b, *J. Asia Pacific Biod.,* 14: 541-542. Inoue, 1997, *Tinea,* (2): 149.

Type locality: Arunachal Pradesh

Diagnosis: Head with frons obliquely rounded; frons dressed with ochreous scales; vertex covered with pale-yellowish scales, white posteriorly; labial palpi with basal and second segments covered with ochreous scales, underside fringed with white, third segment blunt; proboscis well developed, basally scaled with white; antennae pale-white. Collar dilated with a narrow band of ochreous scales, followed by a comparatively larger band of shiny-white scales behind eyes; tegula and thorax densely covered with shiny-white scales. Legs long, pure white; foreleg basally, tibia and tarsi edged with ochreous scales; midleg having a pair of tibial spurs with inner one twice the length of outer one; hindleg having two pairs of tibial spurs with upper pair having inner one almost five times the length of outer one and lower ones with inner one more than double the length of outer one.

Wing maculation: Forewing pure white; costal band dark-brown; two notable specks below costal band in antemedial area; two dark-brown specks on upper and lower angles of cell; fringe white; underside similar. Hindwing pure white; specks at both angles of cell, upper one indistinct; fringe white; underside similar.

Wing venation: Forewing with costal margin straight, delicately curved near apex; discal cell closed, half the length of wing; vein 3A curved forward forming a loop and joining with 2A+1A; latter two veins fused from base of wing, reaching margin; Cu_2 from beyond three-fourth of cell; Cu_1 well before lower angle of cell; M_3 from lower angle of cell; M_2 from well above lower angle of cell; M_1 from upper angle of cell; R_5 free, curved toward R_{4+3} near base; latter two veins well stalked; R_2 from before common stalk of R_{4+3}; R_1 from well before two-third of cell; Sc from base of wing, not reaching apex. Hindwing with costal margin delicately curved; discal cell closed, less than half the length of wing; veins 3A and 2A from base of wing, reaching tornus; 1A from base, reaching margin; Cu_2 from well beyond two-third of cell; Cu_1 well before lower angle of cell; M_3 from lower angle

of cell; M_2 from well above lower angle of cell; M_1 and Rs stalked from upper angle of cell; $Sc+R_1$ anastomosing with Rs after its bifurcation from M_1 and ends at costa near apex.

Wing expanse: Male: 32mm; Female: 33-35mm

Body length: Male: 14-15mm; Female: 16-18mm

Male genitalia: Uncus long, narrow with drooping distal end, dorsally setosed with backwardly directed setae; tuba analis longer and broader with basal half well sclerotized and distal half membranous; tegumen straight; juxta rod-shaped, well sclerotized; vinculum "V" shaped, well sclerotized, ending into broad triangular well sclerotized saccus. Valva simple, nearly rhomboidal; costa well sclerotized, distally setosed with short and long setae; sacculus broad, well sclerotized; saccular process projected, angled with point apex; fibula long, spine-like, reaching inner margin of costa, curved near middle. Aedeagus of moderate size, well sclerotized; vesica armed with a long well sclerotized cornutus, distally terminating into a spine.

Female genitalia: Corpus bursae membranous; a pair of long spine-like signa with pointed apices; ductus bursae of same size, with well sclerotized walls; anterior apophyses twice the length of posterior ones with rounded apices; posterior apophyses narrower than anterior ones; papilla analis oblong with rounded ends, decorated with long and short setae.

Material examined: Jammu and Kashmir: Aru, 06.vi.2018, 5♀; Phraslun, 19.vi.2018, 8♂, 5♀; Ashmuqam, 25.vi.2018, 6♂, 3♀.

Distribution: India: Arunachal Pradesh, Assam; Australia; China; Indonesia; Laos; Myanmar; Nepal; Papua New Guinea; Philippines; Taiwan; Thailand; Vietnam.

Remarks: While sorting out *Palpita* species-complex, Kirti and Rose (1992) described this species for the first time from North-East India. Ko *et al*. (2021b) reported this species for the first time from Laos. The collection of this species from Aru, Ashmuqam and Phraslun is its first record from North India and particularly from Jammu & Kashmir. This species belongs to the *unionalis* subgroup of Species-group B.

The genus *Palpita* was proposed by Hübner (1806) as a monotypic genus for *Pyralis unionalis* Hübner. In 1821, he placed it under another new genus *Conchia* (Hübner, 1821). Later, he established another new genus *Margaronia* and considered both genera i.e., *Palpita* and *Conchia* under it (Hübner, 1825). Zeller (1852) placed *Margaronia* Hübner under his new genus *Paradosis*. Eventually, Guenée (1854) placed all these genera under genus *Margarodes* Hübner. Hampson (1896) synonymized all these genera under genus *Glyphodes* Guenée. Klima (1939) synonymized *Glyphodes* Guenée under genus *Diaphania* Hübner. Munroe (1950) revived genus *Palpita* Hübner with *unionalis* Hübner as its type species. Kirti and Rose (1992) followed its revival and described four new species under it from North-East India. Kirti and Singh (1992) transferred three more species under it from genus *Diaphania* Hübner from North-East India. Inoue (1996, 1997, 1999) described old world relatives of this genus in a revisionary series and discussed genus *Palpita* Hübner in two groups i.e., *annulifer* and *unionalis* group. He assigned the species with large conspicuous spots on both fore and hind wings to *annulifer* group and those without such spots to *unionalis* group. While dealing with *Palpita* species from Laos, Ko *et al*. (2021a, 2021b) recorded a total of fifteen species and also provided key to the species of this genus along with taxonomic notes and illustrations of adults and male and female genitalia. The genus *Palpita* Hübner is a species rich genus and represented globally by 160 species (Nuss *et al*., 2024) with 13 species from India (Inoue, 1999). During present studies, one species namely *P. kiminensis* Kirti and Rose referable to *annulifer* species-group having distinct conspicuous spots on wings and strongly outwardly curved submarginal fascia and three species namely *P. warrenalis* (Swinhoe), *P. pajnii* Kirti and Rose and *P. asiaticalis* Inoue without such spots and parallel submarginal fascia with termen pertaining to *unionalis* group respectively have been studied and illustrated.

The present investigation provides a detailed taxonomic account of four species namely *P. kiminensis* Kirti & Rose, *P. warrenalis* (Swinhoe), *P. asiaticalis* Inoue, and *P. pajnii* Kirti & Rose from Jammu and Kashmir, India. All these four species are recorded for the first time from Jammu and Kashmir, representing significant extensions of their known geographical distributions within India. The presence of species belonging to both the *annulifer* and *unionalis* species groups reflects the biogeographical complexity of the north-western Himalayan region. The documentation of these new records contributes valuable baseline data to the systematics, distribution, and biodiversity assessment of the genus *Palpita* in India.

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Evaluation of antagonistic *Pseudomonas* and *Bacillus* spp. against *Meloidogyne incognita* causing root-knot disease in okra

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ABSTRACT: Efficacy of antagonistic bacteria *Pseudomonas* and *Bacillus* against the root-knot nematode (*Meloidogyne incognita*) in okra as a sustainable biological control strategy was evaluated. All the bacterial treatments significantly reduced nematode populations and root galling while enhancing plant growth and yield compared to the control. Nematode-related parameters showed strong positive correlations among themselves: egg mass per plant (r = 0.998), nematode population (r = 0.968), and root-knot index (r = 0.907), while plant growth parameters showed significant negative correlations with nematode infestation: plant height (r = -0.983), fresh shoot weight (r = -0.988), dry shoot weight (r = -0.990), root length (r = -0.960), fresh root weight (r = -0.996), and dry root weight (r = -0.983). Seed treatment with *P. fluorescens* at 10g kg^{-1} was the most effective, giving the lowest root-knot index (3.33) and the greatest reduction in galls, egg masses, and larvae per 200 cc soil. Although carbofuran showed comparable efficacy, its environmental and health hazards limit its use; thus, seed treatment with *P. fluorescens* at 10g kg^{-1} is recommended as an effective, eco-friendly alternative for managing *M. incognita* in okra.
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KEY WORDS: Seed treatment, yield loss, nematode population, root-knot index, galls

INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is economically important annual herbaceous vegetable belonging to the family Malvaceae, widely cultivated across tropical, subtropical, and warm temperate regions of the world. Plant parasitic nematodes are among the most significant biotic constrains limiting okra productivity

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worldwide. Leveraging the bio-synthesis of nanomaterials to combat with root knot nematodes also tested by Ghareeb *et al*. (2024). Notable nematodes that infect okra include species of *Meloidogyne*, *Hoplolaimus*, *Helicotylenchus*, *Tylenchorhynchus*, *Aphelenchus*, and *Rotylenchulus.* Root knot nematodes (RKN) being the most widespread and harmful (Bhosle *et al*., 2004; Muthulaxmi *et al*., 2010). RKN (*M. incognita*) are sedentary obligate endoparasites with a broad host range, infecting a wide diversity of vegetable crops and causing substantial yield losses (Kiewnick and Sikora, 2006; Sahebani and Hadavi, 2008; Elling, 2013). Crops such as tomatoes, okra, brinjal, chili, cluster beans, potatoes, and cucumbers are among the severely affected (Jain *et al*., 2007). In okra, the nematode can complete its life cycle in just 28 days (Al-Sayed *et al*., 2011). Symptoms of infestation include significant root galling and distortion, which can lead to yield reductions of as much as 90 per cent (Rao *et al*., 2014). In India, the economic repercussions of this nematode are estimated to be around $7.8 million (Jain *et al*., 2007). Managing *M. incognita* in intensive agriculture poses challenges, as early signs of infestation are often overlooked, and leads to swift and severe losses in yield. Despite their prevalent use of the pesticides, there was reduction in yield losses (Oerke, 2006). This underscores the need for sustainable, eco-friendly alternatives to chemical nematicides (Copping and Menn, 2000; Lee and Kim, 2016; Patil *et al*., 2020; Abd-Elgawad, 2021). Among these, antagonistic bacteria have emerged as highly promising biocontrol agents for plant-parasitic nematodes (Giannakou *et al*., 2004; Ali *et al*., 2023; Bhat *et al*., 2023; Saharan *et al.,* 2023). These nematophagous bacteria utilize various strategies, including the secretion of toxins or enzymes, parasitizing hosts, triggering systemic resistance in plants, and promoting overall plant health (Gamliel and Katan, 1993; Tian *et al*., 2007). Many species of *Bacillus* are effective in reducing nematode populations, particularly *M. incognita*. Species like *B. pumilus*, *B. megaterium*, *B. cereus*, and *B. thuringiensis* produce metabolites that significantly elevate mortality rates in the J2 stage of *Meloidogyne incognita* (Padgham and Sikora, 2007). However, systematic evaluation of specific bacterial antagonists under field-relevant conditions in okra remains limited (Yadav *et al.,* 2022). The present study was undertaken to evaluate the *Pseudomonas* and *Bacillus* in suppressing *M. incognita* infestation and improving the growth and yield of okra.

MATERIAL AND METHODS

During the 2019–2020 Rabi season, the study was carried out at the net house of the Department of Nematology at the College of Agriculture, O.U.A.T., Bhubaneswar, under pot culture conditions using earthen pots of 30cm diameter filled with 5kg of sterilized sandy loam soil, with two plants of okra genotype Utkal Gaurav (BO2), maintained per pot. The experiment was conducted using a Complete Randomized Design with eight treatments, each replicated three times (Table 2).

Freshly extracted second-stage juveniles (J2) of *M. incognita* were inoculated at the rate of 2000 J2 per pot by pipetting the inoculum suspension into holes made around the root zone at the time of transplanting. Seedlings were inoculated 10 days after sowing by pouring the nematode suspension near the root zone. The experiment was maintained for 60 days after inoculation, after which plants were uprooted for assessment of nematological and morphological parameters. All bioagents, *P. fluorescens*, *B. pumilus*, *B. subtilis*, *B. megaterium*, *P. putida*, and *B. amyloliquefaciens,* were used as talc-based formulations with a minimum viable count of 1 × 10x CFU/g. Seeds were coated with the bioagents at 10 g kg^{-1} of seed using a small quantity of water as a sticking agent and shade-dried for 30 minutes before sowing. The morphological characteristics assessed for the okra varieties comprised plant height (cm), fresh shoot weight (g), dried shoot weight (g), root length (cm), fresh root weight (g), dry root weight (g), the count of galls per plant, the number of egg masses per plant, and the root knot index.

One kg sample of soil was taken from each pot and blended thoroughly, after which 200 cc of soil was extracted from each pot. The nematode populations in these soil samples were evaluated using Cobb's sieving method (Cobb, 1918) and the

modified Baermann funnel method (Schinder, 1961). The root galling index (1–5-point scale, Table 1) was conducted following the method outlined by Taylor and Sasser (1978).

Statistical analysis was done through OP stat**.** Correlation coefficients (Johnson *et al*., 1955) were calculated to analyse the relationship between yield and other factors, as well as among yield components, physiological indicators, and quality attributes.

RESULTS AND DISCUSSION

Impact of bacterial antagonists seed treatment with 10 g kg^{-1} on shoot growth of okra infected by root knot nematode:

The heights of the plants in different treatments descending order were - T1 *P. fluorescens* (53.6cm), T7 Carbofuran 3G @ 1kg a.i. ha^{-1} (51.3cm), T3 *B. subtilis* (50.1cm), T2 *B. pumilus* (47.7cm), T5 *P. putida* (44.9cm), T6 *B. amyloliquefaciens* (40.7cm), and T4 *B. megaterium* (36.8cm), with respective increases of 65.3, 58.2, 54.5, 47.3, 38.6, 25.5, and 13.5% respectively, over control (T8). However, T1 and T7 were statistically similar. The highest fresh shoot weight was observed in T1 (88.8g, with 42.7% increase over the control), followed by T7 (86.4g, with 38.9% increase), T3 (85.6g, 37.6%), T2 (80.3g, 29.1%), T5 (77.1g, 23.9%), and T6 (73.2g, 17.6%).

Although T4 (68.4 g) had the least increase (10.0%), it was still statistically significant in compare with non-treated control (T1).

The dry shoot weight was notably high in T1 *P. fluorescens* (15.38g), followed by T3 *B. subtilis* (14.85g) but were statistically similar to T7 Carbofuran (14.71g). These were followed by T2 (13.31g), T5 (12.46g), and T6 (11.72g), showing increases of 45, 35.8, and 27.6 over the control (T8), respectively (Table 2, Fig. 1).

The study revealed that all bacterial antagonists used as seed treatments increased plant growth considerably. Using *P. fluorescens* resulted in notable increases in height of the plants, both fresh and dry shoot weight, length of the root, and fresh and dry root weight. These findings align with those of Mahalik *et al*. (2020), who found that *P. fluorescens* showed better performance than other bacterial antagonists, such as *P. putida* and *Bacillus* spp., when applied individually. Similarly, Sharma *et al.* (2021) reported that seed and soil application of *P. fluorescens* along with mycorrhizal fungi reduced root-knot nematode infestation and enhanced growth in eggplant. Ali *et al.* (2023) recorded similar growth-promoting and nematode-suppressive benefits of microbial antagonists in okra under greenhouse conditions.

Impact of bacterial antagonists on root growth of okra infected by root knot nematode:

The recorded root lengths of okra plants were: T1 (23.1cm), T2 (18.2cm), T3 (21.8cm), T4 (15.2cm), T5 (17.1cm), T6 (15.8cm), and T7 (22.7cm). All treatments showed significant improvement over the untreated check. Among them, T1 (*P. fluorescens* treatment of seeds at an application rate of 10 g kg^{-1} of seed) had the highest percentage increase in mean root length at (76.6%), followed by T7 (73.6%) and T3 (66.1%). The smallest increase was seen in T4 (16.4%), compared to the untreated check T8. Results revealed with of fresh root weight data showed that T1 had the highest weight (18.74g), followed by T7 (17.91g), T3 (17.32g), and T2 (15.48g), with T8 having the lowest weight (10.42g). Treatment T1 was significantly different from all other treatments in fresh root weight except T7 (Utilizing Carbofuran 3G at 1 kg a.i.ha^{-1}). The percentage increase in fresh root weight, in ascending order, was T4 (31.5%), T6

Table 1. Nematode gall indexing through 5-point scale

No. of galls	Gall rating	Response
0	0	Non-responsive/control
1-2	1	Extremely tolerant (ET)
3-10	2	Tolerant (T)
11-30	3	Partially Tolerant (PT)
31-100	4	Susceptible (S)
>100	5	Extremely Susceptible (ES)

Table 2. Impact of bacterial antagonists on shoot growth of root knot nematode infected okra

Treatments (T1 – T6 - Seed treatment with —-@ 10 g kg^{-1})	Height (cm)	Increase (%)	Fresh weight (g)	Increase (%)	Dry weight (g)	Increase (%)
T1 *P. fluorescens*	53.6	65.3	88.8	42.7	15.38	67.5
T2 *B. pumilus*	47.7	47.3	80.3	29.1	13.31	45.0
T3 *B. subtilis*	50.1	54.5	85.6	37.6	14.85	61.8
T4 *B. megaterium*	36.8	13.5	68.4	10.0	11.14	21.4
T5 *P. putida*	44.9	38.6	77.1	23.9	12.46	35.8
T6 *B. amyloliquefaciens*	40.7	25.5	73.2	17.6	11.72	27.6
T7 Carbofuran 3G @ 1kg a.i. ha^{-1}	51.3	58.2	86.4	38.9	14.71	60.2
T8 Untreated control	32.4		62.2		9.18	
SE (m) ±	1.54		1.24		0.35	
$CD_{(0.05)}$	3.27		2.64		0.73	

(36.2%), T5 (45.3%), T2 (48.5%), T3 (66.2%), T7 (71.8%), and T1 (79.8%). None of the treatments were statistically non-significant compared to the untreated check (T8).

A comparative analysis of the average dry root weight data revealed that the weights varied from 1.80g in T8 to 3.25g in T1. There was little difference in dry root weight amongst the treatments, even though all of them were noticeably superior to the untreated control (T8). T4 saw a 26.3% rise in dry root weight over the check, while T1 saw an 80.7% increase. Following T1 were, T7 (71.7%), T3 (52.4%), T2 (44.3%), T5 (38.1%), and T6 (34.4%). All treatments were statistically similar, except for T1. This treatment T1 was significantly different from all others, with the sole exception of T7, (Carbofuran 3G at a rate of 1kg a.i. ha^{-1} (Table 3, Fig. 2, 3).

Siddiqui *et al*. (2009) similarly reported that *Bacillus* was less effective than *Pseudomonas* isolates in enhancing plant development and minimizing nematode damage in peas. Mishra *et al.* (2018) found that untreated controls affected by root-knot nematodes significantly increased the height, shoot weight, and root weight of okra when compared to soil treatments containing *P. fluorescens*. Khan and Haque (2011) reported similar benefits in tobacco, while Nama and Sharma (2017), Singh *et al*. (2014), and Mohamed et al. (2009) found that *P. fluorescens* improved growth in cowpea, okra, and faba beans by reducing *Meloidogyne incognita* infestation. Bakker *et al.* (2003) reveal that *P. fluorescens's* capacity to create hormones that regulate plant growth, including as gibberellins, auxins, and cytokinins, which enhance the absorption of nutrients and encourage sprouting of roots, is responsible for its growth-promoting properties. This, in turn, increases the plants' resistance to nematode attacks. More recently, Egan and Kakouli-Duarte (2022) confirmed that plant growth-promoting *P. fluorescens* strains suppress *M. javanica* development while simultaneously increasing host plant biomass, reinforcing the dual growth-promotion and biocontrol role observed in the present study.

Impact of bacterial antagonists on root knot nematode growth on okra:

In analysing the quantity of galls that root-knot nematodes produced per plant was estimated under which treatment T1 had the fewest galls (34.00),

significantly differing from all other treatments except T7 (43.33). The treatments in ascending order were T3 (50.00), T2 (61.67), T5 (69.33), T6 (76.33), and T4 (86.33). The application of *P. fluorescens* resulted in the most significant decrease in galls, (70.4% compared to the untreated control) followd by T7 (62.3%), T3 (56.5%) and T2 (46.4%). T5 (39.7%), T6 (33.6%) and T4 (24.9%) showed only lower reduction of galls. All treatments resulted in a significant drop in egg masses per plant. The most substantial decrease in egg masses (78.4%) was achieved with T1 compared to the check (89.33). This was followed by T7 (71.3%), T3 (64.2%), T2 (49.3%), T5 (41.8%), and T6 (37.3%) in descending order. T1 also recorded the lowest number of egg masses (19.33), followed by T7 (25.67) and T3 (32.00). T1 was significantly more effective than all other treatments, including the chemical check, while the treatments (T2, T5), (T5, T6), and (T6, T4) were statistically similar.

In the root-knot nematode population per 200cc of soil also T1 recorded least population (121.0), followed by T7 (127.7), T3 (139.0), T2 (167.3), and T5 (185.3). The reduction in nematode population compared to the check (326.3) was highest in T1 (62.9%), followed by T7 (60.9%), T3 (57.4%), T2 (48.7%), T5 (43.2%), and T6 (41.3%). Compared to the untreated check (T8), T1 (such as the management of seeds using P. fluorescens @ 10g/ kg of seeds) in order was the most effective treatment which was statistically similar to T7, and T5 was also similar to T6. The percentage decrease in nematode population per 200cc of soil ranged from 62.9 in T1 to 37.1 in T4 compared to the check. All treatments significantly reduced the number of root-knot nematodes compared to the baseline. Additionally, T8 had the maximum root-knot index (5.0), whereas T1 had the lowest (3.33), followed by T7 (3.67). The remaining treatments had a root-knot index of 4.0 (Table 4). Anwar-ul-Haq *et al*. (2011) found that *P. fluorescens* significantly improved plant growth in tomatoes by reducing nematode-related issues like females per root system and galls, performing better than *P. putida*, *Bacillus* spp., or PGPR combinations. Kadam and Khan (2015) similarly showed that treating okra

Table 3. Impact of bacterial antagonists on root growth of root knot nematode infected okra

Treatments (T1 – T6 - Seed treatment with —-@ 10 g kg^{-1})	Length (cm)	Increase (%)	Fresh weight (g)	Increase (%)	Dry weight (g)	Increase (%)
T1 *P. fluorescens*	23.1	76.6	18.74	79.8	3.25	80.7
T2 *B. pumilus*	18.2	38.8	15.48	48.5	2.60	44.3
T3 *B. subtilis*	21.8	66.1	17.32	66.2	2.74	52.4
T4 *B. megaterium*	15.2	16.4	13.70	31.5	2.27	26.3
T5 *P. putida*	17.1	30.4	15.14	45.3	2.49	38.1
T6 *B. amyloliquefaciens*	15.8	20.6	14.19	36.2	2.42	34.4
T7 Carbofuran 3G @ 1kg a.i. ha^{-1}	22.7	73.6	17.91	71.8	3.09	71.7
T8 Untreated control	13.1		10.42		1.80	
SE (m) ±	0.59		0.47		0.22	
$CD_{(0.05)}$	1.25		1.00		0.46	

Table 4. Impact of bacterial antagonists on root knot nematode growth in the infected okra

Treatments (T1 – T6 - Seed treatment with — @ 10 g kg^{-1})	**Galls/ plant (no.)**	**Decrease (%)**	**Egg mass/ plant (no.)**	**Decrease (%)**	**Population/ 200 cc soil**	**Decrease (%)**	**RKI**
T1 *P. fluorescens*	34.00(5.79)	70.4	19.33(4.39)	78.4	121.0(11.00)	62.9	3.33
T2 *B. pumilus*	61.67(7.85)	46.4	45.33(6.73)	49.3	167.3(12.93)	48.7	4.00
T3 *B. subtilis*	50.00(7.07)	56.5	32.00(5.65)	64.2	139.0(11.79)	57.4	4.00
T4 *B. megaterium*	86.33(9.29)	24.9	63.00(7.93)	29.5	205.3(14.33)	37.1	4.00
T5 *P. putida*	69.33(8.32)	39.7	52.00(7.21)	41.8	185.3(13.61)	43.2	4.00
T6 *B. amyloliquefaciens*	76.33(8.74)	33.6	56.00(7.48)	37.3	191.7(13.84)	41.3	4.00
T7 Carbofuran 3G @ 1kg a.i. ha^{-1}	43.33(6.53)	62.3	25.67(5.06)	71.3	127.7(11.30)	60.9	3.67
T8 Untreated control	115.00(10.72)		89.33(9.45)		326.3(18.06)		5.00
SE (m) ±	0.44		0.23		0.16		0.24
$CD_{(0.05)}$	0.92		0.48		0.34		0.50

Note: The values in parentheses are "(x + 0.5) transformed mean. RKI - Root-knot index was scored on a 0–5 scale

seeds with *P. fluorescens* (10g kg^{-1}) effectively reduced root-knot nematode populations, while Mishra *et al*. (2017) found that tuberose had higher shoot weight and less gall development. Other studies, such as Kalairasan *et al*. (2008) and Abo-Elyousr *et al*. (2010), supported *P. fluorescens*' effectiveness in lowering nematode populations and boosting plant resistance. This suppression is thought to occur through the production of nematode-inhibiting metabolites (Siddiqui and Shaukat, 2003), degradation of root exudates, and activation of systemic resistance (Jonathan and Umamaheswari, 2006). Enzymes like peroxidase, polyphenol oxidase, and chitinase also enhance plant defenses (Osman *et al*., 2011), leading to fewer galls and improved plant growth. These findings are further corroborated by Stucky *et al.* (2023), who identified several *Pseudomonas* strains as reliable antagonists of *M. incognita*, and by Zhao *et al.* (2021), who showed that endophytic *Pseudomonas* and *Serratia* isolates significantly lowered root-knot nematode reproduction through similar antagonistic mechanisms.

Association of galls/ plant and yield components:

Analysing correlations helps breeders understand the relationships between traits, providing insights for genetic improvement through selective breeding. Various economically important plant traits often display different interconnections. Significant positive correlations were observed between galls per plant and several factors, including egg mass per plant (0.998), nematode population (0.968) and root not index (0.907). In contrast, plant height (0.983), fresh shoot weight (0.988), dry shoot weight (0.990), root length (0.960), fresh root weight (0.996) and dry root weight (0.983) showed negative correlations that were statistically significant (Table 5).

The *P. fluorescens* based seed treatment achieved the lowest root knot index of 3.33, indicating relatively better performance in reducing nematode galls, egg masses, and larval population per 200 cc of soil, under pot experiment conditions with limited

Table 5. Estimates of correlation coefficients among galls/plant and yield components in Okra

	PH	FSW	DSW	RL	FRW	DRW	RNI	EM	NP	GP
PH	1.000	0.997**	0.986**	0.959**	0.972**	0.955**	-0.829*	-0.974**	-0.924**	-0.983**
FSW		1.000	0.995**	0.974**	0.982**	0.961**	-0.834*	-0.984**	-0.934**	-0.988**
DSW			1.000	0.978**	0.989**	0.960**	-0.848**	-0.990**	-0.947**	-0.990**
RL				1.000	0.968**	0.956**	-0.795*	-0.968**	-0.886**	-0.960**
FRW					1.000	0.982**	-0.908**	-0.998**	-0.968**	-0.996**
DRW						1.000	-0.922**	-0.985**	-0.936**	-0.983**
RNI							1.000	0.907**	0.936**	0.907**
EM								1.000	0.969**	0.998**
NP									1.000	0.968**
GP										1.000

*Note: PH = Plant height; FSW = Fresh shoot weight; DSW = Dry shoot weight; RL = Root length;FRW = Fresh root weight; DRW = Dry root weight; RNI = Root-knot index; EM = Egg mass/plant;NP = Nematode population/200 cc soil; GP = Galls/ plant. * = Significant at p d" 0.05; ** = Significant at p d" 0.01.*

replications. Additionally, it tended to improve plant height, both dry and fresh shoot weights, fresh and dry root weights, and a number of other growth metrics. Nevertheless, in most of the measured metrics, its performance was statistically similar to that the application of carbofuran 3G kg a.i.ha^{-1} for combat with root knot nematode (*Meloidogyne incognita*) in okra. Treating seeds with the bacterial antagonist *P. fluorescens* appears promising. These preliminary findings, while encouraging, are based on a pot experiment with limited replications; field-level validation with adequate replications is warranted before firm recommendations can be made. The potential environmental benefits over chemical nematicides, including reduced residual toxicity, health hazards, and groundwater contamination, further support the need for continued investigation of this approach. As recently reviewed by Bhat *et al.* (2023), microbial antagonists such as *Pseudomonas* and *Bacillus* species continue to represent one of the most promising and sustainable alternatives to chemical nematicides for the management of root-knot nematodes across a range of vegetable crops.

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Influence of seasonal fluctuations on eyespot variation in *Ypthima asterope* Klug and *Y. inica* Hewiston (Nymphalidae, Satyrinae)

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ABSTRACT: The eyespots of certain brown butterflies vary in response to seasonal changes. The present study mainly focuses on the variation in eyespot patterns of two *Ypthima* species (*Y. asterope* and *Y. inica*) under the influence of seasonal fluctuations. Morphologically similar, both species differ in number, size, and the ocelli (eye spot) arrangements present on both forewings and hindwings, indicating how the species vary from each other, with seasonal forms mostly noticeable in the wet season and reduced or absent in the dry season. Seasonal monitoring showed *Y. asterope* had limited activity in winter, while *Y. inica* showed more abundance, had a wider range of seasonal presence, and was more resilient overall. A correlation study for both species revealed that their abundance decreased as temperatures increased, but their numbers rose with higher air pressure, while relative humidity had no significant effect. © 2026 Association for Advancement of Entomology

KEY WORDS: Satyrini, abundance, eyespot (ocelli), polyphenism, temperature, air pressure

INTRODUCTION

Butterflies are admired for their graceful flight, vibrant wing coloration, and prominent wing markings, among which eyespots are considered one of the most crucial features. These eyespots are highly diverse in size, shape, number, and colour combinations, contributing significantly to butterfly diversity. Members of the family Nymphalidae possess well-defined circular eyespots on their wings, which play an important role in predator deterrence, mimicry, and survival (Stevens, 2005; Monteiro, 2008). The subfamily Satyrinae comprises medium-sized butterflies generally characterized by dull brown to dark brown coloration, transverse white or tawny bands, and conspicuous ocelli on both dorsal and ventral wing surfaces (Sharma and Goswami, 2020). Commonly referred to as "Browns," these butterflies exhibit weak and erratic flight close to the ground and are frequently associated with bushes, grasses, bamboo patches, and shaded habitats. Adults primarily feed on tree sap, fermented liquids such as toddy, and juices from rotten fruits, while larvae mostly develop on grasses and bamboos, except in *Elymnias* spp., which utilize palms as larval host plants (Mathew and Soumya, 2013). Many satyrid butterflies display pronounced seasonal polymorphism, particularly on the ventral wing surface, representing an adaptive response to varying environmental conditions. Wet-season forms generally possess brighter brown coloration with distinct eyespots, whereas dry-

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season forms are comparatively dull brown with reduced or obscure eyespots, often showing cryptic coloration that enhances camouflage.

Among Satyrinae, the present study focuses on two species of the tribe Satyrini, namely *Ypthima asterope* Klug, 1832 (Common Three-ring) and *Ypthima inica* Hewitson, 1865 (Lesser Three-ring), both belonging to the family Nymphalidae. *Ypthima asterope* is distributed throughout most parts of India except Ladakh, whereas *Y. inica* occurs from Punjab southwards to Maharashtra and eastwards through Uttarakhand, West Bengal, and Tripura (Varshney and Smetacek, 2015). In Uttar Pradesh, *Y. asterope* has been recorded throughout the state, while *Y. inica* has been reported from several regions (Van Gasse, 2021; Pandey *et al.,* 2024). Although both species resemble each other morphologically, they can be distinguished by the arrangement of eyespots on the ventral wing surfaces. In *Y. asterope*, the underside of the forewing bears one large eyespot, while the hindwing contains a small eyespot in vein space 6 along with two tornal ocelli in vein spaces 1b and 2. In contrast, *Y. inica* possesses a large eyespot in vein space 6 of the hindwing and two small tornal eyespots in vein spaces 1 and 2, whereas the forewing bears a large sub-apical eyespot that does not form a complete loop. Additionally, the apical and tornal eyespots are misaligned, and the eyespot in vein space 2 is shifted outward. These diagnostic markings are more distinct in wet-season forms but become reduced or occasionally absent during the dry season (Evans, 1932; Bhakare and Ogale, 2018). The diversity in butterfly wing variation and seasonal variation reflects important ecological adaptations and species resilience under different environmental conditions. Therefore, the present study investigates the eyespot variation with respect to seasonality effect in both *Ypthima* species with special emphasis on understanding the influence of environmental factors such as temperature, humidity, and air pressure.

