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Morphological and comparative study of Oxyinae egg pods in relationship to the soil type for farming of grasshopper

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ABSTRACT: The study on grasshoppers, *Oxya velox* (Fabricius) and *Hygracris palutris* (Uvarov) (Orthoptera, Acrididae, Catantopinae, Oxyinae), egg pod and egg morphometric, with two soil types (loamy sand and sandy loam) in Nagaland, India, revealed that egg pods of *H. palutris* showed higher values than *O. velox* in all the parameters measured except the width value. Whereas, regarding eggs of *H. palutris* showed maximum in weight as well as in morphometrics variation, when compared to *O. velox*. Both morphometric variation and weight for egg pods and eggs were observed that sandy loam is preferred over loamy sand in both selected acridid species, indicating that soil types have a crucial impact on it. Though the morphometric variations are recorded, *O. velox* recorded higher biomass. © 2026 Association for Advancement of Entomology

KEY WORD: Ovipositional behavior, soil types, pod length, weight, width, entomophagy

INTRODUCTION

The substrate played an important role for oviposition. Complete behavioral involvement of insects during oviposition is pre-planned site selection by female adults (Cury *et al.*, 2019). The egg pods studies determine the favorable abundance depending on different morphological and bio-ecology factors which varies from species to species (Katiyar, 1957; Shaikh and Sultana, 2018). The loose sandy soil types played a favorable role for oviposition site selection in different grasshopper such as *Atractomorpha crenulata* (F.) (Srivastava, 1957), *Phlaeoba infumata* (Brunner von Wattenwyl) and *Acrida exaltata* (Walker) (Priyadarshini and Singh, 2016). *Romalea guttata* (Stoll) ovipositional behavioral responded at 150 watts, 14:10 L:D cycle and 26±2 °C showed group ovipositional site with a preference for sand in

deposition of the egg pods (Stauffer *et al.*, 1998). Senegalese grasshopper as a pest, gave the complete biological involvement for its abundances when egg pods procured in August had higher result of no diapauses stages than the September collection (Maiga *et al.*, 2010). The laboratory studies on *Oxya japonica* (Thunberg) showed the impact of soil health and the major role in its survival and development (Trisnawati *et al.*, 2015). The egg pods of Indian Acrididae and the ecology of egg laying substrate i.e. moisture for xerophilic, mesophilic, hydrophilic soil and the egg arrangements also determined the identification keys for Acrididae (Katiyar, 1957). The present study was prompted to comprehend and examine the morphometric variation of egg pods among the two selected Oxyinae species, *Oxya velox* (F.) and *Hygracris palutris* (Uvarov) (Orthoptera, Acrididae, Catantopinae). Additionally, different soil

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types were analyzed for their soil preferences among the selected species. Therefore, the study contributes to the lacunae of existing knowledge about the acridids egg pods under laboratory conditions.

MATERIALS AND METHODS

The studies on the acridids egg pods with relationship to soil type study was carried out in Kohima district, Nagaland at coordinates 25°31'44" N - 25°51'45" N latitude and 93°54'30" - 94°14'09" E longitude. Firstly, acridids species were collected through random sampling methods from agricultural areas and surrounding vegetation with the employment of insect aerial net and hand picking. Two acridid species namely, *O. velox* and *H. palutris* belonging to the sub-family Oxyinae, were selected for the study. In laboratory conditions, the minimum and maximum temperature was recorded as 8.42 and 28.2°C respectively along with relative humidity 72.4 to 92.83 per cent. The collected species were reared as stock culture for further experimentation under laboratory conditions with the modified technique proposed by Fry (1927); Hinks and Erlandson (1994); Haldar *et al.* (1998). Inside the cage, the Oxyinae species was fed *ad libitum* with fresh host leaves. For oviposition purposes, an oviposition tray filled with different sterilized soil types was provided, and distilled water was sprayed to keep it moist. The egg pods laid are checked regularly and the retrieved egg pods was cleaned and sorted. The egg pods were weighted and later the frothy material was removed for counting and measurement of the individuals' eggs within a single egg pod. The morphometric analysis such as weight (mg), width (mm) and length (mm) for both egg pods and eggs were measured with the aid of a digital weighing balance and digital vernier calipers. The different types of soil are determined through soil texture methods of USDA textural triangle using a hydrometer by Estefan *et al.* (2013). Statistical data analysis was conducted using MS. Excel 2021 and XLSTAT 2024.

RESULTS AND DISCUSSION

The current work was carried out to determine the ovipositional behavior with association to the egg

pods and soil type preferences namely loamy sand and sandy loam. The Oxyinae egg pods determined that *O. velox* were comparatively smaller than *H. palutris* in all the morphometric analysis even under the same substrate. When the same species were introduced to two different substrate the egg pods showed variation among the morphometric measurements. In *O. velox* egg pods, length was measured about 10.12±1.44 and 10.91±1.51mm whereas width measured about 6.02±0.80 and 6.07±0.8mm for loamy sand and sandy loam respectively. Subsequently, *H. palutris* egg pods for loamy sand and sandy loam length were 11.02±0.77mm and 11.81±1.60mm while width was about 5.60±0.33 and 5.64±0.49mm respectively. The egg pods retrieved under different substrate exhibited different weightage in both Oxyinae species. In *O. velox*, egg pods retrieved from sandy loam had more weight (103.67±16.16mg) as compared to loamy sand (96.90±18.21mg). Similarly, *H. palutris* also showed more egg pods weightage from sandy loam (109.33±19.95mg) then the loamy sand (97.03±5.52mg) (Table 1, 3).

The comparative Oxyinae eggs was studied on the basis of weight, length and width. In case of *O. velox* the eggs retrieved from sandy loam soil takes higher advantages in all the analysis. The weight in sandy loam is recorded as 4.00±0.45mg whereas loamy sand is 3.97±0.71mg. While, the length and width in loamy sand is 4.18±0.10 and 1.20±0.06mm which is smaller than the eggs retrieved from sandy loam recorded as 4.21±0.17mm and 1.24±0.06mm respectively. For *H. palutris* eggs weight in loamy sand (4.37±0.49mg) is comparatively lighter than eggs retrieved from sandy loam (4.47±0.57mg) soil. Followingly, both the length and width of sandy loam eggs are 4.46±0.27mm and 1.85±0.08mm respectively. Though, the loamy sand eggs length (4.38±0.26mm) and width (1.84±0.08mm) are slightly smaller when compared to eggs retrieved from sandy loam soil type. Overall, *O. velox* eggs showed lower value than *H. palutris* eggs in all the parameters analyzed for the consideration to determined that comparative morphometric variation among the two species (Table 2, 3).

The Oxyinae morphometric assessment on egg pods

and eggs showed slight similarity with Sultana *et al.* (2020) and Doulo and Sote (2024) findings. In case of *O. velox* egg pods, width and length shows more or less similar to Sultana *et al.* (2020) findings where width and length was 5.40 ± 1.07 and 14.5 ± 1.35 mm. During the study period, it was noted that the soil types lead to a variation in the number of the eggs even among the same species. In *O. velox*, number of eggs in loamy sand and sandy loam soil type has a slight variation with 19.17 ± 3.71 eggs and 20.80 ± 2.35 eggs within a single egg pod respectively. While in *H. palutris*, loamy sand has 21.20 ± 2.17 eggs and sandy loam have 21.50 ± 2.29 eggs within a single egg pod were also recorded.

It was determined that the freshly deposited egg pods are soft later become hardened with similar result reported by Shaikh and Sultana (2018). In both the two soil types, it was observed that freshly deposited egg pods were ivory color but once subjected to environmental condition the color changes to saddle brown which were alike to Sultana *et al.* (2020) and Doulo and Sote (2024) findings. The sculpturing display of the two selected

Oxyinae egg pods were quite similar and display distinctive subcylindrical shape. Both the egg pods were pointed at the anterior end and blunted on the posterior end. Due to its blunted end, greater number of eggs exist and least number of eggs were witnessed in the anterior end. Eggs were arranged tightly and systematically within the pods protected by the frothy material. Both the two selected Oxyinae eggs were banana shaped where similar conclusion was reported by Sultana *et al.* (2020) and Doulo and Sote (2024).

Substrate availability has a major driving factors for oviposition success among the acridid species. Soil preferences for oviposition behavior are selected based upon the different individuals even on relationship to moisture content of the soil (Katiyar, 1957; Pande *et al.*, 1990; Torto *et al.*, 2024). The relationship between the morphometric study showed that even among the same species variation depend on the substrate provided which also agreed with Sultana and Wagan (2009) reason. Comparatively among the other grasshopper species Senegalese grasshopper also showed preferences

Table 1. Comparative morphometric egg pods measurement (Mean $\pm$ SD) for *O. velox* and *H. palutris*

Species	Length (mm)		Width (mm)	
	Loamy sand	Sandy loam	Loamy sand	Sandy loam
<i>Oxya velox</i>	10.12 ± 1.44	10.91 ± 1.51	6.02 ± 0.80	6.07 ± 0.81
<i>Hygracris palutris</i>	11.02 ± 0.77	11.81 ± 1.60	5.60 ± 0.33	5.64 ± 0.49

Table 2. Comparative morphometric eggs measurement (Mean $\pm$ SD) for *O. velox* and *H. palutris*

Species	Length (mm)		Width (mm)	
	Loamy sand	Sandy loam	Loamy sand	Sandy loam
<i>Oxya velox</i>	4.18 ± 0.10	4.21 ± 0.17	1.20 ± 0.06	1.24 ± 0.06
<i>Hygracris palutris</i>	4.38 ± 0.26	4.46 ± 0.27	1.84 ± 0.08	1.85 ± 0.08

Table 3. Comparative weight of both egg pod and eggs (Mean $\pm$ SD) for *O. velox* and *H. palutris*

Species	Egg pod weight (mg)		Egg weight (mg)	
	Loamy sand	Sandy loam	Loamy sand	Sandy loam
<i>Oxya velox</i>	96.90 ± 18.21	103.67 ± 16.16	3.97 ± 0.71	4.00 ± 0.45
<i>Hygracris palutris</i>	97.03 ± 5.52	109.33 ± 19.95	4.37 ± 0.49	4.47 ± 0.57

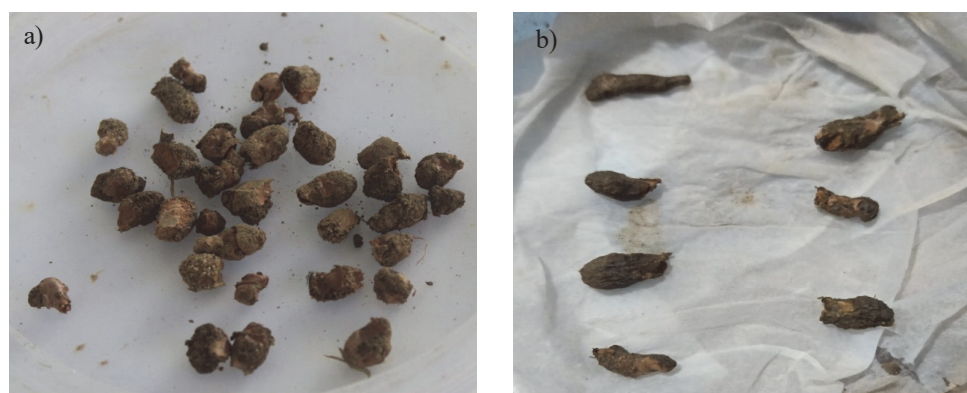


Fig. 1 Retrieved egg pods of - a) *O. velox*; b) *H. palutris* under lab-based farming

on sandy-loamy soil types for oviposition (Maiga *et al.*, 2010). Grasshopper preferred on moderately firm soil for oviposition about 3cm in *Oxya hyla hyla* (Serville) (Pande *et al.*, 1990) with moderate compactness rather than very tight or loose soil type (Toleubayev *et al.*, 2007). Studied on *Aulocara elliotti* (Thomas) and *Opeia obscura* (Thomas) egg pods subjected to fire in the ecosystem resulted to depth of the egg pods in the soil however find out that the *A. elliotti* has higher mortality rate due to narrow egg pods location as compared to *O. obscura* (Branson and Vermeire, 2013). Under experimental condition different soil moisture and soil types correspondingly contributes to the oviposition preference for the same species (Umrani *et al.*, 2007). The soil moisture also contributes to the egg pods as well as the nymphal development (Mukerji and Gage, 1977). It was reported that about 10-11 percent egg undergo diapause stages for around 20 to 23 months (Shaikh and Sultana, 2018). The key feature for grasshopper farming led to the preferences of multivoltine species which ultimately contributes to higher biotic biomass production (Das *et al.*, 2012a, b; Das, 2014). The population incidence of the individual's species can also refer for farming (Sote and Doulo, 2024). However, in environmental condition climatic factors, seasonal factors, elevation, soil moisture and soil type contribute to the availability of the grasshopper species (Katiyar, 1957; Pradhan and Peswani, 1961; Pande *et al.*, 1990; Wei *et al.*, 2023).

Presently, edible insects are highly recognized due to its rich nutritional value as a supplementary diet.

Though edible insects are considered as a conventional food source but figuratively about 92 per cent of edible insect are collected from wild (Yen, 2015) which lead to insect farming under laboratory as significant contribution in food security (Das *et al.*, 2012a, b; Das, 2014). Specifically, acridids are known for rich protein content (Anand *et al.*, 2008; Ganguly *et al.*, 2009, 2013; Das and Mandal, 2014; Ghosh *et al.*, 2016; Das, 2018; Gosh and Mandal, 2019; Samira *et al.*, 2019) and as alternative diet or even animal feeds (Das and Mandal, 2014; Ghosh *et al.*, 2016; Gosh and Mandal, 2019). The high biomass production is the key leading aspects for insect farming. Studies also concluded that selection of certain host plants for specific acridid species also contributed to high yielding biomass in laboratory condition (Das *et al.*, 2012a). Temperature influences the developmental stages of grasshopper (Ali, 1982). For mass acridid production, certain environmental condition is taken into consideration whereby at 35 ± 2 °C and 12:12 light and dark respectively are closely considered to be optimum temperature and light intensity for *O. hyla hyla* (Das *et al.*, 2012b). Additionally, the space utilization by acridids showed that 25 adults of *O. fuscovittata* and 35 adults of *Spathosternum prasiniferum prasiniferum* (Walker) required $10 \times 10 \times 25$ cm³ to prove higher potential of mass rearing (Das *et al.*, 2009). The present study concluded that, sandy loam has more edge over loamy sand in both Oxyinae egg pods and eggs. The study comprehends the different soil type preferences which lead to the morphometric variation among the two selected Oxyinae species.

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Diversity and foraging behaviour of pollinators in cashew (*Anacardium occidentale* Linn.) in Kerala, India

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ABSTRACT: Among the flower visitors in cashew 12 species were documented as pollinators. These belong to Apidae (four), Halictidae (four), Megachilidae (three) under Hymenoptera, and Syrphidae (one) under Dipteran. Species abundance was highest for Halictidae (49%), followed by Apidae (42.38%). In Halictidae, *Halictus* sp.1 was more abundant (18.54%), followed by *Lasioglossum* sp. (16.56%); while in Apidae, *Apis cerana indica* (21.85%) followed by *Tetragonula* sp. (17.22%). In diversity indices, Simpson diversity index (D) showed 0.16, Shannon-Weiner index (H) of 2.03 and Berger-Parker index (d) of 0.22. Foraging activity peaked between 10:00 and 13:00 h, where *A. c. indica* was most prevalent (12-35%). Mean foraging rate was highest for *A. c. indica* (15.4 flowers/trip), followed by *Lasioglossum* sp. (7.2 flowers/trip), *Helophilus* sp. (6.2 flowers/trip) and lowest for *Tetragonula* sp. (2.8 flowers/trip); while mean foraging speed was highest for *Tetragonula* sp. (6.19 s/flower), followed by *Halictus* sp. 1 (5.74 s/flower), *Helophilus* sp. (5.64 s/flower) and lowest for *Hoplonomia* sp. (1.49s/flower). Nesting sites of *Ceratina hieroglyphica* and *C. binghami* were also observed during the study.

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KEY WORDS: Apidae, Halictidae, diversity, *Apis cerana indica*, *Tetragonula* sp., *Lasioglossum* sp., nesting sites, bee pasturage

INTRODUCTION

Pollination is a crucial process in nature. Only five per cent of crops in nature are self-pollinated; the other 95 per cent are cross-pollinated, with 10 per cent relying on wind and 85 per cent on animal

pollination (Tewari, 1983; Vanitha and Raviprasad, 2019). Insects do 90 per cent of animal pollination (Buchmann and Nabhan, 1996). Nowadays, the worldwide demand for pollination services has increased as a result of a net growth in the value, demand, and land dedicated to insect-pollinated

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crops in recent decades (Aizen *et al.*, 2019). Cashew, *Anacardium occidentale* L., is a significant export crop in the tropics and supports the social and economic advancement of several nations globally (Bezerra *et al.*, 2007; Masawe 2009). Portuguese explorers brought it to India, the East Indies, and Africa in the sixteenth century (Mitchell and Mori, 1987). Cashew inflorescence contains two types of flowers; staminate (male) and hermaphrodite (complete). Staminate flower can be identified by a long stamen surrounded by short ones within the corolla tube while in hermaphrodite flower a stigma is also present, which is longer than the stamen. Cashew flower consists of five sepals, and five petals. Flowers are white during anthesis but changes to pink or shades of red after a day. Most of the flowers dry up gradually. Typically, it takes three to five months for various cashew varieties to finish the sequential anthesis in an inflorescence. Less than 10 per cent of the flowers in an inflorescence are bisexual, despite the fact that the overall number of blooms ranges between 200 and 1600 over the course of 70 to 90 days of flowering (Pavithran and Ravindranathan, 1974). Due to its andromonoecious nature, cashew is particularly attractive to insects for cross-pollination. The longer stamen and shorter style of the hermaphrodite flower make self-pollination challenging. Numerous studies have shown that low productivity in cashew is largely due to insufficient pollination (Freitas *et al.*, 2002; Holanda-Neto *et al.*, 2002; Eradasappa and Mohana., 2016). Studies have also shown the influence of pollinators in fruit set of cashew (Reddi, 1993; Freitas and Paxton, 1996; Vanitha and Raviprasad, 2019). Bees (Freitas *et al.*, 2002), flies (Roubik, 1995), moths (Kevan, 1975) and even ants (Khoo *et al.*, 1982; Dantas de Araujo, 1994) are considered as cashew pollinators in many studies. However, information about efficient pollinators in cashew and their foraging behaviour is limited. Documentation of pollinators in different locations is very important as it helps to address pollination deficiency thereby increasing productivity of cashew. Thus, the present study was undertaken to document diversity, species richness and foraging behaviour of pollinating insects in cashew plantation.

MATERIALS AND METHODS

The present investigation was carried out at cashew orchards of Kerala Agricultural University (KAU)-Regional Agricultural Research Station, Pilicode, Kasaragod. The site is geographically located at 13°N; 75°E at an elevation of 15m MSL. Soil type is laterite with a pH of 5.5 to 6.5. The mean maximum and minimum temperature of the location is 33°C and 23°C respectively, and average annual rainfall is 3379mm. The staminate and hermaphrodite flowers in cashews were collected and familiarised with the help of a microscope. Various cashew germplasms were selected to document the diversity of pollinators in cashew. Pollinator diversity was assessed in selected cashew germplasms maintained under All India Coordinated Research Project on Cashew. Germplasm identities are withheld in accordance with project guidelines. Observations were recorded on ten randomly selected inflorescences from November 2023-March 2024. Insects visiting the inflorescences were collected using fine mesh aerial nets and were identified with the help of experts from National Bureau of Agricultural Insect Resources, Bengaluru and KAU. Abundance of pollinators were recorded by observing for ten minutes at hourly intervals during 08:00 to 15:00 h. Relative abundance of each species with respect to total species was also calculated and each species were placed in respective dominance classes given by Engelmann (1978), *i.e.* Eudominant (>32 -100 %), Dominant (32 - 10%), Subdominant (10 – 3.2 %), Recedent (3.2 – 1 %), Subrecedent (1 - 0.32%) and Sporadic (< 0.32%). Diversity indices like species richness (S), Shannon-Weiner diversity index (H), Simpson diversity index (D) and Berger-Parker index (d) were estimated using PAST software.

Foraging rate and foraging speed: Foraging rate and foraging speed of important species were also recorded by careful examination. Foraging speed is the time spent by a species on each flower. It was noted by using a stopwatch. The stopwatch was started when a pollinator sits on a flower and stopped when it flew away. Flying time from one flower to another was excluded. Foraging rate is the number of flowers visited by a species per trip

in the vicinity. The number of flowers visited by important species per trip was recorded at peak foraging hour. To find out the importance of pollinators in fruit set in cashew, bagging experiment was done, where ten inflorescences at bud stage was bagged to exclude any insect from visiting them and per cent fruit set was calculated.

RESULTS AND DISCUSSION

Diversity and abundance: Among all the insect species seen visiting cashew inflorescences, only twelve are considered as pollinators. Out of the 12 pollinators identified, four belongs to Apidae, four to Halictidae, three to Megachilidae and one to Syrphidae. In a study conducted by Sundararaju (2011), seven species such as *Pseudapis oxybeloides*, *Lasioglossum* sp. and *Halictus* sp. from family Halictidae and *Braunsapis* sp., *Ceratina smaragdula* and *Ceratina* sp. from family Apidae were identified from coastal Karnataka while *Ceratina (Pithitis) binghami* Cockerell, *C. smaragdula*, *Braunsapis* sp. and *P. oxybeloides* from coastal Tamil Nadu. According to Heard *et al.* (1990) *Apis mellifera* L., *Ligyra* sp. and *Helophilus hilaris* were efficient pollinators of cashew. Vanitha and Raviprasad (2019) identified

A. c. indica, *A. florea*, *Braunsapis picitarsus*, *Ceratina hieroglyphica*, *Tetragonula* sp., *Lasioglossum* sp., *Pseudapis oxybeloides* and *Seledonia* sp. as important cashew pollinators. Similarly, in this study 12 species were identified as pollinators and are listed in Table 1. Many species of ants were observed throughout the study, which may not be efficient pollinators. Bhattacharya (2004) states that ants are unlikely to act as pollinators as they search mainly for honeydew secretions by aphid or extra-floral secretions. Accidental pollination may occur due to their erratic movement but further studies are needed to confirm this.

Detailed observations regarding the abundance and foraging activity of pollinators at hourly intervals were also made (Table 2). During 08:00 to 09:00 h, the activity of only three species was observed, and *Tetragonula* sp. was most abundant with 64.29 per cent followed by *Helophilus* sp. (21.43%). The activity of pollinators was more significant when sunshine was bright. Foraging activity was highest from 10:00 -13:00 h when *A. c. indica* was most abundant (12-35%), followed by *Halictus* sp. 1 (16-25%), *Lasioglossum* sp. (16-25%), *Tetragonula* sp. (2-16%) and *Hoplonomia* sp. (2-13%).

Table 1 Relative abundance (RA) and dominance status of each pollinator species

No	Species	RA (%)	Dominant class	Family	Order
1	<i>Apis cerana indica</i>	21.85	Dominant	Apidae (42.38%)	Hymenoptera
2	<i>Tetragonula</i> sp.	17.22	Dominant		
3	<i>Ceratina hieroglyphica</i>	1.99	Recedent		
4	<i>C. binghami</i>	1.32	Recedent		
5	<i>Halictus</i> sp. 1	18.54	Dominant	Halictidae (49%)	
6	<i>Halictus</i> sp. 2	7.29	Subdominant		
7	<i>Lasioglossum</i> sp.	16.56	Dominant		
8	<i>Hoplonomia</i> sp.	6.62	Subdominant		
9	<i>Megachile cephalotes</i>	1.32	Recedent	Megachilidae (2.65%)	
10	<i>M. lanata</i>	0.66	Subrecedent		
11	<i>M. laticeps</i>	0.66	Subrecedent		
12	<i>Helophilus</i> sp.	5.96	Subdominant	Syrphidae (5.96%)	Diptera

Table 2 Foraging activities of pollinators in cashew during 2023-24

Time (h)	Relative abundance of pollinators (%)												Total no. Observed
	<i>Apis cerana indica</i>	<i>Tetragonula</i> sp.	<i>Halictus</i> sp. 1	<i>Halictus</i> sp. 2	<i>Helophilus</i> sp.	<i>Lasioslossum</i> sp.	<i>Hoplonomia</i> sp.	<i>Megachile cephalotes</i>	<i>M. lanata</i>	<i>M. laticeps</i>	<i>Ceratina hieroglyphica</i>	<i>C. binghami</i>	
8:00-9:00	14.29 (2)	64.29 (9)	0.00 (0)	0.00 (0)	21.43 (3)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	14
9:00-10:00	21.74 (5)	39.13 (9)	4.35 (1)	0.00 (0)	8.70 (2)	17.39 (4)	8.70 (2)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	23
10:00-11:00	12.90 (4)	16.13 (5)	16.13 (5)	6.45 (2)	3.23 (1)	25.81 (8)	12.90 (4)	3.23 (1)	0.00 (0)	3.23 (1)	0.00 (0)	0.00 (0)	31
11:00-12:00	19.44 (7)	5.55 (2)	25.00 (9)	8.33 (3)	2.77 (1)	16.66 (6)	8.33 (3)	2.77 (1)	2.77 (1)	0.00 (0)	5.55 (2)	2.77 (1)	36
12:00-13:00	34.29 (12)	2.86 (1)	22.86 (8)	8.57 (3)	5.71 (2)	17.15 (6)	2.86 (1)	0.00 (0)	0.00 (0)	0.00 (0)	2.86 (1)	2.86 (1)	35
13:00-14:00	27.27 (3)	0.00 (0)	45.45 (5)	18.18 (2)	0.00 (0)	9.09 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	11
14:00-15:00	0.00 (0)	0.00 (0)	0.00 (0)	100 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	1
Total (Nos)	(33)	(26)	(28)	(11)	(9)	(25)	(10)	(2)	(1)	(1)	(3)	(2)	151

Note: Figures in brackets are number of bee pollinators recorded

Table 3 Foraging speed, foraging rate and peak foraging hours of important pollinators in cashew

No	Species	Foraging rate (nos/trip)		Foraging speed (s)		Peak foraging hours (h)
		Mean	Range	Mean	Range	
1	<i>A. c. indica</i>	15.4	9-22	2.15	1-3	11:00-13:00
2	<i>Helophilus</i> sp.	6.2	4-9	5.64	4-7	8:00-10:00
3	<i>Lasioglossum</i> sp.	7.2	4-10	2.03	1-4	10:00-13:00
4	<i>Halictus</i> sp.1	5.4	3-8	5.74	4-7	10:00-13:00
5	<i>Tetragonula</i> sp.	2.8	2-4	6.19	4-9	8:00-11:00
6	<i>Hoplonomia</i> sp.	4.4	2-7	1.49	1-3	10:00-12:00
7	<i>Halictus</i> sp.2	4.2	3-6	2.17	1-4	11:00-13:00

Pollinator activity decreased afterwards, especially during evening hours. Heard *et al.* (1990) also revealed that nocturnal insects have no role in pollinating cashew. As per Singh and Adhikary (2021) honeybee pollination can enhance both the quantitative and qualitative traits of many fruit crops depending on the site conditions. Anthesis in cashew starts at 06:00 and is completed by 10: 00 h, but stigma receptivity is maximum after 6 hours, at around 12:00 noon (Pushpalatha, 2000). Since the peak foraging of pollinators occurs during forenoon when flowers are open and receptive, effective pollination takes place.