MATERIALS AND METHODS

The study was conducted within the heterogenous habitats like Grasslands, Shrublands and forest patches of Lucknow district which includes the Reserve forests of Rasoolpur Iduria ($26^{0}44'40.9"$N; $80^{0}56'01.6"$E), Musabagh ($26^{0}52'26.1"$N; $80^{0}52'12.8"$E), Kukrail Reseve Forest ($26^{0}54'45.5'"$N; $80^{0}59'32"$E) during three distinct seasons, summer, monsoon (pre- and post-monsoon), and winter during September 2024 to August 2025. Two species of the *Ypthima* genus were chosen and spotted their morphological characteristics. The survey of the butterflies was based on opportunistic sightings, and the collection of butterfly specimens is avoided strictly, although we performed Catch and Release method by using an aerial sweep net. These butterfly numbers were counted during the morning hours 8: 00 AM to 11:00 AM and again in the afternoon from 2:00 to 4:00 PM.

The species identification relied on photographic evidence captured by a Nikon D5600 with a 70-300 lens, and it was further identified by available field guidebooks (Kehimkar, 2016; Smetacek, 2017; and Bhakare and Ogale, 2018) and additional literature data. The environmental factors, such as temperature, humidity, and air pressure were recorded by using AccuWeather app. Pearson's correlation coefficient (r) has been used on the relationship between weather parameters and abundance of the selected species. The correlation was applied because the variables were continuous and assumed to have linear relationships. All statistical calculations were performed using Microsoft Excel for the analysis of recorded monthly data for both species.

RESULTS AND DISCUSSION

The findings demonstrate a season-wise analysis of both species, exhibiting clear variation. *Ypthima asterope* was moderately active, with the most sightings in September, December, and February, with fewer sightings in October, November, January, April, and August and were clearly absent in March, May, June, and July. This is an indicative of clear limited seasonal presence confined to the post-monsoon and winter. There were a higher total abundance and a wider range of times in *Y. inica* and was often seen in greater numbers from September through October and November, peaked in December and February, and remained high in

January and again in May and August. There were no sightings in March, April, June, or July, suggesting sensitivity to the extreme hot summer. Both species were most active during the post-monsoon (September–November) and winter (December–February) seasons, but they experienced a sharp decrease in numbers or complete absence during early summer (March–July) due to adverse climatic conditions, such as high temperatures and scarcity of their vegetation, which affected their activity, survival, and detectability. As a whole, *Y. inica* (with Lesser three-ring) showed greater resilience and adaptability to seasonal environmental variation compared to *Y. asterope* (with Common three-ring). Its broader seasonal incidence and higher abundance suggest a stronger tolerance to fluctuating ecological conditions, whereas *Y. asterope* appeared more seasonally constrained (Fig. 2).

The correlation analysis indicated that weather parameters such as temperature, humidity, and air pressure influenced the abundance of both species. The results found a strong negative correlation between average temperature and the abundance of *Y. asterope* ($r = –0.70$, $p < 0.05$) and *Y. inica* ($r = –0.77$, $p < 0.01$). However, the air pressure shows a significant positive correlation with *Y. asterope* ($r = 0.74$, $p < 0.01$) and *Y. inica* ($r = 0.77$, $p < 0.01$). The relative humidity of the species revealed a mild and non-significant correlation with *Y. asterope* ($r = 0.18$, $p > 0.05$) and *Y. inica* ($r = 0.27$, $p > 0.05$) (Fig. 3).

The findings indicated that both species are highly affected because of the seasonal and environmental fluctuations. The abundance of both species is high in the winter season when the temperature is cool, humid, and steady. Their abundance decreases during hot and dry weather conditions. The atmospheric variables play a crucial role in affecting their behavior, survival, eyespot variation, distribution, and seasonal occurrence. Earlier studies on other satyrine butterflies done by Brakefield (1987) and Prasannakumar *et al.* (2026) revealed that the seasonal forms and ecological adaptation are mostly affected by the climatic factors. The eyespot size varied due to environmental factors that highly affect both wing morphology and pattern formation during larval and pupal development. Favorable seasons are beneficial in growth and development, resulting in the visibility of big and clear eyespots (Fig. 1). Apart from these factors,

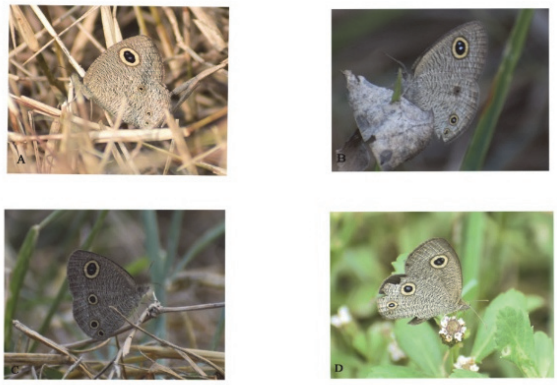


Fig. 1 (a) 2024 and (b) 2025 Wet season form of Common three ring (*Y. asterope*); (c) 2024 and (d) 2025 Wet season form of Lesser three ring (*Y. inica*)

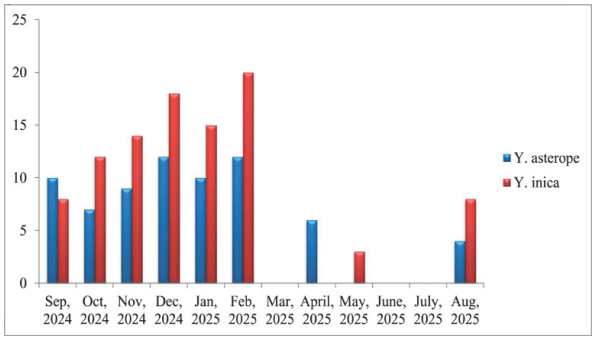


Fig. 2 Seasonal (monthly) observation of *Y. asterope* and *Y. inica* across heterogenous habitats of Lucknow

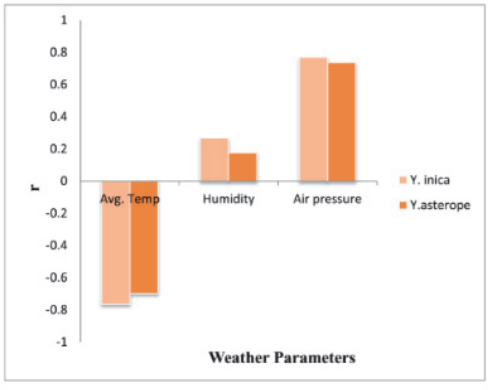


Fig. 3 Correlation between weather parameters and seasonal abundance of both *Y. asterope* and *Y. inica*

hot and dry weather negatively impacts eyespot development, whereas both eyespot size on the forewing and hindwing will be reduced. The previous findings in satyrine butterflies, where temperature-mediated developmental plasticity significantly impacts the size of eyespots and seasonal morph expression (Ramos *et al.,* 2013; Prasannakumar *et al.,* 2025). Humidity is another environmental factor that also affects the eyespots and seasonal forms of butterflies. On satyrine butterflies studied by Prasannakumar *et al.* (2026), it was suggested that humidity alone and with temperature helps to regulate seasonal polyphenism and wing pattern plasticity in tropical butterflies. The maintenance of natural vegetation and the protection of moist microhabitats support the sustainability of the diversity and butterfly population. Of these, long term monitoring programs should be initiated to understand how seasonal changes influenced the diversity and adaptation of butterflies.

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Spider (Arachnida, Araneae) diversity and distribution in the Gandhigram Rural Institute campus, Dindigul district, Tamil Nadu, India

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ABSTRACT: Spiders are key arthropod predators that regulate insect populations and respond sensitively to habitat heterogeneity, making them effective indicators of ecosystem condition. This study assessed spider diversity, distribution, and functional guild structure across six habitat types within The Gandhigram Rural Institute campus, Dindigul district, Tamil Nadu, India. A total of 61 species representing 5,719 individuals, 19 families, and 46 genera were recorded during a three-month survey (January 2025 - March 2025), during the dry season. Salticidae was the most species-rich family, while Oecobiidae, Pholcidae, and Lycosidae were numerically dominant. Alpha diversity varied among sites, with highest richness and Shannon diversity observed in heterogeneous agricultural habitats, whereas built-up areas exhibited greater dominance. Moderate beta diversity (Whittaker = 0.64126; Bray-Curtis similarity) reflected species turnover across contrasting microhabitats. Gamma diversity indices (H' = 3.053; 1–D = 0.9341) indicated a diverse campus-wide assemblage. Diurnal and nocturnal sampling revealed comparable richness, highlighting complementary activity patterns. Guild analysis demonstrated functional diversity dominated by active hunters and web-builders. © 2026 Association for Advancement of Entomology

KEY WORDS: Alpha and beta diversity, campus ecology, habitat heterogeneity, guild analysis

INTRODUCTION

Arachnids (Arthropoda, Chelicerata) comprise a diverse assemblage of terrestrial invertebrates represented by 13 extant orders, including spiders, scorpions, mites, ticks, harvestmen, and solifuges (Ballesteros *et al*., 2022). Among them, spiders (order Araneae) constitute one of the largest and most ecologically significant predatory groups. With more than 53,600 described species worldwide (World Spider Catalog, 2026), spiders occupy a wide range of microhabitats and exhibit diverse foraging strategies, including active hunting and web-building (Foelix, 2011). As generalist predators, they regulate insect populations and contribute substantially to trophic dynamics in both natural and managed ecosystems (Nyffeler and Birkhofer, 2017). Their sensitivity to microclimatic conditions,

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vegetation structure, and habitat disturbance also makes them reliable bioindicators of environmental change (Marc *et al*., 1999).

Despite their ecological importance, spider diversity remains insufficiently documented in many tropical and subtropical regions. Globally, a considerable proportion of arthropod diversity is yet to be described, with tropical landscapes representing major knowledge gaps (Cardoso *et al*., 2011). In India, arachnological research has predominantly focused on biodiversity hotspots such as the Western Ghats, whereas semi-arid and human-modified habitats are comparatively understudied (Siliwal *et al*., 2005). Although a recent checklist enumerates 1,984 spider species from India (Caleb and Sankaran, 2026), only about 19.9 per cent have been reported from Tamil Nadu (Sen *et al*., 2025), highlighting the need for systematic regional surveys.

University campuses represent heterogeneous landscapes comprising gardens, agricultural plots, built structures, and remnant vegetation patches. Such environments function as transitional zones between natural and anthropogenic habitats and can support substantial invertebrate diversity. Documenting spider assemblages in these semi-managed systems provides baseline data for understanding species distribution patterns and the influence of land-use practices. In rural institutions promoting sustainable agriculture and reduced chemical inputs, spiders may play an important role in natural pest regulation, thereby contributing to ecological management strategies.

The Gandhigram Rural Institute (GRI), Tamil Nadu, integrates organic farming and environmentally conscious practices within its campus landscape. However, the diversity and distribution of spiders within this semi-arid, human-modified ecosystem remain undocumented. Assessing species composition and spatial distribution across different habitat types within the campus will generate baseline data essential for biodiversity monitoring and sustainable land-use planning. Therefore, the present study aims to document the spider diversity and distribution within the GRI campus and evaluate patterns in relation to habitat heterogeneity.

MATERIALS AND METHODS

The present study was conducted at GRI campus, located in Dindigul District, Tamil Nadu, India (10°27’96" N; 77°93’45" E), at an elevation of approximately 265 m above mean sea level. The campus lies about 10 km south of Dindigul town and 55 km from Madurai city, positioned in the valley between the Sirumalai and Kodaikanal hill ranges, close to the foothills of Sirumalai. The terrain comprises a mosaic of plains and gentle elevations. The region experiences a tropical savanna climate, characterised by a wet season from June to September and a pronounced dry season from January to May.

Owing to its proximity to the Sirumalai hills, the campus supports varied vegetation and habitat types. Microhabitats include irrigated agricultural fields, deciduous vegetation patches, grasslands, and clusters of academic, administrative, and residential buildings (Fig. 1). For the purpose of this study, the area was divided into six sites (A–F). Sites A, B, and C comprised agricultural landscapes, while Sites D, E, and F represented built-up zones associated with institutional and residential infrastructure (Fig. 2). This heterogeneity provides an opportunity to examine spider assemblages across contrasting land-use types.

The survey was conducted over a three-month period during the dry season (January 2025 - March 2025), with occasional rainfall events. Although the campus landscape is relatively managed, factors such as pesticide application, construction activities, and other anthropogenic disturbances may influence spider distribution and community structure. No previous comprehensive study on spider diversity has been undertaken within the campus, making this survey a baseline assessment for future ecological monitoring and conservation planning.

The study focused on documenting species composition, abundance, guild structure, diurnal and nocturnal occurrence, and spatial distribution across the selected microhabitats.

Spiders were sampled using standardised field techniques including line transects, visual searching,

hand collection, and the inverted umbrella method. Specimens were temporarily collected in Petri dishes, photographed using a Samsung A54 mobile device, and subsequently released at the point of capture to minimise disturbance. Sampling was carried out at all six sites during morning (06:00–09:00 h) and evening (18:00–21:00 h) sessions to record both diurnal and nocturnal species.

Each site was visited 6 times, 3 hours in the morning, per session, totalling 18 hours per site. The same 18 hours effort was carried out in the evening per site/replication. There was 36 hours sampling each day per site replication. Over all there were 216 hours field sampling in all 6 sites. Transect length for this study was not fully structured. But followed zig-zig transect across all sites except buildings where surveyed around the walls.

Specimens were identified based on available taxonomic literature (Tikader, 1970, 1982, 1980; Tikader and Malhotra, 1980; Majumder and Tikader, 1991; Kronestedt, 2010; Huber, 2011; Caleb and Sanap, 2016; Caleb *et al.*, 2018; 2020; Tyagi *et al.*, 2019; Caleb, 2020; Caleb and Wijesinghe, 2022; Jager *et al*., 2022; Sudhin *et al.*, 2023). Identified species were categorised into nine ecological guilds following the framework proposed by Cardoso *et al.* (2011).

Species diversity was evaluated using Dominance (D), Simpson's Index (1–D), Shannon–Wiener Index (H'), Evenness (e^H/S), Whittaker's beta diversity, and the Chao-1 estimator. All diversity analyses were performed using the Paleontological Statistics (PAST) software package.

Geospatial mapping and habitat-based visualisation were carried out using GIS software, with base imagery derived from Google Earth (Version 10.78.02) (Fig. 3). Graphical representations, including bar and pie charts, were prepared using Microsoft Excel (Version 2021).

RESULTS

A total of 61 spider species, comprising 5,719 individuals and representing 19 families and 46 genera, were recorded across the six study sites of the GRI campus (Fig. 4; Tables 1, 4). Salticidae was the most species-rich family (13 species), followed by Araneidae, Lycosidae, Oxyopidae, and Thomisidae. Numerically, Oecobiidae, Pholcidae, and Lycosidae contributed the highest number of individuals.

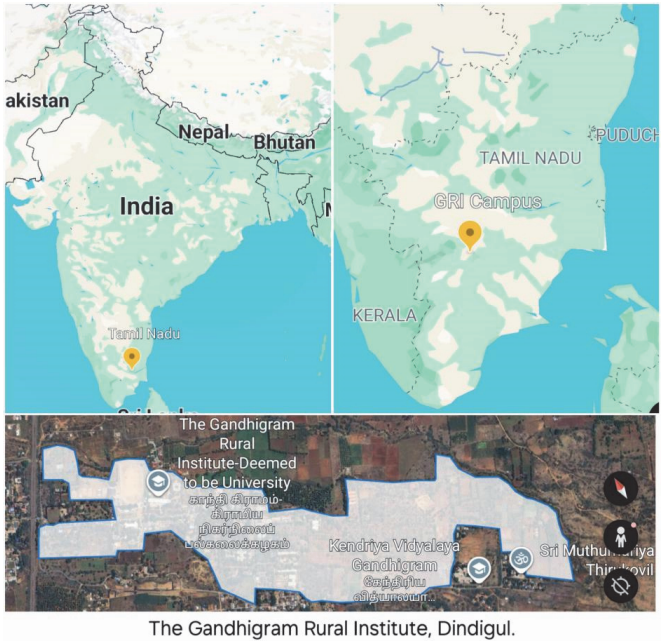


Fig. 1 Study area map of the Gandhigram Rural Institute



Fig. 2 Site map of GRI campus (Green = Agricultural land, Red = Human habitation)

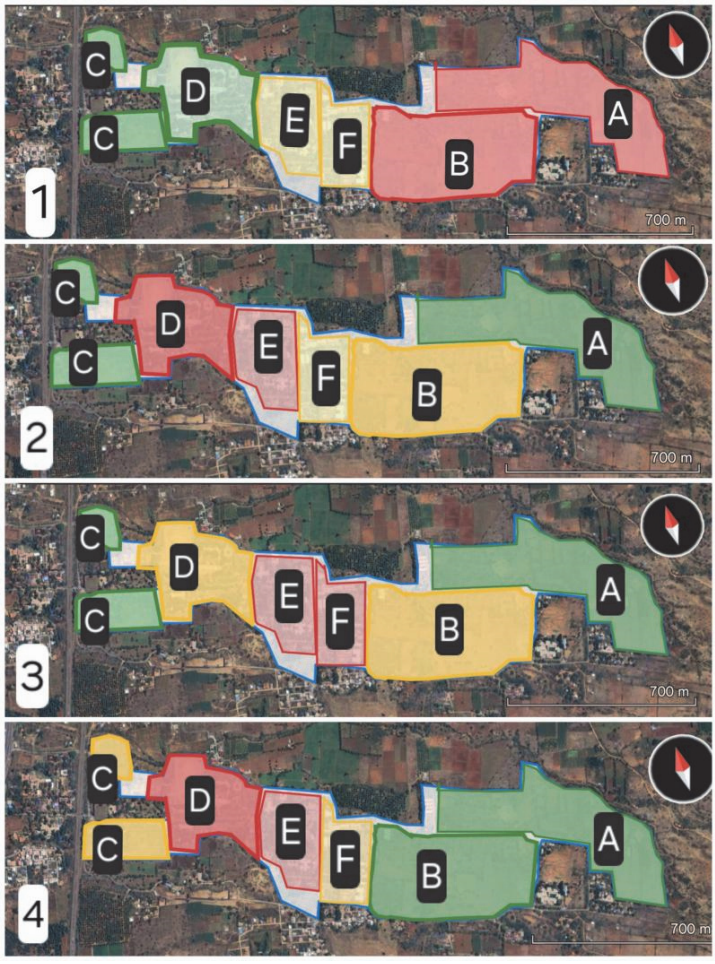


Fig. 3 GIS representation of species richness and 3 diversity indices across 6 sites in GRI. 1. Species richness; 2. Simpson index; 3. Shannon index; 4. Evenness index (Green - High; Yellow - Medium and Red - Low)

Table 1. **List of species identified in GRI campus**

No.	Family/Guild	Name	No. of individuals
1	Araneidae (Orb web weavers)	*Argiope aemula* (Walckenaer, 1841)#	29
2		*Argiope anasuja* Thorell, 1887	66
3		*Argiope pulchella* Thorell, 1881	15
4		*Cyrtophora cicatrosa* (Stoliczka, 1869)	187
5		*Cyrtophora citricola* (Forsskål, 1775)#	3
6		*Eriovixia excelsa* (Simon, 1889)#	23
7		*Eriovixia laglaizei* (Simon, 1877)#	53
8		*Gasteracantha geminata* (Fabricius, 1798)	8
9		*Neoscona* sp.	20
10		*Neoscona vigilans* (Blackwall, 1865)#	21
11	Cheiracanthiidae (Other hunters)	*Cheiracanthium melanostomum* (Thorell, 1895)#	15
12		*Cheiracanthium* sp.*	7
13		*Cheiracanthium trivittatum* Simon, 1906#	2
14	Clubionidae (Other hunters)	*Clubiona filicata* O. Pickard-Cambridge, 1874#	11
15	Corinnidae (Ground hunters)	*Castianeira* sp.*	5
16		*Cambalida flavipes* (Gravely, 1931)#	5
17	Eresidae (Sheet web weavers)	*Stegodyphus sarasinorum* Karsch, 1892	451
18	Idiopidae (Ambush hunters)	Genus and species indet.	2
19	Lycosidae (Ground hunters)	*Draposa* sp. 1	86
20		*Draposa* sp. 2	189
21		*Hippasa pantherina* Pocock, 1899#	441
22		*Hippasa madraspatana* Gravely, 1924	2
23		*Lycosa* sp.	6
24		*Wadicosa* sp.	198
25	Oecobiidae (Sensing web weavers)	*Oecobius putus* O. Pickard-Cambridge, 1876#	474
26		*Oecobius* sp.*	572
27	Oxyopidae (Other hunters)	*Hamataliwa* sp.1*	12
28		*Hamataliwa* sp.2*	9
29		*Oxyopes hindostanicus* Pocock, 1901#	217
30		*Oxyopes shweta* Tikader, 1970	24

31		*Oxyopes javanus* Thorell, 1887[#]	18
32		*Peucetia viridana* (Stoliczka, 1869)[#]	141
33	Philodromidae (Other hunters)	*Thanatus* sp.*	2
34	Pholcidae (Space web weaver)	*Artema atlanta* Walckenaer, 1837[#]	753
35		*Pholcus phalangioides* (Fuesslin, 1775)	160
36	Pisauridae (Other hunters)	*Hygropoda* sp.*	1
37	Salticidae (Other hunters)	*Afraflacillia* sp.*	2
38		*Brettus* sp.*	1
39		*Carrhotus viduus* (C. L. Koch, 1846)	1
40		*Carrhotus sannio* (Thorell, 1877)[#]	2
41		*Chrysilla volupe* (Karsch, 1879)[#]	5
42		*Hasarius adansoni* (Audouin, 1826)[#]	10
43		*Hyllus semicupreus* (Simon, 1885)	146
44		*Menemerus bivittatus* (Dufour, 1831)[#]	18
45		*Myrmarachne* sp.*	10
46		*Plexippus paykulli* (Audouin, 1826)	124
47		*Stenaelurillus megamalai* Sudhin, Sen & Caleb, 2023[#]	264
48		*Stenaelurillus lesserti* Reimoser, 1934[#]	217
49		*Telamonia dimidiata* (Simon, 1899)	53
50	Scytodidae (Other hunters)	*Scytodes fusca* Walckenaer, 1837[#]	5
51	Sparassidae (Other hunters)	*Gnathopalystes flavidus* (Simon, 1897)[#]	13
52		*Heteropoda venatoria* (Linnaeus, 1767)[#]	42
53		*Olios* sp.	12
54	Tetragnathidae (Orb web weavers)	*Leucauge decorata* (Blackwall, 1864)	33
55	Theridiidae (Space web weavers)	*Rhomphaea projiciens* O. Pickard-Cambridge, 1896[#]	4
56	Thomisidae (Ambush hunters)	*Henriksenia hilaris* (Thorell, 1877)[#]	11
57		*Indoxysticus minutus* (Tikader, 1960)[#]	507
58		*Thomisus* sp.	3
59		*Tmarus* sp.*	1
60		*Xysticus* sp.*	2
61	Uloboridae (Orb web weavers)	*Uloborus* sp.	5
Total	**19**	**61**	**5,719**

Note: [#]New species record; [*]new genus record to Dindigul district

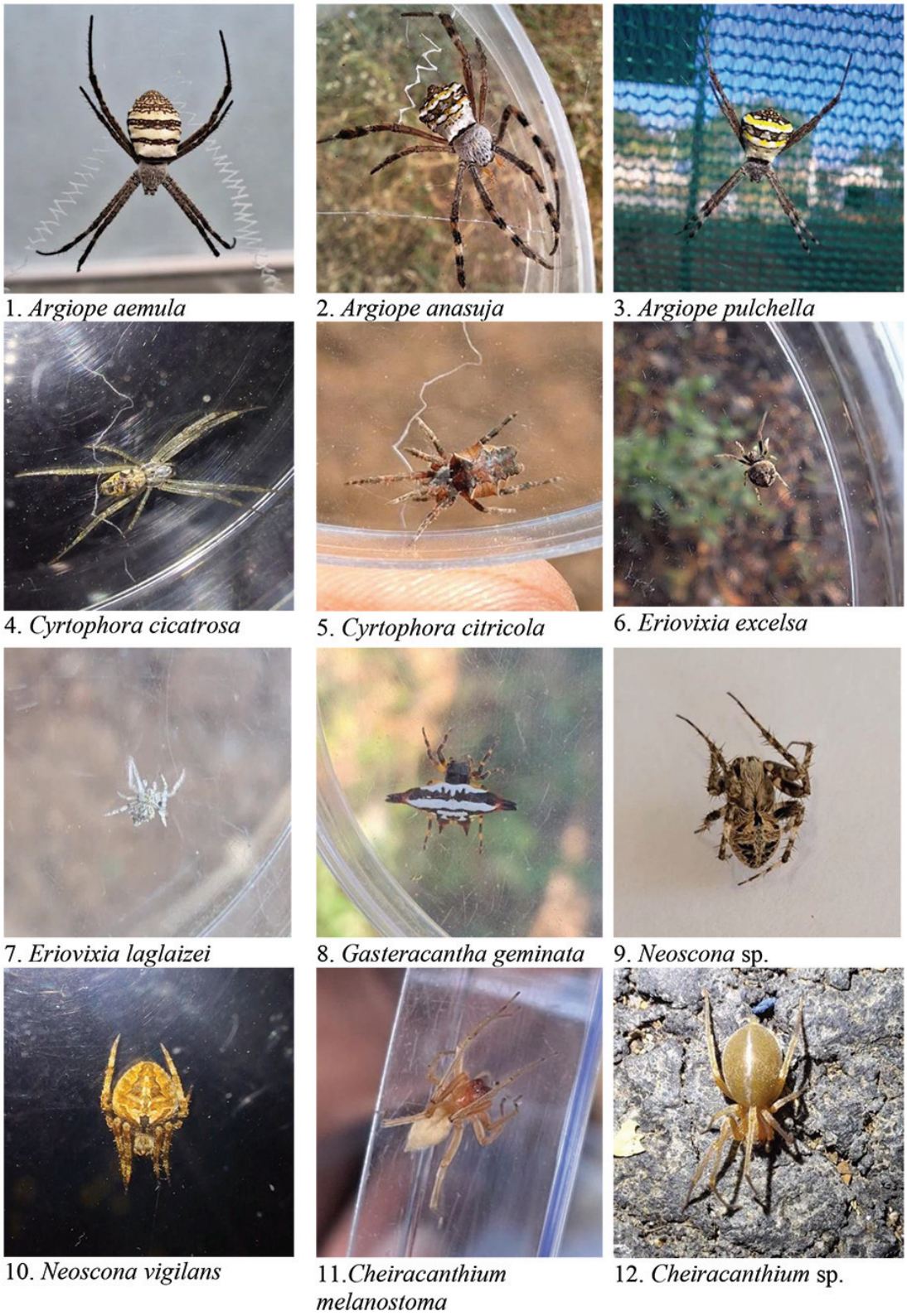


Fig. 4 Spider species recorded in the GRI campus



Fig. 4 Spider species recorded in the GRI campus (Contd.)

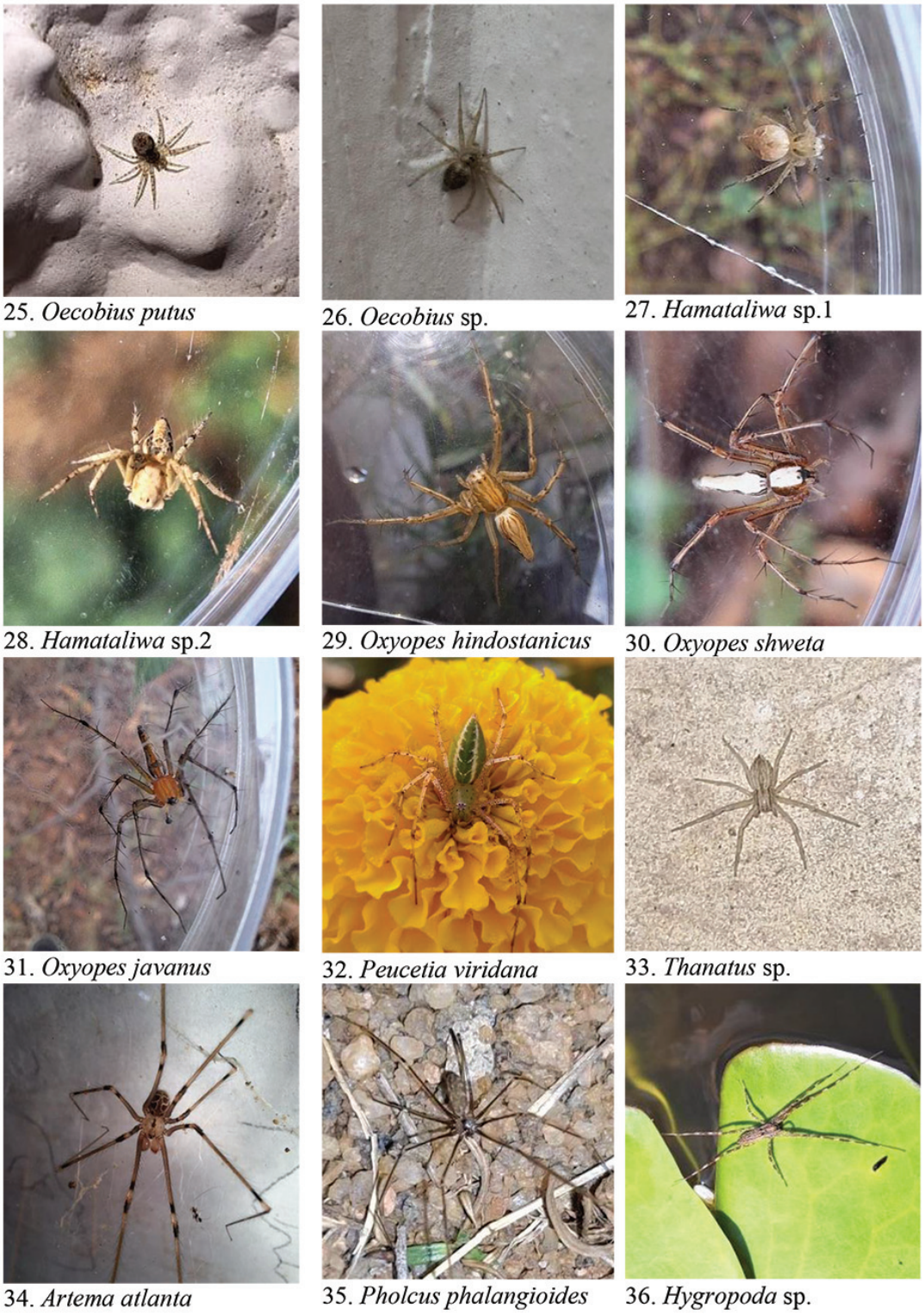


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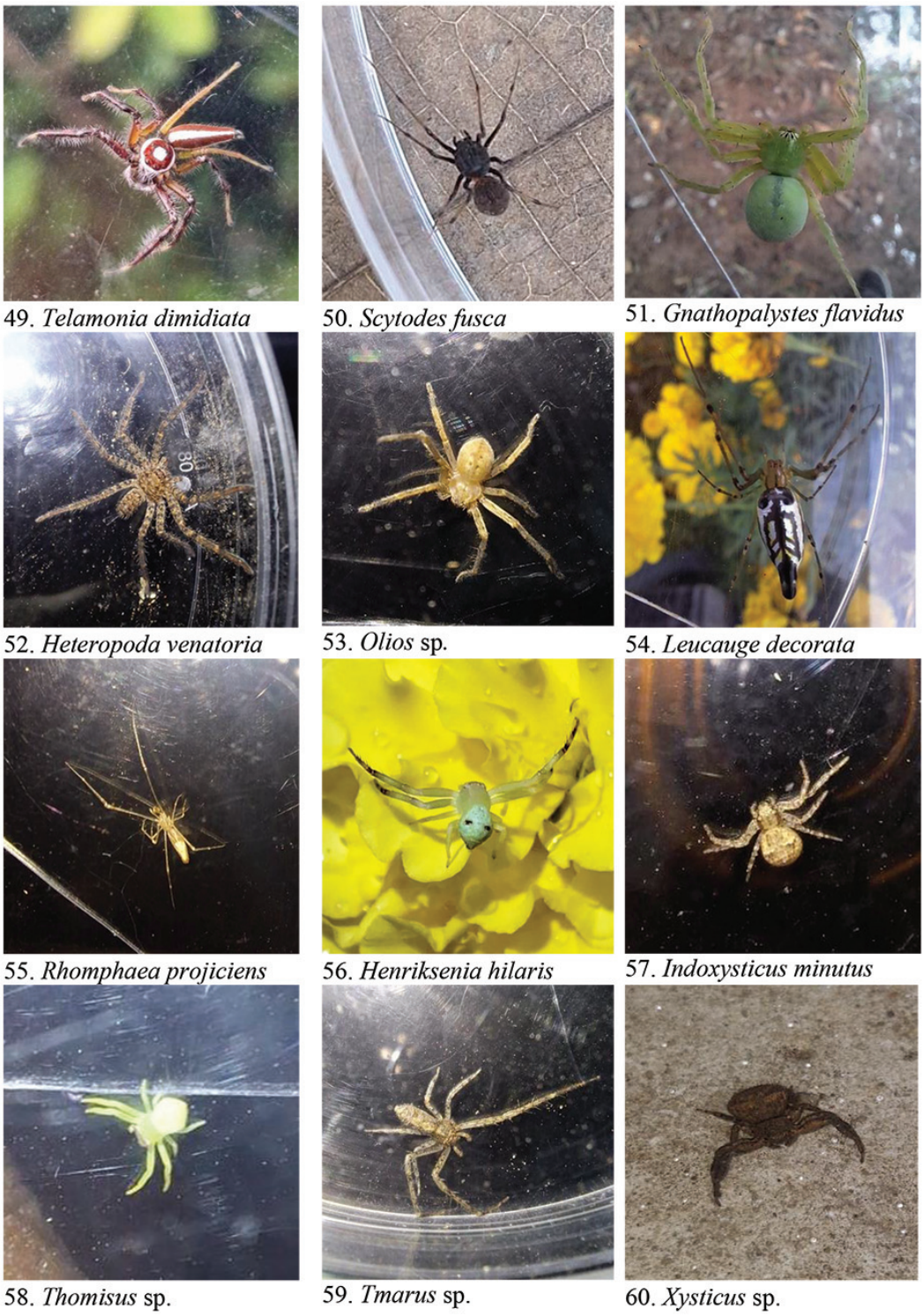


Fig. 4 Spider species recorded in the GRI campus (Contd.)



61. *Uloborus* sp.

Fig. 4 List of images of species identified in GRI campus

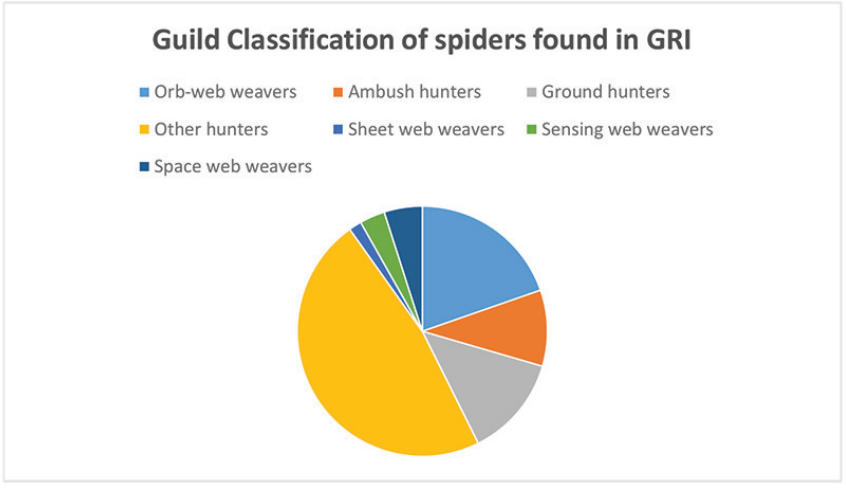


Fig. 5 Guild classification of spiders found in GRI

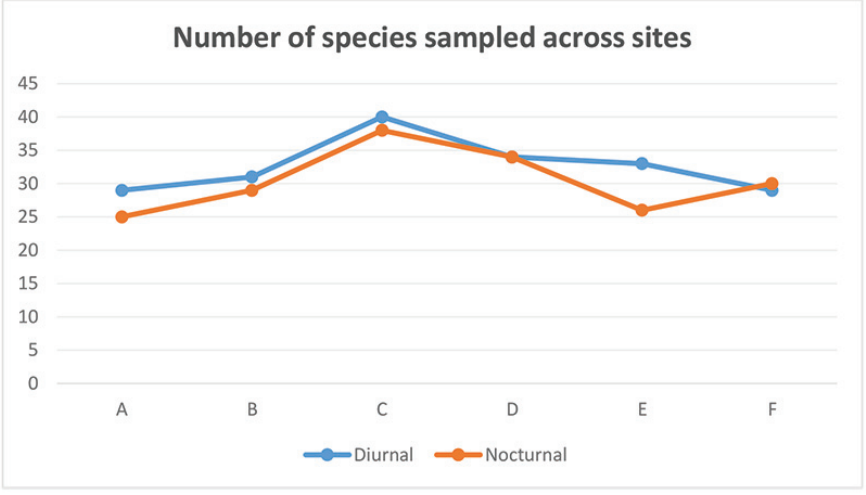


Fig. 6 Number of species sampled across sites

Table 2. **Alpha diversity and evenness indexes of all six sites in GRI**

Sites	A	B	C	D	E	F
Taxa_S	30	31	46	44	37	35
Individuals	934	769	961	1288	825	942
Dominance_D	0.08128	0.0979	0.08573	0.1408	0.1434	0.1375
Simpson_1-D	0.9187	0.9021	0.9143	0.8592	0.8566	0.8625
Shannon_H	2.761	2.691	2.951	2.425	2.396	2.435
Evenness_e^H/S	0.5272	0.4759	0.4158	0.2569	0.2968	0.326
Chao-1	31.2	31	47.88	51.86	44.5	37.14

Alpha diversity indices were calculated among all 6 sites (Fig. 3; Table 2). Species richness ranged from 30 (Site A) to 46 (Site C). Site C exhibited the highest Shannon diversity (H2 = 2.951), indicating a comparatively even distribution of individuals among species. In contrast, Sites D, E, and F showed higher total abundance but lower evenness, with dominance values peaking in Sites D (0.1408) and E (0.1434). Observed richness closely matches with the Chao-1 estimates.

Whittaker's beta diversity was (0.64126) and Bray-Curtis Similarity dendrogram indicate species turnover across the campus and the similarity

Table 3. **Gamma diversity and evenness indexes of GRI**

Site	GRI
Species	61
Individuals	5719
Dominance_D	0.06589
Simpson_1-D	0.9341
Shannon_H	3.053
Evenness_e^H/S	0.3471
Chao-1	61.75

Table 4. **Diversity indexes of Diurnal sampling in GRI**

Site	A	B	C	D	E	F
Taxa_S	29	31	40	34	33	29
Individuals	445	411	488	613	433	402
Dominance_D	0.08264	0.09303	0.08585	0.1348	0.1352	0.1569
Simpson_1-D	0.9174	0.907	0.9142	0.8652	0.8648	0.8431
Shannon_H	2.76	2.707	2.9	2.467	2.437	2.334
Evenness_e^H/S	0.5446	0.4833	0.4543	0.3466	0.3466	0.3558
Chao-1	32.75	34.5	41.25	40	40.86	30.43

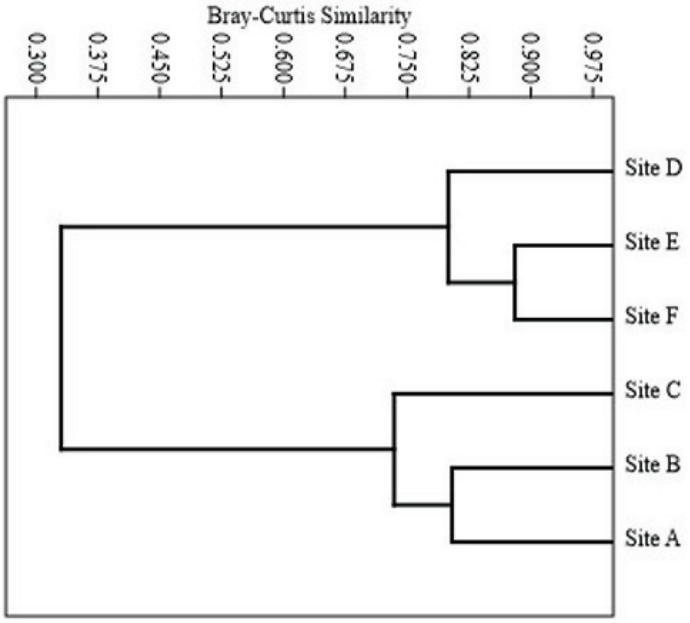


Fig. 7 Bray-Curtis Similarity for Beta Diversity.

Table 5. **Diversity indexes of Nocturnal sampling in GRI**

Site	**A**	**B**	**C**	**D**	**E**	**F**
Taxa_S	25	29	38	34	26	30
Individuals	489	358	473	675	392	540
Dominance_D	0.0881	0.109	0.08783	0.1497	0.1681	0.1287
Simpson_1-D	0.9119	0.891	0.9122	0.8503	0.8319	0.8713
Shannon_H	2.697	2.613	2.924	2.314	2.248	2.448
Evenness_e^H/S	0.5937	0.4704	0.4901	0.2976	0.3643	0.3854
Chao-1	25.5	32	39.67	37	27.43	35.25

between sites (Fig 7). Gamma diversity comprised 61 species, with Shannon index (H') = 3.053 and Simpson's (1–D) = 0.9341 (Table 3).

Diurnal sampling recorded up to 40 species (Site C), with relatively higher evenness (0.3466–0.5446). Nocturnal sampling produced slightly greater abundances in several sites (e.g., 675 individuals at Site D), though dominance values were marginally higher at night, particularly in Site E (D = 0.1681) (Fig. 6; Tables 4, 5)

Guild analysis revealed a functionally diverse community (Fig. 5; Table 6). "Other hunters" constituted the most species-rich guild (29 species), followed by orb-web weavers (12 species), ground hunters (8 species), and ambush hunters (6 species).

Table 6. **Guild classification of spiders in GRI**

Guild	Species	Families
Orb-web weavers	12	Araneidae, Tetragnathidae, Uloboridae
Ambush hunters	6	Idiopidae, Thomisidae
Ground hunters	8	Corinnidae, Lycosidae
Other hunters	29	Cheiracanthiidae, Clubionidae, Oxyopidae, Philodromidae, Pisauridae, Salticidae, Scytodidae, Sparassidae
Sheet web weavers	1	Eresidae
Sensing web weavers	2	Oecobiidae
Space web weavers	3	Pholcidae, Theridiidae

DISCUSSION

The high richness observed in the study demonstrates that semi-managed institutional landscapes can sustain substantial spider diversity. Similar diversity levels have been reported from university campuses and semi-urban habitats in India, highlighting the ecological importance of mosaic landscapes within human-dominated regions (Adarsh and Nameer, 2015; Prajapati *et al.*, 2016; Asima *et al.*, 2020; Caleb 2020; Swapna and Babitha, 2022; Dave and Trivedi, 2024).

The prominence of salticids is consistent with studies from tropical and campus ecosystems, where jumping spiders successfully exploit heterogeneous vegetation and built environments (Adarsh and Nameer, 2015; Prajapati *et al.*, 2016; Asima *et al.*, 2020; Caleb 2020; Dave and Trivedi, 2024). Although species-rich, Salticidae was not the most abundant family. Numerically, Oecobiidae, Pholcidae, and Lycosidae contributed the highest number of individuals, largely due to aggregation of *Oecobius* spp., *Artema atlanta*, and *Hippasa pantherina*. This distinction between species richness and numerical dominance underscores the structural complexity of the campus, where synanthropic web-builders and ground-dwelling hunters coexist.

Alpha diversity indices revealed marked spatial heterogeneity among sites. These built-up areas were characterised by concentrations of a few highly successful species, likely reflecting simplified vegetation structure and localized disturbance. Simpson's diversity values (1–D = 0.8566–0.9187) nevertheless indicate generally stable assemblages across sites. The close agreement between observed richness and Chao-1 estimates suggests adequate sampling coverage.

Beta diversity indices (Whittaker's beta diversity and Bray-Curtis similarity) show moderate species turnover across the campus (Table 3; Fig. 7), reflecting habitat heterogeneity among agricultural fields, open grounds, and built structures. Such turnover is typical of mosaic landscapes, where variation in vegetation complexity, soil conditions, and microclimate shapes community composition (Zhang *et al.*, 2018). The abundance of certain spiders is higher in one site than other is due to niche preference and resource availability and higher adaptability to artificial structures (Priyadharshini *et al*., 2015).

The overall dominance (D = 0.06589) is low indicates a diverse and relatively balanced spider community across the campus. Comparable gamma diversity values have been documented from dry

deciduous forests and urban green spaces (Kayal *et al.*, 2024; Kulkarni *et al.*, 2024), reinforcing the conservation value of semi-natural institutional habitats.

Diurnal and nocturnal sampling yielded comparable richness, confirming the importance of time-stratified surveys. Shannon diversity remained consistently high across both periods, suggesting complementary activity patterns rather than strong temporal segregation. Such temporal partitioning likely reduces interspecific competition and enhances coexistence within the assemblage.

Although hunting guilds dominated in species count, web-building guilds, especially space web and sensing web weavers, were numerically abundant due to high densities on building walls and sheltered microhabitats.

Ground hunters such as Lycosidae were strongly associated with open soil and agricultural plots, whereas ambush hunters (Thomisidae) were linked to flowering vegetation. Orb-web weavers reflected adequate vertical stratification within shrubs and trees. Rare guild representatives, including Idiopidae morphospecies, *Scytodes fusca*, and *Hygropoda* sp., indicate the persistence of specialised microhabitats within the campus.

The coexistence of habitat specialists and widespread generalists highlights the ecological significance of the GRI campus as a semi-natural refuge. Species tolerant of anthropogenic settings (e.g., *Plexippus paykulli*, *Oxyopes javanus*) occurred alongside taxa indicative of relatively undisturbed microhabitats. Overall, the integration of spatial, temporal, and functional analyses demonstrates that habitat heterogeneity within the campus sustains a diverse and structurally complex spider community.

The study also reports a total of 27 species and 11 genera which are new distributional records for Dindigul district, highlighting the poorly explored spider diversity in this region. The occurrence of several rarely reported species, including *Stenaelurillus megamalai*, *Eriovixia excelsa*, and *Gnathopalystes flavidus*, indicates that the campus supports diverse spider assemblage.

These findings provide essential baseline data for long-term biodiversity monitoring and emphasise the importance of incorporating invertebrate conservation into institutional land-use planning and sustainable habitat management strategies.

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Investigation of P-glycoprotein (P-gp) ATPase gene variation in temephos resistant *Aedes albopictus* (Skuse, 1894), Chidambaram, India

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ABSTRACT: The study investigated the role of P-glycoprotein (P-gp) ATPase gene expression in mediating resistance to the organophosphate larvicide temephos in *Aedes albopictus*. Larval populations were subsequently classified into control, susceptible, and resistant groups based on their differential response to temephos following laboratory-induced selection pressure. Total RNA was isolated, and relative gene expression was quantified using RT-PCR, with â-actin serving as the endogenous control for normalization. An upregulation of P-gp expression was observed in resistant larvae compared to susceptible and control groups. Densitometric analysis revealed P-gp expression levels of 35,863.67 AU in resistant larvae, 30,761.16 AU in susceptible larvae, and 33,786.40 AU in control larvae, whereas â-actin expression remained consistent across all groups. The elevated expression of P-gp indicates its involvement in temephos resistance, likely through enhanced xenobiotic efflux. The finding underscores the potential of ATP-binding cassette (ABC) transporters as molecular targets for improving vector control strategies.
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KEY WORDS: Vector, arbovirus, insecticide resistance, RT-PCR, ABC transporter

INTRODUCTION

Insecticide resistance in medically significant vectors present a critical obstacle to vector control strategies (WHO, 2022; Ibrahim *et al*., 2024). Principal resistance mechanisms include the upregulation of detoxification enzymes and target-site mutations (Han *et al*., 2024; Wang *et al*., 2024). However, contemporary studies implicated permeability glycoprotein (P-gp), a plasma membrane-associated ATP-binding cassette (ABC) transporter, in conferring multidrug resistance (Rani *et al*., 2024; Bharathiraja *et al*., 2024; Lespine *et al*., 2024; Elbahnsi *et al*., 2024). Structurally, P-gp is composed of two repeating units, each containing a nucleotide-binding region and transmembrane helices that together form the functional transporter (Allikmets *et al*., 1996; Ayrton and Morgan, 2001; Buss and Callaghan, 2008). The ATP-driven expulsion of various substrates from cells is primarily mediated by P-glycoprotein (See *et al*., 1974; Guiral *et al*., 1994; Schlemmer and Sirotnak,

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1994), contributing to reduced intracellular accumulation and therapeutic failure (Lanning *et al*., 1996). It is ubiquitously expressed across cell types in diverse organisms, including insects, where it protects against toxic compounds (Podsiadlowski *et al*., 1998; Xu *et al*., 1998; Buss and Callaghan, 2008). Notably, presence of P-gps hamper entry of xenobiotics into protected compartments complicating treatment efficacy (Abu-Qare *et al*., 2003; Giacomini *et al*., 2010; Kido *et al*., 2024; Johnston *et al*., 2024; Huang *et al*., 2024)." Functional inhibition or silencing of P-gp in resistant insect species, including *Aedes aegypti* and *Helicoverpa armigera*, has been shown to increase susceptibility to insecticides, underscoring its role in resistance mechanisms (Figueira-Mansur *et al*., 2013). The present study investigates P-gp gene expression in temephos-resistant *A. albopictus* larvae to elucidate its contribution to resistance and inform molecular strategies for vector control.

MATERIALS AND METHODS

For the present study, temephos-resistant *A. albopictus* populations were derived from a susceptible (Manyu *et al*., 2022), through cyclic mass rearing under temephos pressure in a controlled insectary environment in accordance to protocols prescribed by the International Atomic Energy Agency (IAEA, 2017). Samples of resistant larvae (RL), susceptible larvae (SL) and control larvae (CL) were chosen. Total RNA was isolated from each sample (RL, SL, and CL) using the TRIzol method and centrifuged in RNase-free tubes at 5000 rpm for 10 minutes to obtain cell pellets (1X 10^7 cells). Each pellet was lysed with 700µL of TRIZOL, and the lysate was mixed with 300 µL of chloroform. It was then centrifuged (12,000 rpm) for 20 minutes at 4°C, to collect the RNA. RNA precipitation was done by adding isopropanol (700µL), followed by centrifugation to obtain a visible pellet. The RNA pellet was washed with 70 per cent ethanol and dried. The RNA was diluted in 30µL of double-distilled, autoclaved water, stored at 80 °C, and quantified with Labman UV–V is spectrophotometer (Labman, India).

To remove DNA contamination, RNA samples were treated with DNase-I in a 20µL reaction volume at 37°C for 30-45 minutes. Following addition of 2µL of 20µM EGTA, the solution was incubated for 10 minutes at 66°C. RNA was precipitated by adding sodium acetate (1/10 volume) and absolute ethanol (2 volumes), incubated for 60 minutes at -20°C and centrifuged for 20 minutes in 4°C at 12,000 rpm. RNA pellet was extracted and ringed with 500µL 75 **per cent** ethanol. Air dried the sample and 20µL double-autoclaved nuclease-free Milli-Q water was added.

First-strand complementary DNA (cDNA) was synthesized from 1.5ìg of total RNA using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT). Reverse transcription was performed at 25°C for 10 min, followed by 42°C for 59 min. The reaction was terminated by incubation at 99°C for 5s to denature RNA–cDNA hybrids and inactivate the reverse transcriptase enzyme, followed by holding at 4°C. The synthesized cDNA was stored at "20°C until use.

Analysis of P-glycoprotein (P-gp) gene expression (Table 1): The synthesized cDNA was used as a template for amplification of the target P-glycoprotein ATPase (P-gp ATPase) gene by reverse transcription polymerase chain reaction (RT-PCR). Amplifications were performed using 2× Taq PCR Master Mix (BioLit, India) according to the manufacturer's recommendations. Gene expression levels were normalized against the endogenous reference gene ß-actin, which served as the internal control. PCR amplification of the P-gp ATPase gene was carried out under the following thermal cycling conditions: an initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30s, annealing at 56°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min and a hold at 4°C. Amplification of the ß-actin gene was performed using an initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min and a hold at 4°C.

Agarose gel electrophoresis and densitometric analysis: PCR amplicons were resolved by electrophoresis on a 1 **per cent** agarose gel prepared in 1× Tris-acetate-EDTA (TAE) buffer. Agarose (0.3g) was dissolved in 30mL of 1× TAE buffer, heated until completely solubilized, cooled to approximately 50°C, and stained with ethidium bromide (1.5µL). The gel was cast using an appropriate comb and allowed to solidify at room temperature. Electrophoresis was performed in 1× TAE running buffer. PCR products (3iL) mixed with an equal volume of loading dye were loaded into the wells and electrophoresed at 70 V for 15–20 min. Amplified DNA fragments were visualized under ultraviolet illumination using a gel documentation system. Band intensities were quantified by densitometric analysis using ImageJ software (National Institutes of Health, USA). Relative expression of the P-gp ATPase gene was determined after normalization against the corresponding ß-actin expression levels.

Relative expression analysis: Semi-quantitative assessment of P-gp ATPase gene expression was performed by comparing the densitometric intensity of the target gene amplicons with that of the endogenous ß-actin control. Relative expression levels were calculated from normalized band intensities obtained using ImageJ software and expressed as fold change relative to the control group.

RESULTS AND DISCUSSION

P-glycoprotein (P-gp) gene expression: There was a significant upregulation of P-gp gene expression in temephos-resistant *A. albopictus* (Figs. 1, 2). Densitometric analysis indicated overexpression, with levels recorded as, 35,863.67AU in RL, 30,761.16AU in SL, and 33,786.40AU in CL. The findings are consistent with those of Figueira *et al*. (2013), who reported similar evidence in resistant *Aedes aegypti*. Identical overexpression of P-gp is reported in *Anopheles gambiae* (Roth *et al*., 2003), *Heliothis virescens* and *Helicoverpa armigera* (Lanning and Carter, 2006, *Drosophila melanogaster* (Grailles *et al*., 2003), *Teleogryllus commodus* (Leader and O'Donnell, 2005), *Rhodnius prolixus* (Rheault *et al*., 2006), *Coptis japonica* (Buss and Callaghan, 2008), and *Aedes caspius* (Porretta *et al*., 2008).".The elevation of P-gp is correlated to insecticide resistance (Bourguinat *et al*., 2007; Aurade *et al*., 2012).

A comparative analysis of â-actin gene expression (Fig. 1), alongside P-gp gene expression (Fig. 3), indicated minimal variation, confirming its suitability as a reference gene. Expression values were 49,340.06AU (RL), 49,340.97AU (SL), and 49,342.35AU (CL), demonstrating consistent expression across all groups. P-glycoprotein (P-gp), first reported by Juliano and Ling (Gottesman and Ling, 2006), is known to actively effluxes xenobiotics from cells using ATP hydrolysis (Buss and Callaghan, 2008; Aurade *et al*., 2012; Figueira-Mansur *et al*., 2013; Thevenod and Lee, 2024; Devi *et al*., 2024), that results in reduced drug delivery efficacy (Abu-Qare *et al*., 2003; Veider *et al*., 2024). Empirical studies have demonstrated its role in conferring resistance to multiple classes of insecticides (Buss and Callaghan, 2008; Szpila *et al*., 2008; Xu *et al*., 2023; Tseng *et al*., 2024). The P-glycoprotein is a potential molecular target to enhance insecticide efficacy and manage resistance (Akhtar *et al*., 2011; Ma *et al*., 2013; Parida *et al*., 2024).

Table 1. Primers for P-glycoprotein (P-gp) gene

Genes	Direction	Sequence (5′ – 3′)
P-gp	Forward	52 - TAAAATGAAGTTGAACGTATCGT-3'
	Reverse	52 - AGATGGTCAGCGGTGATTTC-3'
ß-actin	Forward	52 - ATGGTCGGTATGGGGCAGAAGGACTC-3'
	Reverse	52 - TCGCACTTCATGATAGAGTTGTA -3'

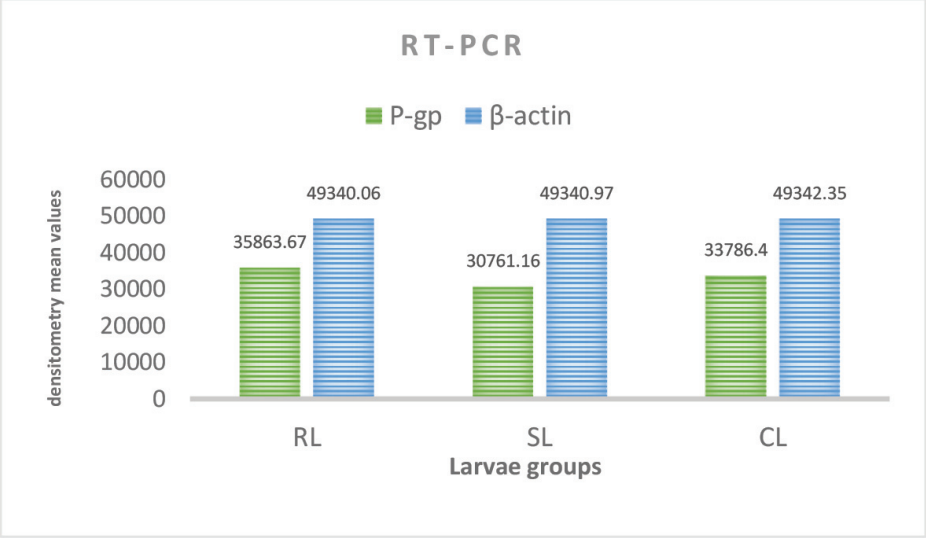


Fig 1. Densitometric analysis of P-gp and â-actin gene expression values in control and experimental *Aedes albopictus* larvae.
Densitometric analysis: All the data are expressed as the mean ± S.D measured by RT-PCR. The values are statistically significant and different at $p<0.05$. â-actin was used as loading control; RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae

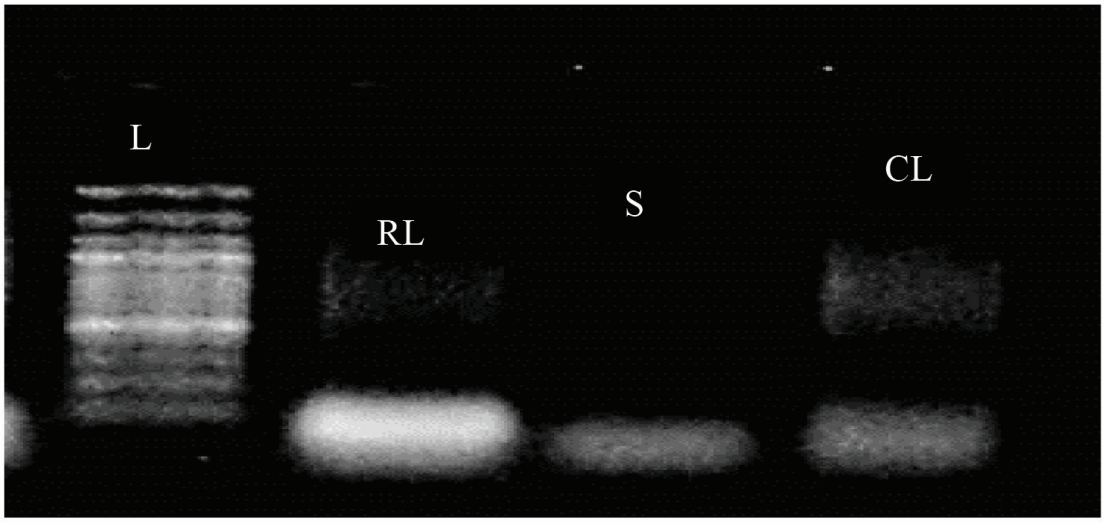


Fig 2. Gene expression profile of P-gp using SDS electrophoresis in control and experimental *Aedes albopictus* larvae. RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae; L: ladder-100 bp

Reports have been necessitated the investigation of P-glycoprotein inhibitors to improve the efficacy of therapeutic agents and pesticides (Shukla *et al*., 2008). Documented P-gp inhibitors include curcumin, flavonoids, kaempferol, quercetin, tariquidar, verapamil, cyclosporin A, quinidine, reserpine, and tamoxifen (Xu *et al*., 1998; Akhtar *et al*., 2011; Lima *et al*., 2014; Sankar and Kumar, 2023). Among these, verapamil has demonstrated a significant increase in mortality rates through effective inhibition of P-gp activity (Kundu *et al*., 2024). This strategy may also be relevant for addressing insecticide resistance by facilitating improved drug delivery and directly targeting

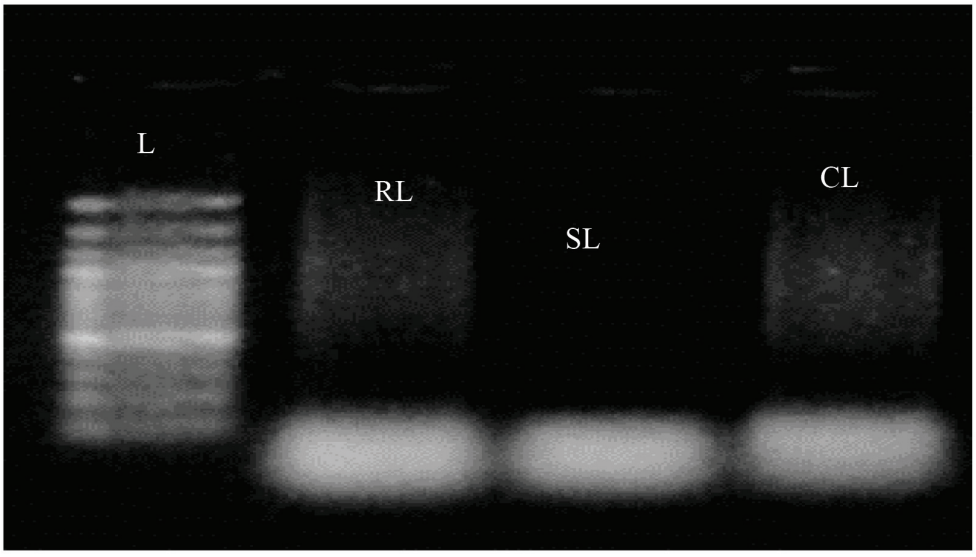


Fig 3. Gene expression profile of â-actin using SDS electrophoresis in control and experimental *Aedes albopictus* larvae. RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae; L: l adder-100 bp

resistance mechanisms (Tapadia and Lakhotia, 2005). Elucidation of such molecular study in insecticide resistance is highly significant to formulate effective management strategies (Akbar *et al*., 2014). Analysis of â-actin gene expression indicated its suitability as a reference gene. The elevated expression of P-gp indicates its involvement in temephos resistance, likely through enhanced xenobiotic efflux. The finding underscores the potential of ATP-binding cassette (ABC) transporters as molecular targets for improving vector control strategies.

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Parasitoids of butterflies: Diversity and host associations in Kolhapur district, Maharashtra, India

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ABSTRACT: This study describes the diversity of parasitoids associated with different life stages of the butterfly. During the study, 14 parasitic Hymenopteran and Dipteran species belong to seven families, *viz*. Braconidae (five species), Encyrtidae (two species), Scelionidae (two species), Ichneumonidae (two species), Pteromalidae (one species), Chalcididae (one species), and Tachinidae (one species) were recorded. Both, the braconid larval parasitoid *Distatrix papilionis* (Viereck) and the pupal parasitoid *Philomacroploea* sp. are gregarious, while other parasitoid species are solitary, except the egg parasitoid *Ooencyrtus* sp., from which three individuals emerged. Although parasitism on egg and pupal stages has been reported, most parasitic insects target the larval stages. Besides highlighting the ecological importance of parasitic insects, this study provides fundamental data useful for studies on butterfly ecology and conservation.
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KEY WORDS: Papilionoidea, Hymenoptera, Diptera, ecology, biocontrol

INTRODUCTION

Many biological factors, including parasitoids, control the populations of ecologically significant insects, the butterflies (Ehrlich and Raven, 1964). Parasitoids, which are primarily members of the orders Hymenoptera and Diptera, are powerful natural enemies of butterflies because they develop in or on a single host and eventually induce host mortality (Godfray, 1994). Eggs, larvae, and pupae are all attacked by butterfly parasitoids; reports of larval parasitoids are the most common. According to Stireman *et al.* (2006), important parasitoid families include Braconidae, Ichneumonidae, Chalcididae, Tachinidae, and Trichogrammatidae. Despite their ecological importance, butterfly parasitoids remain poorly documented, particularly in biodiverse tropical regions. Therefore, research on parasitoid diversity and host associations is crucial for understanding butterfly population regulation and guiding conservation initiatives.