Diversity indices such as Shannon-Weiner Diversity Index (H), Simpson Diversity Index (D), Species Richness (S), and the Berger-Parker Index (d) was calculated for pollinators observed during the study period. A total of 12 pollinator species were recorded during the study, indicating a species richness of 12. The Simpson diversity index ($D = 0.155$) and the Shannon–Wiener index ($H = 2.029$)

reflect a high level of pollinator diversity in cashew at the study site. Furthermore, a Berger–Parker index value of 0.22 suggests that the pollinator community was relatively evenly distributed, with no single species exerting dominance.

Foraging rate and foraging speed: Mean foraging rate was highest for *A. c. indica* (15.4 flowers/ trip) followed by *Lasioglossum* sp. (7.2 flowers/ trip), *Helophilus* sp. (6.2 flowers/trip), and *Halictus* sp. 1 (5.4 flowers/trip). It was least for *Tetragonula* sp. (2.8 flowers/trip). Increased foraging rate ensures the delivery of pollen grains thereby increase the fruit set (Mitchell and Waser, 1992). Mean foraging speed was found to be maximum for *Tetragonula* sp. (6.19 s/flower) followed by *Halictus* sp. 1 (5.74 s/flower) and *Helophilus* sp. (5.64 s/flower); and the least for *Hoplonomia* sp. (1.49s/flower). Peak foraging hours also differ with species. Sundararaju (2011) recorded the time spent by pollinators on cashew flowers in the range of 0.8 - 9.0s for *A. c. indica*, 1.2- 22.2s for *A. florea*,

5.0- 25.1s for *Ceratina binghami* and 2.0- 22.0s for *Braunsapis* sp. while Vanitha and Raviprasad (2019) noted that *A. c. indica* spent 1-4 s, *A. florea* 3-21s, *B. picitarsus* 8-16s and *Pseudapis oxybeloides* 1-4s. The number of flowers visited by these bees falls in the range of 6-20, 3-11, 4-7 and 2-5 respectively (Table 3). Foraging speed may vary between species based on their foraging behaviour or flower structure (Free, 1993).

Fruit set: From the bagging experiment done to take observation regarding fruit set in cashew, no fruit development was recorded in bagged inflorescences, as all flowers dried and became damaged. Fruit set was observed in all unbagged, open-pollinated inflorescences, highlighting the crucial role of pollinators in cashew yield. Despite successful pollination in unbagged inflorescences, the overall fruit set was low (reaching only 7.17%). This finding agrees with Vanitha and Raviprasad (2020), who reported a comparable fruit set (7.96%) under open pollination. They also emphasized that hermaphrodite flowers require pollination within four hours of peak stigma receptivity, indicating the necessity of adequate pollinator populations for improved cashew yield.

Nesting sites: Even though *A. c. indica* was observed in the field; bee hives were not on site. Nevertheless, this study identified nesting sites of two pollinators, *Ceratina hieroglyphica* and *C. binghami*, inside dried branches of cashew trees. Mostly, they occupy stumps left after the pruning of branches. A typical nest has a clean circular entrance hole, which helps identify it. The adult insects scoop out the soft central pith region to make the nests. The entrance hole of *C. hieroglyphica* nest was about 3.1mm wide, and the nest was about 15cm deep. Different life stages were observed inside the nest, separated by cells made of powdered wood. Cells containing younger stages were seen towards the entrance, while older stages were in deep cells. The nest of *C. binghami* was 3.3mm wide and about 13cm deep. These findings are in line with those observed by Vanitha (2023). Even though nests of these two species were observed in the field, they rarely visited the cashew inflorescences.

Bee pasturage/ bee flora: Vegetation that provides pollen and nectar to the bees are collectively called bee pasturage/ bee flora. Understanding flowering plants that are valuable as bee pasture will help in maintaining the bee colonies. In our preliminary study during limited period, mainly three species such as *Stachytarpheta* sp., *Mimosa pudica* and *Rungia* sp. were noted as bee pasture. *Ceratina* spp. were attracted to *Stachytarpheta* sp., *Mimosa pudica* and *Rungia* sp. while *Megachile* sp., *A. c. indica* and *Tetragonula* sp. were attracted to *Mimosa pudica*. Sundararaju (2011) reported the presence of cashew pollinators on *Mimosa pudica*, *Blumea* sp., *Acacia pennata*, *Rungia repens*, *Lindernia antipoda*, *Leucas aspera*, *Muntingia calabura* etc. Further studies on different bee pasturage existing near the plantations, flowering phenology and pollinator abundance will help to understand the foraging range of bees, their choice of flora, survival during non-flowering period etc. and thereby make sure the conservation of pollinators during off season.

Documentation of cashew pollinators is fundamental for elucidating their importance in pollination and ecosystem functioning. In the study location, twelve species of insects were identified as pollinators of cashew, among which the Indian honeybee, *A. c. indica*, was most abundant. Knowledge regarding important pollinators, nesting sites, and bee pasturage can help researchers and farmers conserve efficient pollinators, thereby increasing the productivity of cashew plantations. Understanding peak hours of foraging can ensure proper conservation of these pollinators by protecting them from pesticide sprays. A proper understanding of their activity and bee flora can provide favourable conditions for pollinators during the off-season.

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Morphological and molecular characterization of two species of *Laccotrephes* (Hemiptera, Nepidae) from Eastern Ghats, India

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ABSTRACT: This study presents an integrative approach combining morphological and molecular techniques to identify and characterize two species of the genus *Laccotrephes* Stål — *L. griseus* (Guérin-Ménéville) and *L. ruber* (Linnaeus) from the Eastern Ghats, India. Morphological differentiation was based on key characters such as body length, respiratory siphon length, prosternal structure, and male parameres. For molecular analysis, partial mitochondrial COI gene sequences (~620 bp) were amplified and analyzed using the Maximum Likelihood method in IQTREE. The resulting phylogenetic tree supported species-level divergence, with pairwise genetic distances ranging from 15.5 to 17.7 per cent between *L. griseus* and *L. ruber*, confirming their distinct taxonomic status. Intraspecific variation was minimal (<1%), while interspecific distances among other congeners showed clear genetic gaps. The integration of morpho-taxonomy and DNA barcoding underscores the value of a combined approach for accurate species delimitation and reveals potential cryptic diversity within the genus. This work enhances the understanding of aquatic Hemiptera biodiversity and provides a foundation for further taxonomic revisions in *Laccotrephes*. © 2026 Association for Advancement of Entomology

KEY WORDS: Water scorpion, taxonomy, DNA barcodes, mtCOI gene, Eastern Ghats

INTRODUCTION

Among true bugs (Heteroptera), the infraorders Nepomorpha and Gerromorpha have a close association with aquatic habitats, with the former completely submerged and the latter prospering on the water surface or along shorelines. *Laccotrephes* Stål, 1866 is the major genus in the subfamily Nepinae of the family Nepidae in the insect suborder Heteroptera. It is a large genus (roughly 60 species, Keffer 2004) that is primarily

restricted to the Old-World tropics (Neiser *et al.*, 2009). Poisson (1965) published a catalogue of African Nepidae, which included African *Laccotrephes* parameres. Polhemus and Keffer (1999) published an overview of the *L. grossus* subgroup, however, there has been no recent survey of Asian species (Neiser *et al.*, 2009).

The water scorpion (Nepidae), notably the species *Laccotrephes*, is a crucial ecological predator of crustaceans, fish, amphibians, and mosquito larvae.

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The long-term predation experiment (up to 30 days) revealed that *L. griseus* is an excellent predator of mosquito larvae such as *C. quinquefasciatus* (Ghosh and Chandra, 2011). The majority of *Laccotrephes* species, like other members of the Nepinae, reside near the shallow border of stagnant waters, even virtually static bays of streams, where they maintain contact with atmospheric air by respiratory siphons. In India, seven *Laccotrephes* species have been recorded (Thirumalai, 2007), with only three species, *L. elongatus*, *L. griseus*, and *L. ruber*, being spread throughout the country, while the remaining species are uncommon and have no documentation after Thirumalai (2007). Traditional taxonomy, while valuable, can be subjective and limiting, especially when specimens are damaged or missing. DNA barcoding based on mitochondrial COI gene sequences has emerged as a dependable and effective approach for species identification, allowing for correct classification with minimal tissue samples (Hebert *et al.*, 2003; Ward *et al.*, 2005). This molecular technique, paired with morphological taxonomy, provides a solid framework for distinguishing *Laccotrephes* species with precision. The present study integrates morpho-taxonomy and DNA barcoding to establish a definitive identification of *Laccotrephes* species in India, addressing taxonomic ambiguities and contributing to a deeper understanding of aquatic Hemiptera diversity.

MATERIALS AND METHODS

Laccotrephes specimens have been collected from several locations in Eastern Ghats, India (Fig. 1, Table 1). The collected specimens were kept in ethanol (90%) and examined with Leica EZ4W and Olympus SZX10 stereo-zoom microscopes, as well as photographed with a Nikon D500 Nikon AF-S DX Micro Nikkor 40mm f/2.8G Lens. Morphological identification is done using relevant literature (Thirumalai, 2007; Chandra and Jehamalar, 2020). The identified specimens were placed in the National Zoological Collection of the Zoological Survey of India, Freshwater Biological Regional Centre, Hyderabad. DNA was extracted from tissues from the base of the chosen specimens' mid and fore legs using the Dneasy®

Blood & Tissue kit (Qiagen, Germany).

Amplification done through primer LCO-1490 5'-GGTCAACAAATCATAA AGATATTGG-3', HCO-2198 5'-TAAACTTCAGGGTGACCAAA AAATCA-3' for the mitochondrial Cytochrome Oxidase Subunit I (COI) gene (~620bp) (Folmer *et al.*, 1994). Polymerase chain reaction prepared for 25µl which contains 12.5µl of 2x PCR Taq mixture, 0.5µl (10pmol Con) of each primer, 10.5µl of molecular grade water and 1µl of template DNA used for both genes. The thermal cycle profile was set as 95°C for 1 min, 94°C for 30sec, 48°C for 30 sec 35 cycles, 72°C for 1 min. Agarose gel (0.5%) was used to check the DNA which also contains Ethidium bromide (10mg ml⁻¹). The PCR products were purified using NucleoSpin® Gel and PCR clean up kit protocol were followed. Forward and reverse sequences were generated using the Sanger method by Eurofins Genomics (Ebersberg, Germany). Generated chromatogram files of three species have been handled in MEGA 11 (Kumar *et al.*, 2018) phylogenetic analyzing software. The generated sequences were searched in NCBI (<https://www.ncbi.nlm.nih.gov>) to prepare final dataset. A total of 4 sequences were acquired from the NCBI database to compare and prepare final database for the study. MEGA 11 software was used to align the generated sequences and genetic divergence were calculated by using Kimura-2-Parameter (K2P) model in MEGA 11 (Kumar *et al.*, 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1982) in both MEGA 11 and IQTREE for better results. The tree with the highest log likelihood (-3369.75) is shown. Branch support was estimated using the Bootstrap value analysis with 1000 replicates. *Metrocoris communis* (Distant, 1910) was used as an out-group for better understanding (Table 2).

RESULTS AND DISCUSSION

Class Insecta Linnaeus, 1758; **Subclass** Pterygota Brauer, 1885

Order Hemiptera Linnaeus, 1758; **Suborder** Heteroptera Latreille, 1810

Table 1. Survey locations throughout the Eastern Ghats

No.	Survey locations	Latitude	Longitude	MSL
1	Koyalagudem	17.4985N	81.6043E	410
2	Rollapadu Wildlife Sanctuary	15.4514N	78.2122E	321
3	Ananthavoram	16.5887N	80.4362E	45
4	Kalab Dam	18.6116N	82.6242E	868
5	Mandrikalapadu	17.7226N	80.6224E	137
6	Bairampally	16.1427N	78.6813E	755
7	Bolghatpally	16.4022N	78.8861E	587
8	Octopus viewpoint	16.2227N	78.8313E	817
9	Mallela Theertham waterfalls	16.2657N	78.8541E	729
10	Mudasarova reservoir	17.7641N	83.2962E	65
11	Kasipatnam	18.6521N	83.3034E	123
12	Kondakarla ava lake	17.6035N	82.9981E	33
13	KP Dam	13.7245N	79.3250E	927
14	Cheyeru river	14.1280N	79.0166E	228
15	Adidavaram cheruvu	17.7898N	83.2541E	108
16	AP Secretariat	16.5186N	80.5201E	45
17	Meghadri Gadda reservoir	17.7747N	83.1511E	33
18	Relli Lake	17.8093N	83.3298E	86
19	Muran Dam	19.1058N	82.7618E	643
20	Thatipudi reservoir	18.1722N	83.1913E	120
21	Stream near Maredumilli	17.5518N	81.7273E	342
22	Musurumilli reservoir	17.3881N	81.7691E	128
23	Tadikonda	16.4149N	80.4529E	41
24	Bhadrachalam	17.6743N	80.8812E	58
25	Narsipatnam	17.6652N	82.6047E	88
26	Ethakayala Mandapam	13.6807N	79.3506E	881

Infraorder Nepomorpha Popov, 1971;
Superfamily Nepoidea Latreille, 1802

Family Nepidae Latreille, 1802; **Subfamily** Nepinae Latreille, 1802

Genus *Laccotrephes* Stål, 1865

Laccotrephes is a genus of “water scorpion”. They are carnivorous, hunting at the water surface or

submerged in aquatic plants. They are dark brown to rufous brown in color, with an elongated and flattened body, hooked raptorial forelegs, and a long, thin respiratory siphon extending from the abdomen tip (Polhemus and Keffer, 1999). The respiratory siphon consists of two filaments that extend from the eighth abdominal tergum.

Table 2. Specimen sequences generated in current study, and additional sequences obtained from GenBank

Accession no.	Species	Collection locality as per NCBI GenBank	Source
KY320485	<i>Laccotrephes japonensis</i>	Japan: Kumamoto	GenBank
KF966560	<i>L. japonensis</i>	South Korea	GenBank
KY320469	<i>L. aff. pfeifferiae</i>	China: Tianjin	GenBank
KP274069	<i>L. maculatus</i>	India: Imphal, Manipur	GenBank
OR056392	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
OR056393	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
OR056394	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
PP409484	<i>L. maculatus</i>	Kenya: Nyeri	GenBank
PV041420	<i>L. ruber</i>	India: Umri village, Satara, Maharashtra	Current study
PV603800	<i>L. griseus</i>	India: Kinnerasani Wildlife Sanctuary, Bhadradi Kothagudem, Telangana	Current study
PP032035	<i>L. griseus</i>	India: Chennai, Tamil Nadu	GenBank
PQ436053	<i>Diplonychus annulatus</i>	India: Kinnerasani Wildlife Sanctuary, Bhadradi Kothagudem, Telangana	Current study
PV041409	<i>D. molestus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
KY451618	<i>D. rusticus</i>	India: Dindi reservoir, Koratikal, Nizamabad, Telangana	GenBank
PP885728	<i>D. rusticus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
PV041413	<i>D. rusticus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
MN053270	<i>D. esakii</i>	South Korea: Upo wetlands, Yueo-myeon, Changnyeong-gun, Yibang-myeon, Gyeongsangnam-do	GenBank
MN053261	<i>D. esakii</i>	South Korea: Jeju Islands	GenBank
MN053268	<i>D. esakii</i>	South Korea: Upo wetlands, Yueo-myeon, Changnyeong-gun, Yibang-myeon, Gyeongsangnam-do	GenBank
PV041414	<i>Metrocoris communis</i>	India: Goutami Dam, Nashik, Maharashtra	Current study

Key to the species of *Laccotrephes* from Eastern Ghats of India

1. Small sized with body size 13.7-15.21mm in males and 15-16.43mm in females, respiratory siphon shorter than body in both sexes, fore femora with blunt process, apex of paramere hooked and sub-apex with blunt protrusion..... (Fig. 3A-F) ***L. griseus***
2. Elongated species with body size 27.41-31.23mm in males and 28.79-33.33mm in females, respiratory siphon longer than body length, respiratory siphon longer than body length, paramere with hooked apex and a

small process at tip..... (Fig. 3G-L) ***L. ruber***

A. *Laccotrephes griseus* (Guérin-Méneville, 1835)

1844. *Nepa griseus* Guérin-Méneville, *Iconogr. Regne. Anim. Ins.*, 352.

1910. *Laccotrephes griseus* (Guérin-Méneville, 1844): Distant, *Fauna of Brit. India*, 5: 314.

Material examined: 1♂1♀, FBRC/ZSI/INS/2479, 15.vii.2021, Koyalagudem; 1♂, FBRC/ZSI/INS/2510, 16.viii.2024, Rollpadu Wild life somctuary; 2♂, FBRC/ZSI/INS/2770, 7.iv.2024, Ananthavorum; 2♂1♀, FBRC/ZSI/INS/2840,

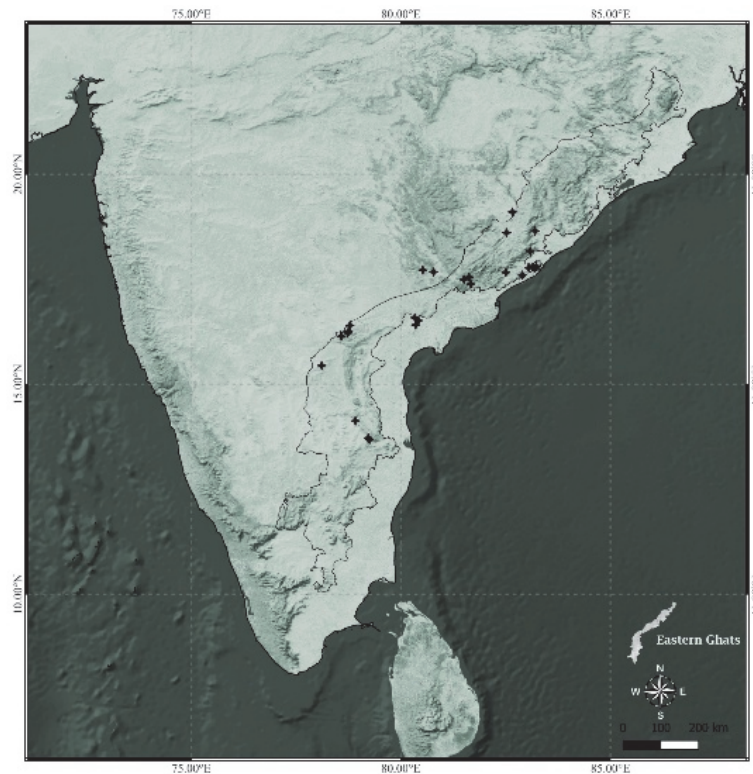


Fig. 1 Survey locations throughout the Eastern Ghats (parts of Odisha, Andhra Pradesh and Telangana)

18.i.2022, Kalab dam; 1♂, FBRC/ZSI/INS/2844, 20.i.2022, Saradapur; 2♂1♀, FBRC/ZSI/INS/2855, 22.xii.2021, pond near Bairamrampally;

2♂, FBRC/ZSI/INS/2883, 20.xii.2021, Stream near Bolghatpalli; 1♀, FBRC/ZSI/INS/2890, 3.x.2021 Octopus view point; 1♂, FBRC/ZSI/INS/4447, 8.iii.2023, Mudasarlova resarvoir; 1♂, FBRC/ZSI/INS/3612, 29.vii.2022, Kasipatnam, 1♀, FBRC/ZSI/INS/3760, 17.x.2024, Konndakara pava Lake Coll. Deepa J Somesh B.

Diagnostic characters: Small species with body length 13.7-15.21mm in males and 15-16.43mm in females. Abdomen above with bluish tinge. Anterior area of prosternum provided with a strong acute spinelike structure. Respiratory siphon shorter than body length, 10.81-12.07mm in males and 12.46-14.12mm in females. Pygophore subquadrate in ventral view, posterior margin shallowly concave, latero-posterior angles rounded, anal segment short, slightly exposed dorsally. Parameres are

symmetrical and slightly hook shaped at apex, while sub-apex has blunt protrusion, inner margin smooth, lacking teeth. In females' sub-genital plate broad triangular, ovipositor slender, elongate with narrow base. Gonapophyses with two pairs of plate-like structures.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Delhi, Gujarat, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Manipur, Meghalaya, Nagaland, Odisha, Pondicherry, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh and West Bengal (Jaiswal and Somesh, 2023).

Elsewhere: Malaysia, Myanmar, Seychelles, Sri Lanka and Thailand (Jaiswal and Somesh, 2023).

Note: This species can be found in moderate numbers from all types of wetlands, small streams. It inhabits the bottom littoral zone weedy zone of stagnant or slow flowing waters. It is very common

Table 3. Sequence divergence of COI gene (K2P) for *Laccotrephes* species and out groups

Genebank No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
KY320485	<i>Laccotrephes japonensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KF966560	<i>Laccotrephes japonensis</i>	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KY320469	<i>Laccotrephes aff. pfeifferiae</i>	0.15	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KP274069	<i>Laccotrephes maculatus</i>	0.16	0.15	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-
OR056392	<i>Laccotrephes maculatus</i>	0.20	0.19	0.18	0.15	-	-	-	-	-	-	-	-	-	-	-	-
PP409484	<i>Laccotrephes maculatus</i>	0.20	0.19	0.18	0.15	0.00	-	-	-	-	-	-	-	-	-	-	-
PV041420	<i>Laccotrephes ruber</i>	0.16	0.15	0.15	0.02	0.14	0.14	-	-	-	-	-	-	-	-	-	-
PV60380	<i>Laccotrephes griseus</i>	0.18	0.18	0.18	0.16	0.15	0.15	0.17	-	-	-	-	-	-	-	-	-
PP032035	<i>Laccotrephes griseus</i>	0.18	0.18	0.19	0.16	0.15	0.15	0.17	0.01	-	-	-	-	-	-	-	-
PQ436053	<i>Diplonychus annulatus</i>	0.20	0.19	0.20	0.19	0.20	0.20	0.18	0.19	0.19	-	-	-	-	-	-	-
PV041409	<i>Diplonychus molestus</i>	0.18	0.19	0.20	0.18	0.19	0.19	0.19	0.17	0.18	0.19	-	-	-	-	-	-
PP885728	<i>Diplonychus rusticus</i>	0.22	0.22	0.20	0.17	0.18	0.18	0.18	0.21	0.22	0.17	0.15	-	-	-	-	-
PV041413	<i>Diplonychus rusticus</i>	0.21	0.21	0.19	0.17	0.17	0.17	0.17	0.21	0.21	0.17	0.15	0.01	-	-	-	-
MN053270	<i>Diplonychus esakii</i>	0.20	0.20	0.22	0.21	0.19	0.19	0.21	0.21	0.21	0.19	0.15	0.09	0.09	-	-	-
MN053261	<i>Diplonychus esakii</i>	0.19	0.20	0.22	0.21	0.20	0.20	0.21	0.20	0.20	0.19	0.15	0.10	0.09	0.01	-	-
MN053268	<i>Diplonychus esakii</i>	0.19	0.20	0.22	0.21	0.20	0.20	0.21	0.21	0.21	0.19	0.15	0.10	0.09	0.01	0.01	-
PV041414	<i>Metrocoris communis</i>	0.26	0.26	0.25	0.22	0.23	0.23	0.23	0.28	0.29	0.24	0.23	0.22	0.21	0.23	0.23	0.23

species throughout India and occurs at the edges of water bodies.

B. *Laccotrephes ruber* (Linnaeus, 1764)

1764. *Nepa ruber* Linnaeus, *Mus. Lud. Ulr.*, 165.

1906. *Laccotrephes ruber* (Linnaeus): Distant, *Fauna of Brit. India*, 3: 18.

Material examined: 1 ♂, FBRC/ZSI/INS/2471, 22.xii.2020, Gambheeram Gadda reservoir; 1 ♂ 1 ♀, FBRC/ZSI/INS/2478, 15.vii.2021, Koyalagudem; 1 ♀, FBRC/ZSI/INS/2482, 16.viii.2021, Rollapadu Wildlife Sanctuary; 1 ♀, FBRC/ZSI/INS/2837, 18.i.2022, Octopus view point; 1 ♂, FBRC/ZSI/INS/3011, 6.xii.2021, Muran dam; 1 ♂, FBRC/ZSI/INS/3013, 5.xii.2021, Kalab dam; 1 ♂, FBRC/ZSI/INS/3024, 7.xii.2021, Saradapur; 2 ♀ 2 ♂, FBRC/ZSI/INS/3143, 27.vii.2022, Musurumilli reservoir; 2 ♂ 3 ♀, FBRC/ZSI/INS/3377, 19.vii.2022, Kondakarla lake; 1 ♂ 2 ♀, FBRC/ZSI/INS/3597, 26.ii.2023, Thatipudi reservoir, Coll. Deepa J and Somesh B.