MATERIALS AND METHODS

Butterfly eggs, larvae, and pupae have been collected during regular field surveys in Kolhapur district, Maharashtra, from 2022 to 2024. The eggs, larvae, and pupae were hand-picked from host plants and brought to the laboratory in ventilated plastic containers. In the Department of Zoology

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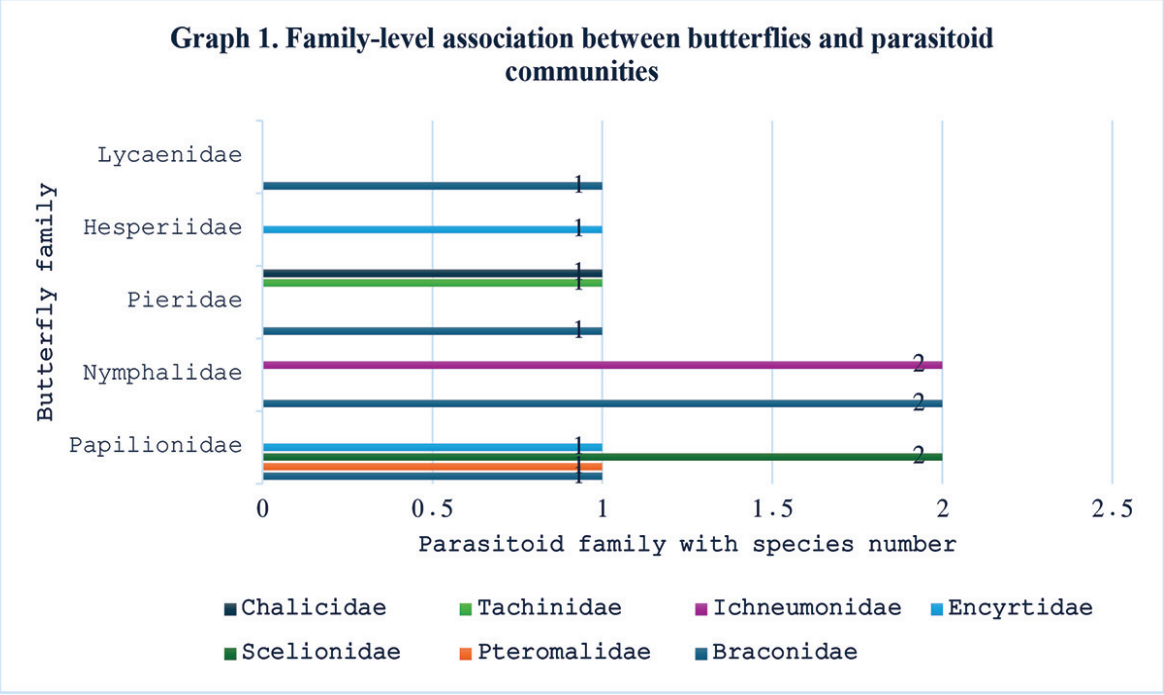
at Shivaji University in Kolhapur, collected eggs, larvae, and pupae were reared under carefully regulated laboratory conditions (25 ± 2 °C and RH 60–70%). Every day, the caterpillars were provided with fresh host plant material. Daily signs of parasitism, parasitoid emergence, and pupation were observed with eggs, larvae, and pupae. Additionally, while studying the life cycles of various butterfly species in the laboratory, parasitoids that emerged at different stages of their life cycle were collected and included in this study. An aspirator was used to collect emerging parasitoids as soon as they appeared. For subsequent studies, adult parasitoids were either dry-mounted or kept in 70–95 per cent ethanol after being killed with ethyl acetate. The host species, host developmental stage, and emergence date were all noted on the labels of each specimen. Using reference collections and established taxonomic keys, *viz*. Boucek and Rasplus (1991); Rousse and Villemant (2012); Rousse and Gupta (2013); Gupta *et al.* (2015); Narendran and Achterberg (2016); Gupta and Mandal (2019); Oktaviani *et al.* (2021) and Chen *et al.* (2021), the parasitoid species associated with various stages of butterfly life cycle were identified to the lowest possible taxonomic level.

RESULTS AND DISCUSSION

During the study period, 14 parasitoid species from 10 genera and seven families of Hymenoptera and Diptera were recorded. Of these, most species (13 species) belonged to Hymenoptera and only one belonged to Diptera. Among the hymenopterans, the family Braconidae was represented by the maximum number of species (five species) while the families Encyrtidae, Scelionidae, and Ichneumonidae were represented by two species each, and Pteromalidae and Chalcidae by one species each. The family Tachinidae was represented by a single dipteran parasitoid species (Table 1).

The egg, larval, and pupal life stages of a total of 12 butterfly species were found to be parasitized by parasitoids. Specifically, the eggs of butterfly species *Erionota thrax* (Linnaeus), *Graphium agamemnon* (Linnaeus), *Papilio clytia* Linnaeus and *Papilio polytes polytes* Linnaeus were recorded as being attacked by parasitoids *Ooencyrtus* sp. (Fig. 1A), *Ooencyrtus papilionis* Ashmead (Fig. 1B), *Telenomus* sp. (Fig. 1C) and *Telenomus* sp. (Fig. 1D), respectively. The larvae of butterfly species *Euploea core core* (Cramer), *Luthrodes pandava* (Horsfield), *Papilio demoleus* Linnaeus and *Eurema hecabe* (Linnaeus) were found to be attacked by Ichneumonidae parasitoids (Fig. 1E, F), *Parapanteles eros* Gupta (Fig. 1G), *Distatrix papilionis* (Viereck) (Fig. 1H) and *Cotesia* nr. *effrenus* (Fig. 1I), respectively. The pupae of butterfly species *E. core core* and *Danaus chrysippus* (Linnaeus) were parasitized by *Philomacroploea* sp. (Fig. 1J, K), while *Papilio agenor polymnestor* Cramer, and *Belenois aurota* (Fabricius) were recorded as being parasitized by *Pteromalus puparum* (Linneaus) (Fig. 1L), and *Brachymeria* nr. *minuta* (Fig. 1M), respectively. Only one butterfly, *Catopsilia pomona* (Fabricius) pupa, was found to be parasitized by a Tachinid fly (Fig. 1N).

Overall, regarding the parasitoid population, it was observed that three individuals of *Ooencyrtus* sp. emerged from the eggs of *E. thrax* through three different holes, while only one wasp of *Telenomus* sp. emerged from the egg of *P. polytes polytes*. The highest number of wasp cocoons (72), of *D. papilionis* was recorded from the fifth instar of *P. demoleus*, which were previously attached to its body in the form of cocoons. A single individual of indeterminate Ichneumonidae was observed emerging by making a hole in the head of the *E. core core* larva, and one individual of *Cotesia* nr. *effrenus* was recorded emerging from the dorsolateral side of the abdominal end of the *E. hecabe* larva. In the laboratory, 63 wasps of *Philomacroploea* sp. were observed emerging from a single hole in the pupa of *E. core*, while 55 wasps of the same species were reared from the pupa of *D. chrysippus*. One individual of *B.* nr. *minuta* was reared from the pupa of *B. aurota*, and a single dipteran parasitoid, a tachinid fly species, was found in the form of a maggot in the pupa of *C. pomona pomona*, and it transformed into a pupa the next day. Among all the recorded parasitoid families, Braconidae was the most dominant,



attacking the larval and pupal stages of five butterfly species. The members of families Encyrtidae and Scelionidae parasitized the egg stage, while Ichneumonidae species parasitized only the larvae. Both, Pteromalidae and Chalcididae species were pupal parasitoids (Table 1).

In relation to the parasitic link between parasitoids and butterfly families (Graph 1), it was observed that the family Papilionidae is parasitized by species from four parasitoid families: Scelionidae (2 species), Encyrtidae (1 species), Braconidae (1 species), and Pteromalidae (1 species). Additionally, one species each from Encyrtidae and Braconidae was recorded as a parasitoid for the families Hesperiidae and Lycaenidae, respectively. Among the remaining families, two species each from Ichneumonidae and Braconidae were recorded parasitizing Nymphalidae, while one species each from Braconidae, Chalcididae, and Tachinidae (Diptera) was recorded parasitizing Pieridae. It was found that species of Braconidae parasitize various life stages of butterfly families other than Hesperiidae (Table 1).

Considering the studies on Lepidoptera and their parasitoids, mainly the lepidoptera pests found on crops and their associated parasitoids have been studied. For example, Mahavar *et al.* (2017) studied the larval parasitoid *Compoletes chloridae* of *Helicoverpa armigera*, Bhosale (2020) studied the larval parasitoid *Diadegma argenteopilosa* of *Spodoptera litura*, and Sharmitha *et al.* (2020) studied the egg parasitoid of *Scirpophaga incertulus* in the rice ecosystem. Many such references can be given, which are mainly based on parasitoids of crop pests. However, the references on parasitoids of butterflies are relatively few. The current study highlights the significance of parasitoids in controlling butterfly populations.

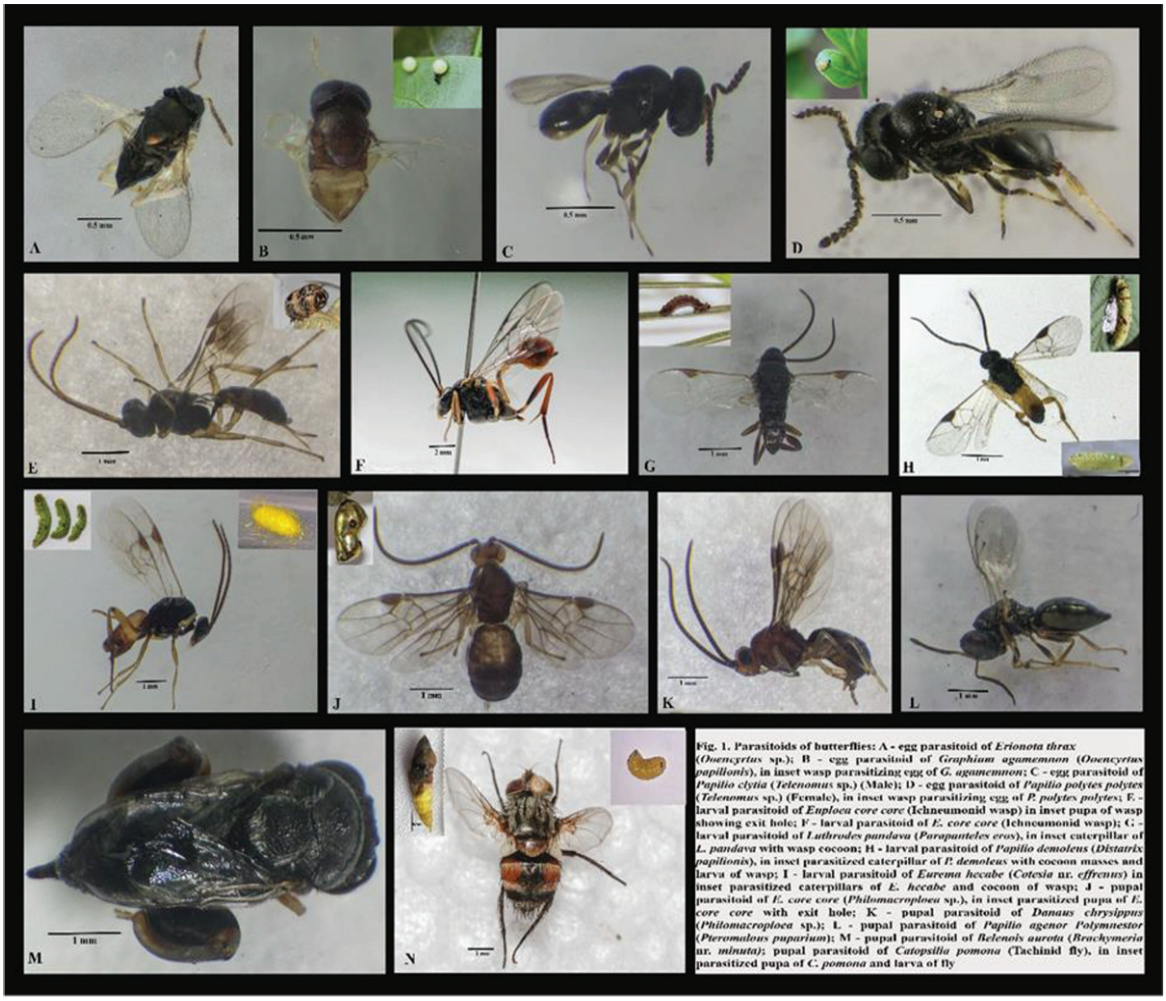


Fig. 1 A) *Ooencyrtus* sp.; B) *Ooencyrtus papilionis* Ashmead; C) *Telenomus* sp.; D) *Telenomus* sp.; E, F) Ichneumonidae parasitoids; G), *Parapanteles eros* Gupta; H) *Distatrix papilionis* (Viereck,); I) *Cotesia* nr. *effrenus*; J, K) *Philomacroploea* sp.; L) *Pteromalus puparum* (Linneaus); M) *Brachymeria* nr. *minuta*; N) Tachinid fly

Butterflies are subjected to severe biotic pressure from a variety of natural enemies throughout their life cycle. Our findings are consistent with previous work by Hawkins *et al.* (1997), which showed that mortality due to natural enemies, especially in the egg and larval stages, is often much higher than mortality due to abiotic factors. Hymenopterans (Braconidae, Ichneumonidae, Trichogrammatidae) and dipterans (Tachinidae) have become highly specialized and efficient natural enemies of butterfly eggs, larvae and pupae. Like the previous study by Stearman *et al.* (2006), the parasitoid assemblage associated with butterflies in the present study was dominated by Hymenoptera and Diptera. Butterfly eggs, larvae, and pupae are frequently parasitized by hymenopteran families such as Braconidae,

Table 1. Parasitoids associated with different life stages of butterflies

Life stage	Host species/ Family	Parasitoid species/ Family	Solitary/ gregarious
Egg	1. *Erionota thrax* (Linnaeus, 1767) (Hesperiidae)	*Ooencyrtus* sp. (Encyrtidae)	Gregarious
	2. *Graphium agamemnon* (Linnaeus, 1758) (Papilionidae)	*O. papilionis* Ashmead, 1905 (Encyrtidae)	Gregarious
	3. *Papilio clytia* Linnaeus, 1758 (Papilionidae)	*Telenomus* sp. (Male) (Scelionidae)	Gregarious
	4. *P. polytes polytes* Linnaeus, 1758 (Papilionidae)	*Telenomus* sp. (Female) (Scelionidae)	Gregarious
Larva	5. *Euploea core core* (Cramer, [1780]) (Nymphalidae)	Indeterminate (Ichneumonidae)	Solitary
	6. *E. c. core* (Cramer, [1780]) (3rd instar) (Nymphalidae)	Unidentified Ichneumonid wasp	Solitary
	7. *Luthrodes pandava* (Horsfield, [1829]) (5th instar) (Lycaenidae)	*Parapanteles eros* Gupta, 2014 (Braconidae)	Solitary
	8. *P. demoleus* Linnaeus, 1758 (5th instar) (Papilionidae)	*Distatrix papilionis* (Viereck) (Braconidae)	Gregarious
	9. *Eurema hecabe* (Linnaeus, 1758) (Pieridae)	*Cotesia* nr. *effrenus* (Braconidae)	Solitary
Pupa	10. *E. core core* (Cramer, [1780]) (Nymphalidae)	*Philomacroploea* sp. (Braconidae)	Gregarious
	11. *Danaus chrysippus* (Linnaeus, 1758) (Nymphalidae)	*Philomacroploea* sp. (Braconidae)	Gregarious
	12. *P. agenor polymnestor* Cramer,1775 (Papilionidae)	*Pteromalus puparum* (Linnaeus, 1758) (Pteromalidae)	Gregarious
	13. *Catopsilia pomona* (Fabricius, 1775) (Pieridae)	Tachinid Fly (Tachinidae)	Solitary
	14. *Belenois aurota* (Fabricius, 1793) (Pieridae)	*Brachymeria* nr. *minuta* (Chalcididae)	Gregarious

Ichneumonidae, Chalcididae, and Trichogrammatidae, whereas dipteran parasitoids are primarily represented by Tachinidae, that attack larval and pupal stages (Scoble, 1995; Feener and Brown, 1997). Gupta *et al.* (2016) reported six species of wasps belonging to three families, *viz*. Braconidae, Chalcididae and Ichneumoindae parasitic on various life stages of seven butterfly species of three families: Hesperiidae, Lycaenidae and Papilionidae. In the present study, as mentioned by Scoble (1995), Feener and Brown (1997) and Gupta *et al*. (2016), members of the families Braconidae, Ichneumonidae, and Chalcididae were found to parasitize the larvae and pupae of butterflies. Verza *et al.* (2024) recorded the first case of gregarious parasitism by *Brachymeria pandora* (Crawford, 1914) in the pupae of *Danaus eryppus* (Cramer, 1775). Patil and Gaikwad (2024) have also newly reported *Brachymeria euploeae* (Westwood), a chalcid wasp, as a pupal parasitoid of the butterfly *Acarea terpsicore* (Linnaeus). Simoneli *et al.* (2025) reported that the Chalcididae pupal parasitoids, the undescribed gregarious *Conura* (*pygmaea* group) and *Brachymeria*

mnestor (Walker), are parasites of the butterflies *Dione vanilla* (Linnaeus) (Nymphalidae) and the *Melete lycimnia flippantha* (Fabricius) (Pieridae), respectively, and their parasitization rate is high. However, in the present study, instead of Trichogrammatidae, members of Encyrtidae and Scelionidae were recorded as egg parasitoids. Additionally, along with Tachinidae and Braconidae, members of Pteromalidae were also found to be parasitizing the pupa of a butterfly.

According to Gupta and Mandal (2019), *D. papilionis* is a gregarious endoparasitoid of *P. demoleus*, and 21-34 wasp cocoons are associated per caterpillar. Similarly, in the present study, the gregarious larval parasitoid *D. papilionis* was found to be associated with the fifth instar caterpillar of *P. demoleus*, with a record of 72 wasp cocoons. Moreover, another gregarious pupal parasitoid, *Philomacroploea* sp., is reported in association with the pupa of *E. core*, with an emerged individual number of 63.

Parasitic insects show a clear preference for specific stages of the butterfly's life cycle. Godfray (1994) states that, particularly, families Trichogrammatidae and Scelionidae often prevents the host's development at an early stage. Similarly, in the present study, a member of the Scelionidae family was found to parasitize the eggs of *P. polytes polytes* but not Tichogrammatidae. Polaszek *et al.* (2024) reported that the Scelionidae parasitic wasp *Telenomus niszwensis* Polaszek, is responsible for high levels of egg mortality in the pomegranate butterfly *Deodorix* (= *Virachola*) *livia* Klug. We have also found two species of genus *Telenomus* of the Scelionidae, parasitizing on the eggs of butterflies, *P. clytia* and *P. polytes polytes*. The parasitism on larvae was the most frequently recorded category, as they are more exposed with longer lifespan and are nutritionally more suitable for the development of parasites (Shaw, 1988). The present findings is in consistent with Shaw (1988), such as members of the Ichneumonidae and Braconidae families parasitizing the larvae of a total of five butterfly species. According to Hawkins (1994), pupal parasitoids, although less diverse, make a significant contribution to controlling host populations. Feener and Brown (1997) reported that tachnid parasitoids (Diptera), play a significant role in regulating lepidopteran host populations. However, current studies showed that parasitoids from the three families Braconidae and Pteromalidae (Hymenoptera), and Tachinidae (Diptera) attack and control butterfly pupae. Overall, the present study indicated that parasitoids are a crucial part of butterfly natural enemy complexes, displaying a variety of host exploitation tactics and considerable potential for population regulation. Improved knowledge of these relationships will certainly benefit lepidopteran and parasitoid ecology.

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Influence of weather parameters on the seasonal incidence of shoot fly (*Atherigona soccata* Rondani) on sorghum in Gujarat, India

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ABSTRACT: A field study was conducted at the Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, during the *summer*, *kharif* and *rabi* seasons of 2024-25 to investigate the seasonal incidence of sorghum shoot fly, *Atherigona soccata* Rondani and its relationship with prevailing weather parameters. The incidence of shoot fly varied significantly among seasons. During summer, infestation commenced in the 13th Standard Meteorological Week (SMW) with 6.67 per cent dead hearts and reached a peak of 36.67 per cent during the 15th SMW. In *kharif*, infestation began in the 28th SMW with 10.00 per cent dead hearts and attained a maximum of 43.33 per cent during the 32nd SMW, representing the highest seasonal infestation. During *rabi*, shoot fly incidence started in the 47th SMW with 3.33 per cent dead hearts and peaked at 30.00 per cent during the 50th SMW. Spider populations appeared shortly after crop establishment and fluctuated throughout the crop season. Correlation analysis revealed a significant negative association between wind speed and shoot fly infestation during summer (r = -0.627*), while bright sunshine hours showed a highly significant negative relationship during *kharif* (r = -0.792**). No weather parameter significantly affected shoot fly incidence during *rabi*. Multiple regression analysis indicated that weather parameters exerted a greater influence on shoot fly incidence during *kharif* ($R^2 = 0.83$) than during summer ($R^2 = 0.56$) and *rabi* ($R^2 = 0.42$).
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KEY WORDS: Seasons, infestation, dead hearts, abiotic factors, correlation, shoot fly, spider

INTRODUCTION

Sorghum *Sorghum bicolor* (L.) Moench is a warm-season cereal whose origin is from Africa (Rosentrater and Evers, 2018). It is grown globally for human consumption and animals as food, feed,

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fodder, fibre and fuel or bioenergy (Padmaja, 2016). Protein in sorghum is gluten free and it is specialty food for people who suffer from diabetics (Prasad and Staggenborg 2009). In Gujarat, the overall sorghum production across all three seasons *kharif*, *rabi* and summer covered an area of 42,660ha, with a total production of 66,130 tonnes and an average productivity of 1,550kg ha^{-1} (Anonymous, 2025). Sorghum crop production was affected (nearly 32%) with the insect pests menace in India (Borad and Mittal, 1983) of which nearly 5 per cent of the loss has been attributed to sorghum shoot fly (Jotwani, 1983). The yield of sorghum is being affected by a wide variety of biotic and abiotic constraints (Subramanyam *et al*., 2020). Sorghum shoot fly, *Atherigona soccata* Rondani (Diptera, Muscidae) is a major pest of sorghum that mainly infest the crop during seedling stage or early crop growth stages causes dead hearts and leads to killing of entire plant. The incidence of shoot fly is known vary from region to region and season to season (Singh and Verma, 1988). Understanding the population dynamics of pests, natural enemies, and disease incidence in relation to environmental factors is imperative for developing effective pest management strategies (Singh *et al.,* 2023). In this study, the relationship between seasonal weather parameters and shoot fly incidence was analysed and multiple regression equations were developed.

MATERIALS AND METHODS

The sorghum variety GJ-43 was sown following the recommended agronomic practices. The experiment was established in a plot measuring 9m × 7.5m with a spacing of 45cm × 15cm. Observations on shoot fly incidence were recorded at weekly intervals from the 12th to 24th Standard Meteorological Week (SMW) during summer, 27th to 39th SMW during *kharif* and 46th SMW to 6th SMW during *rabi*. Shoot fly infestation was assessed based on the incidence of dead hearts. Observations were recorded from six randomly selected quadrates and five plants from each quadrate were examined for dead heart symptoms. The percentage of dead hearts was calculated.

Meteorological data corresponding to the observation periods were obtained from the meteorological observatory of Sardarkrushinagar Dantiwada Agricultural University. The weather parameters considered included maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, rainfall, wind speed and bright sunshine hours. The relationship between shoot fly infestation and weather parameters was determined using Pearson's correlation analysis (Pearson, 1895). Further, multiple linear regression analysis was performed to quantify the contribution of individual weather variables towards variations in shoot fly incidence and to develop predictive regression models for pest occurrence. Statistical analyses were carried out using appropriate statistical software.

RESULTS AND DISCUSSION

Incidence of shoot fly during summer, 2024: In summer incidence of sorghum shoot fly, *A. soccata*, commenced during the 2nd week after sowing (WAS) corresponding to the 13th SMW, with 6.67 per cent dead hearts. The infestation increased progressively and reached its peak (36.67% dead hearts) during the 15th SMW. Thereafter, the infestation declined gradually, with the lowest incidence (6.67% dead hearts) recorded during the 20th SMW. The population of natural enemies, particularly spiders, was first observed during the 3rd WAS (14th SMW; first week of April) with 0.03 spiders per plant. The spider population increased steadily and attained its maximum abundance (0.27 spiders per plant) during the 8th WAS (19th SMW; first week of May). Subsequently, the population declined gradually but remained active up to the 11th WAS (22nd SMW; third week of May), recording 0.06 spiders per plant (Table 1). Correlation analysis between shoot fly incidence and weather parameters (Table 2) revealed that wind speed exhibited a significant negative association with dead heart incidence (r = -0.627*), indicating a suppressive effect on shoot fly activity. However, the remaining meteorological parameters did not show any significant influence on shoot fly infestation. Similarly, correlation analysis indicated that the spider population was not significantly affected by any of the weather parameters.

Incidence of shoot fly during *kharif*, 2024: During the *kharif* 2024, infestation of sorghum shoot fly commenced during the 2nd WAS, corresponding to 28th SMW (second week of July), with 10 per cent dead hearts (Table 1). The infestation increased progressively with crop growth and reached its peak (43.33% dead hearts) during 6th WAS (32nd SMW; first week of August). Thereafter, the infestation declined gradually and reached 6.67 per cent dead hearts by the 9th WAS (35th SMW; third week of August). No shoot fly infestation was recorded beyond the 35th SMW. A single population peak of shoot fly (43.33% dead hearts) was observed during the *kharif* season. Correlation analysis (Table 2) revealed that bright sunshine hours exhibited a highly significant negative association with shoot fly incidence (r = -0.792**). However, other abiotic factors, including rainfall, wind speed, maximum temperature, minimum temperature and relative humidity, did not exert any significant influence on shoot fly activity. The activity of spiders, was first observed during the 3rd WAS (29th SMW; third week of July) with a population of 0.06 spiders per plant. The spider population increased gradually and attained its maximum density of 0.16 spiders per plant during the 7th and 8th WAS (33rd and 34th SMW; second and third weeks of August). Subsequently, the population declined gradually and remained active up to 11th WAS (37th SMW; second week of September), recording 0.06 spiders per plant. Correlation studies indicated that bright sunshine hours (r = -0.598*) and maximum temperature (r = -0.747*) had significant negative relationships with spider population. In contrast, maximum relative humidity (r = 0.628*) exhibited a significant positive relationship with spider abundance, suggesting that higher humidity favoured spider activity and their potential role in the natural suppression of shoot fly population.

Table 1. Occurrence of *Atherigona soccata* in sorghum during summer, *kharif* and *rabi*

Summer 2024 (March – June)				*Kharif* 2024 (July – September)				*Rabi* 2024-25 (November – February			
WAS	SMW	DH (%)	NE no/ plant	WAS	SMW	DH (%)	NE no/ plant	WAS	SMW	DH (%)	NE no/ plant
1	12	0.00	0.00	1	27	0.00	0.00	1	46	0.00	0.00
2	13	6.67	0.00	2	28	10.00	0.00	2	47	3.33	0.00
3	14	16.67	0.03	3	29	13.33	0.06	3	48	10.00	0.00
4	15	36.67	0.06	4	30	20.00	0.06	4	49	26.67	0.00
5	16	30.00	0.10	5	31	33.33	0.10	5	50	30.00	0.03
6	17	26.67	0.06	6	32	43.33	0.10	6	51	20.00	0.06
7	18	16.67	0.16	7	33	23.33	0.16	7	52	16.67	0.13
8	19	10.00	0.27	8	34	16.67	0.16	8	1	16.33	0.10
9	20	6.67	0.23	9	35	6.67	0.13	9	2	6.67	0.10
10	21	0.00	0.10	10	36	0.00	0.13	10	3	0.00	0.03
11	22	0.00	0.06	11	37	0.00	0.06	11	4	0.00	0.03
12	23	0.00	0.00	12	38	0.00	0.00	12	5	0.00	0.00
13	24	0.00	0.00	13	39	0.00	0.00	13	6	0.00	0.00
Mean		11.54	0.08	Mean		12.82	0.07	Mean		9.97	0.04

WAS: Weeks After Sowing, SMW: Standard Meteorological Weeks; DH – Dead hearts; NE – Natural enemies

Table 2. Correlation of weather parameters with *Atherigona soccata* infesting summer, *kharif* and *rabi* sorghum

Pest/ spider	Sun shine hours	Wind speed (km h^{-1})	Temp - Max °C	Temp - Min °C	RH (%) - Max	RH (%) = Min	Rainfall
Summer 2024							
DH (%)	-0.107^{NS}	-0.627*	-0.340^{NS}	-0.363^{NS}	0.055^{NS}	-0.091^{NS}	Nil
Spider	0.404^{NS}	-0.049^{NS}	0.279^{NS}	0.081^{NS}	-0.109^{NS}	-0.416^{NS}	Nil
***Kharif* 2024**							
DH (%)	-0.792**	0.319^{NS}	-0.416^{NS}	0.161^{NS}	0.170^{NS}	0.238^{NS}	-0.127^{NS}
Spider	-0.598*	0.117^{NS}	-0.747**	-0.275^{NS}	0.628*	0.482^{NS}	0.401^{NS}
***Rabi* 2024-25**							
DH (%)	-0.051^{NS}	0.103^{NS}	-0.240^{NS}	-0.371^{NS}	0.106^{NS}	-0.172^{NS}	Nil
Spider	-0.351^{NS}	0.274^{NS}	-0.613*	-0.545^{NS}	0.375^{NS}	0.282^{NS}	Nil

*Significance at 5 per cent level of significance ('r' = 0.553); ** Significance at 1 per cent level of significance ('r' = 0.684); DH - Dead heart (%); Spider - no/ plant

Incidence of shoot fly during r*abi,* 2024-25: During *rabi* 2024-25 the incidence commenced during the 2nd WAS, corresponding to 47th SMW (third week of November), with 3.33 per cent dead hearts (Table 1). The infestation increased progressively with crop growth and attained its peak level of 30 per cent dead hearts during the 5th WAS (50th SMW; second week of December). Thereafter, the infestation declined gradually and reached 6.67 per cent dead hearts by the 9th WAS (2nd SMW; second week of January). No further development of shoot fly-induced dead hearts was observed after the 3rd SMW. Correlation analysis between shoot fly infestation and prevailing weather parameters (Table 2) indicated that none of the meteorological factors exhibited a significant influence on dead heart incidence during the *rabi* 2024-25 season. The activity of spiders was first recorded during 5th WAS (50th SMW; second week of December) with a population of 0.03 spider per plant. The population increased gradually and reached its maximum level of 0.13 spider per plant during the 7th WAS (52nd SMW; fourth week of December). Subsequently, the spider population declined and remained active up to the 11th WAS (4th SMW; third week of January), with a population of 0.03 spider per plant. Correlation studies between spider population and weather parameters revealed a significant negative association with maximum temperature (r = -0.613*). However, none of the other weather parameters showed any significant effect on spider activity during the cropping period.

Comparison of regression values among the seasons revealed that in *kharif* (R^2 = 0.83) influenced by weather parameters that is explained by the higher explanatory power of the regression model than the other two seasons like summer (R^2 = 0.56) and *rabi* (R^2 = 0.42). Natural enemies like spiders are also influenced by weather parameters during the *kharif* with higher R^2 (0.90) explanatory power than in *rabi* (R^2 = 0.54) and summer (R^2 = 0.28) shown that the weather parameters contribution towards the spider population build up and survival. All the prediction equations have given good R square value.

Shekhar (1995) studied the correlation between dead hearts and maximum temperature and reported significant negative (-ve) relationship with temperature. The present findings are in close conformity with earlier reports on the seasonal incidence of shoot fly in sorghum. Karibasavaraja and Balikai (2006) reported that shoot fly activity commenced during the 27th (SMW) with 43.10 dead hearts and increased steadily to reach a peak of 93.40 per cent during the 33rd SMW. Similarly, Keerthi *et al*. (2017) have also reported that the minimum temperature exhibited non-significant

negative correlation with dead heart formation which is consistent with the current study's findings. Likewise, Rakesh *et al*. (2022) observed the initial occurrence of *A. soccata* at 7 days after sowing (28th SMW) with maximum infestation (11.67% dead hearts) recorded during the 29th SMW. Supporting these observations, Sakshi *et al*. (2022) documented the first incidence of shoot fly during the 27th SMW with 4.12% dead hearts and remained actively till the vegetative stage (34th SMW). The highest infestation (55.43% dead hearts) was noted during the 30th SMW (23th - 29th July), followed by a gradual decline, reaching nil infestation by the 35th SMW. Similarly, Sonawane *et al*. (2022) reported the initiation of dead heart formation (12.10%) during the 28th SMW with peak infestation (15.01% dead hearts) occurring in the 31st SMW.

Among the three seasons, the highest shoot fly infestation was recorded during the *kharif* season with a mean dead heart incidence of 12.82 per cent, followed by summer (11.54%) and *rabi* (9.97%). Analysis between abiotic factors and dead heart incidence revealed that wind speed exhibited a significant and negative correlation. While, bright sunshine hours showed a highly significant negative association with shoot fly infestation in sorghum. The abundance of spiders was comparatively higher during the summer season, recording a mean population of 0.08 spiders/plant, followed by *kharif* (0.07 spiders/plant), whereas the lowest population was observed during the *rabi* season (0.04 spiders/plant). Correlation analysis with weather parameters during summer indicated that none of the abiotic factors exerted a significant influence on spider activity. In *kharif* season, spider activity showed a significant negative correlation with bright sunshine hours and a highly significant negative correlation with maximum temperature. During the *rabi* season, spider activity exhibited a significant negative association with maximum temperature, suggesting its important role in regulating spider activity.

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Potential insecticidal effects of *Valeriana jatamansi* and *Isodon rugosus* against *Acanthoscelides obtectus* (Say) (Coleoptera, Chrysomelidae), a pest of *Phaseolus vulgaris* L.