Diagnostic characters: Medium sized species

with body length 27.41-31.23mm in males and 28.79-33.33mm in females. Respiratory siphon longer than the body length, 29.36-33.83mm in males and 29.76-35.37mm in females. Prosternum convex in the middle. Pygophore elongate than wide, posterior margin deeply emarginate, lateral angles prominent. Parameres stout and strongly curved inward, apex distinctly hooked and broadened (claw-like), inner margin with subapical swelling, basal half thick, apical half abruptly narrowed. In females, subgenital plate broadly rounded posteriorly. Gonapophyses shorter and stouter, apex blunt rather than needle-like.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Odisha, Punjab, Sikkim, Tamil Nadu, Telangana, Tripura, Uttar Pradesh and West Bengal (Jaiswal and Somesh, 2023).

Elsewhere: China, Japan, Nepal, Pakistan and Taiwan (Jaiswal and Somesh, 2023).

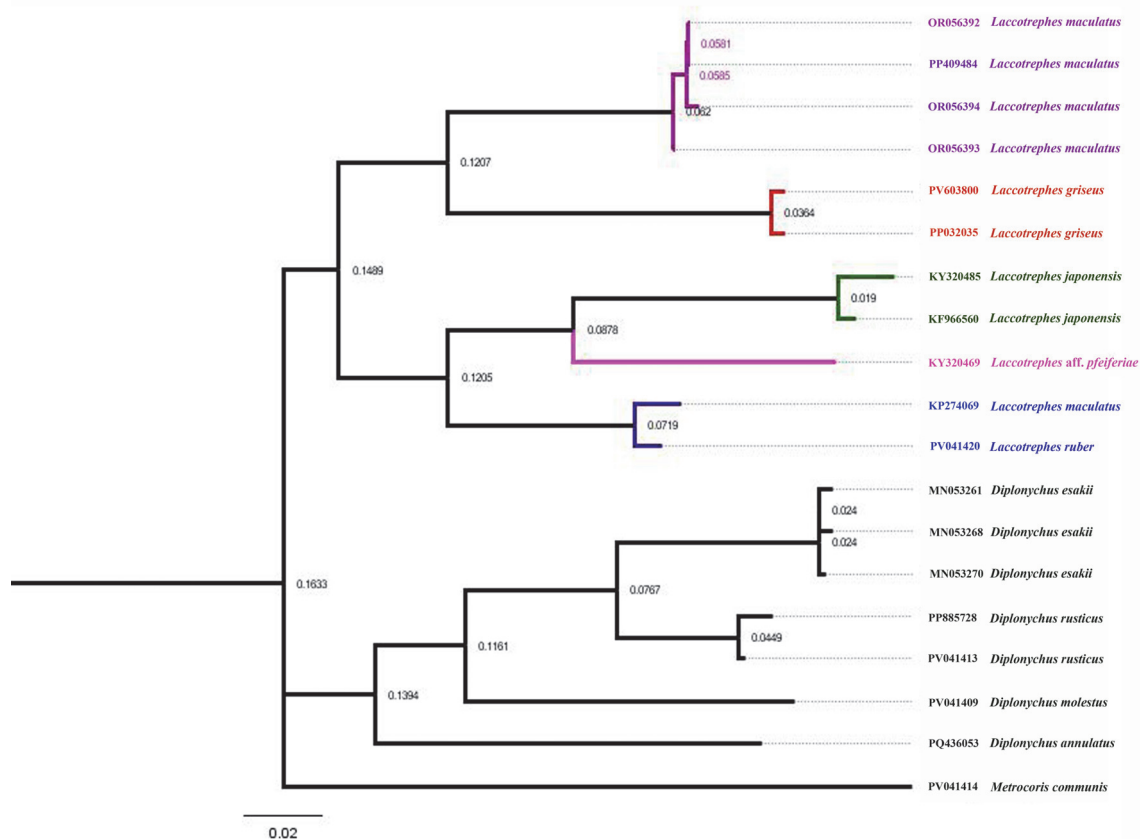


Fig. 2 Maximum likelihood phylogenetic tree for *Laccotrephes* species through mtCOI gene in the present study

Note: It is a common species with wide distribution range throughout the Indo-Australian region and found all most all sorts of freshwater bodies with stagnant or low flowing water entangled with aquatic weeds.

Molecular identification: Pairwise genetic distances were estimated using the mitochondrial COI gene to aid in species identification and the evaluation of genetic divergence within and among *Laccotrephes* species. Table 3 shows the matrix of interspecific and intraspecific distances. Sequences from both GenBank and freshly generated data are included, with GenBank accession numbers and associated species names provided.

The genetic distance matrix shows minimal intraspecific divergence between conspecific sequences but substantial interspecific divergence. For example, two sequences of *L. griseus* (Sl. No.

3 and 4; GenBank No. PV603800 and PP032035) have a minimal divergence of 0.0073, indicating their conspecific identity. In comparison, the divergence between *L. griseus* and *L. ruber* or *L. maculatus* varies from 0.155 to 0.168, indicating a clear species-level distinction.

The morphological and molecular distinction of the genus *Laccotrephes* was evaluated for the first time from the Eastern Ghats. IQTREE and MEGA 11 software were used to examine the conventional mitochondrial DNA barcode (COI) and build a maximum-likelihood tree based on the Kimura 2-parameter (K2P) model. The number labels on internal branches represent K2P genetic distances (substitutions per site) calculated by the ML analysis. The scale bar (0.02) indicates that branch lengths are expressed in terms of predicted substitutions (2% divergence per 0.02). This study's morphological and genetic investigation revealed the identification of two species. Previous

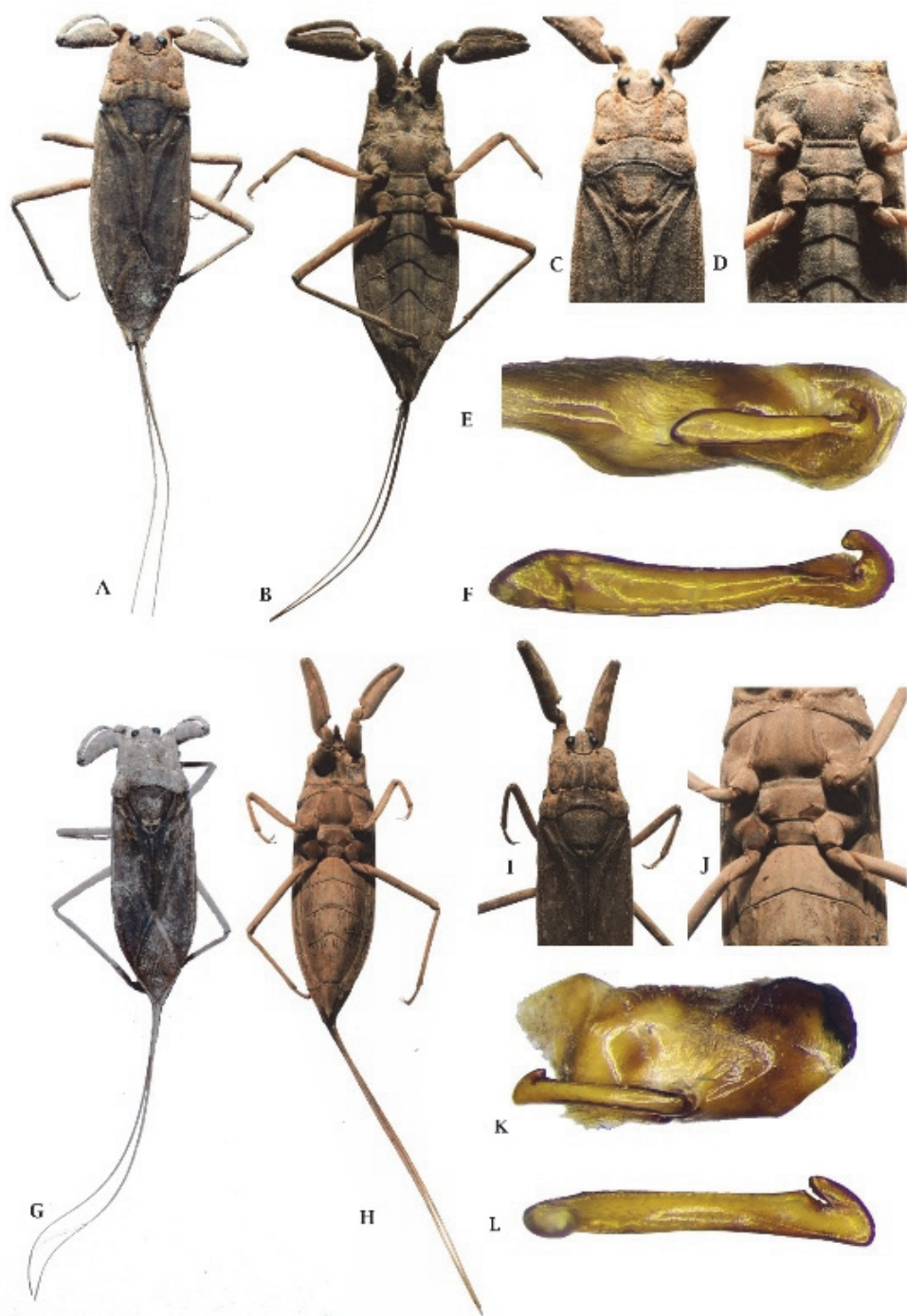


Fig. 3 *Laccotrephes griseus* (Guérin-Méneville, 1835). A – habitus (dorsal view), B – habitus (ventral view), C – head and pronotum, D – metasternum, E – pygophore with paramere, F – right paramere (lateral view); (G -L) *Laccotrephes ruber* (Linnaeus, 1764). G – habitus (dorsal view), H – habitus (ventral view), I – head and pronotum, J – metasternum, K – pygophore with paramere, L – left paramere (lateral view)

morphological investigations focused mostly on male genitalia (Polhemus and Keffer, 1999; Keffer, 2004). This time, molecular data is included to discriminate between the two sister species, *L. griseus* and *L. ruber*.

The tree (Fig. 2, Table 2) mostly supports the current species boundaries, but some changes may be required. *L. maculatus*, *L. japonensis*, and *L. griseus* form monophyletic clades, indicating that these species are genetically separate. In contrast, the nesting of the *L. aff. pfeifferiae* specimen within *L. japonensis* suggests that it may not be a distinct taxon; it could be a cryptic morph or just mislabeled. Similarly, the merger of *L. ruber* and *L. maculatus* indicates that their species borders are ambiguous and should be reconsidered. *Laccotrephes maculatus* (KP274069) does not form its own clade and clusters with *L. ruber* (PV041420) at a modest divergence (~0.0719). This shows that some specimens may have been miss-assigned. Based on partial mitochondrial (COI) sequences, the molecular data provided here show very low genetic divergence (p-distance = 0.0166) between *L. ruber* (PV041420) and *L. maculatus* (KP274069). This degree of divergence, around 1.66%, is well within the range of intraspecific variation seen in aquatic Hemiptera and other insect species (Hebert et al., 2003; Zhou et al., 2009). Interspecific divergence values within the genus *Laccotrephes*, on the other hand, usually approach 15%, as seen in other taxa studied. These discordant placements show areas where morphology and genetics disagree and require further exploration. In contrast, the apparent separation of each *Diplonychus* species in the tree suggests that there is no obvious taxonomic conflict within the genus.

Laccotrephes griseus can be distinguished from its congeners by its larger body size, shorter respiratory siphon, and fine granulation on the pronotum and hemelytra. It is most morphologically like *L. ruber*, but can be distinguished by its stronger forelegs and changes in male genitalia, and the body length with respiratory siphon (Jaiswal and Somesh, 2023). Molecular data indicate *L. griseus* in a distinct clade (blue, Fig. 2), with a pairwise divergence of 0.17-0.18 from *L. ruber*, confirming

its status as a different species. The clade that includes *L. griseus* (blue in Fig. 2) is well-supported and distinct from *L. japonensis* and *L. ruber*. Genetic distances indicate species-level separation, with values above normal intraspecific thresholds. Morphological distinctions remain consistent with prior taxonomic treatments, while subsequent investigations may investigate intraspecific variation across a wider range.

Overall, the COI phylogeny highlights potential cryptic diversity and suggests that an integrative approach (using both morphology along with molecular data) is needed to resolve species limits in these water bugs. These findings demonstrate the utility of DNA barcoding for uncovering unexpected relationships and guiding future revisionary work. The identified specimen mentioned in the publication is deposited at the National Zoological Collection of Freshwater Biology Regional Center, Zoological Survey of India and available on request.

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Intraspecific variations in wing venation pattern of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera, Nymphalidae) from Mohali, Punjab, India

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ABSTRACT: The wing venation of *Danaus chrysippus* (Linnaeus, 1758) was assessed and vein atrophy as well as the presence of spurs at the middle discocellular of both forewings and hindwings were observed. This is perhaps the first evidence of intraspecific variations in the wing venation of this species. The forewing spurs varied in number and positioning on the middle discocellular. In the hindwings, variation occurred with respect to the presence or absence of a spur, as well as a protuberance at the base of the third anal vein (3A). These findings enhance the understanding of morphological diversity within *D. chrysippus*, and also underscore the potential significance of intraspecific venational variation in refining the taxonomy of this butterfly. © 2026 Association for Advancement of Entomology

KEY WORDS: Wing veins, nymphalid butterfly, first evidence, vein atrophy, vein spurs, morpho-taxonomy

INTRODUCTION

Wing venation patterns are significant taxonomic characters that are useful for identifying and classifying insects, including butterflies (Comstock and Needham, 1898a, b; Nath and Devi, 2009; Khaing *et al.*, 2022). Their taxonomic utility was brought to light over a century ago by Comstock and Needham (1898a, b), who argued that all extant insect groups evolved distinct venation patterns that could be traced back to a common ancestor. They further reported on the disappearance of the main stem of the Median vein in numerous Lepidoptera families, leading to the formation of vein spurs that project into the discal cell. The typical venation

pattern of butterflies comprises eleven or twelve veins in the forewings and eight or nine veins in the hindwings (Bingham, 1905; Revathy and Mathew, 2013; Khaing *et al.*, 2022). In the forewings of *Danaus* butterflies, from the costal to the dorsal margin, these veins are the Subcosta (Sc), Radius (R_1 - R_5), Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A) veins, and in the hindwings, the Subcosta and the Radius veins coalesce, giving rise to the $Sc+R_1$ vein and an unbranched Radial sector (R_s); the other hindwing veins, in similar order to that of the forewings, are the Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A and 3A) veins (Bingham, 1905; Comstock and Needham, 1898a; Nath and Devi, 2009; Khaing *et al.*, 2022).

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Furthermore, the discal cell (DC) of both fore- and hindwings is closed by three cross veins: the upper discocellular (UDC), middle discocellular (MDC), and lower discocellular (LDC) (Bingham, 1905). Despite the significance of wing venation patterns in insect taxonomy, the factors that lead to their development are poorly understood (Bingham, 1905; Das Shimmi *et al.*, 2014; Banerjee *et al.*, 2020).

Danaus chrysippus (Linnaeus, 1758) (Fig. 1a, b), commonly known as the Plain Tiger, African Queen, or African Monarch, is an acrid and migratory butterfly that is prevalent throughout the continent of Africa, as well as some parts of Europe and Asia, including India (Tolman and Lewington, 1997; Perkovic, 2006; Liu *et al.*, 2022; Goswami *et al.*, 2024; Omanakuttan *et al.*, 2024). This butterfly is characterised by a tawny ground colouration bearing black and white markings (Lushai *et al.*, 2005; Smith *et al.*, 2005; Braby *et al.*, 2015; Vane-Wright, 2020). According to Vattakaven *et al.* (2016) the Plain Tiger prevails across India, from Punjab in the north to Kerala and Tamil Nadu in the south. It exhibits sexual dimorphism, with differences in the number of black spots at the centre of the hindwings. Females possess three spots, and males have four spots, with the additional fourth functioning as a specialised scent gland (Vattakaven *et al.*, 2016; Alam *et al.*, 2019).

The wing venation pattern of *D. chrysippus* has been demonstrated to conform to the general pattern observed in butterflies (Bingham, 1905; Mal *et al.*, 2015; Patil and Magdum, 2017). However, Bingham (1905) reported a forking of the 2A vein at its base occurring in all species of the genus *Danaus*; in addition, two vein spurs occur on the MDC of the forewings in *D. chrysippus*, and in the hindwings, the MDC lies perpendicular to the M_2 vein. Bingham (1905) used this perpendicular feature as one of the diagnostic characters to discriminate *D. chrysippus* from *D. genutia*, whose MDC curves towards the M_2 vein. Interestingly, in more recent studies, neither the forking of the 2A vein nor the occurrence of vein spurs has been reported (Mal *et al.*, 2015; Patil and Magdum, 2017). The inconsistencies between Bingham (1905) and more recent studies (Mal *et al.*, 2015; Patil and Magdum,

2017) suggest that either certain diagnostic features of venation may have been overlooked or that they have undergone alterations due to regional or phenotypic variability. The present study aimed to document and analyse the detailed wing venation pattern of *Danaus chrysippus* and assess its variability within populations. Particular attention was paid to the presence or absence of specific veins and vein spurs on the middle discocellular (MDC), which have been inconsistently reported in the literature.

MATERIALS AND METHODS

Specimens of *D. chrysippus* were collected from semi-urban landscape of Gharuan village, Mohali, Punjab, India (30.773°N; 76.556°E). Gharuan experiences a temperate climate, characterised by hot summers (mid-April to June), monsoon rains (July to September), and cool winters (December to February). The vegetation comprises mainly crops, trees, and shrubs, including *Lantana camara*, which serves as one of the nectar plants. The butterfly specimens were captured with insect aerial nets during the day, between 0900 and 1600 hours, from March to August 2023. After capturing, they were narcotised, stretched, pinned, dry preserved, labelled and stored using standard entomological procedure (Beige and Noetzel, 1978). Thirty-two permanent slides were prepared: 16 of the forewings and 16 of the hindwings. A standard protocol advocated by Lee and Brown (2015) was followed. The slides were examined under a compound light microscope (Labomed) at $\times 40$ magnification and were photographed with a digital camera (Huawei Y6S). For wing venation nomenclature, the system adopted by Comstock and Needham (1898a) was followed. Each wing specimen was examined for the number, branching pattern, and position of veins, crossveins, and discal cell configuration. Observations were compared with standard descriptions by Bingham (1905) and later studies (Mal *et al.*, 2015; Patil and Magdum, 2017) to assess intraspecific variation. All observations were repeated to confirm consistency in the configuration of veins and to minimise observational errors.

RESULTS AND DISCUSSION

Family: Nymphalidae; **Subfamily:** Danainae;
Genus: *Danaus* Kluk, 1780

Type species: *Danaus plexippus* (Linnaeus, 1758)

Species: *Danaus chrysippus* (Linnaeus, 1758)
Bold (Fig.1)

Material examined: India: Punjab, Mohali district, Gharuan; 4 ♀♀, 06.iv.2023; 2 ♀♀, 27.iv.2023; 2 ♀♀, 10.v.2023; 3 ♀♀, 04.viii.2023; 1 ♂, 06.iv.2023; 1 ♂, 27.iv.2023; 1 ♂, 10.v.2023; 2 ♂♂, 04.viii.2023; coll. Aaron Mboma.

Distribution: India: Punjab (Mohali district: Gharuan). This species is recorded from Gharuan for the first time.

Description: Detailed examination of the forewing and hindwing venation in sixteen specimens of *D. chrysippus* revealed a generally consistent pattern characteristic of the genus *Danaus*, although individual variations were also observed.

General venation pattern: The venation of *D. chrysippus* conforms to the typical venation pattern of butterflies, with twelve veins in the forewings, i.e., Subcosta (Sc), Radius (R_1 - R_5), Median (M_1 - M_3), Cubitus (Cu_1 and Cu_2), and Anal (2A); and nine veins in the hindwings, i.e., Sc+ R_1 , Radial sector (Rs), M_1 - M_3 , Cu_1 and Cu_2 , 1A+2A, and 3A. The discal cell (DC) of each wing is closed by three discocellulars: upper discocellular (UDC), middle discocellular (MDC), and lower discocellular (LDC). A detailed pattern for the forewing and hindwing is provided below.



Fig.1a



Fig.1b

Fig. 1 *Danaus chrysippus* - **a** - Male with four black spots on each hindwing; **b** - Female with three black spots on each hindwing

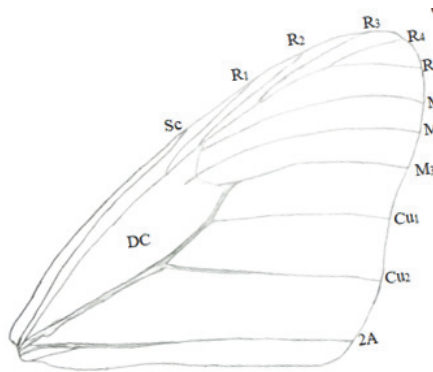


Fig.2a

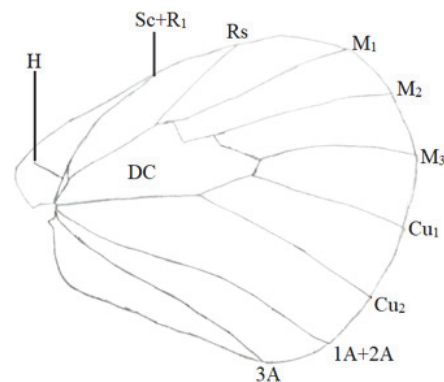


Fig.2b

Fig. 2 *Danaus chrysippus* - Generalised venation pattern - **a** - forewing; **b** - hindwing



Fig. 3 Forewing of *Danaus chrysippus* showing MDC and LDC (encircled in red to highlight variation)

Forewing venation (Fig. 2a): The forewing has four principal veins originating from the wing base: Subcosta, Radius, Cubitus, and Anal; discal cell closed by three cross veins: UDC, MDC, and LDC; UDC very short, MDC slanted inwards, upper half of LDC faded; Radius branches into five veins, R_1 to R_5 ; R_1 - R_3 branch to the costa, R_4 runs to the apex, and R_5 runs to the termen; the three median veins arise from the DC; M_1 and M_2 emanate from the MDC, M_3 emanates from the base of the LDC; Cu vein branches into Cu_1 and Cu_2 veins; a single anal veins (2A) is forked at the wing base; vein atrophies occur at the MDC and project as spurs into the DC.

Hindwing venation (Fig. 2b): In the hindwing, the four principal veins originating from the base of the wing are a coalescence of the Sc and R ($Sc+R$),

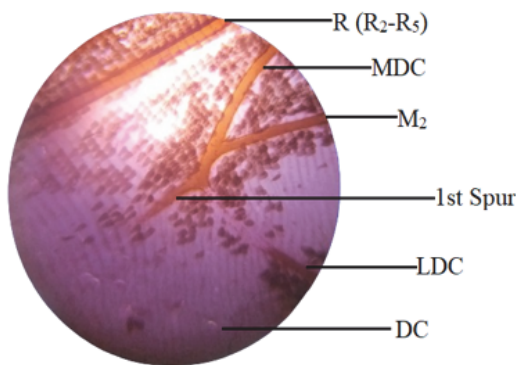


Fig.4a

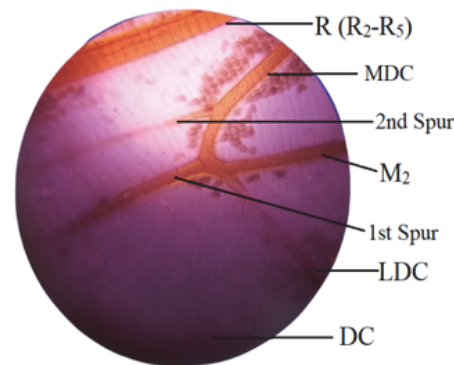


Fig.4b

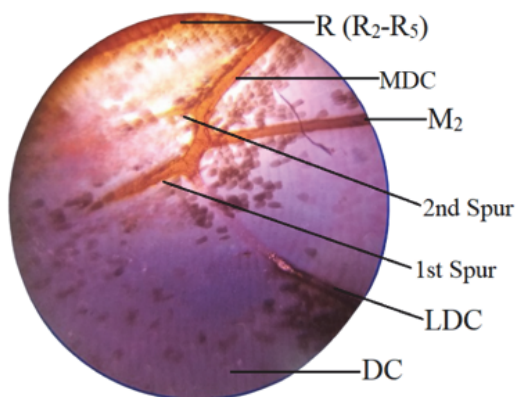


Fig.4c

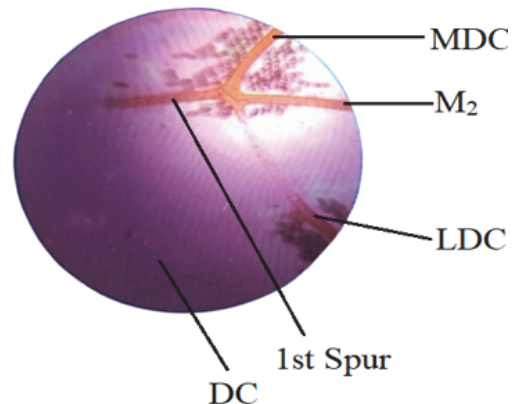


Fig.4d

Fig. 4 *Danaus chrysippus*: **a** - Forewing showing a single spur below the vein M_2 ; **b** - Forewing showing two spurs, with the first slightly above the vein M_2 ; **c** - Forewing showing two spurs, with the first spur below the vein M_2 ; **d** - Forewing showing a single spur occurring on the vein M_2

Cu, a coalescence of 1A+2A, and an independent 3A vein; DC closed by three cross veins: UDC, MDC, and LDC; a short humeral (H) vein branches out from the Sc+R, terminating before the costal margin; Radial sector (Rs) branches out of the Sc+R and runs to the costa; M_1 - M_3 veins arise from the DC; Cu branches into Cu_1 and Cu_2 ; a single vein atrophy occurs at the junction of the MDC and M_2 vein (MDC/ M_2 junction) and projects into the DC.

Intraspecific variations (Figs. 3, 4a, b, c, 5, 6a, b, 7a, b): The forewings exhibited variation in the number and position of vein atrophy at the MDC as spur(s). Of the examined specimens, 62.5 per cent (%) displayed two atrophied spurs on the MDC, one above the other. The lower spur occurred at or slightly above the M_2 vein. In these specimens, the upper spur varied in prominence, from conspicuous to significantly reduced to a notch; the remaining 37.5 per cent displayed only a single spur. Positionally, 12.5 per cent of the specimens displayed a spur below the M_2 vein instead of at or slightly above this vein.

The hindwings likewise exhibited variation in the form of vein atrophy at the MDC/ M_2 junction. About 56.25 per cent of the specimens displayed a very short spur, which was absent in the rest. The MDC of specimens with this spur lay almost perpendicular to the M_2 vein, while the MDC of specimens lacking this spur curved towards the M_2 vein. Additionally, 37.5 per cent of the specimens exhibited a protuberance at the base of the 3A vein, giving this vein a hooked appearance. However, this protuberance was absent in 43.75 per cent of the specimens, and inconclusive in the remainder due to damage to the area during preparation.

Perusal of the literature reveals that such intraspecific variations in the wing venation patterns have been reported across several insect taxa, including Lepidoptera (Albrecht and Kaila, 1997; Nath and Devi, 2009; Solonkin *et al.*, 2021, 2024). And in view of the diagnostic role of wing venation patterns, recording of such intraspecific variation is of considerable significance to insect systematics. Presently, the authors revisited the venation pattern of *D. chrysippus* to record intraspecific variations.

The ensuing account describes intraspecific variations in the examined specimens: vein atrophy in the forewing, altered angularity of discocellulars in the hindwing, and additional protuberance at the base of the 3A vein in the hindwing.

The presence of two vein spurs in the forewings of 62.5 per cent of the specimens corroborates earlier observations made by Bingham (1905). However, 37.5 per cent of the specimens exhibited only a single spur, which was not reported before. Furthermore, 12.5 per cent of the specimens exhibited displacement of the first spur, indicating developmental instability during wing morphogenesis; this feature has not been previously documented.

Bingham (1905) distinguished *D. chrysippus* from *D. genutia* (Cramer, 1779) primarily based on the angularity of the middle discocellular in the hindwing, arguing that it was perpendicular to the M_2 vein in *D. chrysippus* and curved in *D. genutia*. However, in this study, 43.75 per cent of *D. chrysippus* specimens displayed the MDC of the hindwing curving towards the M_2 , indicating that this feature is variable and not taxonomically stable.

Furthermore, in the hindwing, the presence of a protuberance at the base of the 3A vein in 37.5 per

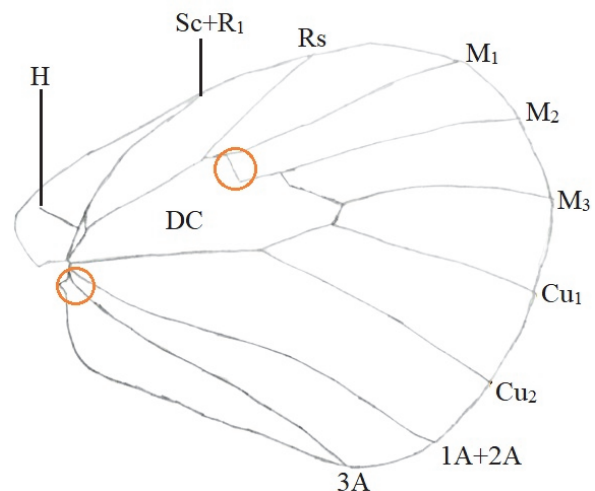


Fig. 5 *Danaus chrysippus* - Hindwing showing MDC and the base of the Anal veins (encircled in red to highlight the variation)

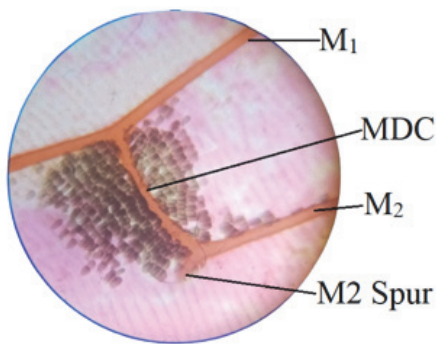


Fig. 6a

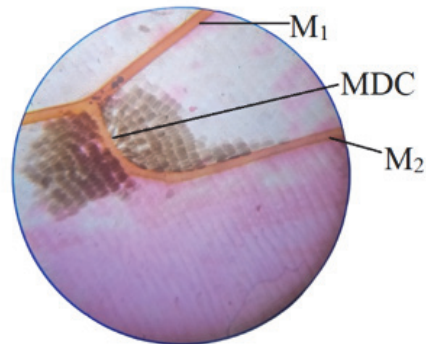


Fig. 6b

Fig. 6 *Danaus chrysippus*: **a** - Hindwing showing a short spur with the MDC perpendicular to the vein M_2 ; **b** - Hindwing in which the short spur is lacking and the MDC curves towards the M_2 vein

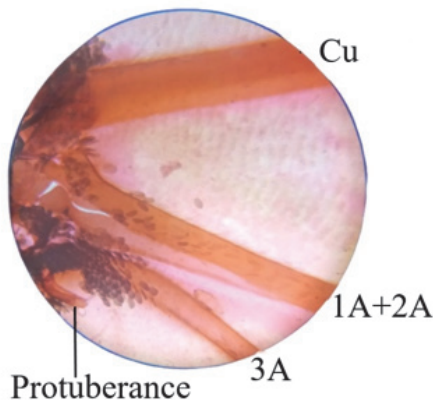


Fig. 7a

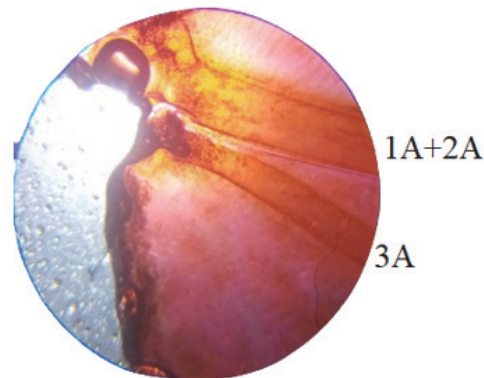


Fig. 7b

Fig. 7 *Danaus chrysippus*: **a** - Hindwing showing a hook-shaped 3A vein; **b** - Hindwing in which the protuberance in 3A vein is lacking

cent of the specimens represents a previously unrecorded structural deviation. This protuberance does not correspond to an atrophied vein; its formation may reflect localised sclerotisation or vein thickening during wing expansion, rather than a modification of the venation pattern. There is a further need for detailed morphometric and microstructural analysis to conclude its origin and whether it is aberrant or taxonomically relevant.

The absence of the presently reported variation in recent studies (Mal *et al.*, 2015; Patil and Magdum, 2017) could result from differences in the landscapes where specimens were collected, slide preparation methods, low magnification power of the

microscope, or simply a missed observation. Alternatively, vein spurs may have undergone significant reduction or have been completely lost in certain populations. This argument advocates for broader geographic sampling and the use of standardised modern imaging techniques. Although this study did not investigate the underlying causes of variability in venation patterns, which was beyond the scope of the present study, the observed vein atrophy is indicative of developmental instability. Solonkin *et al.* (2024) reported the influence of environmental factors, such as temperature and precipitation, on the development of wing veins in *Aporia crataegi* (Pieridae). This observation suggests that similar ecological stresses

could affect *D. chrysippus*. Thus, future studies must integrate morphometric, environmental, and genetic data from multiple populations of the species to understand the developmental mechanisms and adaptive significance of venation variability in *Danaus*. This study highlights that even morphologically 'stable' venational traits in *D. chrysippus*, or other species, may exhibit subtle yet significant variations, reinforcing the need for re-assessment of traditional diagnostic characters vis-à-vis taxonomy in Lepidoptera.

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Distributional records of the families Eupelmidae and Neanastatidae (Hymenoptera, Chalcidoidea) from Madhya Pradesh, India

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ABSTRACT: The parasitic hymenopteran families Eupelmidae and Neanastatidae in the superfamily Chalcidoidea have been documented in Madhya Pradesh of India. *Anastatus* (*Anastatus*) *acherontiae* Narayanan, Subba Rao & Ramachandra, 1960, *Eupelmus* (*Eupelmus*) *atus* Narendran and Anil, 1998, *E. (E.) urozonus* Dalman, 1820, *E. (E.) vermai* (Bhatnagar, 1952), *E. australis* Girault, 1915, *E. (Episolidelia) testaceiventris* (Motschulsky, 1863), *Neanastatus cinctiventris* Girault, 1913, *N. turneri* Ferriere, 1938, and *Neanastatus* sp. (species identity undetermined) are reported from Madhya Pradesh, India.

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KEY WORDS: Taxonomy, parasitoids, host, new state records

INTRODUCTION

The superfamily Chalcidoidea is one of the most biologically diverse groups of parasitic Hymenoptera. Several members have significant potential as natural enemies of various agricultural pests. Despite their importance as biocontrol agents, this group has not been thoroughly surveyed and studied in many states of India. The families Eupelmidae and Neanastatidae are small and relatively obscure within the chalcidoids. These families have been reported as primary and secondary parasitoids of various insect orders, including Hemiptera (mostly the family Pentatomidae), Lepidoptera, Coleoptera, Diptera, Hymenoptera, Orthoptera, Blattodea and even some spiders. Most of the species in these families are solitary and typically parasitize their hosts'

immature stages, such as eggs and larvae. Worldwide, numerous successful attempts have been made to utilize eupelmids against troublesome crop pests. Different species of *Anastatus* Motschusky have proved to be significant as potential control agents of pentatomid bugs. *A. bifasciatus* is among the few egg parasitoids that can develop on live brown marmorated stinkbug, *Halyomorpha halys* (Stål) eggs (Haye *et al.*, 2015), and can be mass-reared as a candidate for augmentative biological control against *H. halys*. *Anastatus bangalorensis* Mani and Kurian and *Anastatus acherontiae* Narayanan, Subba Rao and Ramachandra were recorded as egg parasitoids of the litchi stink bug, *Tessaratoma javanica* Thunberg (Choudhary *et al.*, 2015). The family Eupelmidae Walker is currently divided into three subfamilies: Eupelminae Dalman, 1820, Calosotinae

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Boucek, 1958, and Eusandalinae Fusu, 2022. Globally, it includes approximately 980 species across 39 genera (Fusu and Gibson, 2025), while in India, 87 species are distributed among 12 genera (UCD Community, 2024; Rameshkumar *et al.*, 2025; Ghosh *et al.*, 2025; Binoy *et al.*, 2025). The genus *Eupelmus* Dalman is the most speciose among the Eupelmidae globally and in India, with 30 recorded species. Most *Eupelmus* species are solitary and act as primary or secondary larval or pupal ectoparasitoids of a wide range of holometabolous insects, often in concealed environments (Gibson, 2011). The family Neanastatidae Kalina, which has been elevated from subfamily Neanastatinae of Eupelmidae to family status (Burks *et al.*, 2022), contains 47 species in two extant genera globally (UCD Community, 2024; Upadhyay *et al.*, 2025). In India, 11 species are known under genus, *Neanastatus* Girault (Narendran *et al.*, 2006; Noyes, 2024; Upadhyay *et al.*, 2025) and members of the genus are primarily parasitoids of gall midges (Cecidomyiidae, Diptera) and psyllids (Psyllidae, Hemiptera) (Narendran *et al.*, 2006). Survey on the distribution of Eupelmidae and Neanastidae was under taken in the Eastern Ghats of Madhya Pradesh.

MATERIALS AND METHODS

Collection surveys were conducted at 21 locations throughout Madhya Pradesh; Eupelmidae and Neanastatidae were identified at eight locations across Indore, Bhopal, Raisen, and Jabalpur districts. The specimens were collected using various methods, including sweep nets, yellow pan traps (YPT), and Malaise traps from different locations across the state. Notably, the sweep net collections yielded the highest number of specimens. The collected parasitoids were preserved and processed following the standard protocol (Noyes, 1982) and identified with the appropriate keys (Narendran and Anil, 1998; Narendran, 2009; Narendran *et al.*, 2006). The eupelmids were sorted, mounted, identified, and photographed using a Leica DMC4500 camera attached to a Leica M205A microscope. The photos were retouched using Adobe Photoshop CS3 Software. The specimens have been deposited in the National

Zoological Collection at the Zoological Survey of India in Kolkata, each with a registration number. A map of the study areas was prepared using QGIS software (Fig.1).

Abbreviations used: BMNH = Natural History Museum, London, UK; DZUC=Department of Zoology, University of Calicut, Kerala, India; IARI = Division of Entomology, Indian Agricultural Research Institute, New Delhi, India; NRS = Naturhistoriska Riksmuseet Stockholm, Sweden; NZCZSI = National Zoological Collection, Zoological Survey of India; QMB = Queensland Museum, Brisbane, Australia; SEA = School of Entomology, St. John's College, Agra, India; ZMMU = Zoological Museum of Moscow State University, Moscow, Russia.

RESULTS AND DISCUSSION

The study documents Eupelmidae and Neanastatidae species for Madhya Pradesh, with two genera and eight species identified as new records for the state.

Order **Hymenoptera**; Superfamily **Chalcidoidea**;
Family **Eupelmidae**;

Subfamily **Eupelminae**

Genus *Anastatus* Motschulsky, 1859; Subgenus *Anastatus* Motschulsky, 1859

1. *Anastatus (Anastatus) acherontiae* Narayanan, Subba Rao & Ramachandra, 1960 (**Fig. 2a**)

Anastatus acherontiae Narayanan, Subba Rao & Ramachandra, 1960: 171, Proceedings of the National Institute of Sciences of India (B), Holotype Female, India, Delhi (IARI).

Materials examined: 1 ♀, India: Madhya Pradesh, Bhopal, Indian Institute of Science Education and Research, 22.2246°N; 77.2729°E, 27.ix.2024, Sweep Net, Coll. A. Rameshkumar; 1 ♀, Raisen, Banskwar, 22.9659°N; 77.6072°E, 29.ix.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33897/H3, NZCZSI).

Distribution: India: Delhi, Himachal Pradesh, Punjab, Kerala, Tamil Nadu, Madhya Pradesh (**New record**).

Hosts: *Acherontia styx* (Westwood) (Lepidoptera, Sphingidae) (Narayanan *et al.*, 1960); Pentatomidae (Hemiptera) eggs (Narendran, 2009).

Genus *Eupelmus* Dalman, 1820; Subgenus *Eupelmus* Dalman, 1820.

2. *Eupelmus (Eupelmus) atus* Narendran and Anil, 1998 (Fig. 2b)

Eupelmus atus Narendran and Anil, 1998: 9, Journal of the Zoological Society of Kerala, Holotype Female, India, Kerala, Parambikulam (DZUC).

Materials examined: 1 ♀, India: Madhya Pradesh, Indore, Indian Institute of Soybean Research, 22.6826°N; 75.8695°E, 21.ix.2024, YPT, Coll. A. Rameshkumar; (Reg. No. 33898/H3, NZCZSI).

Distribution: India: Kerala, Madhya Pradesh (**New record**).

Host: Unknown.

Remarks: After the discovery of this species in 1998 from Kerala (Narendran and Anil, 1998), it is for the first time this species is found again with its extended distribution in Madhya Pradesh.

3. *Eupelmus (Eupelmus) urozonus* Dalman, 1820 (Fig. 2f)

Eupelmus urozonus Dalman, 1820: 378, Kungliga Svenska Vetenskapsakademiens Handlingar, Holotype Female, Sweden (? NRS).

Materials examined: 1 ♀, India: Madhya Pradesh, Indore, Umrikheda, 22.6118°N; 75.8876°E, 24.ix.2024, Sweep Net, Coll. A. Rameshkumar; 1 ♀, Indore, Malendi Village, 22.4985°N; 75.7741°E, 22.ix.2024, YPT, Coll. A. Rameshkumar; (Reg. No. 33899/H3, NZCZSI).

Distribution: India: Delhi, Gujarat, Tamil Nadu, West Bengal, Madhya Pradesh (**New record**).

Host: Coleoptera: Apionidae (Islam and Hayat, 1986), Attelabidae, Chrysomelidae, Coccinellidae, Curculionidae (Herting, 1973); Diptera: Agromyzidae (Abdul-Nasr and Assem, 1969),

Cecidomyiidae (Narendran and Anil, 1998), Tephritidae (Noyes, 2024); Hemiptera: Aphididae, Coccidae (Herting, 1972); Hymenoptera: Cynipidae, Diprionidae, Eurytomidae, Pteromalidae, Siricidae, Tenthredinidae, Torymidae (Noyes, 2024); Lepidoptera: Cecidosidae, Coleophoridae, Gelechiidae, Gracillariidae, Lasiocampidae, Lymantriidae, Momphidae, Notodontidae, Pieridae, Pyralidae, Tortricidae, Yponomeutidae (Noyes, 2024).

4. *Eupelmus (Eupelmus) vermai* (Bhatnagar, 1952) (Fig. 2e)

Solindenia vermai Bhatnagar, 1952: 162, Indian Journal of Agricultural Science, Holotype Female, India, Kanpur (? SEA).

Materials examined: 2 ♀, India: Madhya Pradesh, Indore, Umrikheda, 22.6118°N; 75.8876°E, 24.ix.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33900/H3, NZCZSI).

Distribution: India: Uttar Pradesh, Goa, Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh (**New record**).

Host: Coleoptera: Coccinellidae (Herting, 1973).

Subgenus *Episolinodelia* Girault, 1914

5. *Eupelmus (Episolinodelia) australis* Girault, 1915 (Fig. 2c)

Eupelmus australis Girault, 1915: 6, Memoirs of the Queensland Museum, Holotype Female, Australia, Brisbane (QMB).

Materials examined: 3 ♀, India: Madhya Pradesh, Indore, Umrikheda, 22.6118°N; 75.8876°E, 24.ix.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33779/H3, NZCZSI).

Distribution: India: Kerala, Madhya Pradesh (**New record**).

Host: Unknown.

Remarks: The species was so far known only from Kerala (Narendran and Anil, 1998)

6. *Eupelmus (Episolindelia) testaceiventris* (Motschulsky, 1863) (Fig. 2d)

Roptrocerus testaceiventris (Motschulsky, 1863: 49), Byulleten' Moskovskogo Obshchestva Ispytateley Prirody (Otdel Biologicheskii), Holotype Female, Sri Lanka, Mt. Nuara Eliya (ZMMU).

Materials examined: 2♀, India: Madhya Pradesh, Indore, Umrikheda, 22.6118°N; 75.8876°E, 24.ix.2024, Sweep Net, Coll. A. Rameshkumar; 6♀, Jabalpur, Tropical Forest Research Institute, 23.1106°N; 79.9402°E, 3.x.2024, Sweep Net, Coll. A. Rameshkumar; 3♀, Jabalpur, Bhamki Village, 23.1435°N; 79.6734°E, 5.x.2024, Sweep Net, Coll. A. Rameshkumar; 2♀, Jabalpur, Tripur Sundari Temple Road, 23.1468°N; 79.8186°E, 5.x.2024, Sweep Net, Coll. A. Rameshkumar; 1♀, Bhopal, Indian Institute of Science Education and Research, 22.2246°N; 77.2729°E, 27.ix.2024, Sweep Net, Coll. A. Rameshkumar; 1♀, Raisen, Bhimbetka, 22.9395°N; 77.6124°E, 29.ix.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33781/H3, NZCZSI).

Distribution: India: Maharashtra, West Bengal, one report of this species from India without mention of state in CBIC Annual Report 1984-1985: 30. Commonwealth Agricultural Bureaux, Slough, U.K., Madhya Pradesh (**New record**).

Host: Diptera: Cecidomyiidae (Askew and Nieves-Aldrey, 2000); *Contarinia sorghicola* (Coq.) (Herting, 1978); *Procontarinia matteiana* Kieffer and Cecconi (CIBC, 1985).

Remarks: Since many specimens were collected from different localities of the study area, this species seems to be a common species. The distribution of this species was reported to be limited to Maharashtra and West Bengal so far.

Family Neanastatidae; Genus *Neanastatus* Girault, 1913

7. *Neanastatus cinctiventris* Girault, 1913 (Fig. 3a).

Neanastatus cinctiventris Girault, 1913: 35, Bulletin of the Wisconsin Natural History Society (new series). Syntypes Female, Australia, Queensland (QMB).

Materials examined: 1♀, India: Madhya Pradesh, Jabalpur division, Mandla, Paprikalam, 22.9581°N; 79.9676°E, 4.x.2024, Sweep Net, Coll. A. Rameshkumar; 1♀, Jabalpur, Tropical Forest Research Institute, 23.1106°N; 79.9402°E, 3.x.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33901/H3, NZCZSI).

Distribution: India: Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Odisha, Tamil Nadu, Uttar Pradesh.

Host: *Orseolia oryzae* (Wood-Mason) (Diptera, Cecidomyiidae) (Narendran *et al.*, 2006).

8. *Neanastatus turneri* Ferriere, 1938 (Fig. 3b)

Neanastatus turneri Ferriere, 1938: 65, Annales de la Société Entomologique de France. Holotype Female, South Africa, (BMNH).

Materials examined: 1♀, India: Madhya Pradesh, Jabalpur, Tropical Forest Research Institute, 23.1106°N; 79.9402°E, 3.x.2024, Sweep Net, Coll. A. Rameshkumar; (Reg. No. 33903/H3, NZCZSI).

Distribution: India: Andhra Pradesh, Kerala, Uttar Pradesh, Uttarakhand, Madhya Pradesh (**New record**).

Host: *Asphondylia punica* Marchal (Diptera, Cecidomyiidae) (Askew & Nieves-Aldrey, 2006).

9. *Neanastatus* sp. (Fig. 3c)

Materials examined: 1♂, India: Madhya Pradesh, Jabalpur, Hirapur Village, 23.0724°N; 79.5231°E; 5.x.2024, Sweep Net, Coll. A. Rameshkumar; 1♂, Jabalpur, Bhamki Village, 23.1435°N; 79.6734°E, 5.x.2024, Sweep Net, Coll. A. Rameshkumar; 1♂, Bhopal, Udaygiri, 23.5360°N; 77.7718°E, 28.ix.2024, Sweep Net, Coll. A. Rameshkumar.

Remarks: This species looks very close to *Neanastatus turneri* but differs from it in having bright yellow colour in the anterior part of acropleuron and an orange-yellow metasoma with black stripes. Since only male specimens were found in the collections, the identity of the species could not be ascertained.