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ABSTRACT: The insecticidal efficacy of *Valeriana jatamansi* and *Isodon rugosus* was evaluated against the bean weevil *Acanthoscelides obtectus* (Say), a pest of *Phaseolus vulgaris* L. The rhizome and leaves extracts from *V. jatamansi* and *I. rugosus* were prepared by using both acetone and methanol solvents and five concentrations of each extract were evaluated for its effectiveness in inducing adult mortality of the adult weevils. Acetone and methanol extracts of *V. jatamansi* rhizome and *I. rugosus* leaves at 12 per cent concentration exhibited 100 per cent mortality of adult bean weevils on the 7th and 8th day, respectively. As the concentration of the extracts escalated from 4 to 12 percent, the death rate also correspondingly increased. © 2026 Association for Advancement of Entomology

KEYWORDS: Bean weevil, adult mortality, biopesticide, dose-dependent mortality, post-harvest storage

INTRODUCTION

The global population is to reach 9.8 billion by 2050, with global food demand anticipated to rise by a minimum of 50 per cent, but the requirement for protein-rich products may escalate at an even faster rate (Mesterházy *et al*., 2020). Pulses provide essential protein, especially for India's large vegetarian population (Hans *et al*., 2024) encompasses various crops, including peas, many bean varieties, fava beans, chickpeas, lentils, lupins, and others. Beans (*Phaseolus* spp.) are essential for poor communities, providing protein, carbohydrates, vitamins, and minerals (Celmeli *et al*., 2018). *Acanthoscelides obtectus* Say (Coleoptera, Chrysomelidae), infest stored common beans, *Phaseolus vulgaris* L. (Thakur, 2012). Infestation typically initiates in the field and progresses during post-harvest storage. Oviposition occurs superficially amid the seeds, and the first instar larvae pierce the seed coat and infect the cotyledons. The endophytic larval stages demonstrate vigorous feeding behavior, finishing their development within the endosperm (Thakur and Renuka, 2014). Post-harvest food loss (PHL) denotes the food that is lost during the supply chain from the moment of harvest to eating or other ultimate applications (Aulakh *et al*., 2013). Postharvest loss includes both direct physical losses and quality deterioration that degrades the economic

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value of crops or renders them unsuitable for human consumption. In extreme cases, these losses may reach to 80 per cent of total production (Fox and Fimeche, 2013). The management and supervision of this pest (*A. obtectus*) in large storage facilities predominantly depend on the application of phosphine, pyrethroids, organophosphates, and various other synthetic pesticides (Daglish *et al*., 1993). Considering the detrimental impacts of chemical pesticides on agricultural soils, water resources, human health, and the environment, there is an urgent need for enhanced pest management strategies. Utilizing biopesticides is a primary strategy for controlling these pests in an environmentally sustainable way (Khursheed *et al*., 2022). The various elements of medicinal plants, such as stems, flowers, leaves, and roots, are ecologically sustainable and provide an alternative for pest management. These may eradicate pests and inhibit their growth and reproduction (Mohammad *et al.*, 2024). The current study aims to examine the biopesticidal effects of *Isodon rugosus* (Wall. ex Benth. Codd) (Lamiaceae family) and *Valeriana jatamansi* Jones ex Roxb. on the bean weevil, *A. obtectus*. *Valeriana jatamansi*, (Valerianaceae family), Indian valerian is a wild herb located in the subtropical and temperate areas of the Himalayas (Raina and Negi, 2015). It is beneficial due to its insect repellent and antihelminthic properties. Similarly, traditional pharmaceutical systems, particularly in Asia, exhibit sedative, neurotoxic, cytotoxic, antidepressant, antioxidant, and antibacterial activities (Jugran *et al*., 2019). The toxicity of *V. jatamansi* has been demonstrated on cowpea aphid (*Aphis craccivora*) and cigarette beetle *Lasioderma serricorne* (Feng *et al*., 2020; Tewary *et al*., 2005). *Isodon rugosus* is an important medicinal plant is primarily found in the country's eastern and peninsular regions. (Khanal *et al*., 2025). This herb is well-known in ethnomedicine and has been used to treat analgesia and pain (Zeb *et al*., 2016). According to Zeb *et al*. (2017) *I. rugosus* has potent antibacterial, insecticidal, and anthelmintic properties. It also has antinociceptive, antioxidant, and anti-cholinesterase properties (Sadiq *et al*., 2018). Therefore, research was done on the insecticidal efficacy of extracts of rhizome and leaves from *V. jatamansi* and *I. rugosus* against the bean weevil *A. obtectus*, a serious pest.

MATERIALS AND METHODS

Sample collection and maintenance of the insect culture: Infested seeds of *P. vulgaris* were procured from Kotkhai, Shimla district, Himachal Pradesh, India. The culture has been sustained under controlled conditions of temperature (28.00±1 ºC) and relative humidity (65.00 ± 2) in a BOD incubator. Culture of bean weevils was maintained on the identical host grain (*P. vulgaris*) to provide uniformity in feeding substrate and developmental circumstances. The culture was sustained in the laboratory for 11–14 generations before the start of experiment. Periodic sub-culturing was carried out by transferring newly emerging adults to fresh beans. The leaves and rhizomes of *I. rugosus* and *V. jatamansi* respectively were collected from Prospectus Hills and Ghanahatti of Shimla District of Himachal Pradesh, India. They were washed and dried in the shade at ambient temperature in the lab. The dried leaves and rhizomes were crushed into a fine powder. For extraction, 20g of powdered material was subjected solvent extraction (1:5 w/v) using acetone and methanol (Talukdar and Howse, 1993). After 48 hours, the soaked powdered material (leaves and rhizome) was filtered using Whatman No. 1 filter paper and re-extracted to make sure that as much as possible was recovered. About one gram more or less crude extract was obtained after the evaporation of solvents at their respective boiling points in water bath. Subsequent to processing, the extracts were preserved at 4°C for future investigations.

Fumigant toxicity test: The toxicity of the fumigant was evaluated using the filter paper impregnation method. Crude extracts were serially diluted in acetone and methanol to achieve concentrations of 4, 6, 8, 10, and 12 per cent (w/v). An aliquot (1.0 mL) of each concentration was uniformly dropped onto Whatman No. 1 filter paper discs (9 cm diameter) and air-dried for 20–30 minutes ensuring full solvent evaporation. The treated discs were positioned in Petri dishes, into which ten newly emerging adults were put in each Petri dish separately. Each treatment was

replicated thrice under controlled conditions. Filter papers treated with acetone and methanol in Petri dishes served as controls. All Petri dishes were inspected and mortality was recorded every 24 hours until the insects were completely eradicated. The corrected percentage mortality was recorded after the application of extracts through the Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

Effect of rhizome extracts *of V. jatamansi* on the adult mortality of *A. obtectus*: With the increase in concentration of the extracts from 4 to 12 per cent, the adult mortality rate also increased. Methanol extract of *V. jatamansi* rhizome showed 100 per cent mortality at 4, 6 per cent concentration on 11th day of exposure, while 8 per cent concentration showed on 9th day, 10 per cent concentration showed on 8th day and 12 per cent concentration showed on 7th day. In the case of acetone extract the adult mortality was cent per cent at 4, 6, 8 and 10 per cent concentration, on 10th, 9th ,8th and 7th day respectively. Both acetone and methanol extracts of *V. jatamansi* rhizome demonstrated total elimination of the bean weevil by the seventh day of treatment. Whereas, mortality in control on the respective days (11th and 10th) was 33.1 and 29.9 per cent in methanol and acetone treatments respectively (Figs. 1a, b).

These findings align with those of Mohammad *et al*. (2024), who assessed the methanolic extracts of five medicinal plants viz. *Lantana camara* (leaves), *Carica papaya* (seeds), *Ricinus communis* (leaves), *Calotropis gigantea* (flowers), and *Gliricidia sepium* (leaves) for their insecticidal efficacy against the rice weevil through mortality assays conducted in laboratory settings. The leaves of *G. sepium* exhibited the 100 percent insecticidal activity after a duration of seven days.

Effect of leaves extracts *of I. rugosus* on the adult mortality rate of *A. obtectus*:

As the concentration of the extracts increased from 4 to 12 percent the mortality rate was also increased. Methanol leaves extract at 4, 6, 8, 10 and 12 per cent concentration showed 100 per cent mortality on 10th each, 9th and 8th each day of exposure respectively and in case of acetone extract the mortality at respective concentrations was on 12th,

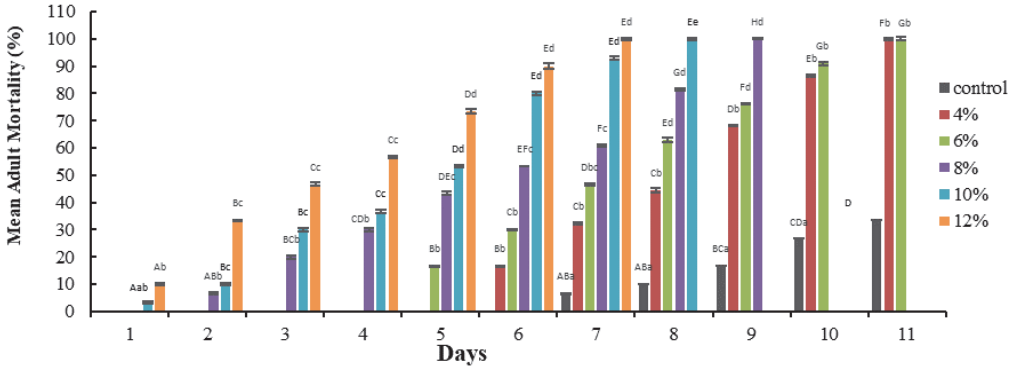


Fig1a. Mean adult mortality of *A. obtectus* by rhizome extract of *V. jatamansi* in methanol

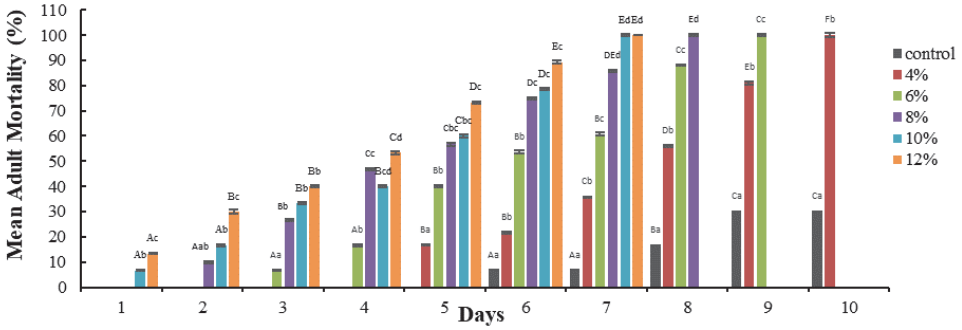


Fig.1b Mean adult mortality of *A. obtectus* by rhizome extract of *V. jatamansi* in acetone

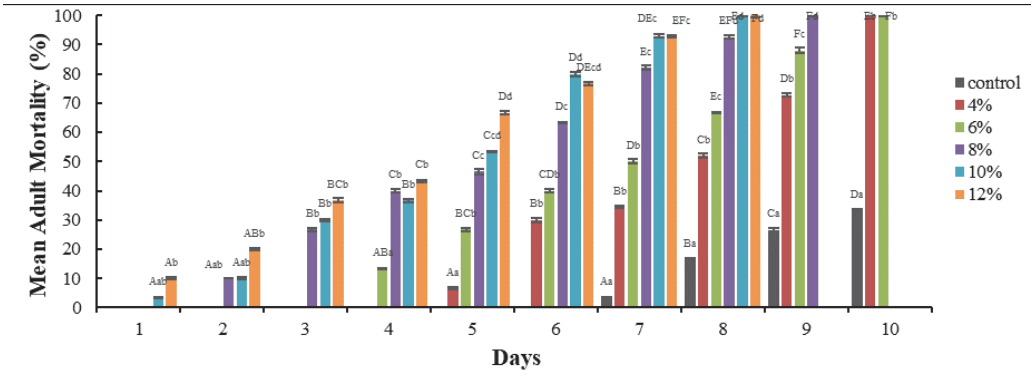


Fig.2a Mean adult mortality of *A. obtectus* by leaves extract of *I. rugosus* in methanol

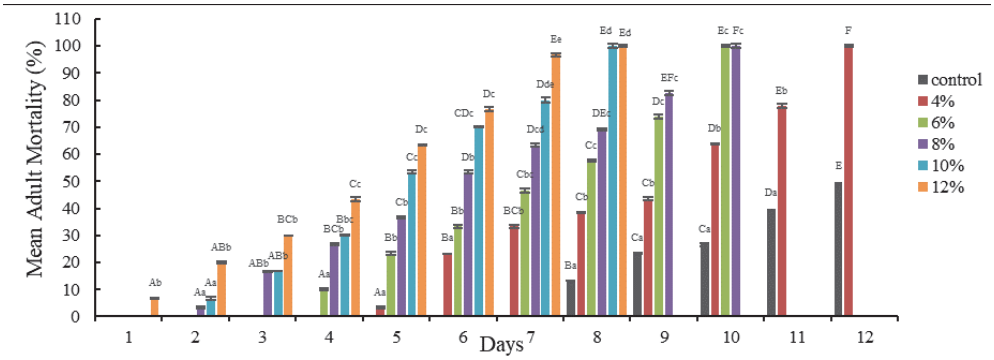


Fig.2b Mean adult mortality of *A. obtectus* by leaves extract of *I. rugosus* in acetone

10th each and 8th each day respectively. Whereas, mortality in control on the respective days (10th and 12th) was 31.1 and 49.9 percent in methanol and acetone treatments respectively (Figs. 2a, b).

Khan *et al.* (2017) discovered corresponding findings, indicating that among the seven plant extracts evaluated, the crude extract from *I. rugosus* exhibited the highest mortality rate in pea aphids. Rosmarinic acid has been identified as the bioactive compound in *I. rugosus* and is considered the principal substance exhibiting insecticidal properties (Khan *et al.*, 2019). The methanol extract of *I. rugosus* exhibited marginally greater efficacy than the acetone extract. This result aligned with the findings of Songwe *et al.* (2021), which indicated that the methanol extract of *Moringa oleifera* L. seeds exhibited a greater death rate against *A. obtectus* as compared to the ethanol and acetone seed extracts.

The insecticidal efficacy enhanced when the concentration of extracts increased from 4 to 12 per cent for both *I. rugosus* and *V. jatamansi* extracts. The average adult mortality of *A. obtectus* on the 7th day was 35.76 and 100 per cent at 4 and 12 per cent concentrations of *V. jatamansi* rhizome acetone extract, respectively. Aslan *et al.* (2006) also observed that the insecticidal efficacy of *Sideritis trojana* acetone extract against *A. obtectus* increased with the dosage administered.

The current investigation revealed that the rhizome extracts of *V. jatamansi* and leaves extracts of *I. rugosus* in both solvents (acetone and methanol) were effective in causing adult bruchid mortality. However, acetone rhizome extract of *V. jatamansi* and methanol leaves extract of *I. rugosus* were more effective against *A. obtectus.* The mortality of the bean weevil observed on 7th and 8th day of exposure in acetone rhizome extract of *V. jatamansi* and methanol leaves extract of *I. rugosus* respectively was 100 per cent. The current findings indicate that a 4 percent concentration of all investigated extracts caused *A. obtectus* mortality on the 10th, 11th, and 12th days post-treatment. Rodríguez-González *et al.* (2019) noted that *Ocimum basilicum* and *Cymbopogon*

winterianus had comparable insecticidal efficacy against *A. obtectus.* The essential oils from these two plants at a dosage of 120µL resulted in 100 percent mortality of *A. obtectus* by the 11th day of exposure. These results are closely related to the current study. The extracts of both plants were evaluated at varying concentrations of 4, 6, 8, 10, and 12 per cent in the selected solvents. The insecticidal efficacy enhanced when the concentration of extracts increased from 4 to 12 per cent for both *I. rugosus* and *V. jatamansi* extracts. Clove and cinnamon essential oils exhibited comparable toxicity towards *A. obtectus*, resulting in a dose-dependent reduction in the growth rate of this pest (Jumbo *et al*., 2014). Ethanolic extracts from the leaves of *Ocimum gratissimum*, *Sida acuta*, *Telfaria occidentalis*, and *Vernonia amygdalina* were assessed for their secondary metabolite composition and insecticidal efficacy against the bean weevil (*A. obtectus*), demonstrating that the effectiveness of each extract increased with both concentration and exposure time (Adeniyi *et al*., 2010). Previous research predominantly focuses on the therapeutic characteristics of *V. jatamansi* and *I. rugosus*, whereas their insecticidal potential remains underexplored. *A. obtectus* is a significant pest of *P. vulgaris*. The insecticidal efficacy of *V. jatamansi* and *I. rugosus* against this pest has not yet been investigated. The findings of the current investigation indicate that both *I. rugosus* and *V. jatamansi*, particularly their leaves and rhizomes, have insecticidal efficiency. The acetone extract of *V. jatamansi* rhizome and the methanol extract of *I. rugosus* were more effective in accelerating the adult mortality of *A. obtectus*. The insecticidal efficacy increased as the concentration of extracts rose from 4 to 12 percent for both plants. The research advocates for the utilization *of I. rugosus* and *V. jatamansi* for the management of stored grain pests.

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Evaluation of larvicidal efficacy of *Clerodendrum infortunatum* Linn extracts against *Culex quinquefasciatus* Say (Diptera, Culicidae)

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ABSTRACT: *Clerodendrum infortunatum* Linn (Family, Lamiaceae), extracts of acetone, methanol and aqueous evaluated against larvae of *Culex quinquefasciatus* Say, showed promising results. Acetone leaf extract recorded an LC_{50} value of 345.41ppm, and was found effective for the management of *Cu. quinquefasciatus*. In vitro cytotoxic analysis of L929 mouse cell lines using the plant extract proved safe for non-target organisms, higher vertebrates. Phytochemical screening of the acetone extract revealed the presence of secondary metabolites that may have contributed to its insecticidal activity.
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KEY WORDS: Acetone leaf extract, LC_{50}, cytotoxicity, MTT assay, secondary metabolites

INTRODUCTION

The application of synthetic pesticides and chemicals is detrimental to all ecosystems, and nowadays they are being replaced by green or bio-pesticide methods to manage pollution hazards. Phytochemicals and other secondary metabolites produced in plant parts protect themselves by defensive mechanisms and serve humanity as larvicides, insecticidal agents, weedicides, medicines, nutraceuticals, anticancer agents, anti-feedants, etc., since they are safe for non-target organisms, readily available and environmentally benign (Ghosh *et al*., 2012; Kekuda *et al*., 2019). The spread of mosquito-borne diseases such as fevers and filariasis in both humans and animals is caused by *Culex quinquefasciatus* (Diptera, Culicidae). Additionally, it may spread the West Nile virus and St. Louis encephalitis (Ragavendran *et al*., 2017). It breeds widely in unclean water sources, such as burrow pits, cesspools, septic tanks with leaks, sluggish drains, and practically all organically damaged water sources (Suleiman *et al*., 2014). Common weed plant in tropical plains, *Clerodendrum infortunatum* Linn (Family, Lamiaceae), locally referred to as Indian Bhant tree or peruvelam in Kerala, is a terrestrial shrub with an unpleasant stench that has several therapeutic and insecticidal uses (Abbaszadeh *et al*., 2014; Kekuda *et al*., 2019). Because of its accessibility and alleged health benefits, different components of *C. infortunatum* are employed for different purposes (Uddin *et al*., 2021). The tribal community in Kokrajhar (Assam) uses *C. infortunatum* as a mosquito-repellent plant. It is a customary practice to use the leaves of this plant to keep flies and

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mosquitoes away from food (Patil *et al*., 2014; Swargiary *et al*., 2021). The present investigation was carried out to analyse the bioefficacy of different extracts of *C. infortunatum* leaves against fourth-instar larvae of *Cu. quinquefasciatus*. The study also evaluates cytotoxicity against the L929 cell line.

MATERIALS AND METHODS

The experiment was conducted in the Research Laboratory of the Zoology Department, University College, Thiruvananthapuram, Kerala. Five replicas of toxicity bioassay and different tests for different solvents of plant material were conducted with fourth-instar mosquito larvae under standard laboratory conditions. *Culex* egg rafts were collected by placing a plastic bucket (10L) filled three-fourths with water and two crushed domestic fowl eggs to attract oviposition (Ayana Gayathri and Evans, 2018). After 3 to 4 days, a foul smell was produced from the bucket, and the mosquito began to lay eggs. The eggs were laid in rafts and collected from the bucket with a soft brush. The rafts were transferred into small bottles containing de-chlorinated tap water and a small amount of egg solution from the bucket. After 24 hours, small, slender first-instar larvae emerged from the eggs, exhibiting wriggling movements. Larvae that emerged were pooled and identified using standard manual (Barraud, 1934) and maintained in distilled water for further experiments. During this time, crushed dog biscuits were provided to the medium to nourish the larvae.

Fresh leaves of *C. infortunatum* were washed thoroughly with de-chlorinated tap water and shade-dried for two weeks. The dried leaves were pulverised in a domestic grinder. According to the polarity, from low to high, different solvents such as acetone, methanol and distilled water were subjected to Soxhlet extraction (Madhu *et al*., 2011). The extracts obtained were dried and used for further analysis. Ten per cent stock solution was prepared using the dried extract of acetone, methanol, and distilled water, with distilled water as the solvent. From the stock solution, concentrations ranging from 100 to 1000ppm were prepared.

Evaluation of larvicidal activity: Larvicidal activity was evaluated on fourth-instar larvae of uniform size, randomly selected from the stock culture in accordance with WHO guidelines (WHO, 2005). Larvae (20 Nos.) were exposed for a 24-hour bioassay to different concentrations of *C. infortunatum* extracts, ranging from 100 to 1000ppm, prepared from the stock, along with distilled water as a control. Larvae were considered dead when they showed no movement or when they were touched with a needle on their siphon. The lethal concentration (LC_{50}) and its 95 per cent upper and lower confidence limits were determined using probit analysis (Finney, 1971).

Ovicidal and pupicidal activity: Ovicidal and pupicidal activity were studied using the acetone extract of *C. infortunatum*. Eggs were separated carefully from the egg raft for ovicidal activity, and pupae were maintained for pupicidal activity in paper cups containing acetone leaf extracts of sub-lethal concentration 300 ppm and kept for 24 hours. Five replicates, including a control, were maintained and observed for hatchability and pupal emergence, respectively.

$$\text{Hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

$$\text{Pupicidal activity (\%)} = \frac{\text{Number of dead pupae}}{\text{Total number of pupae}} \times 100$$

Cytotoxic analysis of plant extract on non-target cells: The L929 mouse fibroblast cell line cells were procured from National Centre for Cell Sciences (NCCS), Pune, India and cultured in Dulbecco's Modified Eagles Medium (DMEM), supplemented with 10 percent heat inactivated Fetal Bovine Serum (FBS) and one percent antibiotic cocktail containing penicillin (100 U/mL), streptomycin (0.1 mg/mL) and amphotericin B (0.25 µg/mL).

Cell viability assay: The assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) was performed to determine the IC_{50} (Mosmann, 1983; Joseph *et al*., 2012). The L929 cells were seeded on 96-well plates and

allowed to acclimate to the culture conditions. The samples were prepared in DMEM and filter-sterilised. The samples were further diluted in DMEM and added to the wells containing cultured cells at a concentration of 300µg mL^{-1}. Control wells were kept untreated. After treatment, the plates were incubated for 24 hours. After the incubation period, the media were discarded. 100µL MTT solution in PBS (Phosphate-Buffered Saline) was added to the wells. The plates were incubated for an additional two hours to develop formazan crystals. After incubation, the supernatant was removed, and 100µL of DMSO (Dimethyl Sulfoxide) was added to each well. Absorbance was measured at 570nm. Three wells per plate without cells served as a blank. The formula calculated the cell viability:

$$\text{Cell viability (\%)} = \frac{\textit{Average of absorbance of test}}{\textit{Average of absorbance of control}} \times 100$$

Estimation of phytochemical content in *C. infortunatum*: The phytochemical screening of the acetone leaf extract of *C. infortunatum* was done for phenol, tannin, flavonoids, saponins, terpenoids, alkaloids, glycosides, quinones, fatty acids and steroids using standard protocols (Ejikeme *et al*., 2014; Kumar *et al*., 2014). Statistical analysis of the data was performed to determine the LC_{50} using probit analysis in R (R Core Team, 2025).

RESULTS AND DISCUSSION

The acetone, methanol and aqueous extracts of *C. infortunatum* induced mortality in fourth-instar larvae of *Cu. quinquefasciatus* after 24-hour exposure (Table 1). The acetone extract of *C. infortunatum* showed greater activity against the fourth-instar larvae compared to methanol and aqueous extracts. Low and moderate larvicidal activities were shown by aqueous and methanol extracts when treated with 100 to 1000ppm concentrations against fourth-instar larvae. No larval mortality was observed in the solvent-treated controls or in the control groups maintained in distilled water. The acetone extract caused higher mortality at higher concentrations, at 1000ppm, 100 per cent larval mortality, and the mortality decreased at lower concentrations. At 100ppm, no significant mortality was observed. With increasing concentration, larval mortality increased significantly. Mortality was also increased with exposure duration across all concentrations tested. In the methanol and aqueous extracts 100 per cent larval mortality was not observed after 24 hours of exposure. The acetone extract exhibited the highest larvicidal activity, with an LC_{50} of 345.41ppm, compared to the methanol extract (LC_{50} = 2820.96ppm) and the aqueous extract (LC_{50} = 1637.65ppm) (Table 2). The acetone extract was more effective against the fourth instar larvae in

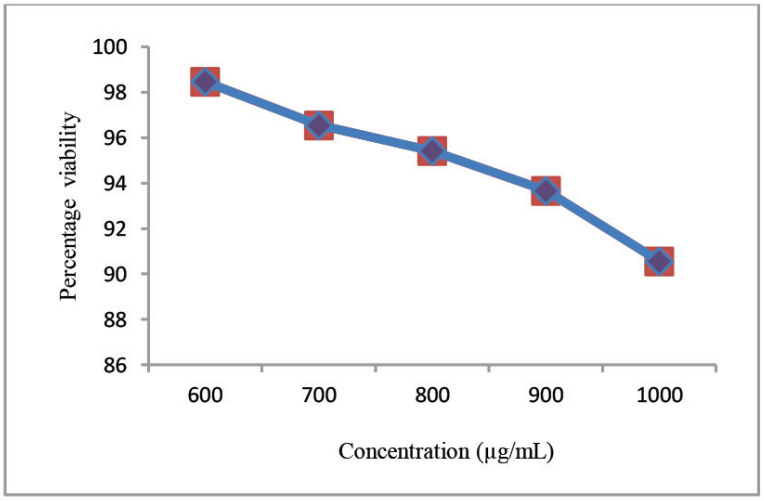


Fig. 1 Cell viability of the L929 cell line treated with a higher concentration of the acetone extract of *C. infortunatum*

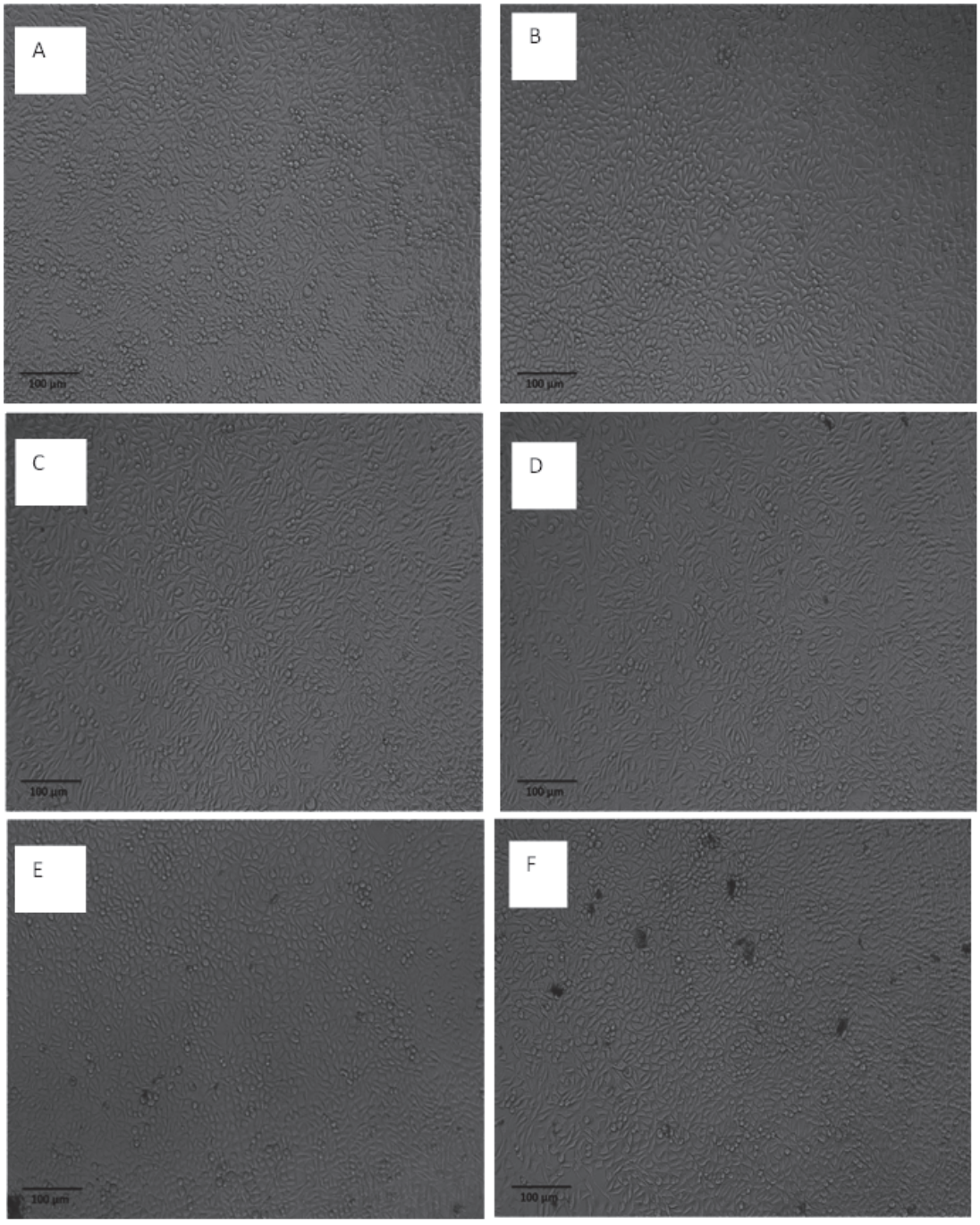


Plate 1. Photomicrographs of cultured L929 cells; A-Control; B, C, D, E, F represent cells exposed to 600, 700,800,900 and 1000 µg ml^{-1} acetone extract. Images were taken using a light microscope at 40X magnification

Table 1. Mortality of *C. infortunatum* extracts against fourth-instar larvae of *Cu. quinquefasciatus*

Treatment concentration	Mean mortality after 24-h exposure %		
	Acetone	Methanol	Aqueous
Untreated	0	0	0
Solvent-treated	0	0	0
100ppm	16.66 ±2.58	5.83±2.04	3.33±5.16
200ppm	28.33±4.08	6.66±2.58	15.0±3.16
300ppm	39.16±5.84	9.16±3.76	16.66±4.08
400ppm	51.66±5.16	12.5±2.73	21.66±4.08
500ppm	54.16±2.04	15.83±4.91	25.0±3.16
600ppm	73.33±6.05	18.33±4.08	28.33±2.58
70ppm	75.83±5.84	20.0±3.16	30.0±3.16
800ppm	82.5±2.73	25.0±4.47	30.83±3.76
900ppm	91.66±6.83	32.5±2.73	32.50±5.24
1000ppm	100±0	34.16±2.04	41.66±4.08

terms of mortality than the methanol and aqueous extracts. Adewoyin *et al*. (2021) reported the insecticidal activity of the methanol leaf extract of *C. polycephalum* against *Aedes*, *Anopheles*, and *Culex* mosquito species at concentrations of 250 to 2000 ppm, and found it effective. Similarly, Nambiar and Ranjini (2018) reported that *C. infortunatum* affects sixth-instar larvae of the *Orthaga* species.

Ovicidal and pupicidal activity analysed using a sub-lethal concentration at 300ppm, showed 89.16per cent mortality in pupae and 57.5per cent mortality in the egg stage. Pupae were unable to emerge as adults and exhibited structural deformities in their wings. Larvae placed in distilled water as a control did not exhibit ovicidal and pupicidal effects. Remia and Lagaswamy (2009) reported the larvicidal efficacy of leaf extracts from two botanicals against the mosquito vector *Aedes aegypti*. Bioactivity of plant extracts on the larval and pupal stages of *A. aegypti* was also reported (Candido *et al*., 2013). Target-specific larvicidal activity of the *Typhonium trilobatum* leaf extract against *Culex* mosquitoes was reported by Haldar *et al*. (2011). The insecticidal properties of leaf extracts of *C. infortunatum* have been reported by various authors (Pavela, 2007; Yankanchi *et al*., 2015; Jadhav *et al*., 2016; Kekuda *et al*., 2019; Swargiary *et al*., 2021).