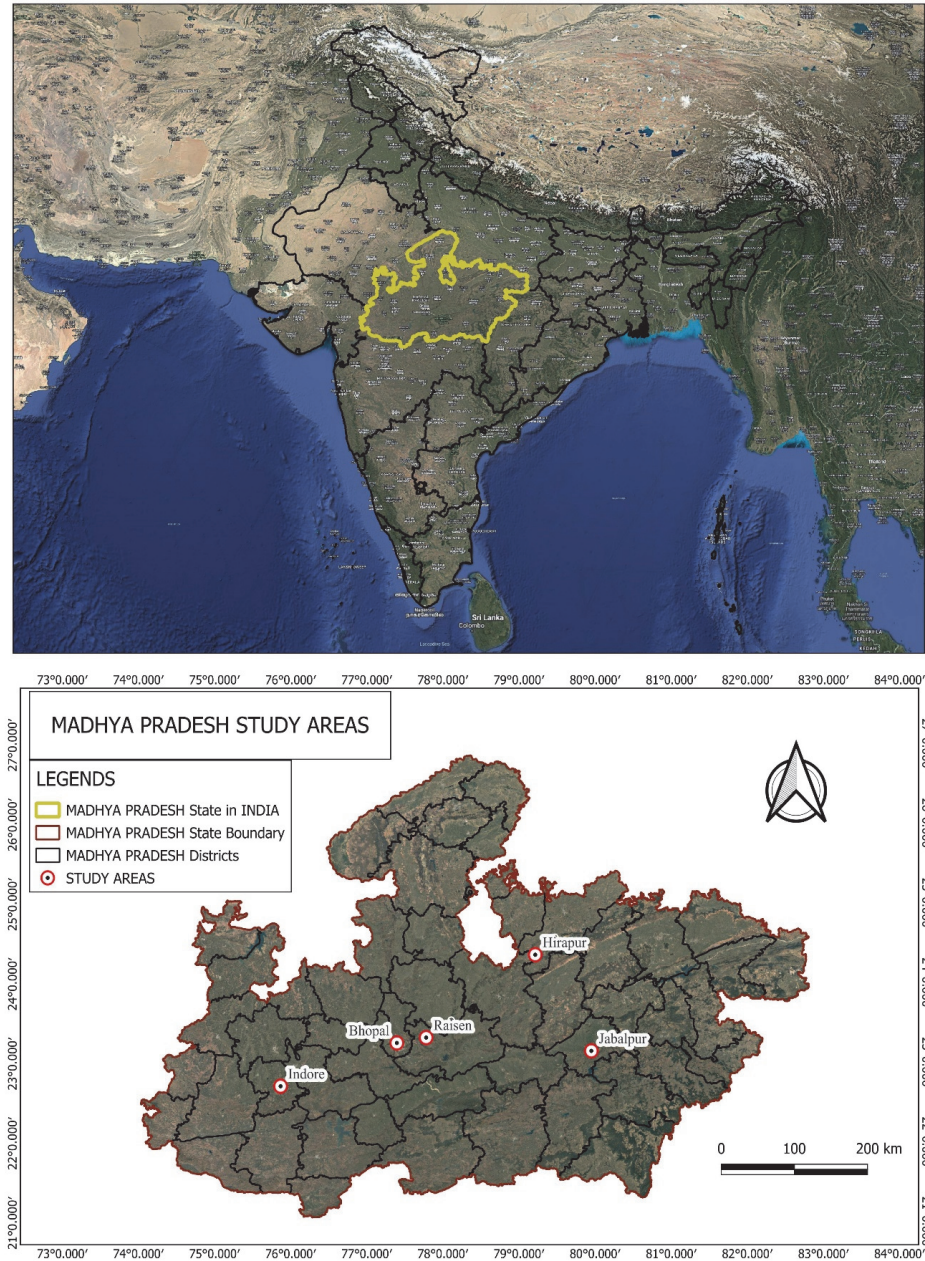


Fig. 1 The study areas in Madhya Pradesh

In India, studies on Eupelmidae and Neanastatidae are limited to the reports of a few species mainly from Kerala, Tamil Nadu and Uttar Pradesh. Extensive surveys were conducted under experts such as T.C. Narendran and M. Hayat who provided valuable insights on Indian fauna of these parasitoids. However, there is limited knowledge about eupelmids in most states of India, including

Madhya Pradesh, where only one species, *Neanastatus cinctiventris* Girault, 1913 has been reported (Islam and Hayat, 1986). The group is considered underdocumented in India despite their economic importance. Therefore, the relatively unexplored central region of the country was chosen as the study area. The dominant vegetation here consists of moist deciduous forests along with

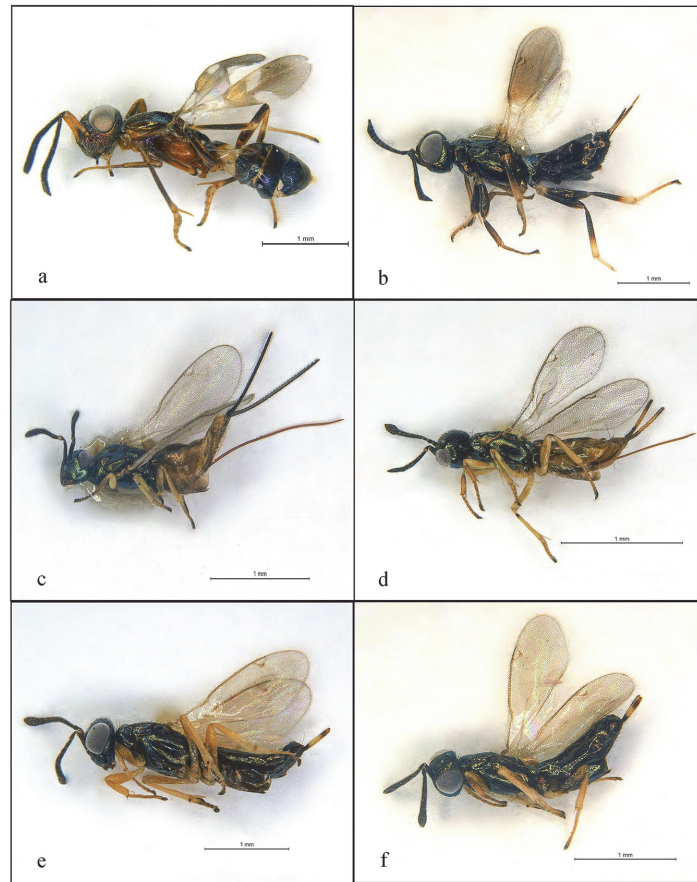


Fig. 2 Family Eupelmidae: a - *Anastatus acherontiae*; b - *Eupelmus atus*; c - *E. australis*; d - *E. testaceiventris*; e - *E. vermai*; f - *E. urozonus*

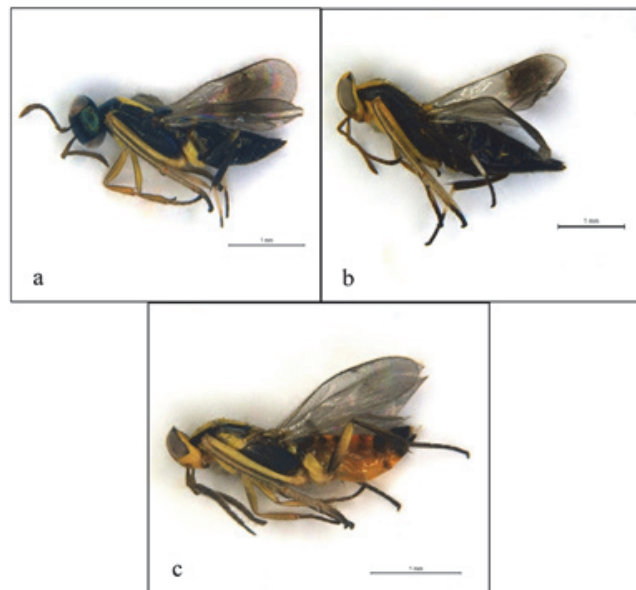


Fig. 3 Family Neanastatidae: a. *Neanastatus cinctiventris*; b. *N. turneri*; c. *Neanastatus* sp. (Male)

bamboo thickets and mixed forests. Though substantial numbers of parasitoids were collected in the survey material, eupelmids were comparatively rare among them in most collection methods. Furthermore, due to significant sexual dimorphism, accurate species identification typically relies on female specimens for most eupelmids. This study presents a list of Eupelmidae and Neanastatidae species for Madhya Pradesh, with two genera and eight species identified as new records for the state. Within the limited collection of the group, one species is reported for the first time here after its discovery and four species known only from a few states show extended distribution. Moreover, common occurrence of one species throughout the collection, which so far showed limited distribution, underlines the lack of proper documentation of the group. Considering their immense potential in biological control, further exploration of these natural enemies is essential to provide comprehensive knowledge on their diversity and distribution in India. Bridging knowledge gaps in host information and association of males with their female counterparts is also imperative.

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Larval odonate assemblage structure in a disturbed rainforest stream of the southern Western Ghats, Kerala, India

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ABSTRACT: Larval odonate assemblages were investigated in the Kallar stream system, a perennial hill stream of the southern Western Ghats in Thiruvananthapuram District, Kerala, India. Monthly sampling was conducted at five sites (Darpha-Kalungu, Pottanchira, Kaliyikkal, Meenmutty and Main Kallar) that differed in riparian cover, land use and substrate composition. Larvae were collected from multiple microhabitats including sand, gravel, cobble, boulder and leaf-litter deposits using hand nets and direct substrate examination and were identified using standard taxonomic keys. A total of 3,090 individuals belonging to 12 families and 39 morphospecies were recorded. Dragonfly larvae dominated the assemblage, with Gomphidae showing the highest abundance. Open-canopy sites associated with settlements and plantations were mainly represented by widespread families such as Libellulidae and Gomphidae, whereas shaded forest reaches containing leaf litter and woody debris supported additional families including Platystictidae, Protoneuridae and Euphaeidae. Damselflies were more frequent in shaded stretches, while sandy reaches supported fewer individuals. The results indicate that riparian canopy cover and substrate heterogeneity strongly influence odonate larval distribution and emphasize the importance of maintaining natural riparian vegetation in Western Ghats hill streams.

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KEY WORDS: Damselflies, dragonflies, hill stream, riparian vegetation, substrate, heterogeneity

INTRODUCTION

The order Odonata, comprising Anisoptera (dragonflies) and Zygoptera (damselflies) are one of the fascinating groups of insects. Owing to their amphibious life history, relatively short generation time, high trophic position and diversity, odonates are considered as an important component of freshwater ecosystems as well as good indicators of ecosystem health (Corbet, 1993; Clark and Samways, 1996, Dijkstra *et al.*, 2014; Cheri and

Finn, 2023). They are valuable as indicators of aquatic and terrestrial ecosystem health and play a vital role as prey and predators to maintain the balance of trophic levels of food chain in freshwater ecosystems. Their aquatic larvae constitute a natural biological control over mosquito larvae and thus help to control several epidemic diseases like malaria, dengue, filariasis etc. (Mitra, 2002; Abdullah and Mamat, 2024)). Odonate taxa are ideal models for the investigation of the impact of environmental warming and climate change due to

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their tropical evolutionary history and adaptations to temperate climates (Hassall, 2015; Olsen *et al.*, 2022). Freshwater insects, including odonates, are widely used in biomonitoring studies to evaluate river health and habitat quality (Kalkman *et al.*, 2008; Termaat *et al.*, 2015; Tiple *et al.*, 2019; Chakravarty and Gupta, 2024). The present work was therefore undertaken to evaluate the diversity and abundance of larval stages of odonates of Kallar stream and its tributaries.

MATERIALS AND METHODS

Kallar is a typical tropical rainforest stream located near Ponnudi in Thiruvananthapuram district, Kerala, the southern part of the Western Ghats. In this study five collection sites were selected in which four are the tributaries of Kallar stream and one is the main Kallar. The selected sites are Darpha-Kalungu (S1), Pottanchira (S2), Kaliyikkal (S3), Meenmutti (S4) and main Kallar (S5). Habitat characteristics such as riparian vegetation, canopy cover, substrate composition and human disturbance were recorded at each site to evaluate habitat influence on larval assemblage structure. At each sampling locality, a stretch of 100 m area was chosen for collection (Fig. 1, Table 1). The sampling was done monthly basis for a period of one year using kick net (1m² area, mesh size 200mm) and D-frame net (mesh size 50mm) and preserved in alcohol (70%). In the laboratory, larvae were sorted, identified and counted under stereoscopic microscope using standard keys (McCafferty and Provonsha, 1981; Morse *et al.*, 1984). The biodiversity indices like Margalef's richness index, Shannon-Weiner diversity index and Simpson dominance index were calculated using the software PAST.

RESULTS AND DISCUSSION

A total of 3,090 odonate larvae were collected from five sampling sites along the Kallar stream and its tributaries during the study period. The collection comprised 12 families and 39 morphospecies belonging to the two odonate groups, dragonflies and damselflies. Dragonfly larvae formed the major component of the assemblage. Four families were recorded: Gomphidae, Corduliidae, Libellulidae and

Macromiidae. Gomphidae was the most dominant family and occurred at all sites, with the highest abundance at Site 2 (Pottanchira) and the lowest at Site 4 (Meenmutty). Libellulidae was the second most abundant family and was recorded from all five sites, showing relatively higher abundance at Site 1 (Darpha-Kalungu), an open-canopy reach associated with agriculture and plantations. Corduliidae occurred in moderate numbers with an irregular distribution among sites, whereas Macromiidae was represented by comparatively few individuals (Table 2).

Damselfly larvae collected belonged to eight families: Chlorocyphidae, Euphaeidae, Platystictidae, Lestidae, Chlorolestidae, Protoneuridae, Platycnemididae and Calopterygidae. Euphaeidae showed relatively high abundance at Sites 2 and 5. Chlorolestidae was particularly abundant at Site 3 (Kaliyikkal), a residential reach with open canopy. Platystictidae and Protoneuridae were more frequent in shaded sections of the stream (Sites 4 and 5), where leaf litter and woody debris were present. Chlorocyphidae and Lestidae were recorded in low numbers across all sites. Platycnemididae occurred mainly at Site 3, while Calopterygidae was more abundant at Site 1 and less frequent at Site 5.

Biodiversity indices varied among the sites. The Shannon–Wiener diversity index ranged from 2.14 to 3.10, with the highest value at Site 1 and the lowest at Site 2. Simpson dominance index values ranged from 0.79 to 0.94, indicating variation in dominance patterns among sites. Margalef's richness index ranged from 3.97 to 5.57, with the highest richness at Site 3 and the lowest at Site 5. Overall, dragonfly larvae were numerically more abundant than damselfly larvae at all sampling locations. Sites characterized by shaded canopy, forest cover, and the presence of leaf litter and woody debris (Sites 4 and 5) supported several families associated with structurally complex habitats, whereas open-canopy sites with nearby human settlement or plantations (Sites 1-3) were dominated by widely distributed families (Table 3).

The present study demonstrated clear spatial variation in the composition and diversity of odonate

larvae among the five sampling sites of the Kallar stream system. Differences in larval assemblages were closely associated with habitat characteristics such as canopy cover, substrate composition, altitude and degree of human disturbance. Odonates are known to respond strongly to local habitat structure and riparian conditions, making them reliable indicators of freshwater ecosystem integrity (Corbet, 1999; Kalkman *et al.*, 2008; Harabis and Dolny, 2021).

Anisopteran larvae dominated all sampling sites, particularly members of the family Gomphidae. Gomphid larvae are burrowers that prefer sandy and gravelly substrates in running waters (Corbet, 1999). Such substrates were common at Sites 1, 2 and 3, which were characterized by open canopy and mixed mineral bed material, providing suitable conditions for burrowing forms. Similar associations between gomphid larvae and sandy substrates have been reported in tropical stream systems (Subramanian, 2009; Tiple *et al.*, 2019). In contrast,

Sites 4 and 5 contained greater amounts of leaf litter, cobbles and woody debris, which supported comparatively fewer burrowing larvae.

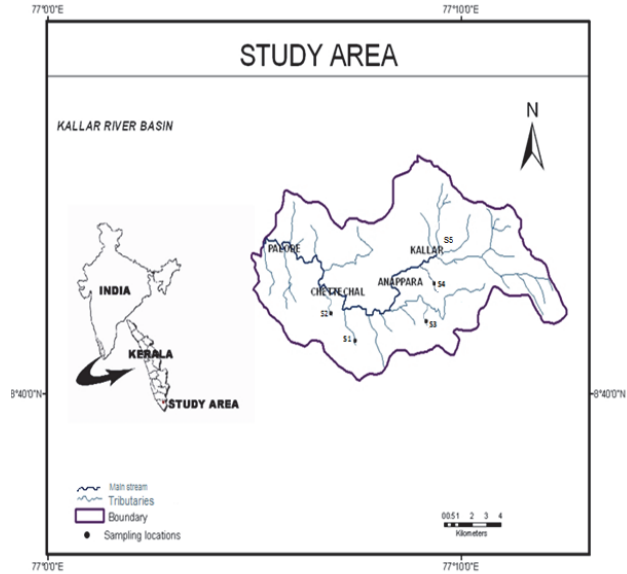


Fig. 1 Location of the study area

Table 1. Habitat characteristics of Kallar stream and its tributaries

No.	Parameter	Site 1 (Darpha-Kalungu)	Site 2 (Pottanchira)	Site 3 (Kaliyikkal)	Site 4 (Meenmutti)	Site 5 (Main Kallar)
1	Latitude	8°402 423N	8°412 313N	8°402 163N	8°422 363N	8°432 423N
2	Longitude	77°042 023E	77°032 093E	77°062 043E	77°072 413E	77°072 373E
3	Altitude (msl)	111	105	116	156	227
4	Flow regime	Perennial	Perennial	Perennial	Perennial	Perennial
5	Riparian vegetation	Mixed riparian vegetation with herbs and grasses	Plantation-associated riparian vegetation	Disturbed riparian vegetation near settlement	Forest riparian vegetation	Dense forest riparian vegetation
6	Land use	Agriculture & plantations	Plantation-Rubber	Residential influence	Forest	Forest
7	Canopy cover	Open (<30%)	Open (<30%)	Open (<30%)	Partially shaded (30–70%)	Shaded (>70%)
8	Water clarity	Clear	Clear	Clear	Clear	Clear
9	Large woody debris	Absent	Absent	Absent	Present	Present
10	Water odour	Odourless	Odourless	Odourless	Odourless	Odourless
11	Human disturbance	Present	Present	Present	Present	Absent
12	Dominant substrate	Sand, gravel, pebble, cobble, small boulders	Sand, gravel, pebble, cobble	Sand, gravel, pebble, cobble	Sand, gravel, cobble, leaf litter	Cobble, boulder, woody debris, leaf litter, rocks

Table 2. Odonates larvae collected from the study sites

Family	Genus	S1	S2	S3	S4	S5
Sub order – Anisoptera (Dragonflies)						
Gomphidae	<i>Gomphidae</i> sp. 1	82	328	85	99	205
	<i>Gomphidae</i> sp. 2	33	131	31	38	73
	<i>Gomphidae</i> sp. 3	8	12	11	14	6
	<i>Gomphidae</i> sp. 4	95	62	62	3	17
	<i>Gomphidae</i> sp. 5	12	5	14	0	6
	<i>Gomphidae</i> sp. 6	16	7	12	3	0
	<i>Gomphidae</i> sp. 7	4	9	1	0	9
	<i>Gomphidae</i> sp. 8	10	1	1	0	0
	<i>Gomphidae</i> sp. 9	0	1	1	0	4
	<i>Gomphidae</i> sp. 10	8	2	5	0	0
	<i>Gomphidae</i> sp. 11	2	1	1	0	0
	<i>Gomphidae</i> sp. 12	0	0	1	0	2
Cordullidae	<i>Cordullidae</i> sp. 1	9	5	7	15	0
	<i>Cordullidae</i> sp. 2	34	4	15	53	5
	<i>Cordullidae</i> sp. 3	0	0	1	5	0
Libellulidae	<i>Libellulidae</i> sp. 1	29	8	29	12	3
	<i>Libellulidae</i> sp. 2	23	1	19	5	0
	<i>Libellulidae</i> sp. 3	12	3	16	3	0
	<i>Libellulidae</i> sp. 4	7	0	2	0	0
	<i>Libellulidae</i> sp. 5	13	0	5	1	0
	<i>Libellulidae</i> sp. 6	3	1	2	0	0
	<i>Libellulidae</i> sp. 7	10	0	2	0	0
	<i>Libellulidae</i> sp. 8	21	2	12	3	0
Macromidae	<i>Macromidae</i> sp.1	3	5	2	3	2
Sub order – Zygoptera (Damselflies)						
Chlorocyphidae	<i>Chlorocyphidae</i> sp.1	0	3	2	0	3
	<i>Chlorocyphidae</i> sp.2	5	0	1	3	5
Euphaidae	<i>Euphaidae</i> sp.1	40	67	60	32	66
	<i>Euphaidae</i> sp.2	15	41	26	4	24
Platystictidae	<i>Platystictidae</i> sp.1	7	7	10	10	16
Lestidae	<i>Lestidae</i> sp.1	5	3	3	1	12
	<i>Lestidae</i> sp.2	1	1	0	1	5
Chlorolestidae	<i>Chlorolestidae</i> sp.1	19	32	56	33	44
	<i>Chlorolestidae</i> sp.2	20	18	37	21	16
Protoneuridae	<i>Protoneuridae</i> sp.1	42	3	54	4	1

Platycnemididae	<i>Platycnemididae</i> sp.1	21	0	24	1	6
	<i>Platycnemididae</i> sp.2	6	1	8	3	4
Calopterygidae	<i>Calopterygidae</i> sp.1	61	35	15	26	6
	<i>Calopterygidae</i> sp.2	7	4	5	20	1
	<i>Calopterygidae</i> sp.3	5	1	0	2	1

SITES: Darpha-Kalungu (S1), Pottanchira (S2), Kaliyikkal (S3), Meenmutti (S4) and main Kallar (S5)

Table 3. Biological indices of odonates

Diversity Indices	Site 1	Site 2	Site 3	Site 4	Site 5
Shannon Weiner index	3.10	2.14	2.99	2.64	2.24
Simpson Index	0.94	0.79	0.93	0.89	0.81
Margalef's richness index	5.20	4.63	5.57	4.47	3.97

Zygopteran families such as Euphaeidae, Platystictidae and Chlorocyphidae were more common in shaded stream sections. These families are typically associated with forested streams having stable flow and well-developed riparian vegetation (Subramanian, 2005; Dijkstra *et al.*, 2014). The relatively higher abundance of these families at Sites 4 and 5 can be attributed to increased canopy cover, organic substrates and reduced disturbance. Shaded habitats buffer temperature fluctuations and provide microhabitats suitable for climbing and clinging larvae (Corbet, 1999; Cheri and Finn, 2023).

The lowest diversity recorded at Site 2 may be related to influence of rubber plantation and greater human activity. Removal or modification of riparian vegetation is known to reduce habitat heterogeneity and shelter availability for aquatic insects (Kalkman *et al.*, 2008; Harabis and Dolny, 2021). In contrast, Site 1 exhibited the highest diversity, possibly due to heterogeneous substrates and moderate disturbance, which often increases niche availability in stream ecosystems.

Altitude also appeared to influence assemblage structure. Site 5, located at higher elevation and surrounded by forest, supported several stream-associated families, indicating relatively stable ecological conditions. Forested headwater streams generally maintain cooler temperatures, higher

dissolved oxygen and increased allochthonous organic input, which favour sensitive odonate larvae (Dijkstra *et al.*, 2014; Olsen *et al.*, 2022).

Overall, dragonfly larvae were more abundant than damselfly larvae across all sites. Dragonflies possess greater dispersal ability and ecological tolerance, enabling them to colonize disturbed habitats more effectively (Corbet, 1999; Kadoya *et al.*, 2004). Damselflies are more habitat-specific and sensitive to environmental alteration, particularly changes in riparian cover and substrate structure (Weir, 1974; Tiple *et al.*, 2019).

The observed distribution pattern supports the use of odonate larvae as effective indicators of freshwater habitat quality. Sites with intact riparian vegetation and heterogeneous substrates supported higher diversity, whereas disturbed locations showed reduced richness and dominance of tolerant groups. Therefore, odonate larval assemblages can serve as a useful tool for ecological monitoring and conservation assessment of tropical hill streams in the southern Western Ghats.

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Evaluation of bio-efficacy of botanical and chemical insecticides for the management of gram pod borer (*Helicoverpa armigera*) infesting chickpea in Bihar, India

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ABSTRACT: A field experiment was conducted to evaluate the efficacy of various botanical and chemical insecticides against *Helicoverpa armigera*. Eight treatments including three botanical and four chemical insecticides were tested. All the botanical and chemical insecticides were showed significantly superior to control. Among the treatments indoxacarb (14.5 SC @ 75 g a.i. ha⁻¹) was found most effective in controlling gram pod borer incidence with less pod damage and maximum yield (7.8% pod damage and 1519.97 kg ha⁻¹ respectively) and which was at par with emamectin benzoate 5 SG @ 11 g a.i. ha⁻¹ (9.2% pod damage, and 1454.11 kg ha⁻¹). The control recorded higher pod damage and low yield (23.87% pod damage and 844.20 kg ha⁻¹). On the basis of economic parameters, the highest incremental benefit-cost ratio (IBCR) was recorded in the treatment of indoxacarb 14.5 SC (7.2:1), followed by emamectin benzoate 5 SG (7.0:1), chlorantraniliprole 18.5 SC (4.6:1), fipronil 5 SC (4.0:1), NSKE 5 (%) (2.1:1), neem oil (2%) (1.5:1) and karanj oil (5%) (1.2:1). © 2026 Association for Advancement of Entomology

KEY WORDS: Incremental benefit cost ratio, indoxacarb, pod damage, yield

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a member of the family Fabaceae, is an ancient, self-pollinated leguminous crop widely cultivated across the world. Commonly known as Bengal gram, ceci bean, chana, and garbanzo bean (Patil *et al.*, 2017), it holds significant importance as a major *rabi* season pulse crop in India, where it is often referred to as the king of pulses. Globally, chickpea ranks as the

third most important grain legume after dry beans and peas (Sai *et al.*, 2021). Chickpea seeds are highly nutritious, containing 18-22 per cent protein, 52-70 per cent carbohydrates and 4-10 per cent fat. The crop residue or straw also serves as valuable livestock forage (Shrestha *et al.*, 2011). Moreover, chickpea contributes significantly to soil fertility through biological nitrogen fixation, up to 140 kg N ha⁻¹ Year⁻¹ to the soil (Mustafa *et al.*, 2023). In India chickpea was grown on area of

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10.74 m ha⁻¹ and production was 13.54 million tons with productivity of 1261 kg ha⁻¹. In Bihar chickpea cultivated on area of 0.051 million hectare and production of 0.054 million tons with productivity of 1053 kg ha⁻¹ (Anonymous, 2023). However, a vast number of insect species limit chickpea yield in both field and storage conditions. Among these *Helicoverpa armigera* (Hubner) (Lepidoptera, Noctuidae) is a multivoltine, very polyphagous, and worldwide pest known for its high reproduction rate and short life cycle (Kumar and Singh, 2014). The destructive stages of the insect are its larval stages, which feed on over 182 different host plants, including pigeon pea, chickpea, cotton, maize, and tomato (Gowda *et al.*, 2005). The damaging potential of *H. armigera* on chickpea ranges from 75 to 90 per cent losses in seed yield, despite the application of insecticides (Divija *et al.*, 2024)). Farmers frequently rely extensively on old-generation pesticides, and their indiscriminate use has contributed to the development of pest resistance, as well as a variety of environmental and health issues. Therefore, early and climate-smart pest management interventions are essential to protect pods and prevent significant losses in seed yield (Balraj *et al.*, 2025; Prajapati and Patel, 2025).

To address these challenges, the use of botanical and new generation insecticides that give satisfactory results with minimal effect on the natural enemies, human beings, and the environment. The new generation insecticides act as an effective alternative source with their unique mode of action and less impact on the environment. Overall, studying the efficacy of both botanical and chemical insecticides against gram pod borer is essential for sustainable pest management practices that balance agricultural productivity, environmental protection, human health and economic viability.

MATERIALS AND METHODS

Agricultural research farm at Dholi, positioned at 25.9° N latitude and 85.5° E longitude, with an elevation of 53 meters above sea level. It lies within agro-climatic zone-1, specifically the North West Alluvial Plain Zone of Bihar and is part of the command area of Gandak River. The soil in this

region varies in texture from loamy to sandy loam. The experiment was setup with chickpea variety P-3043 and eight treatments replicated thrice in Randomised Block Design. Each plot is 2 × 2m with a spacing of 30cm×10cm. Two successive pesticide sprays were applied at 15-day intervals, beginning with the blossoming stage. The efficacy of botanical and chemical insecticides, Emamectin benzoate 5 SG, Chlorantraniliprole 18.5 SC, Indoxacarb 15.8 SC, Fipronil 5 SC, Neem Seed Kernal Extract (5%), Karanj oil (5%), and Neem oil (2%), was compared to a control plot against the gram pod borer, *H. armigera*. The agronomic package of strategies is being carried out in accordance with the recommendations of RPCAU, Pusa, Bihar.

Observations on larval population were recorded by counting number of larvae on five randomly selected plants per each replication, with data collected one day prior to the spray and at 1, 3, 7, and 14 days after both first and second spray.

At crop maturity, pods from five randomly selected plants in each treatment and replication were taken to determine the pod damage level of *H. armigera*. The proportion of pod damage was calculated using the following formula. Chickpea grain yields were calculated for each treatment and then converted to ha⁻¹.

$$\text{Pod damage (\%)} = \frac{\text{No of infested pods}}{\text{Total no of pods}} \times 100$$

The gross return was determined by multiplying the total yield by the market price of the produce. and the cost of treatment imposition were then subtracted from the gross returns to calculate additional income over control and the Incremental benefit cost ratio (IBCR) using the following formula:

$$\text{IBCR} = \frac{\text{Additional income over control}}{\text{Cost of protection}}$$

RESULTS AND DISCUSSION

First spray: The mean analysis for the overall effect demonstrated that all the botanicals and chemical insecticidal treatments were significantly

effective in reducing the larval population of *H. armigera* compared to the control plots (Table 1). Among the tested treatments, indoxacarb 14.5 SC (0.92 larvae plant⁻¹) was found to be the most effective in controlling the incidence of the gram pod borer. This was followed by emamectin benzoate 5 SG (1.08 larvae plant⁻¹), chlorantraniliprole 18.5 SC (1.25 larvae plant⁻¹), fipronil 5 SC (1.42 larvae plant⁻¹), neem seed kernel extract (5%) (1.60 larvae plant⁻¹), neem oil (5%) (1.65 larvae plant⁻¹) and karanj oil at 5 per cent (1.72 larvae plant⁻¹). The current findings are in line with the results reported by Tripathi *et al.* (2023), who noted the lowest larval density of the gram pod borer in plots treated with indoxacarb at 79 g a.i. ha⁻¹ (0.59 larvae plant⁻¹). Similarly, Meena *et al.* (2018) found that indoxacarb 14.5 SC was the most effective treatment for reducing the larval population of the gram pod borer, while neem seed kernel extract (NSKE) exhibited moderate efficacy in controlling the gram pod borer. In terms of botanical treatments, the present study aligns closely with Yerrabala *et al.* (2021), who reported that neem seed kernel extract 5 per cent achieving the highest reduction in pod borer population, followed by neem oil 5 per cent. Furthermore, Kumar *et al.* (2019) identified NSKE (5%) as the most efficacy in controlling the gram pod borer, followed by neem oil (2%) significantly reduced pod damage.

Second spray: All insecticidal treatments were significantly reduced the larval population of the gram pod borer compared to the control (Table 1). Among all the treatments, indoxacarb 14.5 SC was the most effective against gram pod borer (0.57 larvae plant⁻¹). The effectiveness of the other treatments was ranked as follows: emamectin benzoate 5 SG (0.75 larvae plant⁻¹), chlorantraniliprole (0.83 larvae plant⁻¹), fipronil (1.13 larvae plant⁻¹), neem seed kernel extract (NSKE) (1.38 larvae plant⁻¹), neem oil (1.50 larvae plant⁻¹), and karanj oil (1.62 larvae plant⁻¹). The present findings consistent with Tripathi *et al.* (2023), who found that indoxacarb applied @ 79 g a.i ha⁻¹ was the showing more efficacy, resulting in only 0.27 larvae per plant. Furthermore, Das *et al.* (2022) evaluated the effectiveness of various

treatments for reducing the larval population of the gram pod borer and reported that neem oil (2 %) and karanj oil (2%) achieved percentage reductions of 45.81 per cent and 37.53, respectively.

Pod damage: The pod damage caused by the gram pod borer across various treatments were significantly lower compared to the control (Table 1). Among all treatments, application of indoxacarb 14.5 SC showed the greatest efficacy, resulting in the least pod damage at 7.8 per cent, which was statistically similar to that of emamectin benzoate 5 SG, which recorded a damage level of 9.2 per cent. Conversely, the highest percentage of pod damage was noted in the plot treated with karanj oil (5%), which exhibited a damage rate of 17.8 per cent. This was closely followed by plots treated with neem oil (2%) at 16.2 per cent and neem seed kernel extract at 14.1 per cent, both of which showed similar levels of damage. The findings of the present study are in close agreement with Tripathi *et al.* (2023) who revealed the efficacy of chemical and botanical insecticides to gram pod borer. Among them indoxacarb 15.8 EC at the rate of 79 g a.i ha⁻¹ showed the highest effectiveness, resulting in lower pod damage at (7.33%), followed by emamectin benzoate showed a pod damage rate of (9.20%) and chlorantraniliprole exhibited a pod damage rate of (10.25%). fipronil (12.3%) and neem oil showed a pod damage rate of (18.35%). Similarly, Meena *et al.* (2018) recorded the per cent pod damage of gram pod borer which was ranged from 4.69 to 20.95 per cent.

Yield: The data regarding chickpea grain yield, as presented in (Table 2), indicated that all treatments resulted in a significant increase in grain yield compared to the control. The grain yield for chickpeas varied from (844.20 to 1519.97 kg ha⁻¹) across the different treatments, while the control (844.20 kg ha⁻¹). The highest yield was observed in the plot treated with indoxacarb 14.5 SC (1519.97 kg ha⁻¹), followed closely by emamectin benzoate 5 SG, (1454.11 kg ha⁻¹), which were statistically similar. Conversely, the minimum grain yield was noted in karanj oil (1021.45 kg ha⁻¹), followed by neem oil (1064 kg ha⁻¹) and NSKE (1098 kg ha⁻¹).

The findings of the present study are in close agreement with Meena *et al.* (2018), who reported yield data (ranging from 8.63 to 14.83 q ha⁻¹). The plot treated with indoxacarb 14.5 SC achieved the highest yield, while the lowest yield was recorded in the control plot.

Determination of incremental benefit cost ratio: The IBCR analysis for the various botanical and chemical insecticides used against the chickpea gram pod borer are shown in Table 2. Among all the treatments, the highest incremental benefit-cost ratio was attained with indoxacarb 14.5 SC (7.2:1),

Table 1. Efficacy of botanical and chemical insecticides against pod borer in chickpea during *Rabi*, 2023 -2024

Treatments/ Dosage (g a.i. /ha)	Mean incidence after			Pod damage (%)
	DBS	I spray	II spray	
Fipronil 5% SC @50	1.93	1.42	1.13	12.84
Chlorantraniliprole 18.5% SC @25	2.00	1.25	0.83	11.4
Indoxacarb 14.5% SC @75	1.93	0.92	0.57	7.8
Emamectin benzoate 5% SG @11	1.87	1.08	0.75	9.2
Neem oil @2%	1.93	1.65	1.50	16.2
Neem seed kernal extract @5%	2.00	1.60	1.38	14.1
Karanj oil @5%	1.93	1.72	1.62	17.8
Control	2.00	2.25	3.03	23.87
SEm(±)	0.11	0.06	0.08	0.83
CD ($p=0.05$)	(NS)	0.19	0.23	2.50
CV (%)	9.73	8.50	11.79	10.11

Table 2. Economics of treatments against gram pod borer on chickpea during *Rabi*, 2023 – 2024

Treatments	Yield (kg ha ⁻¹)	Yield over control (kg ha ⁻¹)	Income over control (Rs ha ⁻¹)	Cost of PP (Rs ha ⁻¹) (2 Sprays)	Income over control (Rs ha ⁻¹)	IBCR
Fipronil 5 % SC	1249.99	405.79	21507	4300	17207	4.0:1
Chlorantraniliprole 18.5 % SC	1365.00	520.80	27602	4966	22636	4.6:1
Indoxacarb 14.5 % SC	1519.97	675.77	35816	4380	31436	7.2:1
Emamectin benzoate 5 % SG	1454.11	609.91	32325	4032	28293	7.0:1
Neem oil 2%	1064.00	219.80	11649	4600	7049	1.5:1
Neem Seed Kernal Extract 5%	1098.00	253.80	13451	4400	9051	2.1:1
Karanj oil 5%	1021.45	177.25	9394	4260	5134	1.2:1
Control	844.20	-	-	-	-	-

followed by emamectin benzoate 5 SG (7.0:1), chlorantraniliprole 18.5 SC (4.6:1), fipronil 5 SC (4.0:1), NSKE (2.1:1), neem oil (1.5:1), and karanj oil (1.2:1). The current findings are closely linked to those of Meena *et al.* (2018) and Tripathi *et al.* (2023), who indicated that the insecticide indoxacarb 14.5 SC had the highest cost-benefit ratio. Similarly, Yerrabala *et al.* (2021) revealed that fipronil was the most cost-effective treatment, followed by NSKE (5%), and neem oil (2%).

The study found that all insecticidal treatments considerably reduced *H. armigera* infestations in chickpea compared to the control. Indoxacarb 14.5 SC consistently demonstrated to be the most effective, with the lowest larvae population, minimal pod damage, and greatest grain production. Emamectin benzoate and chlorantraniliprole also worked well, with botanical treatments providing moderate control. Economic study revealed that indoxacarb had the greatest IBCR, followed by emamectin benzoate. Overall, indoxacarb 14.5 SC proved to be the most effective and cost-effective solution for gram pod borer control.

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Study of wing venation in *Zygogramma bicolorata* Pallister (Coleoptera, Chrysomelidae) in Dehradun of Uttarakhand, India

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ABSTRACT: Costa and sub costa veins are found in the wings of *Zygogramma bicolorata*. They appear as dark coloured markings. The wing comprises three margins namely anterior margin, posterior margin and Apex. Coastal margin is another name for anterior margin. There are coastal margins on the wings' front or first side. The posterior margin is located towards the back of the wing, whereas the apex or apical margin is located at the pointed end of the wing. The anal margin is another name for the posterior edge. The veins in the wing are further divided into two parts which is proximal and distal. The veins are denoted with the help of vein coordinates ranging from one to thirty-six. Vein coordinates help in denoting the exact location of the veins which form the basic framework of the hind wings. From the current analysis it was concluded that the veins found on the hind wing of *Z. bicolorata* are as follows costa, subcosta anterior, subcosta, radius, radius basal, radius anterior, subcosta posterior, radius posterior, radius (2-3), radius (4-5), media anterior (1-2), media posterior (1-2), media posterior (3-4), medial, cubital, cubitus anterior (1-2), base of cubitus (3-4), auxillary (1-3) and radio-medial cross vein. Costa is represented by C and represents the rib portion, subcosta is represented by Sc and is found below the rib portion, radius is represented by R representing the forearm region, media is represented by M which represents the middle portion, cubitus is represented by Cu, which represents the elbow region and anal veins is represented by A, which represents the posterior position. All these are the main longitudinal veins found from the anterior margin towards the posterior margin of the wing.

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KEY WORDS: Hind wings, Mexican beetle, vein coordinates, costa, sub costa

INTRODUCTION

Parthenium hysterophorus is an internationally, widespread, hazardous weed and it has an exclusive host called *Zygogramma bicolorata* Pallister 1953 (Coleoptera, Chrysomelidae), known as Parthenium beetle or Mexican beetle (Jayanth, 1987). The origin

of the beetles goes back to Mexico. It is a small beetle comprising of a brown coloured head, yellow coloured graduated pronotum and has yellow or cream coloured elytra marked with long brown stripes which also happens to be a its characteristic feature (Mehta and Raghuraman, 2019). This beetle has been named as *Zygogramma bicolorata*

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by Pallister in 1953. It is host specific and is an exclusive feeder of *P. hysterophorus* (Tripathi and Tripathi, 2021) and can be used as an effective bio control agent against *P. hysterophorus*.

Wings of any insect play a crucial role in their identification, differentiation from other insects and studying about the characteristic features. The hind wing of *Z. bicolorata* is also known as the flight wing as it is mainly used by the beetle for flight mode. It is found in pairs below the elytra (forewings) which are hard coverings and provide protection to the hind wings and abdomen region of the beetle. The sub coastal and coastal veins present in the hind wings of *Z. bicolorata* appear as dark brown, the red coloured markings when observed. The hind wing is divided into two parts proximal and distal. Proximal parts represent the anterior portion of the wing and distal part represents the posterior portion of the wing. The wing is also divided into three margins which are as follows Anterior margin which is known as Costal margin which lies on the front side, Apex is called the Apical margin which is found at the edge pr pointed end of the wing and Posterior margin which is also known as the Anal margin and is found at back side. Variation in the venation pattern helps to study the evolution and life-cycle parameters of the insect with respect to its environment. Wing venation not only provides the basic structural framework to the wings, but also helps in circulation, gaseous exchange, navigator help and sensory aid to the body of the beetle.

MATERIALS AND METHODS

Parthenium beetle is widely available in India during the months of July to October. It has a characteristic identifying feature elytra, which are forewings modified into hard protective outgrowths and is creamish in appearance with prominent deep brown markings. The study area is Dehradun District of Uttarakhand state of India. It is a valley region surrounded by the Himalayan foothills. It is situated between 30° 01'N and 31° 2'N latitude and 77° 34'E and 78° 18'E longitude. The identification of the beetle was done from the department of entomology at Forest Research Institute, Dehradun by Dr. Arvind Kumar.

Three beetles were collected from each of the selected sites (Garhi Cantt, Kaulagarh, Raipur, Rajpur, Doiwala, Sahastradhara, Prem Nagar, Jamunwala, Anarwala and Jhajra) all over Dehradun region and labelled separately. Cotton plugs dipped in 30 percent absolute alcohol are used to kill the collected beetles. After being killed, the beetles were dissected using needle and forceps. Hind wings were gently removed using forceps and placed on a slide. As a fixative, Canada Balsam was used. The slides were observed under stereo zoom microscope. Pictures of high resolution were captured using Nikon D5600 camera. Coloured images of the hind wings are then scanned to study the venation system (Ren *et al.*, 2017; Sun and Bhushan, 2012; Bethoux, 2005). The entire experimental work was done at the Department of Zoology, Shri Guru Ram Rai University, Dehradun, Uttarakhand.

RESULTS AND DISCUSSION

The hind wings or flight wings comprise of three margins, namely, anterior margin, posterior margin and apex (Fig. 2). Anterior margin is also known as coastal margin. Coastal margins present on the first or front side of the wings. Apex or apical margin is found at the pointed end of the wing while the posterior margin is found towards the backside of the wing. Posterior margin is also known as the anal margin. Margins of insect wings are basically edges which give shape to the structure and interior margin or coastal margin is primarily the leading edge of the wings. As the insect flies, the leading part of the wings forms the coastal margin. Apical margin or apex forms the edge of the wing or the outermost part at the tip of the wing. Anal margin, also known as posterior margin is the part of the wing, which lies closest to the insects' body. The venation system of *Z. bicolorata* (Fig. 1, Table 1) comprises of an intense network of veins, comprising of Costa, represented by "C", sub costa anterior represented by "ScA", sub costa represented by "Sc", radius represented by "R", radius basal represented by "RB", radius anterior represented by "RA", sub costa posterior represented by "ScP", radius posterior represented by "RP", radius (2-3) represented by R(R2-R3),

Table 1. Abbreviations used in Fig. 1

Symbol	Vein name
C	Costa
ScA	Subcosta Anterior
Sc	Subcosta
R	Radius
RB	Radius Basal
RA	Radius Anterior
ScP	Subcosta Posterior
RP	Radius Posterior
R(R2-R3)	Radius (2-3)
R(R4-R5)	Radius (4-5)
Ma (Ma1-Ma2)	Media Anterior (1-2)
Mp(Mp1-Mp2)	Media Posterior (1-2)
Mp(Mp3-Mp4)	Media Posterior (3-4)
M	Medial
Cu	Cubital
CuA (CuA1-CuA2)	Cubitus Anterior (1-2)
CuB (CuB3-CuB4)	Base of Cubitus (3-4)
A (A1-A3)	Auxillary (1-3)
Rp-Mp	Radio-Medial cross vein

radius (4-5) represented by R(R4-R5), media anterior (1-2) represented by Ma(Ma1-Ma2), media posterior (1-2) represented by Ma(Ma1-Ma2), media posterior (3-4) represented by Mp(Mp3-Mp4), medial represented by “M”, cubital represented by “C”, cubitus anterior (1-2) Represented by CuA (CuA1 - CuA2), Base of cubitus (3-4) represented by CuB (CuB3-CuB4), auxillary (1-3) represented by A(A1-A3) and radio medial cross vein represented by (Rp-Mp). The wings venation of *Z. bicolorata* is analysed in two portions. The veins have been depicted via Numbers ranging from 1 to 36 (Fig. 3, Table 2, 3). The veins lying in the proximal part are as follows, 1 representing humeral plate’s proximal anterior point, 2 representing Costa and sub Costa’s crossing point, 3 representing sub Costa bifurcating into sub costa anterior and sub costa posterior, 4 representing crossing point of sub Costa posterior and radius anterior, 6 representing crossing

point radio medial cross vein and radius anterior, 23 representing anterior point of media cubital, cross vein , 24 representing crossing point of radio medial Cross vein and media posterior, 26 representing posterior point of media cubital cross vein, 27 representing point of anal anterior bifurcation, 28 representing point of anal anterior (1+2) almost joining with cubitus anterior (3+4), 29 representing anal anterior (3+4)’s posterior point, 30 representing medial Cross vein , 31 representing posterior part of anal anterior (1+2), 32 representing cubitus anterior (1+2) and media posterior 4’s anterior point, 33 representing medial cross vein’s distal point, 34 representing cubitus anterior (1+2) and media posterior 4’s posterior point, 35 representing anal posterior (3+4)’s base point and 36 representing posterior point of anal posterior (3+4). The veins lying in the distal part of the wing are as follows, 5 representing crossing point of sub costa anterior and radius anterior, 7 representing radial cell’s proximal anterior point, 8 representing radial cell’s distal anterior point, 9 representing radial cell’s distal posterior point, 10 representing radial Cross vein 4’s anterior point , 11 representing radial cell’s proximal posterior point, 12 representing radial Cross vein 3’s proximal point, 13 representing apical hinge point, 14 representing anterior point of triangular area of radial cells’ distal side, 15 representing posterior point of triangular area of radial cells’ distal side, 16 representing proximal point triangular area of radial cells’ distal side, 17 representing distal point of radius anterior four, 18 representing distal point of radius anterior one, 19 representing distal point of radius posterior two, 20 representing point of medial posterior (1+2) bifurcation, 22 representing proximal point of radial posterior and 25 representing posterior point of medial spur.

Ren *et al.* (2017), studied about the geometric morphometrics of flight wings (hind wings) found in leaf beetles. Sun and Bhushan (2012) analysed the mechanical and structural properties of beetle wings and how they aid in flight. Bethoux (2005) studied about the wing venation system of Plecoptera order and analysed their hind wings in detail. The analysis on the wing venation of hind wing of *Z. bicolorata* in all the samples collected throughout the region of Dehradun indicated as that

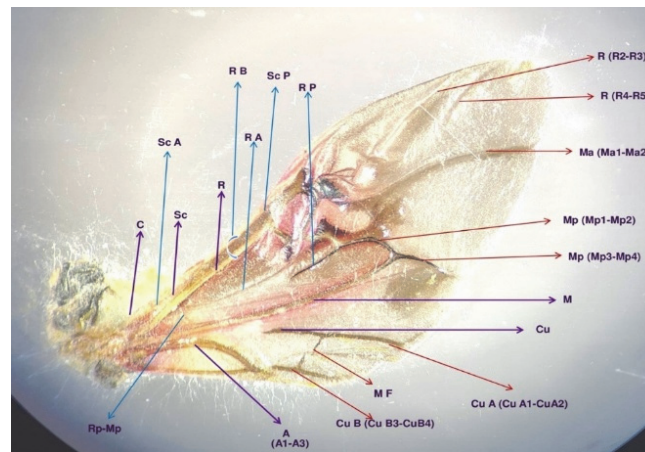


Fig. 1 Morphometric parameters studied in wing venation of *Z. bicolorata*

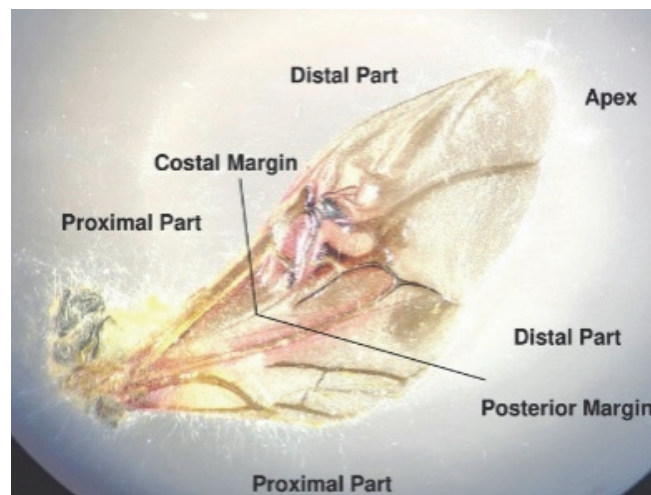


Fig. 2 Different regions in the wing venation of *Z. bicolorata*

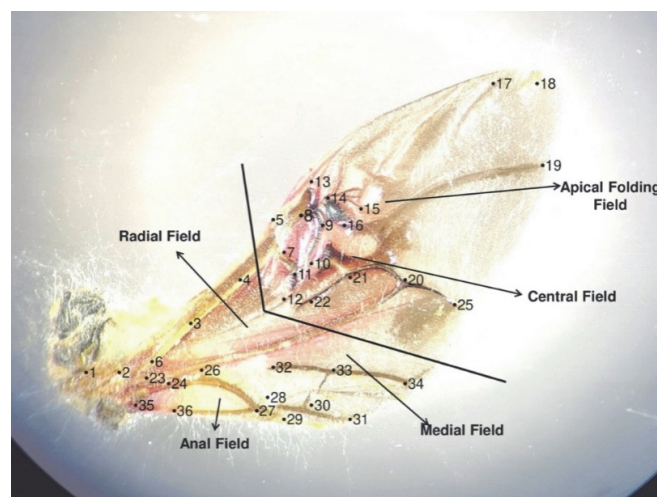


Fig. 3 Geometric morphometric analysis of wing venation of *Z. bicolorata*

Table 2. Description of vein coordinates marked in Fig. 3 veins in proximal part of the wing

coordinates	Vein
1	Humeral plate's proximal anterior point
2	costa and subcosta crossing point
3	Subcosta bifurcating into subcosta anterior and subcosta posterior
4	Crossing point of subcosta posterior and radius anterior
5	Crossing point of subcosta anterior and radius anterior
6	Crossing point of radiomedial cross vein and radius anterior
7	Radial cell's proximal anterior point
8	Radial cell's distal anterior point
9	Radial cell's distal posterior point
10	Radial cross vein 4's anterior point
11	Radial cell's proximal posterior point
12	Radial cross vein 3's proximal point
13	Apical hinge point
14	Anterior point of triangular area of radial cell's distal side
15	Posterior point of triangular area of radial cell's distal side
16	Proximal point of triangular area of radial cell's distal side
17	Distal point of radius anterior 4
18	Distal point of radius anterior 1
19	Distal point of radius posterior 2
20	Point of medial posterior (1+2) bifurcation
21	Posterior point of radial cross vein 4
22	Proximal point of radial posterior
23	Anterior point of media-cubital cross vein
24	Crossing point of radio-medial cross vein and media posterior
25	Posterior point of medial spur
26	Posterior point of media-cubital cross vein
27	Point of anal anterior bifurcation
28	Point of anal anterior (1+2) almost joining cubitus anterior (3+4)
29	Anal anterior (3+4)'s posterior point
30	Medial cross vein

31	Posterior point of anal anterior (1+2)
32	Cubitus anterior (1+2)'s anterior point of and media posterior 4
33	Medial cross vein's distal point
34	Cubitus anterior (1+2)'s posterior point and media posterior 4
35	Anal posterior (3+4)'s base point
36	Anal posterior (3+4)'s posterior point

all of them belonged to the same species *Z.bicolorata*. The study of hind wings of *Zygogramma bicolorata* has been done in accordance with the Comstock- Needham system (Comstock and Needham, 1898; Fraser, 1938). Similar studies have been done on the wing venation of other insects. Rattanawanne *et al.* (2010) and Hadis Gomeh *et al.* (2016) did a comparative study on honey bees by comparing different morphometric methods to differentiate between them in a given population. Namgung *et al.* (2022) analysed the wings of aphids by studying their morphometric features. Analysis of butterflies wing venation was done by Patil and Magdum (2017) in Gujrat region of India. Wahid *et al.* (2023) also studied about the morphology of butterfly wings belonging to the genus *Graphium*. Another remarkable study on the wing venation and shape of Diptera insects was done by Belyaev and Farisenkov (2019). Hind wings of *Plesispa reichei*, beetle found on coconut leaves were also studied by Manseguias *et al.* (2015). The wing venation of mosquito was also studied by Zhou *et al.* (1985) using their digitalized pictures and studied about their veins in detail. Understanding of the venation system is crucial in learning about the structural aspects of the hind wing.