The toxicity of phytochemicals must be investigated *in vitro* because such data enable us to choose plant products with the least environmental impact. An alternate technique for examining toxicity is to use cell lines. To evaluate the potential hazards and unintended effects of insecticidal substances on the environment and human health, toxicity tests on non-target cells are essential. It is essential to determine the safety of a substance when used in the environment. The MTT assay is a widely used colorimetric method for assessing cellular metabolism as an indicator of cell viability, proliferation, and cytotoxicity. Cell lines are a relevant model system for studying the toxic effects of plant extracts. L929 mouse fibroblasts are rapidly proliferating, spindle-shaped, fibroblast-like cells derived from mouse connective tissue.

The cytotoxic activity study of the acetone extract of *C. infortunatum* was assessed using the MTT assay in the L929 cell line at concentrations ranging from 600 to 1000µg mL^{-1}. The cell viability studies indicated that the acetone extract of *C. infortunatum* was non-toxic to the L929 mouse fibroblast cell line at the concentrations tested (Fig. 1). Cell survival analysis showed no significant effect on viability or change in cell morphology (Plate 1). The viability was 98.46 per cent at 600µg mL^{-1}, 96.55 per cent at 700µg mL^{-1}, 95.42 per cent at 800µg mL^{-1}, 93.67 per cent at 900µg mL^{-1}, and 90.7 per cent at 1000µg mL^{-1}. The results showed that the plant extract was least toxic to non-target cells

Table 2. LC_{50} value of different extracts of *C. infortunatum* against *Cu. quinquefasciatus* larvae

Solvent	LC_{50} (ppm)	LCL – UCL (95%)
Acetone	345.41±28.12	299.629 - 391.076
Methanol	2820.96±191.65	1945.370 - 5260.213
Distilled water	1637.66±143.54	1274.735 - 2406.159

with an IC_{50} value above 100µg mL^{-1}. In most toxicological studies, an extract is considered safe or biocompatible if the cell viability remains above 70 or 80 per cent compared to the control group.

The qualitative phytochemical analysis of the acetone extract of *C. infortunatum* revealed the presence of phenol, tannins, flavonoids, saponins, terpenoids, alkaloids, glycosides, quinones, fatty acids and steroids. The phytochemical content and larvicidal properties of *C. infortunatum* were extensively explored (Swargiary *et al*., 2021). Hepatoprotective potential of the methanol extract of *C. infortunatum* was proved by Sannigrahi *et al*. (2009). The bioactivity of the plant's secondary metabolites was reported by Helen *et al*. (2021). The cytotoxicity of *C. viscosum* was extensively reviewed by Srivastava *et al*. (2021), and the results were comparable to those reported here. Numerous studies have evaluated the insecticidal potential of the *Clerodendrum* family (Das *et al*., 2014). Research showed that three diterpenoids isolated from *C. infortunatum* clerodin,15-methoxy-14,15-dihydroclerodin and 15-hydroxy-14,15-dihydroclerodin showed insecticidal activity against *Helicoverpa armigera* (Abbaszadeh *et al*., 2014). Hexane extract of *C. infortunatum* showed strong insecticidal activity against the sand fly *Phlebotomus argentipes* (Dinesh *et al*., 2015). Ethyl acetate extract of *C. capitatum* showed strong repellent activity to adults of three major stored grain insects, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* (Adesina *et al*., 2019). Studies revealed that the aqueous extract of *C. infortunatum* killed third-instar larvae of *Hyposidra talaca* (Geometridae), a new and serious tea pest, using the leaf-dip method (Roy *et al*., 2015).

This study clearly demonstrates the larvicidal potential of *C. infortunatum* against *Cu. quinquefasciatus*, offering a sustainable, eco-friendly alternative to toxic synthetic insecticides. The high larval mortality rates observed, coupled with the plant's natural abundance, suggest its viability as a primary tool in Integrated Mosquito Management (IMM) strategies.

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Annual life history of okra shoot and fruit borer, *Earias vittella* (Fabricius), under mid-hill conditions of Himachal Pradesh, India

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ABSTRACT: Annual life history of *Earias vittella* (Fabricius) (Lepidoptera, Nolidae), was investigated under natural laboratory conditions in the mid-hill region of Himachal Pradesh, India, during 2023–2024. The insect completed five overlapping generations annually. The duration of the active generations ranged from 31.6 to 34.6 days, whereas the overwintering generation exhibited a markedly prolonged developmental period of 200.4–229.5 days. The mean incubation, larval and pupal periods during the active generations were 3.4 ± 0.05, 14.0 ± 0.44 and 8.3 ± 0.24 days, respectively, while the overall mean developmental period was 87.7 ± 38.7 days. Adult females survived longer than males, with mean longevities of 6.1 ± 0.20 and 5.3 ± 0.10 days, respectively. Mean fecundity was 147.8 ± 21.60 eggs per female but declined markedly during the overwintering generation. Egg, larval and pupal survival were also reduced under winter conditions, resulting in lower growth indices than those recorded during the active generations. The findings demonstrate that seasonal temperature profoundly influences the annual life history, survival and reproductive potential of *E. vittella*. The study provides baseline information for forecasting pest incidence and developing timely, location-specific integrated pest management strategies for okra under the mid-hill agroclimatic conditions of Himachal Pradesh.
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KEY WORDS: Overlapping generations, developmental biology, overwintering, survival, temperature

INTRODUCTION

Among vegetable crops, okra, *Abelmoschus esculentus* (L.) Moench (Malvaceae), commonly known as lady's finger or bhendi, is a highly nutritious vegetable rich in dietary fibre, carbohydrates, proteins, vitamins (A, C, K and folate), and essential minerals such as calcium, magnesium and potassium. It is also a valuable source of bioactive compounds, including polyphenols and flavonoids, which contribute to its antioxidant, anti-inflammatory, and nutraceutical properties (Elkhalifa *et al.,* 2021). Linoleic acid, an essential polyunsaturated fatty acid, is the major constituent of okra seed oil (Savello *et al.,* 1980). Besides its nutritional importance, okra is widely consumed as a fresh vegetable and processed into canned, frozen and dehydrated products, while its

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mucilage is used in food processing and pharmaceutical industries (Elkhalifa *et al.,* 2021). India is one of the world's leading producers of vegetables, and vegetable cultivation plays a pivotal role in ensuring food and nutritional security (FAO, 2023). Despite its economic importance, successful cultivation of okra is severely constrained by several insect pests, among which the okra shoot and fruit borer, *Earias vittella* (Fabricius) (Lepidoptera, Nolidae), is considered one of the most destructive species (Chandran *et al.,* 2020). The larvae bore into terminal shoots during the vegetative stage, causing wilting, and subsequently infest developing fruits, rendering them unmarketable due to extensive internal feeding and accumulation of frass. Infestation results in substantial quantitative and qualitative yield losses, with shoot damage ranging from 0.1 to 9.8 per cent (Patait *et al.,* 2023) and fruit damage varying between 12.9 and 90.0 per cent under different agroecological conditions (Chauhan *et al.,* 2023).

A comprehensive understanding of the annual life history and seasonal developmental pattern of *E. vittella* is essential for identifying vulnerable stages of the pest and developing timely and effective management strategies. Although several studies have described the biology of *E. vittella* under laboratory conditions (Shah *et al.,* 2012; Kumar *et al.,* 2014; Sahito *et al.,* 2019; Kulhari *et al.,* 2023), information on its annual life history under the mid-hill agroclimatic conditions of Himachal Pradesh remains scarce. Seasonal variations in temperature and relative humidity prevailing in this region are expected to influence the developmental duration, survival and reproductive potential of the pest. Therefore, the present investigation was undertaken to elucidate the annual life history, generation pattern, developmental biology, survival and growth indices of *E. vittella* under the mid-hill conditions of Himachal Pradesh.

MATERIALS AND METHODS

Annual life cycle of *E. vittella* was studied on okra fruits under natural laboratory conditions at the Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSK HPKV), Palampur, Himachal Pradesh, India, situated at an altitude of 1,268 m above mean sea level (32.11°N; 76.53°E). For the study, a stock culture of the okra shoot and fruit borer, *E. vittella*, was established using larvae collected from unsprayed farmers' fields. The culture was maintained under natural laboratory conditions (25 ± 2°C and 65 ± 5% RH), which were monitored, by providing fresh shoots or fruits of the okra variety 'Palam Komal' in insect-rearing cages. Pupae thus obtained were collected and transferred to separate glass jars for adult emergence. Newly emerged adults were released in pairs into oviposition cages covered with muslin cloth secured with rubber bands for mating and oviposition. The oviposition cages were provided with a 10 per cent honey solution on a cotton swab as a food source and fresh okra fruits as an oviposition substrate, along with crumpled paper strips as resting sites for the moths. A cotton swab soaked in water and placed in a Petri dish was used to maintain humidity inside the cage. Newly hatched larvae (n = 30) were individually transferred to fresh okra fruits using a fine camel-hair brush and reared separately until adult emergence. The stalks of the fruits were wrapped with moist cotton plugs to maintain freshness, and fresh okra fruits were replaced daily throughout the larval rearing period. Proper hygienic conditions were maintained throughout the experiment, and successive generations were reared continuously to study the annual life history of *E. vittella*.

Observations were recorded on the pre-oviposition, oviposition and post-oviposition, incubation, larval, pre-pupal, pupal and total developmental periods, along with adult longevity, fecundity, and survival rate (%) at egg, larval and pupal stages for each generation. Generation I during July 21 - August 22, 2023, generation II during August 23 -September 24, generation III during September 25 - October 29, generation IV during October 30 - June 11, 2024/ October 30 - April 16, 2024 and generation V during June 12 - July 20, 2024.

Growth indices were computed from the developmental duration and survival data using the formula described by Sharma *et al.* (1982). All biological observations were recorded from 30 independently reared individuals and are presented

as mean ± standard error (SE). Descriptive statistics, including means and standard errors, were computed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Developmental biology of during different generations: The annual life history of *E. vittella* was investigated under natural laboratory conditions, and the insect completed five overlapping generations during the study period (Table 1). The duration of the active generations (I, II, III and V) ranged from 31.6 to 34.6 days. In contrast, the fourth generation, which commenced during late autumn and overwintered (during October 30 - June 11, 2024/ October 30 - April 16, 2024), exhibited a markedly prolonged duration of 200.4–229.5 days. The extended developmental period during this generation was attributed to the prevailing low-temperature conditions (19.7-22.5 and 7.6-10.3^{0}C max and min temperature respectively), during the winter months. Similar observations on the prolonged life cycle of *E. vittella* under winter conditions have been reported by Butani and Jotwani (1984). In contrast, Sharma *et al.* (1985) reported that *E. vittella* completed eleven generations annually, under the warmer agroclimatic conditions of Bihar, highlighting the strong influence of environmental conditions on the seasonal development of the pest.

The developmental biology of *E. vittella* varied among different generations (Table 2). The incubation period ranged from 3.3 to 3.5 days during generations I, II, III and V, whereas it increased to approximately 5.0 days in the overwintering generation (IV), indicating delayed egg hatching under low-temperature conditions. Similar delayed incubation periods have been reported by Kumar *et al.* (2014), who observed it as 3.27 and 3.67 days during July–August and October–November, respectively. Likewise, Bhat *et al.* (2005) reported a longer incubation period (4.4–4.8 days) during September–November than during June–August, supporting the present findings.

The total larval period ranged from 10 to 16 days during generations I–III and V, which agrees with the observations of Sagar and Nebapure (2018) and Sahito *et al.* (2019). However, the larval period was prolonged during the overwintering generation (16.2–16.8 days), presumably due to reduced temperatures. Similarly, the pre-pupal period increased from approximately 1 day during the active generations to 2.4 days during winter. The pupal period ranged from 7.9 to 8.8 days during generations I–III and V but extended markedly to 171.1–199.9 days during the overwintering generation. Comparable pupal durations (8–12 days) during the cropping season were reported by Butani and Jotwani (1984).

The total developmental period from egg to adult emergence ranged from 25.5 to 28.4 days during the active generations but increased to 194.8–224.0 days during the overwintering generation. The mean developmental period of the active generations was 26.8 ± 0.72 days, whereas the overall annual mean was 87.7 ± 38.7 days. Kumar *et al.* (2014) reported

Table 1. Mean active duration of *E. vittella* in different generations/ seasons in 2023-24

Generation	Period	Duration (Days)	Temp (^{0}C)		RH (%)
			Max	Min	
I	July 21 - August 22, 2023	31.6	26.7	19.7	88.0
II	August 23 -September 24	32.0	26.2	18.9	85.3
III	September 25 - October 29	34.2	25.4	14.5	73.6
IV	October 30 - June 11, 2024	229.5	22.5	10.3	55.2
	October 30 - April 16, 2024	200.4	19.7	7.6	60.2
V	June 12 - July 20, 2024	34.6	28.7	19.1	76.0

Table 2. Developmental biology of *Earias vittella* during different generations

Generation	Duration (days)									
	Incubation		Total larval		Pre-pupal		Pupal		Total developmental	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
I	3-4	3.4±0.05	11-15	13.1±0.39	1-2	1.0±0.02	7-8	7.9±0.06	22-28	25.5±0.33
II	3-4	3.3±0.08	10-15	13.5±0.10	1-2	1.0±0.03	7-8	7.9±0.06	21-27	25.8±0.68
III	2-4	3.5±0.06	11-16	14.5±0.36	1-2	1.0±0.00	8-9	8.6±0.04	22-29	27.6±0.47
IV	4-6	5.0±0.07	12-19	16.8±0.99	2-3	2.3±0.07	196-210	199.9±0.06	214-232	224.0±0.43
	4-6	5.1±0.20	12-19	16.2±0.53	2-3	2.4±0.03	170-172	171.1±0.01	188-193	194.8±0.38
V	3-4	3.5±0.06	11-16	15.0±0.20	1-2	1.1±0.06	8-9	8.8±0.16	23-32	28.4±0.32
Mean		4.0±0.30		14.9±0.60		1.5±0.30		67.41±37.5		87.7±38.70
Active generations (except IV)		3.4±0.05		14.0±0.44		1.0±0.01		8.3±0.24		26.8±0.72

Values are presented as mean ± standard error ; n = 30 individuals.

Table 3. Adult longevity and fecundity of *Earias vittella* during different generations

Generation	**Duration (days)**									
	Male		Female							
			Pre-oviposition		Oviposition		Post-oviposition		Total longevity	Fecundity
	Range	Mean	Range	Mean	Range	Mean	Range	Mean		
I	2-6	5.1±0.02	1-2	1.0±0.02	2-3	2.8±0.02	2-3	2.4±0.01	6.2±0.22	180.2±0.64
II	3-6	5.2±0.01	1-3	1.0±0.00	2-4	3.1±0.11	2-3	2.2±0.05	6.3±0.05	191.4±0.43
III	2-7	6.0±0.18	1-2	1.0±0.00	2-3	2.8±0.00	2-3	2.8±0.01	6.6±0.30	190.6±0.31
IV	2-8	5.2±0.22	1-2	1.0±0.01	2-3	2.4±0.02	2-3	2.0±0.01	5.5±0.05	80.6±0.68
	2-8	5.3±0.18	1-2	1.0±0.01	2-3	2.5±0.03	2-3	2.1±0.01	5.6±0.02	80.8±0.31
V	2-6	5.1±007	1-2	1.1±0.02	2-3	2.7±0.03	2-3	2.5±0.14	6.2±0.32	163.2±0.52
Mean		5.3±0.10		1.0±0.00		2.7±0.10		2.3±0.10	6.1±0.20	147.8±21.60

Values are presented as mean ± standard error (SE); n = 30 individuals.

a comparable life cycle duration of 25.05 days. Likewise, Bhat *et al.* (2005) observed prolonged incubation, larval and pupal periods during cooler months at Jammu, while Sahito *et al.* (2019) also demonstrated a marked increase in developmental duration under lower temperatures. These findings clearly indicate that temperature is a major factor regulating the developmental rate of *E. vittella*.

Females consistently survived longer than males across all generations, with mean longevities of 6.1 ± 0.20 and 5.3 ± 0.10 days, respectively. Adult longevity exhibited only minor variation among generations (Table 3). Similar longevity of male and female moths were reported by Sahito *et al.* (2019). The mean pre-oviposition and post-oviposition periods were approximately 1.0 and 2.3 days, respectively, whereas the oviposition period varied from 2.4 to 3.1 days across generations. Mean fecundity was 147.8 ± 21.60 eggs per female, ranging from 163.2 to 191.4 eggs during generations I–III and V. However, females emerging after the overwintering period (generation IV) laid considerably fewer eggs (80.6–80.8 eggs per female), indicating that low-temperature conditions adversely affected reproductive potential. Similar

Table 4. Survival of developmental stages of *Earias vittella* during different generations

Generation	Mean survival (%)			
	Egg	Larva	Pupa	Adult emergence
I	57.21	56.31	79.93	25.75
II	58.80	57.10	80.41	27.00
III	55.66	55.15	81.20	24.93
IV	51.83	45.28	60.67	14.24
	52.65	38.49	57.28	11.61
V	56.81	55.84	76.86	24.38
Mean	55.49	51.36	72.73	21.32

fecundity values have been reported by Bhat *et al.* (2005) and Kumar *et al.* (2014), although Syed *et al.* (2011) observed comparatively longer oviposition and post-oviposition periods under different host plant conditions.

Survival of different stages and growth indices during different generations: The survival of *E. vittella* varied considerably among generations (Table 4). Egg survival was lowest during the overwintering generation (IV), ranging from 51.83 to 52.65 per cent, whereas higher survival (55.66–58.80%) was recorded during the remaining generations. Similarly, larval and pupal survival were markedly reduced during the overwintering generation, (from 38.49 to 45.28% and 57.28 to 60.67%, respectively). In contrast, larval survival during the active generations ranged from 55.15 to 57.10 per cent. The reduced survival observed during the overwintering generation indicates the adverse effect of low-temperature conditions on the immature stages of *E. vittella*. Among all developmental stages, the pupal stage exhibited the highest mean survival (72.73%), whereas the larval stage showed the lowest mean survival (51.36%), probably because newly hatched larvae remain exposed to several biotic and abiotic factors before successfully boring into the host shoots or fruits (Subba *et al*., 2023). Adult emergence varied from 24.38 to 27.00 per cent during the active generations but declined markedly to 11.61–14.24 per cent during the overwintering generation. These observations differ from those of Rajveer *et al.* (2018), who reported maximum mortality during the larval stage under laboratory conditions, and Kumar *et al.* (2014), who recorded higher egg hatchability under warmer environmental conditions.

The larval and total growth indices also varied among generations. Larval growth index was maximum in generation I (4.32), followed by II (4.23), III (3.80), V (3.72), and least in IV (2.20/2.38). Similar trend followed in the total growth index, with maximum in generation II (1.05), followed by I (1.01), III (0.09), V (0.86), and least in IV (0.06/0.06). The lowest growth indices were recorded during the overwintering generation, reflecting the combined effects of prolonged developmental duration and reduced survival under low-temperature conditions. Similar variation in larval and total growth indices among different host genotypes has been reported by Singh (1987). *Earias vittella* completed five overlapping generations annually under the mid-hill conditions of Himachal Pradesh, with developmental duration, survival and reproductive performance being strongly influenced by seasonal temperature. The overwintering generation exhibited a markedly prolonged developmental period accompanied by reduced survival and fecundity, whereas the active generations completed development rapidly under favourable environmental conditions. These findings demonstrate the significant influence of temperature on the annual life history of *E. vittella* and provide valuable baseline information for forecasting pest incidence, identifying vulnerable developmental stages and developing timely, location-specific integrated pest management strategies for okra cultivation under mid-hill agroclimatic conditions.

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Efficacy of horticultural mineral oil and its combinations against leafhopper *Amrasca biguttula biguttula* (Ishida) in brinjal and whitefly *Aleurodicus dispersus* Russell in chilli, Kerala, India

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ABSTRACT: Horticultural mineral oils (HMO) and its combinations were evaluated against leafhopper *Amrasca biguttula biguttula* (Ishida) in brinjal and whitefly *Aleurodicus dispersus* Russell in chilli. HMO (3%) in combination with thiamethoxam (25WG 0.1g L^{-1}), was significantly effective in control the leaf hopper on brinjal. While in the case of white fly on chilli HMO (3%) with thiomethoxam (25WG 0.2g l^{-1}) was effective. The results show a reduction in pesticide load to half without compromising pesticide efficacy.
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KEY WORDS: Sucking pests, integrated pest management, population reduction

Horticultural mineral oils (HMOs) act as contact insecticides, disrupting insect respiration through physical means rather than chemical action (Johnson, 1980). HMOs suffocate insects by forming an oil coating that blocks the spiracles. They deter oviposition on the treated surface. They pose minimal risk to groundwater or soil, and no pest resistance has been recorded so far (Helmy *et al*., 2012). HMO could effectively combine with abamectin and exhibited a synergistic effect against *Helicoverpa armigera* (Wang *et al*., 2005). Leaf hopper *Amrasca biguttula biguttula* (Ishida) and whitefly *Aleurodicus dispersus* Russell are major sucking pests of brinjal and chilli respectively. Leaf hoppers cause more than 50 per cent of economic losses due to their continuous feeding of leaves, that ultimately leads to necrosis (Ramzan *et al*., 2020); on the other hand, whiteflies can potentially lead the chilli crop to low productivity with considerable economic loss (Aswathanarayanareddy *et al*., 2006). The issues that arise due to the use of chemical pesticides, *viz*., target pest resistance, residues in the harvested products, minor pest resurgence, mortality of beneficial organisms, and ecosystem disruption, are always a concern among farmers (Halder *et al*., 2014). Brinjal and chilli crops have to be harvested very frequently, wherein adopting a longer waiting period is not possible once yielding starts. This makes the research community inquisitive about safe alternative methods. It was reported that HMOs are safe for mammals (including humans), some birds, and reptiles with low phytotoxicity (Sridharan *et al*., 2015a). In this backdrop, the

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present research has been undertaken to evaluate the efficacy of HMO against the leaf hopper in brinjal and whitefly in chilli.

Field experimente were carried out on brinjal and chilli at the instructional farm of College of Agriculture, Padannakkad from November 2023 to April 2024. Seeds of brinjal variety Surya and chilli variety Vellayani Athulya were sown in pot trays and transplanted to the main field. Crops were raised as per the recommendations (KAU, 2016). The experiment was performed under the layout Randomized Block Design with eight treatments (Table 1 for brinjal experiment, Table 2 for chilli experiment) and three replications. Cristol TSO, commercial horticultural mineral oil obtained from Krishna Antioxidants Pvt. Ltd. Mumbai, which meets NPOP and USDA standards, was used. Treatments were applied at vegetative and reproductive stages as soon as the pest infestation was seen. Observations on the population density of leafhoppers and whiteflies were made a day prior to spraying and post treatment population density at 1, 3, 5, 7 and 14 DAT from six leaves (two top, two middle, and two lower) of tagged plants. The data were analysed using analysis of variance after square root transformation, and significant means were separated by Duncan Multiple Range Test test at 5 per cent level of significance. Grapes version 1.1.0 software (Gopinath *et al*., 2021) was used to compare the significance of each treatment.

***Amrasca biguttula biguttula* in brinjal:** The pest population (mean of 15 plants) on the seventh DAS was only discussed, as the data showed the consistency of population reduction after the treatments (Table 1). On the seventh day of first application, the treatment T_4 [HMO 3% + Thiamethoxam 25WG 0.1g L^{-1}] recorded the lowest leafhopper population (0.00), which was statistically on par with T_5 [Thiamethoxam 25WG 0.2g L^{-1}] and T_2 [HMO 3% + Azadirachtin formulation 1%, 1ml L^{-1}] both recording 0.13, whereas the population of leafhoppers was maximum in T_8 [Absolute control] (8.60). T_1 [HMO 3%], was statistically on par with T_6 [HMO 3% + Chlorantraniliprole 18.5SC 0.15ml L^{-1}] both recording 0.27 and T_7 [Chlorantraniliprole 18.5SC 0.3ml L^{-1}] with 0.33. All treatments were significantly different from the control after the first application. After the second application, on the seventh day after treatment, T_4 recorded the lowest leafhopper population (0.80), which was on par with T_5 (1.40) and T_6 (1.47). T_1 (2.27) was statistically on par with treatments with T_2 (1.60) and T_7 (1.80). The population of leafhoppers was highest in T_8 (9.53). All treatments were significantly different from the control after the second application. The data on the leaf hopper population after the third application, on the seventh day, all treatments were significantly different from the control. The population was highest in T_8 (9.67). T_4 (1.07) exhibited the lowest leafhopper population which was on par with T_5 (1.40), T_2 (1.67) and T_6 (1.80). T_1 (2.47) was statistically on par with T_3 [Azadirachtin formulation 1%, 2ml L^{-1}] (2.27) and T_7 (1.87).

From the data of the leaf hopper *A. biguttula biguttula* population, it is clear that, there is a significant reduction under different treatments of HMO and its combinations compared to the control. HMO at 3 per cent concentration also can effectively control leaf hoppers compared to control. This finding is in line with the observation of Sridharan *et al*. (2015a), who found that HMO at 1 per cent and 2 per cent could effectively reduce the leaf hopper *A. biguttula biguttula* population in bhindi. In the present study, treatment HMO at 3 per cent + thiamethoxam 25WG 0.1g/L (half the recommended dose) combination was superior among all other treatments against leaf hoppers, which was on par with treatment with thiamethoxam alone (full recommended dose). This indicates that, HMO can be effectively used in combination with chemical pesticides, which improves its efficiency. Similar findings were recorded by Malik *et al*. (2020), who found that the combination of HMO @ 50ml/10l + thiacloprid 240SC @ 4ml/10l significantly reduced the leaf hopper *A. biguttula biguttula* population (0.49 leaf hoppers/plant) in potato. It was also found that mineral oil in combinations with karanj oil or neem oil effectively controlled leaf hoppers *A. biguttula biguttula* in bhindi (Sridharan *et al*., 2015a). According to Fernandez *et al*. (2005), the apple

Table 1. Effect of treatments on *Amrasca biguttula biguttula* population in brinjal (mean of 15 plants)

Treatment	First application		Second application		Third application	
	7DAS	14 DAS	7DAS	14 DAS	7DAS	14 DAS
T_1 - HMO 3%	0.27 (1.13)^{b}	0.87 (1.37)^{b}	2.27 (1.78)^{b}	2.40 (1.83)^{b}	2.47 (1.85)^{b}	3.47 (2.11)^{b}
T_2 - HMO 3% + Azadirachtin formulation 1%, 1ml L^{-1}	0.13 (1.06)^{bc}	0.60 (1.26)^{c}	1.60 (1.61)^{b}	1.87 (1.69)^{b}	1.67 (1.63)^{bcd}	2.27 (1.81)^{cd}
T_3 - Azadirachtin formulation 1%, 2ml L^{-1}	0.33 (1.15)^{b}	0.53 (1.24)^{cd}	1.60 (1.61)^{b}	1.80 (1.67)^{b}	2.27 (1.79)^{bc}	2.87 (1.96)^{bc}
T_4 - HMO 3% + Thiamethoxam 25WG 0.1g L^{-1}	0.00 (1.00)^{c}	0.47 (1.21)^{cd}	0.80 (1.34)^{c}	1.53 (1.59)^{b}	1.07 (1.44)^{d}	1.27 (1.50)^{e}
T_5 - Thiamethoxam 25WG 0.2g L^{-1}	0.13 (1.06)^{bc}	0.33 (1.15)^{d}	1.40 (1.55)^{bc}	2.07 (1.75)^{b}	1.40 (1.55)^{cd}	1.67 (1.63)^{de}
T_6 - HMO 3% + Chlorantraniliprole 18.5SC 0.15ml L^{-1}	0.27 (1.13)^{b}	0.47 (1.21)^{cd}	1.47 (1.57)^{bc}	2.13 (1.77)^{b}	1.80 (1.67)^{bcd}	2.40 (1.84)^{cd}
T_7 - Chlorantraniliprole 18.5SC 0.3ml L^{-1}	0.33 (1.15)^{b}	0.53 (1.24)^{cd}	1.80 (1.67)^{b}	2.53 (1.88)^{b}	1.87 (1.69)^{bcd}	2.47 (1.86)^{c}
T_8 - Absolute control	8.60 (3.09)^{a}	8.80 (3.13)^{a}	9.53 (3.25)^{a}	10.33 (3.36)^{a}	9.67 (3.27)^{a}	10.87 (3.44)^{a}

leaf hopper *Typhlocyba pomeria* population considerably reduced through a higher number of applications of mineral oil in a three-year duration pest management programme.

Whitefly *Aleurodicus dispersus* in chilli:

There was no infestation of whiteflies in chilli during the first application of treatments. From the second application only, the observations were taken. The pest population (mean of 18 plants) on the seventh DAS was only discussed, as the data showed the consistency of population reduction after the treatments (Table 2). On the seventh day, the population was lowest in T_4 (0.67), which was on par with T_5 (0.78). T_1 (2.50) was statistically on par with T_2 (2.33), T_6 (1.94) and T_7 (2.00). The population of whiteflies was highest in T_8 (7.78). All treatments were significantly different from the control. The data on the whitefly population after the third application on the seventh day after application, showed the lowest whitefly population in T_4 (0.06), which was on par with T_5 (0.11). T_1 (1.89) was statistically on par with T_3 (1.94), T_6 (1.78) and T_7 (1.83). The population of whiteflies was highest in T_8 (7.94). All treatments were significantly different from the control after the third application.

There was a significant reduction in the whitefly population in HMO and its combination treatments in chilli compared to the control after two sprays (Table 2). In the present study, treatment HMO at 3 per cent + thiamethoxam 25WG 0.1g/L (half the recommendation dose) combination gave the best results among all other treatments against whiteflies, which was on par with treatment with thiamethoxam 25WG 0.2g/L (full recommended dose). So, the

Table 2. Effect of treatments on whitefly *Aleurodicus dispersus* population in chilli (mean of 18 plants)

Treatments	Second application		Third application	
	7DAS	14 DAS	7DAS	14 DAS
T_1 - HMO 3%	2.50 (1.87)^{b}	2.11 (1.76)^{b}	1.89 (1.69)^{b}	2.56 (1.88)^{b}
T_2 - HMO 3% + Azadirachtin formulation 1%, 1ml L^{-1}	2.33 (1.82)^{b}	2.00 (1.73)^{bc}	1.89 (1.69)^{b}	2.33 (1.82)^{b}
T_3 - Azadirachtin formulation 1%, 2ml L^{-1}	2.22 (1.79)^{b}	2.17 (1.78)^{b}	1.94 (1.72)^{b}	2.72 (1.93)^{b}
T_4 - HMO 3% + Thiamethoxam 25WG 0.2g L^{-1}	0.67 (1.29)^{c}	1.33 (1.53)^{d}	0.06 (1.03)^{c}	1.06 (1.43)^{c}
T_5 - Thiamethoxam 25WG 0.4g L^{-1}	0.78 (1.33)^{c}	1.44 (1.56)^{d}	0.11 (1.05)^{c}	1.33 (1.53)^{c}
T_6 - HMO 3% + Fenpyroximate 5EC 0.3ml L^{-1}	1.94 (1.69)^{b}	1.50 (1.58)^{d}	1.78 (1.67)^{b}	2.00 (1.73)^{b}
T_7 - Fenpyroximate 5EC 0.6ml L^{-1}	2.00 (1.71)^{b}	1.56 (1.59)^{cd}	1.83 (1.68)^{b}	2.11 (1.76)^{b}
T_8 - Absolute control	7.78 (2.96)^{a}	8.00 (3.00)^{a}	7.94 (2.99)^{a}	10.61 (3.41)^{a}

combination effect of HMO is clearly evident. This result is concordant with the findings of Horowitz *et al*. (1997), who observed that the combination having 1 per cent mineral oil with abamectin 18 g a.i./ ha reduced the larval population of sweet potato whitefly *Bemisia tabaci* significantly better than the abamectin applied alone. Mineral oil in combination with pongamia oil and neem oil exhibited higher efficacy in checking *Bemisia tabaci* population in bhindi (Sridharan *et al*., 2015b). In current study, HMO at 3 per cent concentration alone can also effectively manage whiteflies which concur with the results of Stansly and Conner (2005) who reported the considerable reduction of silverleaf whitefly *Bemisia argentifolii* by the application of HMO concentrations ranging from 0.5 per cent to 2 per cent in tomato. It was reported that, the effective reduction in oviposition of greenhouse whitefly *Trialeurodes vaporariorum* was achieved by nC24 horticultural mineral oil in tomato (Xue *et al*., 2002). Laboratory studies of silverleaf whitefly *Bemisia argentifolii* on melon leaves proved that mineral oil 1 per cent could successfully repel whitefly adults and significantly reduce the population (Liang and Liu, 2002). HMO in combination with half the recommended dose of thiamethoxam, azadirachtin and chlorantraniliprole were on par with the full dose of respective chemical pesticides in managing leaf hoppers on brinjal and HMO in combination with a half-recommended dose of thiamethoxam, azadirachtin and fenpyroximate were on par with the full dose

of respective chemical pesticides in managing whiteflies on chilli. This indicates the reduction in the chemical pesticide load in both crops without compromising the efficiency. Similar findings were reported by Shilpa (2024), who found that HMO in combination with half recommended dose of chemical pesticides lambda cyhalothrin 25EC 0.0015 per cent and quinalphos 25 EC 0.025 per cent were on par with the full dose of respective chemical pesticides in managing tea mosquito bug on cashew. The combination effect of HMO with half the recommended dose of the chemical pesticides showed the same efficiency on the leafhopper and the whitefly, when the same chemical pesticides were applied alone in the full recommended dose.