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Evaluation of predation potency of predatory nematodes and the influence of organic amendments on nematode communities and growth of tomato

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ABSTRACT: In the experiment designed to evaluate the predation potency of predatory nematodes, *Iotonchus indicus* and *Mylonchulus minor* under laboratory condition against J₂ of *Meloidogyne incognita* (Kofoid and White) Chitwood as prey, mortality of J₂ was highest when fifteen numbers of predators were used. The mortality of *M. incognita* increased with increase in number of predators. Most effective predator was *M. minor*, when compared to *I. indicus*. Second experiment with soil amendments, showed that neem cake @ 50 g pot⁻¹ was the superior in reducing the final population of *M. incognita* (88.38%) followed by poultry manure @ 50 g pot⁻¹ (85.50%), castor cake @ 50 g pot⁻¹ (81.07%), saw dust @ 50 g pot⁻¹ (73.89%) and *Gliricidia* leaves @ 100 g pot⁻¹ (62.99%) compared to untreated. Similar trend was noticed with other plant parasitic nematodes. Neem cake was the most effective treatment in increasing the final population of *I. indicus* (333.33%), *M. minor* (278.90%) and predatory dorylaimids (125.00%) followed by poultry manure. Neem cake was the superior treatment in reducing the formation of root galls (11.25) and egg masses (7.75) in tomato plant infected with *M. incognita* followed by soil amendment with poultry manure. The shoot length (71.55cm), root length (12.93cm), shoot weight (23.15g) and root weight (6.88g) of tomato were highest when amended with neem cake followed by poultry manure. The increase in the population of predatory nematodes due to the effect of organic amendments might have reduced the population of plant parasitic nematodes and increased plant growth.

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KEY WORDS: *Mylonchulus minor*, *Iotonchus indicus*, *Meloidogyne incognita*, organic amendments, root galls, plant growth

INTRODUCTION

Tomato is an important vegetable crop grown worldwide. In India, it is cultivated in major agro ecological zone under an area of 0.846 million hectares with a production of 0.2 m t and yield of 24.0kg ha⁻¹ (Anonymous, 2023). Among the pests and diseases plant parasitic nematodes (PPN) are

important. Nair (1968) reported the occurrence of *Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Rotylenchulus reniformis*, and *Meloidogyne* sp. from the rhizosphere of tomato. Root knot nematodes (RKN) *Meloidogyne* spp. are highly pathogenic to wide array of crop plants due to their capacity to induce galls on roots. (Mekete *et al.*, 2003; Bouchtaoui *et al.*, 2025;

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Gupta *et al.*, 2025). Jain *et al.* (1994) reported yield loss due to infection of *M. incognita* (Kofoed and White) Chitwood to tomato to a tune of 71.9 per cent, whereas Chandan *et al.* (2022) reported it around 49.62 per cent.

Predatory nematodes are an important entity of the soil food web with biological control potential against plant parasitic nematodes (Bilgrami, 1997). Cobb (1917) reported the efficiency of *Mononchus* spp. as hunters and justified their use in biocontrol of PPN. Over 3126 species of dorylaimids, 432 species of mononchids and 4100 species of tylenchids were reported to occur all throughout the world. More than 200 species of PPN were reported from India. Bilgrami *et al.* (1984) studied the predation behaviour of adults and juveniles of *Mononchus aquaticus*, with *Chiloplacus symmetricus* or *Cephalobus* sp. as prey. Kulshrestha *et al.* (1993) reported that *Mylonchulus minor* feed on PPN by cutting the cuticle and shredding the prey in to pieces and the female nematodes were more vigorous predators compared to all other juvenile stages. The predator preferred to kill maximum number of J₂ of *M. incognita* compared to other nematodes. While PPNs pose considerable challenges for crop production, various nematode groups also play beneficial roles within soil ecosystems (Neher, 2001; Pires *et al.*, 2023; Semprucci *et al.*, 2025). Free-living nematodes, including bacterivores, fungivores, and omnivores contribute to soil nutrient cycling by decomposing organic matter and regulating microbial populations (Li *et al.*, 2024; Martin, 2025). Notably, predatory nematodes, such as *Mononchus* spp., have emerged as potential biological control agents against PPNs, including *Meloidogyne* juveniles (Khan and Kim, 2007; Kim, 2015; Shokoohi, 2024; Martin, 2025).

To enhance soil quality and support sustainable agriculture, soil amendments such as compost and soil organic matter incorporation are essential (Diacono and Montemurro, 2011; Matisic *et al.*, 2024). The addition of organic amendments can significantly increase soil fertility, improve soil structure, and boost microbial activity (Aytnew and Bore, 2020; Zhou *et al.*, 2025). Moreover, such practices promote the proliferation of beneficial

organisms, including predatory nematodes, which can help mitigate the effects of PPNs (Akhtar and Malik, 2000; Oka *et al.*, 2007; Thoden *et al.*, 2011; Malik *et al.*, 2025). Research shows that incorporation of compost can stimulate microbial antagonists that suppress nematode populations (Zhen *et al.*, 2014; Tothnee Bogdaanyi *et al.*, 2021; Karanastasi *et al.*, 2024). The use of nematicides was reported to be toxic to non-target soil organisms like earth worms (Wang *et al.*, 2024). Hence, the present investigation was undertaken to elucidate the predation potency of predatory nematodes distributed in Kerala on PPN and the influence of organic amendments on nematode communities in tomato.

MATERIALS AND METHODS

Potential of predatory nematodes against *M. incognita* under laboratory conditions: Culture of *M. incognita* was maintained on tomato plants in pots in net house conditions. Freshly hatched juveniles were used for the inoculation. The juveniles were counted under a stereo zoom binocular microscope, Zeiss Stemi 508, by dilution method and the required number was inoculated. Predatory nematodes *I. indicus* and *M. minor* were extracted from rhizosphere of brinjal plants (8.431147°N; 76.9906167°E) infected with *M. incognita* grown in the C block of Instructional Farm, College of Agriculture, Vellayani by Cobb's sieving and decanting technique followed Baermann's funnel technique. Sufficient numbers of the predators thus collected were surface sterilized using 0.06 per cent NaOCl. The surface sterilized nematodes were washed in sterile distilled water repeatedly for more than five times to remove the traces of NaOCl and used for further laboratory assay.

A laboratory experiment was conducted in Petri plates containing 2mm thick 1.0 per cent water agar. The predatory nematodes *I. indicus* and *M. minor* were introduced in to the surface of agar plates @ 0 (no predatory nematode - check), 5, 10 and 15. Freshly hatched 25 J₂ of *M. incognita* were introduced to the agar filled Petri plates and incubated at room temperature. Four replications of each treatment were maintained. The

observations on dead prey nematodes were recorded after 24, 48, 72, and 96 hours of exposure to the predators. The experiment was laid out in CRD design. The per cent mortality of J_2 of *M. incognita* was computed and the data was subjected to statistical analysis using ANNOVA by using RAISINS software.

Effect of organic amendments on the population of predatory nematodes and growth of tomato infected with *M. incognita*:

A pot culture experiment was conducted to evaluate the effect of organic amendments on the population of predatory nematodes and growth of tomato infected with *M. incognita* under glass house conditions of Department of Nematology, College of Agriculture, Vellayani. The pots were filled with 1 kg soil collected from nematode sick plots of vegetable ecosystem. Tomato seedlings cv. Anagha (30 days old) was planted in the pots. A total of five treatments and an untreated control (Table 2) was laid out in CRD design and replicated four times. Treatments were applied 15 days prior to planting. Initial population of nematodes including different predatory nematodes, PPN and rhabditids were estimated. Plant growth including shoot length (cm), root length (cm), shoot weight (g) and root weight (g) were recorded 45 days after transplanting. The final population of different nematodes including J_2 of *M. incognita*, predatory and rhabditids were estimated from 200 cm³ soil⁻¹ collected from different treatments and untreated control plants. The nematodes were extracted by Cobb's sieving and decanting technique followed Baermann's funnel technique. Data were subjected to analysis of variance (ANOVA) and the means were compared with critical difference. All calculations were performed with the RAISINS (R and AI Solutions in Inferential Statistics) software available online at the website of www.raisins.live (Hisham *et al.* 2025)

RESULTS AND DISCUSSION

Predation potential of predatory nematodes:

All the treatments with *I. indicus* were effective in increasing the mortality of J_2 of *M. incognita*. After 24 h of exposure @ 15 Petri plate⁻¹ showed

increasing the mortality of *M. incognita* (28.00%) followed by @ 10 Petri plate⁻¹ (14.00%) and @ 5 Petri plate⁻¹ (5.00%), with no mortality in the control. After 48 hours of exposure @ 15 Petri plate⁻¹ recorded highest mortality (76.00%), followed by @10 (31.00%) and 5 (11.00%) numbers of *I. indicus* Petri plate⁻¹. In general, after 72 h all the treatments were significantly effective in increasing the mortality compared to control; @ 15 Petri plate⁻¹ showed maximum mortality (83.00%), while the lowest was in 5 numbers Petri plate⁻¹ (17.00%), when compared to control (0.00 %). After 96 h all the treatments were statistically significant in killing the prey nematodes, @15 Petri plate⁻¹ the mortality was maximum (87.00%), followed by @10 Petri plate⁻¹ (59.00%) and the lowest @ 5 Petri plate⁻¹ (19.00 %) (Table 1).

With *M. minor* as a predator all the treatments were significant in the mortality of *M. incognita*. After 24 h of exposure, @ 15 Petri plate⁻¹ exhibited highest mortality (45.00%) followed by @ 10 Petri plate⁻¹ (16.00 %), with least predation @ 5 Petri plate⁻¹ (5.00 %) and no predation in control (0.00 %). After 48 h 15 Petri plate⁻¹ showed highest mortality (68.00%) followed by @10 (38.00%) numbers Petri plate⁻¹ with no predation in control (0.00 %). After 72 h, all the treatments were significantly effective in increasing the mortality At 15 Petri plate⁻¹ was the superior treatment (81.00%) and the lowest mortality in @5 Petri plate⁻¹ (21.00 %). After 96 hours, all the treatments were statistically significant in killing the prey nematodes. At 15 Petri plate⁻¹ the mortality was maximum (89.00%), and the least in 5 Petri plate⁻¹ (27.00%), with no predation in control (0.00 %). Predation potentiality was more in *M. minor* than *I. indicus* (Table 1).

Similar finding was reported by Phonginsee *et al.* (2025) who found that *Mylonchulus hawaiiensis* was an active predator of J_2 of *M. enterolobii*, with an average daily consumption of 16.1 J_2 s and a cumulative total consumption of 80 J_2 s over five days. Kulshrestha *et al.* (1993) reported that *M. minor* preyed on *M. incognita* by cutting the cuticle and shredding it into pieces. Jairajpuri and Azmi (1978) reported that juveniles of *Meloidogyne* sp.

were preyed up on by *Mylonchulus dentatus*. Cohn and Mordechai (1974) reported the predation of *M. javanica* by *Mylonchulus sigmaturus*. Koohkan and Shokohi (2014) found that *M. javanica* was an excellent source of prey for mass culture of *M. sigmaturus* compared to other prey species. Mohandas and Prabhoo (1980) reported that *I. kherai* and *I. monhystera* preferred *M. incognita* and *R. reniformis*. Azmi (1983) showed that *I. monhystera* was well adapted for predacious mode of feeding of *H. dihystra*. Single *I. monhystera* devoured on an average of 74 to 81 per cent J_2 of *M. javanica* (Azmi, 1992). Wang et al. (2015) studied the predatory behavior of *Mononchus* sp. and *Neoactinolaimus* sp. by employing *M. incognita* and *R. reniformis* as prey. Cabos et al. (2013) found that *Mononchus* sp. and other predatory fauna preyed on *M. incognita*, *R. reniformis* and *Radopholus similis*. A single juvenile of *Iotonchus* sp. from rhizosphere of mango was reported to have *Helicotylenchus digonicus* in its gut (Khan and Jairajpuri, 1981).

Effect of organic amendments on the population of PPN: Neem cake @ 50 g pot⁻¹ recorded 24.25 J_2 200 cm³ soil⁻¹ and was superior in reducing the final population of J_2 of *M. incognita* (88.38%). It was followed by the poultry manure @ 50 g pot⁻¹ (with 30.25 J_2 200 cm³ soil⁻¹ and 85.5% reduction of population). Next best

treatments in the order of effectiveness in the population and in reducing the population were, castor cake @ 50 g pot⁻¹ (39.50 J_2 200 cm³ soil⁻¹; 81.07%), followed by saw dust @ 50 g pot⁻¹ (54.50 J_2 200 cm³ soil⁻¹; 73.89%). *Gliricidia* leaves @ 100 g pot⁻¹ was least effective (77.25 J_2 200 cm³ soil⁻¹; 62.99%) in decreasing the population of *M. incognita* compared to untreated control (208.75 J_2 200 cm³ soil⁻¹) (Table 2).

In general, all the organic amendments treatments were significant in the reduction of *H. dihystra*. Neem cake @ 50 g pot⁻¹ was the most effective in reducing the population of *H. dihystra* to 5.75 nematodes 200 cm³ soil⁻¹ (86.39% reduction), followed by poultry manure @ 50 g pot⁻¹ (7.75 nematodes 200 cm³ soil⁻¹, 81.65% reduction), castor (11.50 nematodes 200 cm³ soil⁻¹, 72.78% reduction, saw dust (14.75 nematodes 200 cm³ soil⁻¹, 65.08% reduction). *Gliricidia* leaves @ 100 g pot⁻¹ was least effective in the reduction of *H. dihystra* (23.00 nematodes 200 cm³ soil⁻¹; 45.56%) compared to untreated control (42.25 nematodes 200 cm³ soil⁻¹) (Table 2).

All the treatments were significant in the reduction in the population of *R. reniformis*. Neem cake @ 50 g pot⁻¹ was the most effective (with only 5.50 nematodes 200 cm³ soil⁻¹) in reducing the population (82.25 % over control), followed by poultry manure

Table 1 Predatory potential of *Iotonchus indicus* and *Mylonchulus minor* on J_2 of *M. incognita*

<i>I. indicus</i> (no.)	Mortality % of <i>M. incognita</i> after - hours of exposure*			
	24	48	72	96
0	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d
5	5.00 ± 2.00 (0.25) ^c	11.00 ± 6.00 (0.35) ^c	17.00 ± 3.83 (0.44) ^c	19.00 ± 3.83 (0.46) ^c
10	14.00 ± 4.00 (0.39) ^b	31.00 ± 5.03 (0.60) ^b	45.00 ± 9.45 (0.74) ^b	59.00 ± 6.00 (0.89) ^b
15	28.00 ± 5.66 (0.57) ^a	76.00 ± 0.91 (1.10) ^a	83.00 ± 10.00 (1.17) ^a	87.00 ± 7.57 (1.23) ^a
<i>M. minor</i>				
0	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d	0.00 ± 0.00 (0.10) ^d
5	5.00 ± 2.00 (0.25) ^c	17.00 ± 2.00 (0.44) ^c	21.00 ± 2.00 (0.49) ^c	27.00 ± 3.83 (0.56) ^c
10	16.0 ± 5.66 (0.42) ^b	38.00 ± 7.66 (0.67) ^b	62.00 ± 7.66 (0.92) ^b	77.00 ± 9.45 (1.09) ^b
15	45.00 ± 8.25 (0.74) ^a	68.00 ± 5.66 (0.42) ^a	81.00 ± 3.83 (1.13) ^a	89.00 ± 3.83 (1.25) ^a

Table 2 Effect of organic amendments on the population (200 cm³ soil⁻¹) of PPN on tomato

Treatments @ - g pot ⁻¹	<i>J₂</i> of <i>M. incognita</i>		<i>Helicotylenchus dihystra</i>		<i>Rotylenchulus reniformis</i>	
	Initial	Final	Initial	Final	Initial	Final
Neem cake @ 50	226.75±3.50 (15.06)	24.25± 3.30 (4.92) ^a	46.75± 2.22 (6.84)	5.75± 0.96 (2.39) ^a	35.25± 3.30 (5.94)	5.50± 0.58 (2.34) ^a
Castor cake @ 50	228.25±4.92 (15.11)	39.50 ± 3.42 (6.28) ^c	46.50 ± 1.73 (6.82)	11.50± 1.73 (3.38) ^c	35.00 ± 1.41 (5.92)	8.50 ± 1.29 (2.91) ^c
Saw dust @ 50	228.50±6.95 (15.11)	54.50 ± 4.20 (7.38) ^d	45.25 ± 2.22 (6.73)	14.75±1.71 (3.84) ^d	35.50 ± 2.08 (5.96)	12.00±1.41 (3.46) ^d
Poultry manure @50	228.00±3.56 (15.10)	30.25 ± 3.30 (5.49) ^b	46.25 ± 0.50 (6.80)	7.75 ± 0.96 (2.78) ^b	35.75± 1.71 (5.98)	6.50 ± 0.58 (2.55) ^b
<i>Gliricidia</i> leaves @100	227.75±4.79 (15.09)	77.25 ± 4.79 (8.79) ^c	45.75 ± 2.06 (6.76)	23.00± 0.82 (4.80) ^c	34.75± 1.26 (5.89)	16.75±1.50 (4.09) ^c
Untreated	226.75 ± 7.09 (15.06)	208.75 ± 4.86 (14.45) ^f	45.75 ± 1.71 (6.76)	42.25± 1.50 (6.50) ^f	35.25 ± 1.50 (5.94) ^f	31.00±1.63 (5.57) ^f
CD	NS	(0.41)	NS	(0.28)	NS	(0.25)

Figures in the parenthesis are square root transformed values; *Mean ± SD

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

(6.50 nematodes 200 cm³ soil⁻¹, 79.03 % reduction over control). castor treatment recorded only 8.50 nematodes 200 cm³ soil⁻¹, showing 72.58% reduction, while saw dust treatment recorded 12.00 nematodes 200 cm³ soil⁻¹, with 61.29% reduction of population. *Gliricidia* leaves @100 g pot⁻¹ (16.75 nematodes 200 cm³ soil⁻¹) was least effective in the reduction (45.96 % reduction over control). Untreated control recorded 31.00 nematodes 200 cm³ soil⁻¹ (Table 2).

Similarly, Akhtar (1998) reported that treatment involving neem cake was the most effective treatment in reducing the population of *H. dihystra*, *R. reniformis* and *M. incognita* infected chick pea. In contrast, treatment of neem leaf powder in the rhizosphere of chick pea increased the populations of predatory nematode, *Dorylaimus elongatus* and other free-living nematodes. Khan *et al.* (1974) reported that neem cake and castor cake significantly reduced the population of *R. reniformis* in brinjal. Phenolic contents of neem cake and castor cake were toxic to *R. reniformis* (Alam, *et al.* 1981). Water soluble fractions of oil cakes extracted from neem, mahua, groundnut and castor were toxic to nematodes like *Hoplolaimus indicus*, *R. reniformis* and *Tylenchorynchus*

brassicae and *M. incognita* (Khan *et al.* 1974). Aqueous extracts of neem seed and neem cake used as root dip treatments were reported to be superior treatments in the mitigation of *M. incognita* infection in tomato (Vijayalakshmi, 2000).

Effect of organic amendments on the population of predatory nematodes: All the treatments were significant in enhancing the population of *I. indicus*. However, soil treatment with neem cake @ 50 g pot⁻¹ (19.50 nematodes 200 cm³ soil⁻¹) was the most effective in increasing the final population of *I. indicus* (333.33% increase over the untreated control), followed by poultry manure @ 50 g pot⁻¹ (14.00 nematodes 200 cm³ soil⁻¹, 211.10 per cent increase over the untreated control). castor treatment showed 150.00 per cent increase over the untreated control, while saw dust treatment recorded 77.7 per cent increase over the untreated control. Lowest population of *I. indicus* was recorded in *Gliricidia* leaves treatment (6.50 nematodes 200 cm³ soil⁻¹, 44.40% increase over the untreated control).

In the case of *M. minor* (Table 3) also amendment with neem cake @ 50 g pot⁻¹ (18.00 nematodes 200 cm³ soil⁻¹) was the most effective treatment in

Table 3 Effect of organic amendments on the population (200 cm³ soil⁻¹)* of predatory nematodes on tomato

Treatments @ - g pot ⁻¹	<i>Iotonchus indicus</i>		<i>Mylonchulus minor</i>		<i>Dorylaimid</i>		<i>Rhabditids</i>	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Neem cake @50	7.50± 0.58 (2.74)	19.50 ± 0.58 (4.42) ^a	6.75± 0.96 (2.59)	18.00 ± 0.82 (4.24) ^a	11.25 ± 2.63 (3.34)	24.75±0.96 (4.97) ^a	70.75±5.80 (8.67)	95.25±5.05 (9.75) ^b
Castor cake @50	8.00 ± 0.82 (2.83)	11.25 ± 0.96 (3.35) ^c	8.25 ± 1.26 (2.87)	12.25 ± 0.96 (3.50) ^c	11.00 ± 1.83 (3.31)	21.00±1.41 (4.58) ^c	55.00±4.96 (8.40)	96.25±2.63 (9.81) ^b
Saw dust @50	7.50 ± 1.29 (2.73)	8.00 ±0.82 (2.38) ^d	7.00 ± 0.82 (2.64)	7.50 ± 1.29 (2.73) ^d	10.50 ± 1.29 (3.24)	14.25±0.96 (3.77) ^d	60.25±4.78 (8.38)	69.00±3.36 (8.30) ^d
Poultry manure @50	8.00 ± 0.82 (2.83)	14.00± 1.15 (3.74) ^b	7.00 ± 0.82 (2.64)	15.25 ± 0.96 (3.90) ^b	10.75 ± 0.96 (3.28)	22.75±0.96 (4.77) ^b	70.25±4.78 (7.75)	105.25±6.23 (10.25) ^a
<i>Gliricidia</i> leaves @100	6.50 ± 1.00 (2.54)	6.50 ± 0.58 (2.55) ^e	6.50 ± 0.58 (2.55)	7.00 ± 0.82 (2.64) ^d	10.25 ± 0.50 (3.20)	12.25±3.50 (7.05) ^e	75.25±6.07 (7.41)	85.25±5.56 (9.23) ^c
Untreated	7.50 ± 0.58 (2.74)	4.50 ± 0.58 (2.12) ^f	7.75 ± 0.50 (2.78)	4.75 ± 0.50 (2.18) ^e	12.00 ± 2.83 (3.45)	11.00±3.50 (5.94) ^f	23.75±2.63 (4.86)	29.75±4.03 (5.44) ^e
CD	NS	(0.19)	NS	(0.22)	NS	(0.17)	NS	(0.40)

Figures in the parenthesis are square root transformed values; *Mean ± SD of four replications;

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

increasing (278.9%) the population of *M. minor*. It was followed by poultry manure (15.25 nematodes 200 cm³ soil⁻¹, with increase of 221.05%). Castor treatment recorded 157.89 per cent increase over control, while in the case of saw dust it was 57.89 per cent. Lowest population of *M. minor* was recorded in *Gliricidia* treatment (7.00 nematodes 200 cm³ soil⁻¹, with only 47.36% increase). Untreated control recorded only 4.75 nematodes 200 cm³ soil⁻¹. However, the treatments saw dust and *Gliricidia* leaves were on par.

For predatory dorylaimids soil treatment with neem cake (24.75 nematodes 200 cm³ soil⁻¹) was the superior in increasing the population of dorylaimids (125.00% increase over control), followed by poultry manure (22.75 nematodes 200 cm³ soil⁻¹, 106.81% increase over control). Castor cake treatment recorded 90.90 per cent increase of dorylaimids over control, while in the case of saw dust it was only 29.54 per cent. Dorylaimids population was low in *Gliricidia* leaves treatment (12.25 nematodes 200 cm³ soil⁻¹, 11.36% increase over control). Untreated control recorded 11.00 nematodes 200 cm³ soil⁻¹ (Table 3). Application of castor cake and *Gliricidia* leaves supported more

density of *M. minor* (157.89 and 47.36% respectively) than *I. indicus* (150.00 and 44.40% respectively). This is in accordance with Choudhury and Sivakumar (1997) findings that neem cake, castor cake, mahua cake and groundnut cakes @ 5 g pot⁻¹ encouraged the multiplication of *M. minor* and mitigated the density of *H. dihystra* on tomato. Nisha and Nimisha (2017) found that soil incorporation of *G. maculata* @ 5kg pit⁻¹ at planting of banana reduced the population of *M. incognita* and *P. coffeae* and increased the population of predatory nematode, *Eudorylaimus* sp.

Organic amendments have enhanced the population of predatory nematodes (neem cake 207.40%; poultry manure 156.79%; castor cake 119.75%; saw dust 46.91%; *Gliricidia* leaves 27.16%). However, it reduced the population of PPN (neem cake 87.50%; poultry manure 84.21%; castor cake 78.90%; saw dust 71.18%; *Gliricidia* leaves 58.51%) (Fig. 1).

Poultry manure @ 50 g pot⁻¹ was the superior in enhancing the population of rhabditids (105.25 nematodes 200 cm³ soil⁻¹), followed by castor cake

@ 50 g pot⁻¹, neem cake @ 50 g pot⁻¹, *Gliricidia* leaves @ 100 g pot⁻¹ and saw dust @ 50 g pot⁻¹ compared to untreated control. However, the application of castor cake and neem cake were statistically on par. In the case of bacterivorous rhabditids, application of poultry manure @ 50 g pot⁻¹ increased the population to a tune of 253.78 per cent, followed by castor cake (223.52%), neem cake (220.16%), *Gliricidia* leaves (186.55 %) and saw dust (131.93%) compared to control (Table 3). Hunt *et al.* (1973) found that population of cephalobids and rhabditids were highest in the soil receiving 32 t ha⁻¹ of compost in oats and sorghum grown in Florida. Organic amendments not only create better habitats for nematodes and facilitate their movement through soil pore water, but also improve the ability of nematodes to capture abundant food resources (bacteria) due to the increased soil organic matter (Liu *et al.*, 2016; Jiang *et al.*, 2018). The higher values of enrichment index (EI), structure index (SI), and maturity index (MI) indicated a more mature and stable food web structure, while the low value of channel index indicated dominant bacterial decomposition pathways under organic material amendments (Shi *et al.*, 2023).