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Bio-insecticidal potential of methanolic seed extract of *Datura innoxia* (Solanaceae) against housefly *Musca domestica* L. (Diptera, Muscidae)

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ABSTRACT: A study was undertaken to evaluate the insecticidal potential of methanolic seed extract of *Datura innoxia* against *Musca domestica*. Phytochemical analysis by FTIR spectroscopy revealed the presence of bioactive compounds. The toxicity of the extract assessed, at varying concentrations and exposure times revealed that the mortality rate increased with concentration and exposure time, and a significant effect was observed @31.65 µg mL^{-1} after 24 hours.
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KEYWORDS: Bioactive compounds, alkyl and aromatic groups, cumulative toxic effect, log-probit regression, FTIR, LC_{50}

The housefly *Musca domestica* L. (Diptera, Muscidae) is cosmopolitan, of significant public health importance, and is a primary vector for numerous enteric and systemic pathogens. It is responsible for the mechanical transmission of disease to humans and cattle by spoiling food and water sources (Wanaratana *et al.,* 2013). Management of *M. domestica* has depended heavily on synthetic insecticides and the indiscriminate use of synthetic chemicals poses substantial risks to non-target organisms and environment (Geden, 2012). In response to these challenges, research was shifted toward botanical insecticides (Scott *et al.,* 2017). *Pithecellobium dulce* leaf extract was found to be a potent insecticide against *Pediculus humanus*, while the stem extract is effective in inhibiting egg hatching (Susmita Gudulkar *et al.,* 2025). *Camphor* extract on melon fruit fly (*Zeugodacus cucurbitae*) has significant potential to control the development of the pupa (Mawtham *et al.,* 2020). Silver nanoparticles from *Datura* extract are effective against larval development of *Epilachna vigitioctapunctata* (Sundareswari *et al.,* 2023). Aqueous, methanolic and acetone extract of *Centella asiatica* showed macromolecular alterations, glycogen total body protein increased, and free amino acid concentration decreased in

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Callosobruchus chinensis (Bindhu *et al.,* 2014). Significant inhibition of the activities of acetylcholinesterase, antioxidant enzymes and Glutathione-S-transferase (GST) mediated by *Streblus asper* (PBSA) on *Dysdercus cingulatus* (Anila *et al.,* 2023). The current research was to evaluate the insecticidal efficacy of *D. innoxia* seed extracts against *M. domestica*.

Datura innoxia seeds, collected from Nashik, were washed, shade-dried for 7 days and pulverised. The resulting powder was stored in an airtight container (Ayub *et al.,* 2011). Seed powder (50g) underwent Soxhlet extraction with 500mL of methanol at 60 °C. The crude extract was concentrated and dried using a rotary evaporator under reduced pressure (Ali Eltayeib *et al*., 2021). Potassium bromide pellets (containing 1% extract @2mg) were prepared using a hydraulic press. Fourier Transform Infrared Spectroscopy Spectra were recorded from 400 to 4000 cm^{-1} to identify primary functional groups. Chemical characterisation was achieved by comparing recorded spectra against reference standards

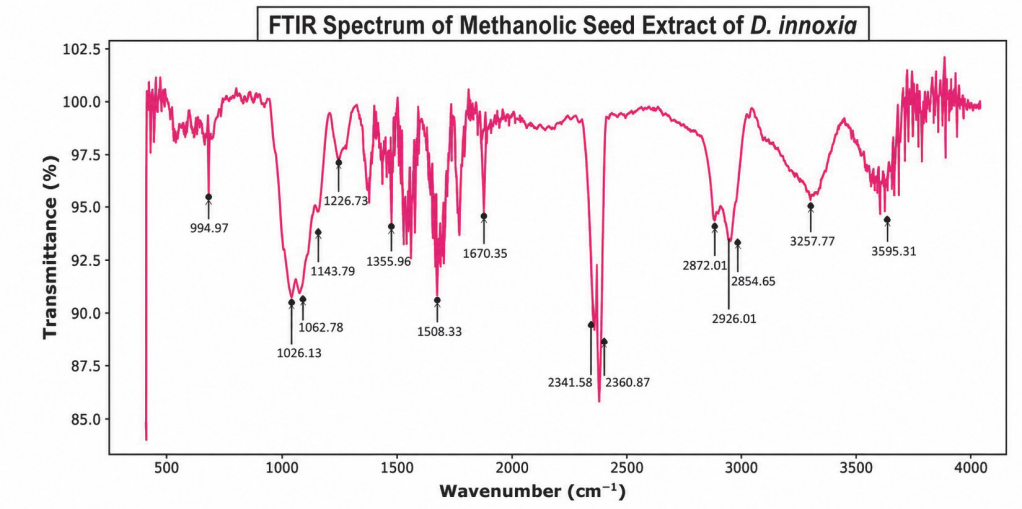


Fig. 1 FTIR transmittance spectrum of the methanolic seed extract

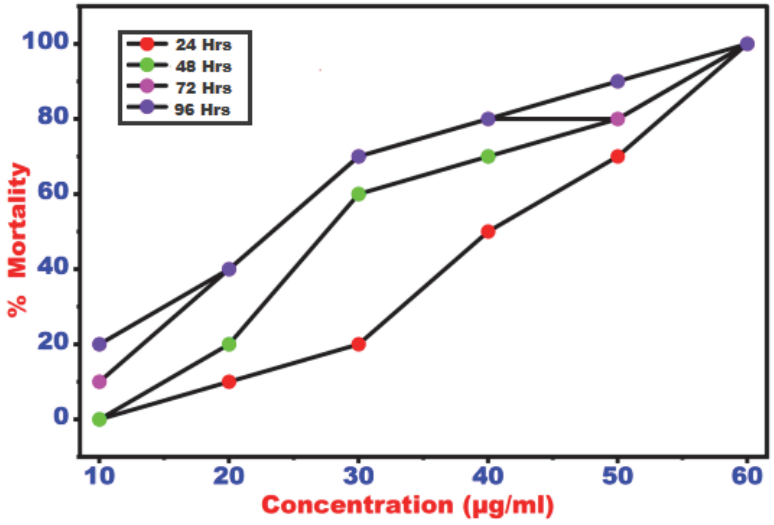


Fig. 2 Mortality (%) of adult *Musca domestica* across varying concentrations of *Datura innoxia* seed extract over a 96-hour exposure

Table 1. Functional groups and FTIR peaks were found in the methanolic *Datura innoxia* seed extract

No.	Wave number (cm^{-1})	Functional group	Compound
1	3595.31	O–H stretch, free hydroxyl	Alcohols, Phenols
2	3277.06	Ca”C –H, C–H stretch	Alkynes (Terminal)
3	3257.77	N–H stretch	Primary, Secondary Amines, Amides
4	2926.01	C–H stretch	Alkanes
5	2872.01	C–H stretch	Alkanes
6	2854.65	C–H stretch	Alkanes
7	2360.87	OH Stretching	Carboxylic acid
8	2341.58	H–C=O: C–H stretch	Aldehydes
9	1670.35	C=O stretch	Alpha, Beta–unsaturated a ldehydes, Ketones
10	1508.33	C–C stretch (in–ring)	Aromatics
11	1355.96	N–O symmetric stretch	Nitro compounds
12	1226.73	C–O stretch	Esters, Ethers, Alcohols, Carboxylic acids,
13	1143.79	C–H wag (–CH_2X)	Alkyl Halides
14	1062.78	C–N stretch	Aliphatic Amines
15	1026.13	C–O stretch	Esters, Ethers, Alcohols, Carboxylic acids,
16	994.97	C–H “loop”	Aromatics

(Durak and Depciuch, 2020). Crude extract (4g) stock solution dissolved in 10mL of 99.9 per cent ethanol, final volume adjusted to 200mL using distilled water. From this stock (20 mg mL^{-1}), specific exposure doses of 1, 2, 3, 4, and 5mL were prepared for the bioassays, following the methodology described by Ali and Siddige (2021). *M. domestica* were laboratory-reared in a 50 cm^3 metallic cage [28±3°C, 55-60(%) RH, 12:12 L:D], in the laboratory. Insecticide-free F_1 generations were maintained to ensure susceptibility. Sugar-milk mixture (10 % w/v) was provided for adult nutrition (Abbas, and Hafez, 2021). Ingestion lethality assays (Mallick *et al.,* 2016) were conducted on 4-to-5-day old flies across six experimental groups (n=10/group). Treatments included *D. innoxia* extract, a 90:10 water-methanol negative control and a pyrethrin positive control. Mortality rates were monitored at 24, 48, 72, and 96h intervals. LC_{50} values were determined via probit analysis (Finney,1971) based on triplicate bioassays. Data processing, including regression (R^2), mean mortality and standard error, was performed in Microsoft Excel 2007. Significance was evaluated using analysis of variance.

FTIR analysis of *D. innoxia* seed extract identified key phytochemical fractions (Table 1). The

Table 2. LC_{50} of *Datura innoxia* seed extract against adult *Musca domestica* at different exposure intervals and log-probit regression parameters

Exposure (hrs)	Regression equation $\{Y = y^- + b(x - x^-)\}$	R^2- values	LC_{50} ($\mu g\, mL^{-1}$)
24	Y=5.7520X-3.63	0.7840	31.65
48	Y=5.8983X-3.4523	0.8821	26.11
72	Y=4.5390X-1.0740	0.8068	21.78
96	Y=4.2697X-0.5297	0.8080	19.74

spectrum illustrates (Fig. 1), the complex chemical fingerprint of the methanolic fraction within the 400-4000 cm^{-1} scanning range. Absorption bands revealed bioactive secondary metabolites with C-H stretching vibrations, indicating alkyl and aromatic groups. The presence of alkaloids and flavonoids was confirmed by C=O stretching in esters and carbonyls, while C-H bending and phosphate groups suggested the presence of terpenoids and anthraquinones (Mardare *et al.,* 2022). The seed extract mortality was directly tied to both dose and exposure time. While the control group showed no mortality throughout the 96-hour study, the LC_{50} value of the extract dropped steadily from 31.65 µg mL^{-1} at 24 hours to 19.74 µg mL^{-1} at 96 hours (Table 2). This progressive decline highlights a cumulative toxic effect; even the lowest dose (10 µg mL^{-1}) eventually achieved significant mortality by the 96-hour mark. The graph (Fig. 2) shows a rise in mortality 24-hour exposure (Red): Indicates the first hazardous reaction; only at greater concentrations does detectable mortality take place. Green and Pink for 48 to 72 hours: shows a progressive change. The highest recorded efficacy is attained after 96 hours of treatment (purple), smaller concentrations (such as 10 µg mL^{-1}) start to show notable mortality. A high R^2 value across all the treatments confirms a strong linear relationship between concentration and mortality, suggesting that the impact of the extract is both predictable and persistent.

Medicinal plants have the ability to kill *M. domestica* through bioinsecticidal effects (Sharma et al., 2011). Physiological alterations in treated *M. domestica,* indicating that the *Datura innoxia* seed extract affects essential metabolic functions and contributes to *M. domestica* mortality (Jangam & Patil, 2026). Late et al., (2026) demonstrated significant larvicidal and repellent activity of ethanolic extracts of *Datura innoxia* and *Datura stramonium* against white grub larvae, the authors attributed the observed toxicity to the presence of bioactive phytochemicals capable of interfering with normal physiological processes in insects by damaging vital digestive tissues. According to Jemberie *et al.* (2016) essential oils *Otostegia integrifolia*, *Echinops kebericho*, *Stephania abyssinica,* and *Millettia ferruginea* show significant repellence against *Aedes arabiensis.* The ethanolic leaf extract of *Calotropis procera* was observed to be more toxic and have notable larvicidal activities at concentrations of 1.923 ppm and 8.83 ppm for LC_{50} and LC_{90}, respectively (Kaushik *et al.,* 2022). Similarly, it was shown by Attaullah *et al.* (2019) that native weeds, such as *Datura stramonium* (62.78%) and *Peganum harmala* (63.87%), showed substantial toxicity at 25 per cent concentration after 72 hours. The finding of the current research provides the first evidence that a methanolic extract of *D. innoxia* seed possesses significant adulticidal potential against *M. domestica*. The bioactivity is likely driven by a synergistic blend of phytochemicals, specifically alkaloids such as atropine and hyoscine, alongside flavonoids, triterpenes and phenolic compounds identified in the analysis. These results position *D. innoxia* as a promising candidate for a

viable, eco-friendly option for the development of biopesticides to manage housefly populations in domestic and livestock settings.

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New records of short-horned grasshoppers (Orthoptera, Acrididae) from Nagaland, India

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ABSTRACT: Three species of short-horned grasshoppers (Orthoptera, Acrididae) - *Patanga succincta*, *Aiolopus thalassinus tamulus*, and *Eucoptacra praemorsa* are reported for the first time from Nagaland, north-east India. These new distributional records are based on specimens collected during surveys conducted in 2024 and examined using standard morphological characters. The findings add to the limited knowledge of Acrididae diversity in the Nagaland region.
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KEY WORDS: Caelifera, morphology, Indo-Burma Biodiversity hotspot, Zunhebeto

Grasshoppers and locusts, members of the family Acrididae, are economically significant orthopteran pests that infest both cultivated and non-cultivated crops.The family Acrididae belongs to the superfamily Acridoidea within the order Orthoptera and is widely distributed throughout India.The superfamily Acridoidea, comprising 11 families, exhibits maximum diversity among orthopteran groups.Acrididae represents the largest and most diverse lineage within this superfamily, encompassing approximately 6,889 species distributed across 28 subfamilies worldwide (Cigliano *et al*., 2025).The Indian acridid fauna comprises 366 species representing 16 subfamilies (Chand *et al.*, 2025). Members of the family Acrididae can be readily identified by distinctive morphological features (Kirby, 1914). The study of Acrididae and Orthoptera in northeastern India has progressed gradually through a series of important taxonomic and faunistic investigations. Early contributions by Serville (1838), Stal (1877), Kirby (1914), Chopard (1921, 1924), Uvarov (1927a, 1927b, 1930, 1942), Ramme (1941), Shishodia and Hazara (1985), Willemse (1932), Uvarov (1942), Varshney (1970) laid the foundation for understanding the orthopteran fauna of the region. Subsequent works by Bhowmik *et al.* (1990), Shishodia and Tandon (2000, 2004), Shishodia and Dey (2006, 2007), Usmani and Khan (2010), and later regional taxonomic revisions significantly expanded the knowledge of Acrididae diversity in northeastern India.

Knowledge of the Acrididae fauna of Nagaland is limited compared to that of other Northeastern states. In the earliest work on Acrididae from Northeast India, Bhowmik *et al.* (1990) reported only two species of Acrididae from Nagaland. No exclusive study on the Acrididae fauna of Nagaland has been conducted in recent years; however,

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Shishodia and Dey (2006) listed 30 species. In this paper, three species of the Acrididae family from the state were reported for the first time.

The specimens were collected from Wokha and Zunhebeto District of Nagaland during a survey conducted in 2024. The specimens were collected during the early hours of the day by repeated sweeping across grasses, shrubs and low vegetation. The comparatively larger or less agile individuals were collected using fingers. The specimens were killed by benzene vapor in a killing jar, pinned and preserved dry. The specimens were examined using the stereo zoom microscope Leica EZ4E. Measurements were done by using a Vernier Calliper and with a reticule attached to the stereomicroscope. The classification of Cigliano *et al.* (2025) and the terminology of Uvarov (1966) are followed. Species identification is largely based on keys provided by Kirby (1914), Uvarov (1923) and Hollis (1968). Following morphometric parameters have been used (Hill and Weissman, 2025):

Body length: dorsally from the fastigium of vertex to the distal end of the genicular lobe of the hind femur in a parallel plane with the abdomen

Antenna length: measured from the scape to the tip of antenna

Head length: measured from the anterior end of the vertex to the anterior margin of the pronotum

Pronotum length: measured along the midline

Tegmina length: measured from the confluence of the C and Sc veins to the apex

Hind femur length: measured from its base to the tip of the knee

Hind tibia length: excluding the condyle, measured from the base to the dorsal insertion of the external spurs.

Male cercus length: laterally, maximum measurement of the left cercus

Male cercus basal width: laterally, along the point of attachment from the dorsal to ventral margin

Male mid cercus width: laterally, at the mid-length of the left cercus

Aedeagus length: caudally from point where ventral portion of the sheath ends to apex of longest valve (dorsal or ventral)

Female dorsal ovipositor valve: Laterally, from the base to the apex

Female ventral ovipositor valve**:** Laterally, from the base to the apex

The specimens have been deposited in the National Zoological collections of North Eastern Regional Centre, Zoological Survey of India, Shillong.

Systematic Account:

Subfamily: Cyrtacanthacridinae; Tribe: Cyrtacanthacridini; Genus: *Patanga* Uvarov, 1923

1. *Patanga succincta* (Johannson, 1763)

1763. *Gryllus locusta succinctus* Johannson, Centuria Insectorum rariorum. Amoenitates Academicae, 6: 383.

2007. *Patanga succincta*, Chandra *et al.*, Zoos' Print Journal, 22(5): 2686.

Diagnosis: A comparatively large species, exhibiting a generally pale appearance, varying from greyish to brownish, sometimes nearly uniform. The vertex bears a broad median yellow to reddish-brown stripe, which extends posteriorly onto the pronotum. Pronotum relatively short and robust, with posterior margin obtusely angular to almost truncate. Hind femur with supero-median carina weakly serrulate, the denticulations few in number and widely spaced. Male cerci conspicuously stout, broad and incrassately compressed.The prosternal tubercle is not recurved posteriorly (Fig. 1).

Materials examined: 1 Male, Nagaland, Wokha Dist., Forest Campus, Doyang Village, 26.22458 N; 94.24871 E, 264 m, 1.8.2024, Coll. Dr. B. Sinha & party; 1 Female, Nagaland, Wokha Dist., Pangti Village, 26.25894 N; 94.31890 E, 932 m, 1.8.2024, Coll. Dr. B. Sinha & party.

Measurements: Male: (mm) - body length = 43.47; antenna length = broken; head Length = 8.53;

pronotum length = 9.01; tegmina length = 38.43; hind femur length = 22.63; hind tibia length = 22.01; cerci length = 1.50; basal width of cercus = 1.01; mid width of cercus = 0.5; Aedegus length = 1.26. **Female:** (mm) - body length = 53.46; antenna length = 21.42; head length = 11.47; pronotum length = 12.27; tegmina length = 51.53; hind femur length = 22.63; hind tibia length = 28.05; dorsal ovipositor valve length = 2.99, ventral ovipositor valve length = 2.98.

Distribution in India: Andhra Pradesh (MandaI and Yadav, 2007), Arunachal Pradesh (MandaI *et al.*, 2006), Assam (Bhowmik *et al.*, 1990), Bihar (Bhowmik *et al.*, 1990), Chhattisgarh (Chandra *et al.*, 2007), Delhi (Shishodia, 2000), Goa (Chandra *et al.*, 2007), Himachal Pradesh (Tandon and Shishodia, 1995), Jammu & Kashmir (Bhowmik *et al.*, 1990), Kerala (Shishodia and Kulkarni, 2001), Lakshadweep Islands (Chandra *et al.*, 2007), Maharashtra (Bhowmik *et al.*, 1990), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), Odisha (Dey and Hazra, 2003), Rajasthan (Chandra *et al.*, 2007), Tamil Nadu (Shishodia and Kulkarni, 2001), Tripura (Shishodia and Tandon, 2000), Uttarakhand (Tandon and Shishodia, 1995), Uttar Pradesh (Tandon and Shishodia, 1995) and West Bengal (Hazra *et al.*, 1993).

Habitat: Common in grasslands, cultivated fields, forest edges and gardens.

Subfamily: Oedipodinae, Tribe: Epacromiini, Genus: *Aiolopus* Fieber, 1853

2. *Aiolopus thalassinus tamulus* (Fabricius, 1798)

1798. *Gryllus tamulus* Fabricius, Entomologia systematica Suppl,: 195

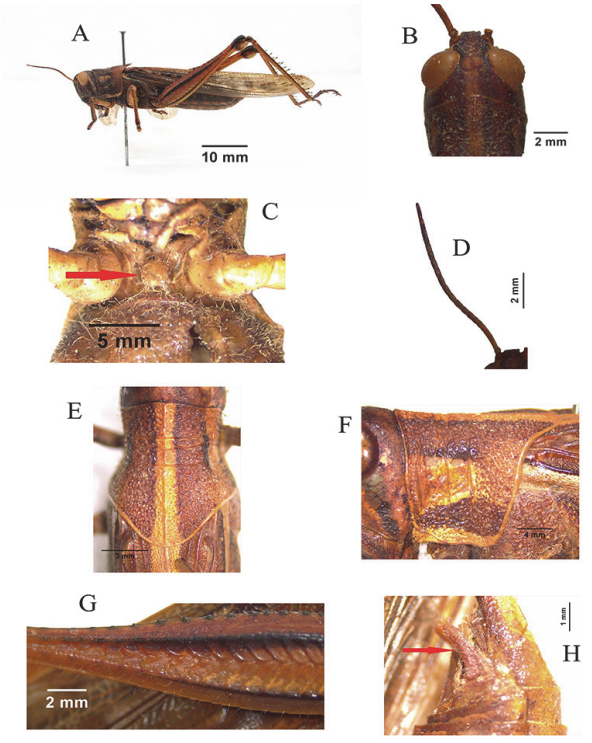


Fig. 1 A- Lateral view of *Patanga succincta* (Male), B-Head and Vertex (Dorsal view), C- Prosternal process, D- Antennae, E- Pronotum (dorsal view), F- Pronotum (lateral view), G- Hind femur, H- Male cerci

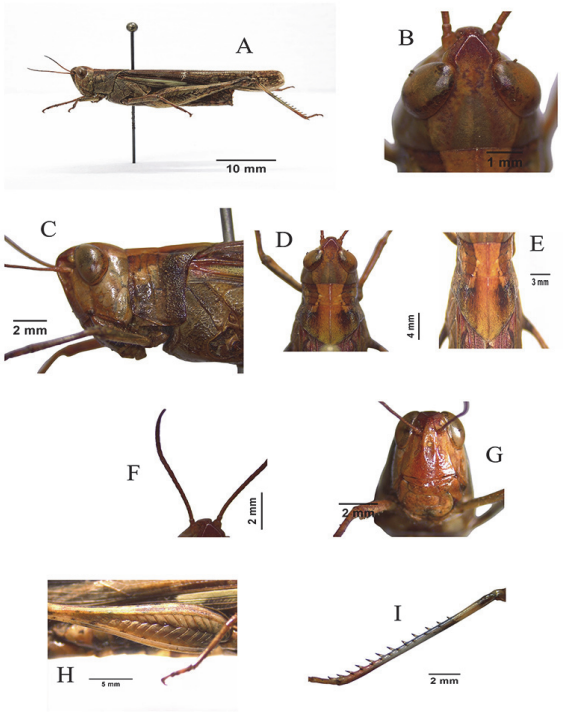


Fig. 2 A- Lateral view of *Aiolopus thalassinus tamulus* (Female), B-Head and Vertex (Dorsal view), C-Head and Pronotum (lateral view), D- Head and Pronotum (dorsal view), E-Pronotum (dorsal view), F- Antennae, G- Frontal ridge, H- Hind femur, I- Hind tibia

2007. *Aiolopus thalassinus tamulus*, MandaI *et al.*, Pictorial handbook on Indian short-horned grasshopper pests, (Acridoidea: Orthoptera) (Zool. Surv. India): 51.

Diagnosis: A species of medium size. Antennae elongate, approximately equal in length to the combined length of head and pronotum. Fastigium with an anterior angle distinctly acute. Pronotum weakly saddle-shaped, posterior margin rounded, disc sparsely punctate.Foveolae are narrowly trapezoidal, about twice as long as broad. Frontal ridge gradually constricted upwards, becoming nearly angular near the fastigial apex. Hind femur is uniformly coloured, lacking oblique fasciae. Posterior tibiae with apical region usually red, this area being widely separated from the basal black band by a broad bluish-grey zone (Fig. 2).

Materials examined: 1 Female, Nagaland, Wokha Dist., Forest Campus, Doyang Village, 26.22458 N; 94.24871 E, 264 m, 1.8.2024, Coll. Dr. B. Sinha & party; 1 Male, Nagaland, Zunhebeto Dist., Aphukhomi Village Forest, 26.07007 N; 94.54482 E, 1525 m, 4.8.2024, Coll. Dr. B. Sinha & party.

Male: (mm) - body length = 14.82; antenna length = 5.82; head Length = 3.91; pronotum length = 2.87; tegmina length = 17.32; hind femur length = 9.57; hind tibia length = 8.94; cerci length = 0.51; basal width of circus = 0.17; mid width of cercus = 0.17; Aedegus length = 0.43. **Female:** (mm) - body length = 21.53; antenna length = 6.13; head length = 5.59; pronotum length = 3.68; tegmina length = 19.39; hind femur length = 10.87; hind tibia length = 10.53; dorsal ovipositor valve length = 1.19, ventral ovipositor valve length = 1.18.

Distribution in India: Andaman & Nicobar Islands (Chandra and Rajan, 2004), Andhra Pradesh (MandaI and Yadav, 2007), Arunachal Pradesh (MandaI *et al.*, 1999), Bihar (Bhowmik *et al.*, 1990), Chhattisgarh (Tandon *et al.*, 1995), Delhi (Shishodia, 1997), Haryana (Shishodia, 1997), Himachal Pradesh (Tandon and Shishodia, 1995), Karnataka (Shrinivasan and Muralirangan, 1992), Kerala (Shishodia and Kulkarni, 2001), Madhya Pradesh (Tandon *et al.*, 1995), Maharashtra (Kulkarni and Shishodia, 2005), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), Odisha (Dey and Hazra, 2003), Punjab (Bhowmik *et al.*, 1990), Rajasthan (Shishodia, 2004), Tamil Nadu (Shrinivasan and Muralirangan, 1992), Tripura (Shishodia and Tandon, 2000), Uttarakhand (Tandon and Shishodia, 1995), Uttar Pradesh (Tandon and Shishodia, 1995) and West Bengal (Hazra *et al.*, 1993).

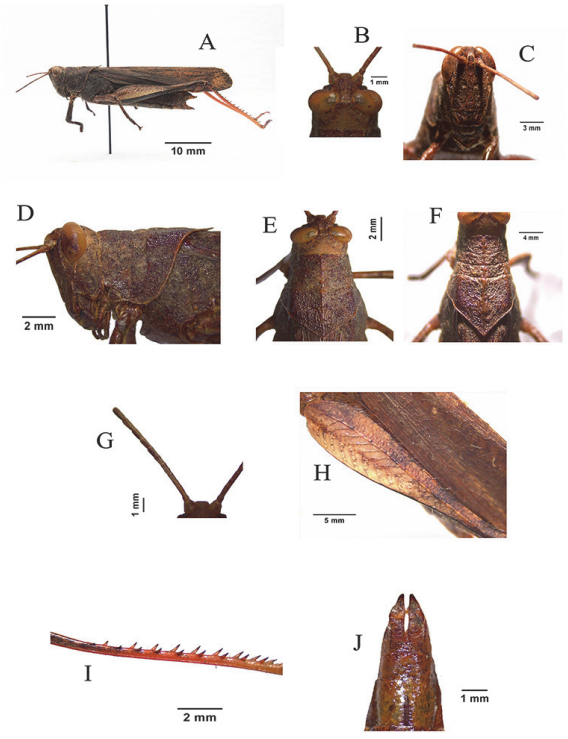


Fig. 3 A- Lateral view of *Eucoptacra praemorsa* (Female), B-Head and Vertex (Dorsal view), C-Frontal ridge, D- Head and Pronotum (lateral view), E- Head and Pronotum (dorsal view), F- Pronotum (dorsal view) , G- Antennae, H-Hind femur, I- Hind tibia with spines, J-Last abdominal tergite showing the subgenital plate of female

Habitat: Inhabits moist grassy habitats (paddy fields, marshes, wet meadows and riverbanks)

Subfamily: Coptacrinae; Genus: *Eucoptacra* Bolivar, 1902

3. *Eucoptacra praemorsa* (Stal, 1861)

1861. *Acridium (Catantops) praemorsum* Stal,

Kongl. Svensk. Fregat Eugen. Res. Omkring Jorden, 3: 330.

2007. *Eucoptacra praemorsa*, Saini & Mehta, Bionotes, 9(3): 76.

Diagnosis: Frontal ridge between the antennae approximately twice as wide as the inter-ocular distance; frontal costa distinctly widened between the antennae and broader than the vertex between the eyes.Principal sulcus of the pronotum is situated slightly anterior to the middle. Tegmina elongate, extending beyond the apex of the hind femora, with a faintly indicated whitish callous fascia near the middle of the median field. General colouration brownish testaceous. Hind femora yellowish brown to dark brown; area supra with two indistinct, faintly marked transverse dark spots and an additional spot at the base; inner area distinctly red; area supra externa without a distinct black spot in or near the median region. Hind tibiae red. Female subgenital plate with posterior margin broadly rounded or triangularly produced (Fig. 3).

Materials examined: 3 Female, Nagland, Wokha Dist., Pangti Village, 26.25894 N; 94.31890 E, 932 m, 1.8.2024, Coll. Dr. B. Sinha & party; 1Male, Nagaland, Zunhebeto Dist., Baimho Village, 26.01367 N; 94.56057 E, 1009 m, 5.8.2024, Coll. Dr. B. Sinha & party.

Measurements: Male: (mm) - body length = 15.29; antenna length = 7.57; head Length = 4.35; pronotum length = 3.68; tegmina length = 17.57; hind femur length = 10.87; hind tibia length = 9.65; cerci length = 0.50; basal width of circus = 0.16; mid width of circus = 0.16; Aedegus length = 0.42. **Female:** (mm) - body length 17.90; antenna length 7.80; head length 5.61; pronotum length 4.55; tegmina length 20.77; hind femur length 11.84; hind tibia length 11.74; dorsal ovipositor valve length 1.0, ventral ovipositor valve length 1.0.

Distribution in India: Andhra Pradesh (MandaI and Yadav, 2007), Arunachal Pradesh (MandaI *et al.*, 2006), Assam (Bhowmik *et al.*, 1990), Chhattisgarh (Kulkarni and Shishodia, 2005), Himachal Pradesh (Tandon and Shishodia, 1995), Karnataka (Bhowmik, 1986), Kerala (Shishodia and Hazra, 1985), Madhya Pradesh (Bhowmik *et al.*, 1990), Maharashtra (Kulkarni and Shishodia, 2005), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), Odisha (Hazra *et al.*, 1993), Sikkim (Shishodia *et al.*, 2003), Tamil Nadu (Bhowmik, 1986), Tripura (Shishodia and Tandon, 2000), Uttar Pradesh (Tandon and Shishodia, 1995), Uttarakhand (Tandon and Shishodia, 1995) and West Bengal (Hazra *et al.*, 1993).

Habitat: Inhabits in dry leaf litter on the forest floor, grassy areas, forest fringes and scrublands.

Bhowmik *et al.* (1990) produced one of the earliest comprehensive faunistic accounts of Acrididae from northeastern India and reported numerous new state records from the region. Later, Shishodia and Tandon (2000) documented 50 species of Acridoidea and Eumastacoidea from Tripura, including several new records for India and the state. Their subsequent work on Manipur recorded 54 species belonging to 41 genera, with many species reported for the first time from the state. Similarly, Shishodia and Dey (2006) reported 34 species under 24 genera and two families from Nagaland based mainly on collections preserved in the Zoological Survey of India, while Shishodia and Dey (2007) documented 40 species from Mizoram. Usmani and Khan (2010) further emphasized the rich but insufficiently explored orthopteran diversity of northeastern India by documenting 53 species representing 36 genera from the region. Later taxonomic investigations demonstrated that northeastern India is an important centre of diversity for Acrididae. Ingrisch *et al.* (2004) described one new genus and several new species from Meghalaya, Manipur, Mizoram, and Tripura. Kumar *et al.* (2018a, b, 2019), Gupta *et al.* (2019), and Chandra *et al.* (2019) described additional new genera and species from the eastern Himalaya and northeastern states, highlighting the presence of highly localized and endemic acridid fauna in the region. Gupta and Chandra (2019), reported 108 species of Acrididae family under 62 genera from the North-East Zone. Kumar *et al.* (2024) documented the first confirmed record of *Oxytauchira aurora* (Brunner von Wattenwyl, 1893) from India, specifically from Nagaland.

The distributional pattern of *P. succincta*, *A.*

thalassinus tamulus, and *E. praemorsa* across northeastern India further reflects the wide ecological adaptability within the Indo-Burma biogeographic region. *Patanga succincta* has previously been reported from Arunachal Pradesh (Mandal *et al.*, 2006), Assam (Bhowmik *et al.*, 1990), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), and Tripura (Shishodia and Tandon, 2000), whereas *A. thalassinus tamulus* is known from Arunachal Pradesh (Mandal *et al.*, 1999), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), and Tripura (Shishodia and Tandon, 2000). Similarly, *E. praemorsa* was documented from Arunachal Pradesh (Mandal *et al.*, 2006), Assam (Bhowmik *et al.*, 1990), Manipur (Shishodia and Tandon, 2004), Meghalaya (Hazra *et al.*, 1995), Sikkim (Shishodia *et al.*, 2003), and Tripura (Shishodia and Tandon, 2000). The present study documents *P. succincta*, *A. thalassinus tamulus*, and *E. praemorsa* as new distributional records from the state of Nagaland.

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Report of *Donda continentalis* Behounek, Han and Kononenko, 2012 (Lepidoptera, Noctuidae) from India

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ABSTRACT: *Donda continentalis*, Behounek, Han and Kononenko, 2012 (Lepidoptera, Noctuidae) earlier known from south China, Thailand, and Vietnam, is herein reported for the first time from India. The species record from Kerala constitutes a part of significant range extension to the Western Ghats of India. A detailed description of male habitus and genitalia with key diagnostic features is provided.
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KEY WORDS: New record, Western Ghats, range extension, genitalia, pantheine

The subfamily Pantheinae Smith, 1898 comprises morphologically diverse species with the greatest diversity in the Holarctic, Oriental, and Neotropical regions (Behounek *et al.*, 2012). *Donda* Moore, 1882, is a typical oriental genus (Behounek *et al*., 2012) characterized by light green ground-coloured forewings with three brown patches. The genus was placed in subfamily Catocalinae by Nye (1975). Later Poole in 1989 shifted the genus into the subfamily Ophiderinae. Kobes (1992) transferred the genus into the sub family Pantheinae of the family Noctuidae. In 2012, Behounek *et al.* revised the genus and listed six species under the genus *Donda*. Out of these species, the taxonomic status of two species is still unclear due to lack of proper description. Later, *D. unispinosa* Bandyopadhyay, Raha and Chandra, 2020 from India (Bandyopadhyay *et al.*, 2019) and *D. hunana* Han, Behounek and Kononenko, 2020 from China (Han *et al.*, 2020) were also described. Joshi *et al.* (2025) listed four species from India. In this study *D. continentalis* is reported for the first time from India.

The adult male specimen was collected using the light trap method. A 160-watt mercury vapor lamp was used for the purpose. A plain long white sheet is tied on a rope, and the mercury vapor lamp was placed in front of the white sheet. The collected specimen was killed using a killing jar containing ethyl acetate-soaked cotton. The killed specimen was then pinned and dried under 60-watt incandescent bulb. The dried specimen was neatly labelled and stored in an airtight insect box. The specimen was duly registered and deposited in the insect collections of Entomo Taxonomy Lab, Christ college (Autonomous), Irinjalakuda, Kerala.

The identification of the collected specimen was carried out using the literature published by Behounek *et al.* (2012) and Bandyopadhyay *et al.* (2019). The species-level identification was confirmed by the genitalia analysis as per the

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Robinson method (1976). The last four abdominal segments were removed and soaked in 10 per cent KOH solution overnight. It was then dissected under a microscope and compared with available genitalia data. The images were taken using a camera associated with the Optika SLX2 Stereozoom microscope.

Systematic account

Superfamily: Noctuoidea Latreille 1809

Family: Noctuidae Latreille 1809

Subfamily: Pantheinae Smith 1898

Genus: *Donda* Moore 1882

***Donda continentalis* Behounek, Han and Kononenko, 2012**

Description: Wing expanse 4.3cm (Fig. 1) Antennae filiform; head covered with light green and brown scales; palpi upright, long yellowish with third segment half the length of the second; thorax with light green and brown scales; abdomen yellowish with dorsal tuft of scales; forewing with light green ground colour; a tiny brown patch present on basal area; a large somewhat triangular patch present hanging from costal part of sub basal area and a brown patch on apical part of terminal and sub terminal area; a small brown patch present on

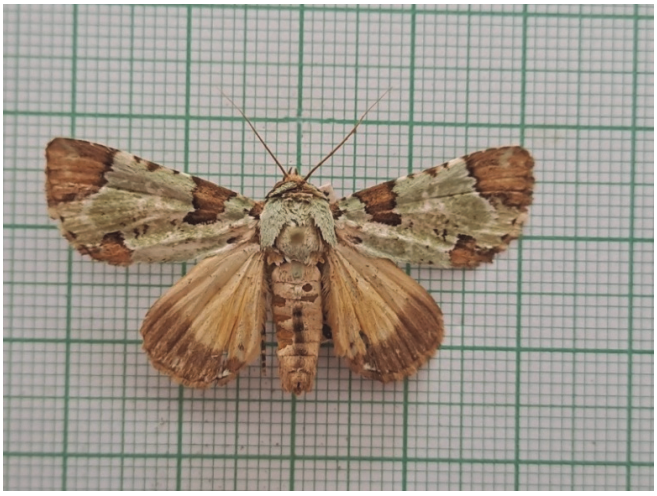


Fig. 1 Habitus of *Donda continentalis*

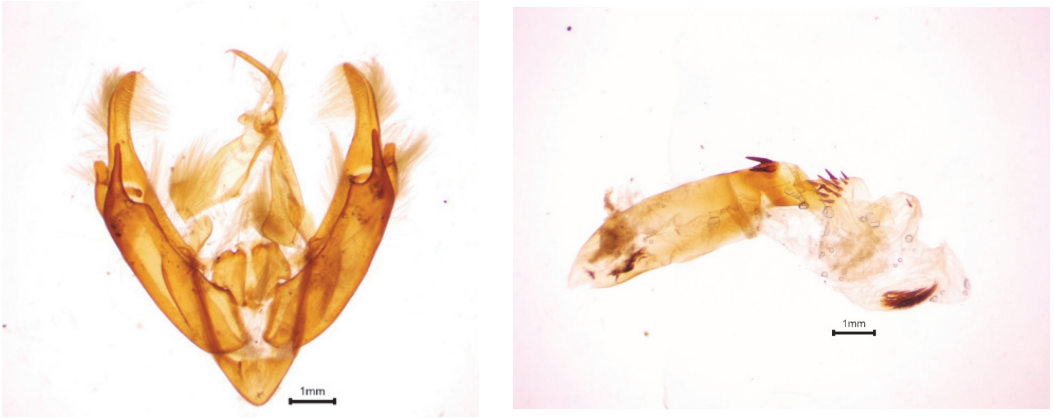


Fig. 2 Male genitalia; Aedeagus of *D. continentalis*

tornal area; slightly more distinct submedial and antemedial lines present. Underside yellowish with two dark bands. Hind wing yellow with broad brown terminal band and with a less distinct medial line. Underside with diffused terminal band and indistinct medial and sub medial wavy lines.

Male genitalia (Fig. 2): Uncus long, basally curved with an apical hook; tegumen broad with peniculus; juxta broad, leaft shaped with wide channel and lateral flaps; valvae long, gradually curved and constricting apically; cucullus setosed; saccus broad U shaped; sacculus long, asymmetric with pointed, slightly curved apical tip; finger-like ventro apical extension present; Aedeagus long with strong spines (Fig. 3); vesica with a patch of spines and two distinct needle-like cornuti.

Material examined: 1 ♂; 30-ix-2024; Shiruvani, Palakkad district, Kerala, India, (10.975N; 76.642E); altitude 960m; Coll. Joslin Treesa Jacob.

Distribution: India [Kerala (New report)], south China, Thailand, Vietnam.

The genus *Donda* includes species that have similar facies and are difficult to be distinguished morphologically. Even though *D. eurychlora* and *D. continentalis* are similar in their external morphology, slight differences on the fascia can be noted. The submedial and antemedial lines on the *D. continentalis* forewing are more visible than in *D. eurychlora*. Also, the medial and submedial lines on the hindwing are distinct in *D. continentalis* than in *D. eurychlora*. Differentiation of these congeners requires careful dissection and analysis of genitalia. The Indian *Donda* species can be identified using the genitalia characters. The juxta of *D. continentalis* species is leaf shaped with a central channel, whereas *D. eurychlora* has lateral flaps and a rod. The shape of juxta and valva can be compared for identification. The species *D. unispinosa* has a comparatively small aedeagus with a single robust spine, whereas *D. continentalis* has 2 distinct spines. The current report of *D. continentalis* from India shows significant range extension of the species to the Western Ghats of India. The presence *D. continentalis* highlights the importance of the less explored biodiversity of the Western Ghats.

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Butterflies of Sasthamkotta Lake – A Ramsar site in Kerala, India

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ABSTRACT: In the study of butterfly diversity in the Sasthamkotta Lake of Kollam, Kerala, a total of 50 species was recorded. The species identifid were of the family Pieridae (17), followed by Nymphalidae (11), Lycaenidae (9), Papilionidae (9), Hesperidae (3), and Riodinidae (1). About eight of these species are listed under the protection category of the Indian Wildlife Act 1972. The findings highlight the ecological importance of Sasthamkotta as an important habitat supporting a rich biodiversity of butterflies.
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KEY WORDS: Diversity, habitat, dominant, conservation

Butterflies are one of the most widely studied insect groups (Robbins and Opler, 1997) and are recognised as a sensitive biological indicator of the ecosystem health because (Gardner *et al.,* 1995; Wood and Gillman, 1998). The adult stages are responsible for pollination, which is essential for environmental stability (Padhye *et al.,* 2006; Tiple *et al.,* 2006). The Western Ghats butterfly checklist contains 337 species, including 42 endemic species, while the Kerala checklist contains 328 species, including 41 endemic species (Sadasivan *et al.,* 2025). Some previous documentation of butterfly species in Kerala and the surrounding areas include Mathew and Rahamathulla (1993), who reported 100 species in the Silent Valley National Park; Sudheendrakumar *et al.* (2000), who reported 124 species of butterflies in the Parambikulam National Park; Prasad *et al.* (2010) identified 52 species in the University of Kerala campus; Aneesh *et al.* (2014) reported 139 butterfly species from six families in the campus of Kerala Agricultural University in Thrissur, and Jobiraj *et al.* (2020) reported 59 butterfly species from Thusharagiri in Kozhikode, Kerala.

A study was launched to study the butterfly diversity of the Sasthamkotta lake of Kollam district, Kerala. As no published checklist of Sasthamkotta butterflies had previously been made available, this work could serve as a basis for further research. Sasthamkotta is a village in Kerala, known for its geography characterized by a mix of highlands and low lands, including the largest fresh water lake in Kerala. situated in the Kollam district of Kerala. The area is geographically located between 9°00'-9°05' N; 76°35'- 76°40' E, at an altitude of 22 to 33m. The area covers an overall geographical area of 3,75ha with a number of sunken and emerging plants, and has a subtropical to warm-temperate climate with an average annual rainfall of 2398mm.

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Table 1 Butterfly species recorded in Sasthamkotta Lake, Kerala

Scientific name	Common name	POP	IUCN	WPA	Endemic
Family: Papilionoidea (Swallow tails)					
Papilio polytes romulus (Cramer, 1775)	Indian common Mormon	C	LC		
P. dravidarum (Wood-Mason, 1880)	Malabar Raven	VR	NE		WG
P. demoleus demoleus (Linn, 1758)	Lime Butterfly	C	NE		
P. paris (Linnaeus, 1758)	Paris Peacock	FR	NE		
*P. helenus (*Linnaeus, 1758)	Red Helen	F	NE		
Graphium teredon	Narrow-banded	C	LC		SI
(Felder & Felder, 1865)	Bluebottle				
Pachliopta aristolochiae aristolochiae (Fab, 1775)	Indian Common Rose	R	LC		
P. hector (Linnaeus, 1758)	Crimson Rose	C	LC	Sch I	PI& SL
Troides minos (Cramer, 1779)	Sahyadri Bird wing	R	LC		WG& SI
Family: Pieridae (Whites and Yellows)					
Eurema blanda silhetana (Wallace, 1867)	Three Spot Grass Yellow	NR	NE		
Euremahecabe hecabe (Linnaeus, 1758)	Oriental Common Grass Yellow	NR	NE		
Catopsilia pomonapomona (Fabricius, 1775)	Oriental Lemon Emigrant	C	NE		
C. pyranthe pyranthe (Linnaeus, 1758)	Oriental Mottled emigrant	R	NE		
Ixias pyrene sesia (Fabricius, 1777)	Dakhan Yellow Orange-tip	R	NE		
Hebomoia glaucippe australis (Linnaeus, 1758)	Sahyadri Great Orange-tip	NR	NE		
Appias libythea (Fabricius, 1775)	Western Striped Albatross	R	NE	Sch IV	
A. (Hiposcritia) indra shiva (Swinhoe, 1885)	Sahyadri Plain Puffin	NR	NE	Sch II	
A. lalage lalage (Doubleday, 1842)	Himalayan Spot Puffin	VR	NE		
A. albina swinhoei (Moore, 1905)	Sahyadri Common Albatross	F	NE	Sch II	
Pieris canidia canis (Evans, 1912)	Sahyadri Cabbage White	VC	NE		
Leptosia nina nina (Fabricius, 1793)	Oriental Psyche	R	NE		

Eurema laeta laeta (Boisduval, 1836)	Indian Spotless Grass Yellow	C	NE		
E. brigitta rubella (Wallace, 1867)	Small Grass Yellow	C	LC		
E. andersoni shimai (Yata & Gaonkar, 1999)	Sahyadri One-spot Grass Yellow	VR	LC	Sch II	
Appias albina swinhoei (Moore, 1905)	Sahyadri Common Albatross	F	NE	Sch II	
Delias eucharis (Drury, 1773)	Indian Jezebel	R	NE		
Hebomoia glaucippe australis _ (Butler, 1898)	Sahyadri Great Orange-tip	NR	NE		
Family: Nymphalidae (Brush-footed butterflies)					
Ypthima baldus baldus (Fabricius, 1775)	Common Five-ring	C	NE		
Y. huebneri (Kirby, 1871)	Common Four- ring	VC	NE		
Junonia hierta hierta (Fabricius, 1798)	Oriental Yellow Pansy	C	LC		
J. iphita iphita (Cramer, 1779)	Chocolate Pansy	C	NE		
Parantica aglea aglea (Stoll, [1782])	Coromandel Glassy Tiger	C	NE		
Tanaecia lepidea miyana (Fruhstorfer, 1913)	Peninsular Grey Count	R	NE	Sch II	
Tirumala septentrionis dravidarum (Fruhstorfer, 1899)	Dakhan Dark Blue Tiger	VC	NE		
Parantica aglea aglea (Stoll, 1782)	Coromandel Glassy Tiger	C	NE		
Euploea core core (Cramer, 1780)	Indian Common Crow	C	LC		
Cupha erymanthis maja (Fruhstorfer, 1898)	Sahyadri Rustic	VR	NE		
Hypolimnas misippus (Linnaeus, 1764)	Danaid Egg fly	R	LC	Sch II	
Family: Lycaenidae (Blues)					
Anthene lycaenina lycaenina (Felder, 1868)	Dakhan Pointed Ciliate Blue	R	NE		
Spalgis epius epius (Westwood, 1852)	Ape fly	E	NE		
Talicada nyseus nyseus (Guérin-Méneville, 1843)	Indian Red Pierrot	R	NE		
Catochrysops strabo strabo (Fabricius, 1793)	Oriental Forget-me-not	R	NE		
Castalius rosimon rosimon (Fabricius, 1775)	Continental Common Pierro	NR	NE		
Pseudozizeeria maha ossa (Swinhoe, 1885)	Dakhan Pale Grass Blue	NR	NE		

Rathinda amor (Fabricius, 1775)	Monkey Puzzle	FR	NE		
Jamides celeno celeno (Cramer, 1775)	Oriental Common Cerulean	VC	NE		
Euchrysops cnejus cnejus (Fabricius, 1798)	Oriental Gram Blue	C	NE	Sch II	
Family: Hesperidia (Skippers)					
Badamia exclamationis (Fabricius, 1775)	Brown Awl	FR	NE		
Celaenorrhinus fusca (Hampson, 1888)	Dusky Spotted Flat	C	NE		PI
Aeromachus dubius dubius (Elwes & Edwards, 1897)	Sahyadri Dingy Scrub Hopper	C	NE		
Family: Riodinidae (Metal marks)					
Abisara echerius prunosa (Moore, 1879)	Lankan Plum Judy	VR	NE		

Note: POP-Population status as VC–Very Common, C– Common, NR–Not Rare, R– Rare, VR–Very Rare and S– Stragglers, WG–Western Ghats, PI–Peninsular India, SL– Sri Lanka, SI–South India, IUCN –IUCN Red List Status, WPA– Indian Wildlife Protection Act Schedule as Sch.

Recognised as a Ramsar site since 2002 has 375-hectare lake famous for its purity. Butterfly fauna was surveyed weekly during 6.00am to 5.00pm, between January and June 2025. The species were photographed with the Nikon D3500 camera from various angles to allow for a positive identification. Butterflies have been identified in the field primarily using field guides. The general taxonomic classification and checklists are based on the works of Evans (1926, 1949), Wynter-Blyth (1957), Larsen (1987, 1988), Gaonkar (1996), Nitin *et al.* (2018), Kunte *et al.* (2022), and Sadasivan *et al.* (2025). The red list status is based on the IUCN website, according to the IUCN 2021 global population inventory, iucnredlist.org, the conservation status is in line with IWPA (1972) and CITES (2012).

The survey documented 50 species, representing six families (Table 1). The species recorded were of the family Pieridae (17), followed by Nymphalidae (11), Lycaenidae (9), Papilionidae (9), Hesperidae (3), and Riodinidae (1). About eight of these species are listed under the protection category of the Indian Wildlife Act 1972. The number of individuals was more in Pieridae, probably because of their affinity for gardens and open spaces (Gupta and Kumar, 2024). Conversely, the Hesperiidae and the Riodinidae have a lower abundance of species, probably due to their specific host preference and limited dispersal capacity (Lodh and Agarwal, 2016). Ten butterfly species found in the area are listed as least concern (LC) in the International Union for Conservation of Nature (IUCN), while the status of the remaining 40 species is not evaluated (NE). According to the IUCN Red List of endangered species, none of the butterflies reported are endangered or threatened worldwide. Four species of Sasthamkotta butterflies are considered to be of an endemic nature. *Papilio dravidarum* was exclusively native to the WG. Eight species were covered by the WPA Schemes and Amendments. There are six species in Schedule II, one in Schedule I and one in Schedule IV. The presence of butterflies in a particular area is of great importance as pollinator and as a biological marker. Their presence or absence may give an indication of the health and stability of the ecosystem.

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Record of pleasant fungus beetle genus *Megalodacne* Crotch (Coleoptera, Erotylidae) on oyster mushrooms from Nainital, Uttarakhand, India

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ABSTRACT: Adults of pleasant fungus beetle, *Megalodacne* species were observed feeding on oyster mushrooms during August- September, 2024 in hilly area of Nainital.
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KEY WORDS: Pleurotus, Megalodacne, pleasant fungus beetle

Pleurotus species, commonly known as oyster mushrooms belong to the class Agaricomycetes, order Agaricales and family Pleurotaceae. They are commonly found in temperate and tropical forests, occurring naturally on decomposing logs. These mushrooms have fruit bodies that are distinctively shell or spatula-shaped and come in different colours, such as white, cream, grey, yellow, pink, light brown and blue, depending on the specific species, they also emit a unique sweet like anise or licorice scent (Bhatia *et al*., 2024). Kues and Gonzalez (2009) and Fraatz and Zorn (2010) have reported that insects are attracted towards polypores and mushrooms due to airborne volatile organic compounds (VOCs) such as alcohols, aldehydes, esters, ketones, acids, and terpenoids released from their fruiting bodies. Among the various insect pests that devour mushrooms, coleopteran beetles are one of the important groups whose adults feed on mushroom mycelia, succulent tissues of stipe, gills and pileus (Rina and Singh, 2016). Members of Erotylidae of Coleoptera are commonly known as "pleasing fungus beetles" due to large size and colourful patterns (Skelley, 2009). Their larvae and adults feed on basidiomycetes fungi (Franc, 2001; Robertson *et al*., 2004; Majka, 2007; Epps and Arnold *et al*., 2010). They are worldwide in distribution with 125genera and 2,500 described species found on various mushrooms and polypore fungi (Goodrich and Springer, 1999, Robertson and Whiting, 2014).

Production of two different species of oyster mushrooms *Pleurotus sajor caju* and *P. florida* was done at Faculty of Agriculture and Agroforestry at Krishnapur, Nainital (Latitude:29.370598, Longitude: 79.464387) on paddy and wheat straw in a mushroom growing room at an average temperature of 22.75ïC and relative humidity of 81.65 per cent. While cultivating oyster mushrooms, many larvae and a few adults of pleasant fungus beetle genus *Megalodacne* were observed feeding on gills and boring into stipes of mushrooms. The grubs, adults were handpicked, collected, photographed and identified at the genus level based on keys, morphological features and available literature (Boyle, 1956; Goodrich and Springer, 1999; Nongkynrih *et al.*, 2017; Chandra *et al.,* 2021;

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Skelley, 2024). The specimen was preserved in the laboratory, Faculty of Agriculture and Forestry, Kumaun University. Larvae were collected and reared further but they could not survive. Earlier studies done by Skelly (1999) have shown that the sluggish larvae are only found associated with the mature host fruiting body and adults are often found on the host fungus in large numbers with the larvae. The beetle species may be inhabitant of adjoining vegetation and pine forests in Krishnapur and may have entered the mushroom growing room through cracks and crevices. Future studies in this aspect may be required to gain knowledge on the life cycle and morphology of the beetle and also devise management strategies as oyster mushrooms are the second largest and most popular cultivated species of economic importance.

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Traditional uses of insects in folk medicine by the Bodo tribe in Baksa District, Bodoland Territorial Region, Assam, India

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ABSTRACT: Bodo tribe use to treat approximately 25 different human ailments with 18 insect species from 15 families and 8 orders that. Among the most commonly mentioned uses were remedies for cough, gastritis, rheumatoid arthritis, stomach ache, fever, and wound healing. Each insect's local name, habitat, parts used, and medicinal applications was given in detail. The Vespidae family (17%) was most prominent, followed by Saturniidae (11%). Orders like Hymenoptera (5 species), Lepidoptera (3 species), and Orthoptera (3 species) were also well-represented. Insects are used in various life stages and as byproducts, with fresh specimens preferred. © 2026 Association for Advancement of Entomology

KEY WORDS: Entomotherapy, medicinal insects, indigenous knowledge, life stages

A medicinal insect is any insect or insect derived product used for therapeutic purposes termed entomotherapy, to prevent or cure various human ailments. These therapeutic practices have a long history across various culture worldwide (Chakravorty *et al.*, 2011). Using of insects for therapeutic purposes, involves utilizing whole insects, their parts, or their secretions to prevent or treat ailments (Devi *et al.,* 2023). Insects derive various bioactive substances, such as cantharidin from blister beetles, which demonstrates their potential as a source of pharmaceuticals for modern medicine (Anal *et al.,* 2025). Baksa district which is home to the Bodo community, has significant traditional knowledge about the medicinal properties of insects, and has potential for more valuable discoveries in the medicinal field. This study focuses on specific insect species and their traditional ethnobiological roles in the Baksa district. Baksa district is situated in the Bodoland Territorial Region of Assam (26.6935° N; 91.5984° E). Total six regions viz., Barama, Baghmara, Anchali, Panbari, Lesra, Bhogpur were selected for the survey.

The study was carried out from January, 2025 to June, 2025. The initial phase of the survey focused on site selection. This involved a preliminary survey of different administrative blocks and ecological zones within the district to ensure a wide range of habitats are covered. Before commencing the field work, permission was taken from the community leaders and local authorities. This qualitative research component involves direct interaction with local communities, particularly the elderly, traditional healers (local *vaidyas* or *ojhas*), and knowledgeable individuals. Semi-structured interviews were employed to gather information on the traditional uses of insects for medicinal purposes.

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Table 1. Insects used to treat different ailments by the Bodo people in Baksa District, Assam

No.	Scientific name	Order/ Family	Common name in English/ Bodo	Habitat	Body part used	Ailments	approach
1	*Cybister japonicas*	Coleoptera/ Dytiscidae	Water beetle/ Bora jalotalaipoka	Aquatic	Nymph and adult	Ear problem	Fried and roasted
2	*Eumeninae*	Hymenoptera/ Vespidae	Potter wasps/ Kanjani	Wood and rock	Adult	Headache	Fried
3	*Lethocercus indicus*	Hemiptera/ Belostomatidae	Giant water bug/ Gangzema	Freshwater	Nymph and adult	Stomach disease	Fried and roasted
4	*Apis indica*	Hymenoptera/ Apidae	Honey bee/ Beree	Tress hollows	Larva and pupa	Mouth ulcer, burns, asthma	Fried and raw
5	*Dolycoris indicus*	Hemiptera/ Pentatomidae	Bamboo bug/ Fiso	Forest, grassland	Adult	Used in paralysis and goiter	Fried and roasted
6	*Vespa magnifica*	Hymenoptera/ Vespidae	Wasp/ Mri	Low mountains, forested areas	Larva and pupa	Curing stomach disease	Fried and raw
7	*Musca nebulo*	Diptera/ Muscidae	Housefly/ Thamfhwi	Environment near humans and animals	Adult	cold fever, used to dye hair black, eye, digestive problem	crushed live and applied
8	*Oecophylla smaragdina*	Hymenoptera/ Formicidae	Red ant/ kujideour	Woodlands, Urban areas	Eggs and young ones	Rheumatism, Asthma, Blood pressure control, Cough, and improve eyesight	Live ones
9	*Antheraea assamensis*	Lepidoptera/ Saturniidae	Muga silk worm/ Muga-latha	leaves of the Som tree	Larva and pupa	Malaria, Tooth ache	Fried, roasted, curry
10	*Acheta domesticus*	Orthoptera/ Gryllidae	House cricket/ Usrenga	Humid and dark environment	Adult	Delicacy	Boiled and fried
11	*Vespa affiniscontinentalis*	Hymenoptera/ Vespidae	Lesser banded hornet/ —	Trees, shrubs and buldings	Adult	Used to cure whooping cough	Fried and roasted
12	*Hieroglypus banian*	Orthoptera/ Acrididae	Grasshopper/ Gijir	Rice growing region	Nymph and adult	Liver disorder	Boiled and roasted
13	*Samia ricini*	Lepidoptera/ Saturniidae	Eri silk worm/ Indi	Cocoon	Pupa	Back pain	Eaten roasted
14	*Gryllotalpa gryllotalpa*	Orthoptera/ Gryllotalpidae	Mole cricket/ Sosroma	Damp, well fertilized fields	Adult	Foot inflamation and fertility	Eaten Cooked
15	*Rhyothemis phyllis*	Odonata/ Libellulidae	Dragon fly/ thamphai	Open ponds or swamp forest	Body part	Curing UTI in young children	Fried and roasted
16	*Microtermis* sp.	Blattodea/ Termittidae	Termite/ Sulung	Moister habitat	Larva	Ulcer	Fried

17	*Bombyx mori*	Lepidoptera/ Bombycidae	Nuni silkworm/ —	Silk farm		Stroke	Boiled and fried
18	*Periplaneta americana*	Blattodea/ Blattidae	Cockroach/ Gumathausi	Humid environments, both indoors	Body part	Asthma, TB	Grinded (Chutney)

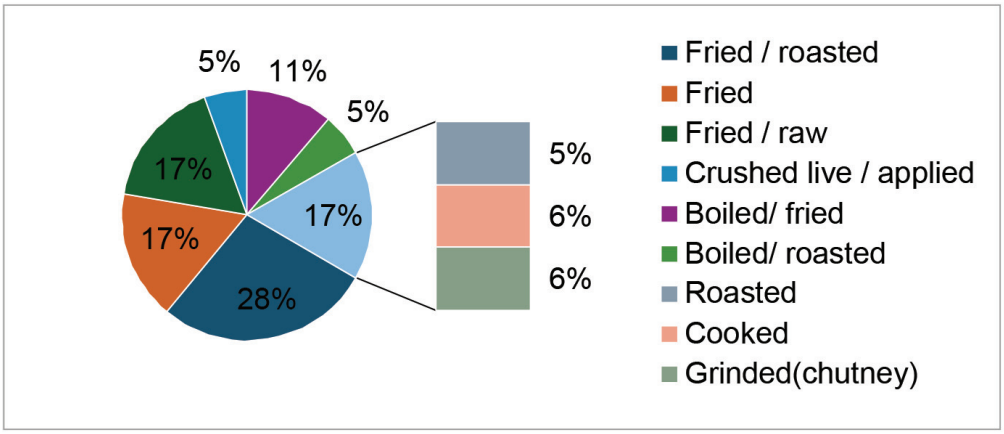


Fig.1 Distribution of species based on consumption method

Questions were designed to elicit details about of the specific insect species used (local names, descriptive characteristics for identification), the ailments they are used to treat, methods of collection, preparation techniques (e.g., raw, powdered, infused, mixed with other ingredients), dosage, administration routes, and perceived efficacy.

The survey recorded 18 different insect species from 15 families and 8 orders that are used by various ethnic groups to treat at least 25 different human ailments. Among these, the most commonly treated ones using insects were cough, gastritis, rheumatoid arthritis, stomach ache, fever, and wound healing. The dominant families reported in the study are the Vespidae (17%) followed by Saturniidae (11%), and six per cent each in Gryllidae, Acrididae, Gryllotalpidae, Bombycidae, Libellulidae, Termitidae and Blattidae; and five per cent each in Dytiscidae, Belostomatidae, Apidae, Pentatomidae, Muscidae and Formicidae. The medicinally used insect species and their local names, habitats, the parts used, and the justifications for their uses are detailed (Table 1). Significant medicinal insect species are utilized as by-products or in their larval, nymphal, pupal, and adult stages. Ninety percent of the informants have used at least one medicinal insect or its derivative product in their lives, and freshly harvested insects are preferred in traditional medicines. The most common methods of using medicinal insects to treat human illnesses are frying, roasting, boiling, cooking, or making chutney (Fig. 1).

These findings align with the previous ethnoentomological research in northeast India. In a study conducted in the Goalpara district of Assam identified 13 insect species across 12 families and 13 genera, with Hymenoptera and Lepidoptera leading the pack in terms of species count, followed closely by Orthoptera and Hemiptera (Das *et al.,* 2015). Similarly, another study in the Dhemaji district found 14 species from 12 families and 14 genera (Doley and Kalita, 2012). As per Kalita *et al.* (2025) insects have historically been used for various health concerns, including pain relief, inflammation, digestive problems, and even serious diseases like malaria and cholera. Tiwa community uses insects especially red ants use to treat different ailments such as Rheumatism and gout, Asthma, Blood pressure control, Cough, and improve eyesight (Dutta *et al., 2025*). The potential for bioactive

compounds in insects to possess anti-inflammatory, antimicrobial, and antiviral properties further bolsters their role in traditional medicine (Sinha and Choudhury, 2024). The study found that the Bodo tribe in Baksa District, Assam, use insects not only as food but also for medicine. The use of insects in medical treatment has been widely practiced among the Bodo community since ancient times. However, there is a need for further research and detailed documentation regarding the experiences and applications of these insects.

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AUTHOR INDEX

Aarati N. Patil, 329
Abhilash Peter, 383
Ajitha V.S., 349
Alakesh Barman, 395
Ali Ashique Iqbal, 395
Amlanjyoti Gautam, 375
Amritpal Singh Kaleka, 275
Anjana Thakur, 357
Ankita Gupta, 329
Arnab Adhikary, 289
Ashok Kumar, 299
Babita Sharma, 299
Balraj G., 337
Basanta Kumar Samal, 289
Basker P., 321
Beena Devi, 343
Bhanavathu Sai, 337
Biman kr. Sarma, 395
Bindu Panickar, 337
Chandaragi M.K., 337
Deepika Vishwanath Jangam, 369
Dhanyaraj D., 387
Dhritismita Talukdar, 395
Dwity Sundar Rout, 289
Elanchezhiyan C., 321
Gandham Sumathi, 337
Hrishikesh Das, 395
Jayanta Kumar Mahalik, 289
Jeet Ram, 393
Jeswin S., 303
John T.D. Caleb, 303
Joslin Treesa Jacob, 383
Jyotika Purohit, 337
Kavya Sukesh, 387
Khushwinder Singh, 357
Kiran P., 387
Koosi Sai Thilak, 337
Manjeet Kumar, 357
Manmeet Pal Kaur, 275
Mohammad Ilyas Motmayen, 357
Neethu Karan, 349
Prashant Bhimrao Koli, 369
Priyanka Bhatt, 393
Rajan M.R., 303
Rajnish Ranjan, 375
Sajay S., 363
Sami-Ul-Hassan Tantary, 275
Sheenu A.S., 349
Siddhartha Das, 289
Soliang Manyu, 321
Sreekumar K.M., 363
Sunil Dagadu Patil, 369
Sunil M. Gaikwad, 329
Sushmita Prakash, 299
Thakur D.R., 343
Vasu Mehta, 357

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