Effect of organic amendments on number of galls and egg masses: Neem cake @ 50 g pot⁻¹ (11.25) was the superior treatment in reducing the formation of root galls in tomato plant infected with *M. incognita*, followed by soil amendment with poultry manure @ 50 g pot⁻¹ (14.0). Treatment with *Gliricidia* leaves @ 100 g pot⁻¹ (26.50) was least effective, compared to untreated control (58.50). Gall index was low in all treatments (index 2). The gall index was three in untreated control. Neem cake @ 50 g pot⁻¹ was the superior in reducing the egg masses of *M. incognita* (7.75), followed by poultry manure @ 50 g pot⁻¹ (9.25). Lowest reduction was noticed in *Gliricidia* leaves (19.50) when compared to untreated control (40.75) (Table 4). Even though treatment involving mulching of *Gliricidia* leaves @ 100 g pot⁻¹ was the least effective in reducing the population of plant parasitic nematodes and enhancing the population of predatory forms, this treatment was significantly effective compared to untreated control.

Pakeerathan *et al.* (2009) recorded that the number of galls produced by *M. incognita* was lowest (35.87) in *G. maculata* @ 25 t ha⁻¹ treated tomato crop compared to control. Nimisha and Nisha (2019) reported that mulching with green leaves of *G. maculata* @ 5 kg pit⁻¹ was equally effective in reducing the population of plant parasitic nematodes. Incorporation of organic amendments and green leaves in soil resulted in an increase in organic matter, water holding capacity and nutrient status of soil which resulted in increased yield of banana. Nisha and Sheela (2002) reported the anthelmintic property of *G. maculata*.

In the present findings (Fig. 11), all the treatments significantly reduced number of root galls formed and egg masses of *M. incognita*. Neem cake @ 50 g pot⁻¹ was the superior treatment in reducing the formation of root galls (80.76%) followed by soil amendment with poultry manure @ 50 g pot⁻¹ (76.06%), castor cake (70.51%), saw dust (62.82%) and *Gliricidia* leaves (54.70%). Similar trend was noticed with number of egg masses. Neem cake was the best treatment in reducing the number of egg masses of *M. incognita* (80.98%) followed by poultry manure (77.33%), castor cake (69.32%), saw dust (60.12%) and *Gliricidia* leaves (52.14%). Similarly, Sidhu and Kanwar (2020) reported that all the treatments including neem cake, poultry manure and the predatory nematode *Fictor composticola* were significantly effective in reducing the gall formation in roots compared to untreated control. The same work also highlighted that minimum egg masses were recorded in case of treatment with *F. composticola* (400 individuals kg soil⁻¹) + poultry manure 5.0 g pot⁻¹ + neem cake 5.0 g pot⁻¹ + neem leaves 5.0 g pot⁻¹ when added to rhizosphere of cucumber infected with *M. incognita*. It was also reported that chemicals like ammonia (Khan *et al.*, 1974) and fatty acids (Sitaramaiah and Singh, 1978) liberated during the decomposition of neem cake could be one of the factors involved in nematode control. Application of poultry manure @ 4t ha⁻¹ significantly reduced both root galling and final nematode population of root knot nematode and significantly increased yield and growth of carrot (Kankam *et al.*, 2015). Prakash and Singh (2014) reported that minimum

Table 4 Effect of predatory nematodes on number of galls, egg masses in tomato infected with *M. incognita* treated with organic amendments

Treatments@ - g pot ^l	No. of galls(5g plant ⁻¹)*	Gall index	No. of egg masses(5g plant ⁻¹)*
Neem cake @50	11.25 ± 1.50 (3.35) ^a	1.00	7.75 ± 1.26 (2.78) ^a
Castor cake @50	17.25 ± 1.50 (4.15) ^c	1.00	12.50 ± 1.29 (3.53) ^c
Saw dust @50	21.75 ± 1.71 (4.66) ^d	1.00	16.25 ± 0.96 (4.03) ^d
Poultry manure@50	14.00 ± 0.82 (3.74) ^b	1.00	9.25 ± 0.96 (3.04) ^b
<i>Gliricidia</i> leaves@100	26.50 ± 1.29 (5.15) ^e	2.00	19.50 ± 1.29 (4.41) ^e
Untreated	58.50 ± 2.52 (7.65) ^f	3.00	40.75 ± 2.50 (6.38) ^f
CD	(0.25)	-	(0.26)

Figures in parenthesis are square root transformed values; *Mean ± SD of four replications;

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

Table 5 Effect of predatory nematodes on the growth parameters of tomato infected with plant parasitic nematodes treated with organic amendments

Treatments @ - g pot ^l	Shoot length (cm)*	Root length (cm)*	Shoot weight (g)*	Root weight (g)*
Neem cake @50	71.55 ± 4.00 ^a	12.93 ± 0.91 ^a	23.15 ± 1.71 ^a	6.88 ± 0.10 ^a
Castor cake@50	54.38± 3.49 ^c	11.35 ± 0.19 ^{bc}	18.79 ± 0.25 ^{bc}	6.46 ± 0.04 ^b
Saw dust @50	51.67 ± 4.24 ^c	10.80 ± 0.18 ^c	17.36 ± 0.23 ^c	6.17 ± 0.02 ^c
Poultry manure@50	64.38 ± 3.27 ^b	11.88 ± 0.17 ^a	19.63 ± 0.33 ^b	6.50 ± 0.05 ^b
<i>Gliricidia</i> leaves@100	50.27 ± 3.55 ^c	9.60 ± 0.18 ^d	15.61 ± 0.13 ^d	4.61 ± 0.04 ^d
Untreated	42.98 ± 3.62 ^c	6.78 ± 0.42 ^c	11.18 ± 1.72 ^c	2.45 ± 0.33 ^c
CD	(0.35)	(0.37)	(1.14)	(0.21)

Figures in the parenthesis are square root transformed values; *Mean ± SD of four replications;

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

number of galls in tomato infected with *M. javanica*, when saw dust was amended in. Sidhu and Kanwar (2024) found that combined application of castor cake @ 15.0 g kg soil⁻¹ + *F. composticola* (400 individuals kg soil⁻¹) was significantly effective in reducing the number of galls and egg masses of *M. incognita* in cucumber plants. This is in accordance with the present findings where castor cake @ 50 g pot⁻¹ was effective in reducing galls and egg masses on tomato plants infected with *M. incognita*. Castor cake contains a lectin (ricin) which inhibits the mobility of *M. incognita* at relatively high concentrations (Rich *et al.*, 1989).

Effect of organic amendments on the growth of tomato infected with nematodes: There was significant variation among treatments on biometric characters of tomato plants at harvest (Table 5). The shoot length of tomato infected with nematodes, was maximum in neem cake treatment (71.55cm) and was statistically superior to other treatments. Poultry manure (64.38cm) was the next best treatment. Treatment with *Gliricidia* leaves (50.27cm) was the least effective compared to untreated control (42.98cm). However, the treatments like castor cake, sawdust and *Gliricidia* leaves treatments were on par in their effect.

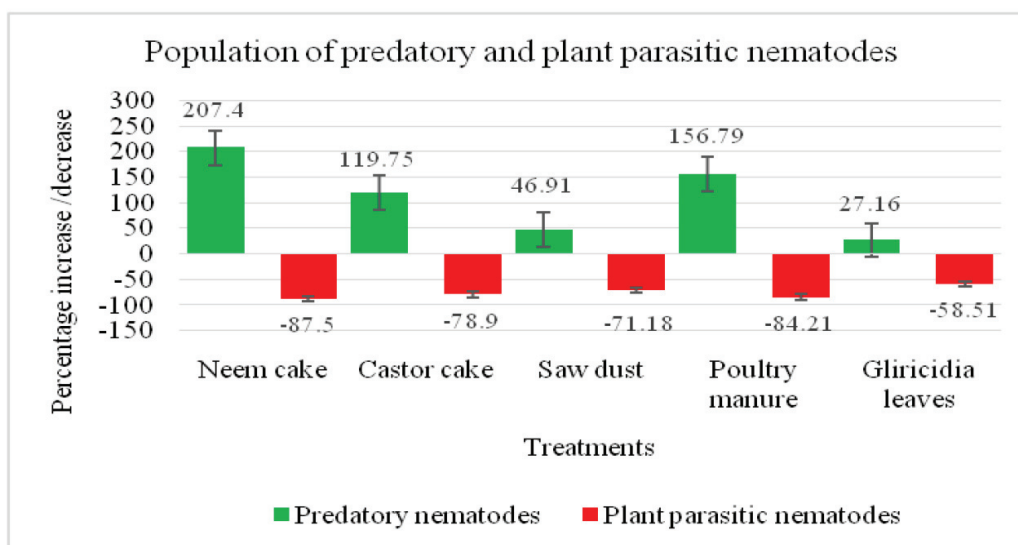


Fig. 1 Influence of organic amendments on nematode communities

The root length of tomato in neem cake treatment was maximum (12.93cm), followed by the poultry manure (11.88cm). Both the treatments were statistically on par. Soil amended with *Gliricidia* leaves (9.60cm) exhibited the lowest root length compared to the untreated control (6.78cm). Perusal of the data on the shoot weight of tomato plants revealed that neem cake (23.15g) was the most effective, followed by the poultry manure (19.63 g). However, application of poultry manure and castor cake was on par. *Gliricidia* leaves showed the lowest shoot weight (15.61g) compared to the untreated control (11.18 g). Neem cake @ 50 g pot⁻¹ (6.88g) was the best treatment in increasing the shoot weight of tomato plants infected with nematodes followed by the treatment with poultry manure @ 50 g pot⁻¹ (6.50 g). The treatments, poultry manure @ 50 g pot⁻¹ and castor cake @ 50 g pot⁻¹ were on par. Soil amended with *Gliricidia* leaves @ 100 g pot⁻¹ (4.61g) showed the lowest root weight compared to the untreated control (2.45g).

Among the treatments, neem cake was the most effective, resulting in an increase in shoot length (66.47%), in root length (90.70%), in shoot weight (107.06%), and in root weight (180.80%), followed by poultry manure (shoot length 49.79%, root length 59.29%, shoot weight 55.27%, root weight 165.30%), castor cake (shoot length 26.52%, root

length 40.26%, shoot weight 112.24%, root weight 163.60%), sawdust (shoot length 20.21%, root length 67.40%, shoot weight 68.06%, root weight 151.00%). *Gliricidia* leaves recorded relatively lower increase (shoot length 16.96%, root length 41.59%, shoot weight 39.62%, root weight 88.16%). These findings are in agreement with the observation of Sidhu and Kanwar (2024) who reported that combined application of *F. composticola* @ 400 individual kg soil⁻¹+ neem cake @ 15g kg soil⁻¹ enhanced plant height (21.63cm), shoot weight (14.91g) and root weight (3.79g) of cucumber infected with *M. incognita* compared to untreated (8.07cm) followed by the treatment with chicken manure @ 15g kg soil⁻¹. Similarly, Akhtar (1998) reported that treatment with neem oil cake significantly improved the dry weight, plant height and number of pods on chickpea plants infected with nematodes.

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Correlating the pheromone-trapped adults of *Spodoptera frugiperda* (J. E. Smith), with the weather parameters in maize fields of Assam, India

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ABSTRACT: Assam, a north-eastern Indian state, has experienced the invasion of fall armyworm (FAW) in recent years. The correlation study of weather factors with the adult population was undertaken with the adults caught in the pheromone traps installed in maize fields, of 3 districts of Assam during 2023-24. There was a positive correlation with the maximum temperature in 4 out of 9 occasions, and with minimum temperature in 7 out of 9 occasions of seasonal correlations across the districts, which led to a positive correlation ($r=0.331$ and $r=0.459$, respectively); however, the effects of minimum temperature on the adult population are more pronounced. Rainfall was positive, but insignificant. Three linear models were developed by the multiple linear regression analysis for weekly adults trapped against these weather parameters for two seasons, as well as their pooled data. All models are statistically significant, and the key factor contributing to variation in weekly trap catch was the temperature variable. The Durbin-Watson value of each season was near 2.0, which indicated that no serious autocorrelation in residuals occurred. These models will help in predicting the pests in the future.

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KEY WORDS: C-strain, adult catches, temperature, linear models, Durbin-Watson

INTRODUCTION

Maize (*Zea mays* L.), is an important staple food crop in India. Siddiqui and Marwaha (1993) reported 12 species as the serious pests of maize in India; however, the recent invasion of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), is a great concern since the pest can cause damage to more than 180 crop species globally (Casmur *et al.*, 2010). FAW is native to America and has recently invaded the

different states of India (Sharanabasappa *et al.*, 2018; Ali *et al.*, 2018; Sisodiya *et al.*, 2018; Babu *et al.*, 2019; Chormule *et al.*, 2019; Maruthadurai and Ramesh, 2019; Meena *et al.*, 2019; Ankita *et al.*, 2020; Kerketta *et al.*, 2020; Patel *et al.*, 2020; Sharma and Sharma, 2020; Suby *et al.*, 2020; Vishwakarma *et al.*, 2020; Paschapur *et al.*, 2021; Soujanya *et al.*, 2024) including North Eastern States (Firake *et al.*, 2019; Sarma *et al.*, 2023). Because of its capability of high migration, FAW has now attained a global pest status (FAO, 2017;

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IPPC, 2021). The pest is predicted to cause up to 53 per cent annual loss in maize (Day *et al.*, 2017) but in India, yield reduction was reported up to 33 per cent (Aruna *et al.*, 2019) and 33-36 per cent (Jagdish *et al.*, 2019); with a predicted annual loss up to 75,000 tonnes (Suby *et al.*, 2020).

Since the long-distance dispersal of FAW mostly occur through winged adults (Garcia *et al.*, 2018), pheromone trap work is an effective tool for monitoring the adults (Akter *et al.*, 2025). FAW relies on the long-distance migration to move to more suitable climates (FAO, 2020), where temperature is a prime mover. North East Region of India is one of the least investigated regions, even after recommendation of pheromone trap for monitoring the FAW (Suby *et al.*, 2020; Nwanze *et al.*, 2021). The study on pheromone assisted trapping at farmers' field in scanty (Sarma *et al.*, 2024). The impacts of weather on FAW have been studied in different parts of India (Anandhi *et al.*, 2020; Ravi and Waghmare, 2021; Ratnam *et al.*, 2023; Prasad *et al.*, 2022; Haroli *et al.*, 2023). In this context, an attempt was made to study the adult population caught in the pheromone traps (installed in farmers' maize fields during 2023-24), based on the prevailing temperature (minimum and maximum) and rainfall in Assam.

MATERIALS AND METHODS

The experiments were conducted in 7-8 maize fields in each of the three districts of North Bank Plain Zone (NBPZ) of Assam, viz., Udalguri, Sonitpur and Biswanath Chariali. The fields of progressive farmers were selected as per the records of the concerned District Agricultural Office. Sonitpur district is located between other two districts and the average distance from the experimental plots of Sonitpur to that of Biswanath is about 43km (towards east) and to that of Udalguri is about 44 km towards west. The coordinates of the centrally located sites of each district are 26.768033°N; 92.098642°E (Udalguri), 26.69376°N; 92.55397°E (Sonitpur), and 26.79393°N; 92.97355°E (Biswanath). The crops were grown under the rainfed production systems and pest data were taken in the same standard meteorological weeks across the locations. The FAW adults were

monitored for 5 weeks a season by 5 pheromone traps installed in each maize field of about 1.5 bigha (0.2 ha) at a height of 1.5m, since trapping of more adults has been reported by Nwanze *et al.* (2021) at this height. Monitoring began at the early whorl stage of the crop, as this stage has been reported as the most preferred stage for oviposition (Harrison *et al.*, 2019) and the observation lasts for 5 weeks per season. The pheromone lures manufactured by Pheromone Chemicals, Hyderabad were used during 2023-24. Lures were changed only once after one month of first installation. The weekly trapped catch (WTC) adults in the traps was correlated with the corresponding week temperature (maximum and minimum), and rainfall. The weather data were collected from Ghogara Tea State, Sonitpur; Chandana Tea State, Udalguri; and Biswanath College of Agriculture, Assam Agricultural University, Biswanath Chariali. Pearson correlation was used for the correlation between the weather parameters and the number of adults captured. To come up with predictive models, different linear regressions were carried out, where trap catch was the dependent variable and three weather variables were used as independent variables in each of two cropping seasons, as well as pooled data. Multiple linear regression was performed using the generalized model ($Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \epsilon$). Statistical validity of each model was determined by R^2 , F-statistic, p-values, Durbin-Watson Statistic, Variance Inflation Factor (VIF), and the verification that model assumptions were true was done by Normal P-P plots of residuals. SPSS 26 version was used for all the analyses.

RESULTS AND DISCUSSION

The number of adults trapped in different districts and years varied. The mean weekly catches generated from 35-40 traps installed in 7-8 locations per district ranged from 1.2 - 6.6 in Udalguri, 0 - 5.6 in Sonitpur, and 0 - 3.2 in Biswanath Chariali during the entire observation period. The mean record of trap catches across the seasons, and places of the three districts was 2.31 adults per week. Diverse correlations of weather parameters with the adult population caught in pheromone traps

across the districts and seasons were noted. Even though the results of different districts were shown, the objective was to draw inferences on the zone as a whole, i.e., for the North Bank Plain Zone (NBPZ) of Assam, India. There was a positive correlation with maximum temperature in 4 out of 9 occasions of seasonal study across the 3 districts ($r=0.331$). Similarly, a positive correlation with minimum temperature was also observed in 7 out of 9 occasions of seasonal study across the 3 districts ($r=0.459$) in the zone as a whole. The correlation of minimum temperature with adult population was more pronounced than that of maximum temperature, which was reflected in 8 out of 10 Pearson's correlation values (Table 1).

Three regression models of the weekly trap catch (viz., number of adults caught weekly in pheromone traps) in relation to the weather parameters (minimum temperature, maximum temperature, and rainfall) were applied. The Normal P-P plots show that the residuals of the Season 1 model are near normal, whereas those of the Season 2 model have some deviations. However, the combined model (Season 1 + Season 2) showed a high level of correspondence with the normal distribution line, which proves that the assumption of normality is met (Table 2).

Interpretation for the season 1 regression model: The multiple linear regression analysis shows that the model has a significant impact in predicting the WTC ($F = 16.498$, $p < 0.001$), and the R^2 of the model is 0.529, which shows that the

given model explains the variance in WTC approximately with 52.9 percent. The adjusted R^2 of 0.509 indicates that the model still fits well even after the consideration of the number of predictors. Minimum temperature is significantly positively correlated with WTC ($p < 0.001$) compared to other predictors, but maximum temperature ($p = 0.843$) and rainfall ($p = 0.313$) fail to become significant predictors. The value of the Durbin-Watson test is 1.976, which is near 2, showing that no serious autocorrelation in residuals occurred. The issue of multicollinearity is to be ignored because the values of the Variance Inflation Factor (VIF) are less than 2, and the distribution of the residuals is normally distributed with a mean near zero (Fig. 1). Thus, it can be stated that the model is statistically significant, and the key factor contributing to variation in WTC among the explored variables is minimum temperature.

Interpretation for season 2: The multiple linear regression reveals that the model is significantly useful in predicting the WTC ($F = 5.139$, $p = 0.002$). The R^2 of 0.527 shows that the amount of variance in WTC explained by the predictors, viz., temperature (max and min) and rainfall, is about 52.7 per cent with adjusted R^2 as 0.502, which is an indication of good model fit when adjusted to account for the number of predictors. The temperature (max) ($p = 0.023$) and temperature (min) ($p = 0.021$) are statistically significant and positively correlated with WTC. However, the factor rainfall is not a statistically significant predictor ($p = 0.908$) in this particular model. The

Table 1. Correlations of weather parameters with weekly trapped catch of FAW during two seasons

Parameters	Udalguri district			Sonitpur district			Biswanath district			NBPZ
	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled	#Pooled
Max - Temp (17.4 - 34°C)	+ve (0.093)	-ve* (-0.388)	-ve (-0.122)	+ve* (0.358)	-ve** (-0.486)	-ve (-0.115)	-ve (-0.024)	+ve (0.072)	+ve (0.062)	+ve** (0.331)
Min - Temp (7.38 - 25°C)	+ve* (0.337)	+ve* (0.376)	+ve* (0.265)	-ve (-0.221)	+ve** (0.661)	+ve (0.232)	-ve (-0.003)	+ve (0.123)	+ve (0.100)	+ve** (0.459)
Rainfall (0 - 70.2mm)	+ve (0.249)	-ve (-0.067)	-ve (-0.006)	+ve (0.009)	+ve (0.236)	+ve* (0.268)	+ve (0.275)	-ve (-0.051)	+ve (.094)	+ve (0.071)

+ve: Insignificantly positive; +ve*: Significantly positive; +ve**: Highly significantly positive.

-ve: Insignificantly negative; -ve*: Significantly negative; -ve**: Highly significantly negative. NBPZ: North Bank Plain Zone (n): The values within parentheses are Pearson Correlation Values. #Pooled across the seasons and districts.

Regression models

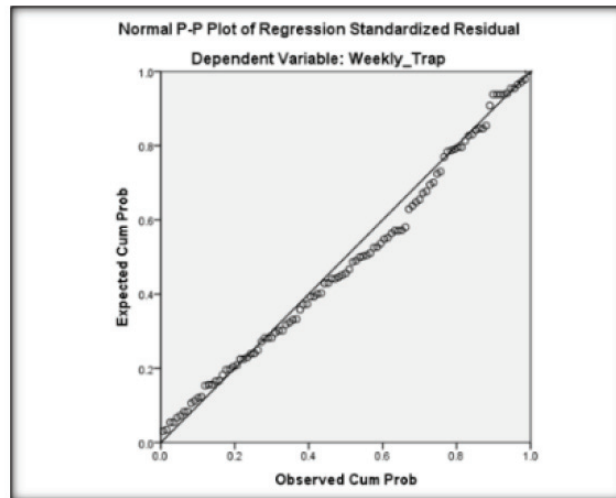
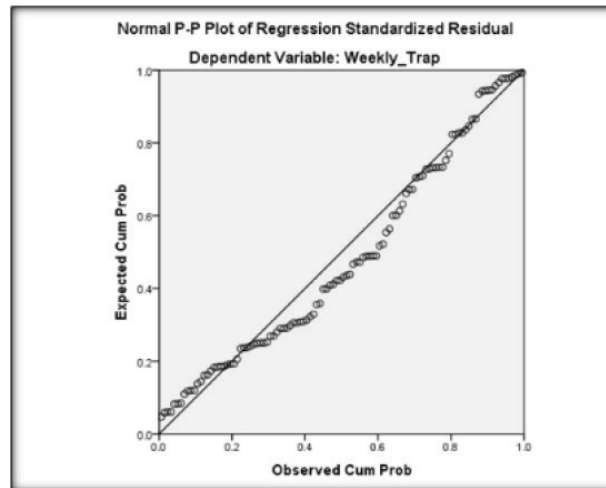
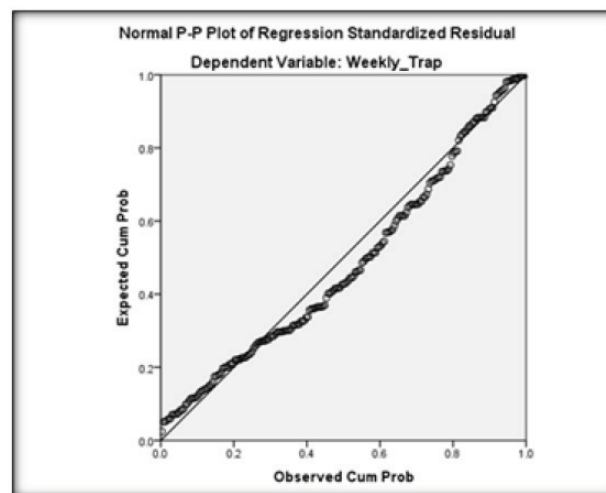
**Fig. 1** Normal P-P Plot for the season 1;**Fig. 2** Normal P-P Plot for the season 2**Fig. 3** Normal P-P Plot for the overall

Table 2. Regression equations for the three models Weekly Trapped Catches (WTC) of FAW adults

Regression models	R ²	SE	F	p-value
Season 1: $WTC = 0.491 + (-0.005) \times \text{Temp_Max} + 0.108 \times \text{Temp_Min} + 0.018 \times \text{Rainfall}$	0.529	0.92827	16.498	< 0.001
Season 2: $WTC = -1.030 + (0.090) \times \text{Temp_Max} + 0.054 \times \text{Temp_Min} + (-0.001) \times \text{Rainfall}$	0.527	1.23678	5.139	0.002
Overall: $WTC = -0.376 + (0.045) \times \text{Temp_Max} + 0.082 \times \text{Temp_Min} + 0.003 \times \text{Rainfall}$	0.626	1.11433	20.536	< 0.001

value of the Durbin Watson test is found to be 1.905. VIF statistics indicate that there is no issue of multicollinearity among predictors because they are as close to 1 as possible (Fig. 2). The developed model is statistically significant and moderately strong, and temperature variables are determinants contributing most to forecasting the WTC.

Interpretation for the overall regression model (Season 1 + Season 2): Multiple linear regression analysis indicates that the model has a significant amount of predictive value for WTC ($F = 20.536$, $p < 0.001$). The model describes 62.6 percent of changes in WTC ($R^2 = 0.626$), the adjusted $R^2 = 0.615$, demonstrating that there is a very strong fit of the model once the number of predictors is considered. When it comes to the independent variables, the maximum temperature and minimum temperature are significant predictors of temperature (maximum) ($p = 0.045$) and temperature (minimum) ($p < 0.001$). The coefficients are positive in both cases. On the other hand, rainfall ($p = 0.595$) is not a significant predictor. The proposed variable (Durbin-Watson test value = 1.863) does not suggest an important autocorrelation of residuals. The value of VIF of all predictors is less than 1.4, which verifies the insignificance of multicollinearity (Fig. 3). In general, the model indicates a statistically significant and relatively strong association of temperature variables with WTC.

The overall mean weekly adult catch record across the locations and seasons is 2.31 per trap. Different sowing dates of maize to varied levels of infestation of FAW in Nagaland (Longkumer *et al.*, 2023), which is also an indication of the differential impact of prevailing weather. Differential seasonal effect of rainfall has also been reported by Haroli *et al.* (2023) in commensuration with our result; their

study conducted in Coimbatore revealed that the rainfall exhibited a positive relation and a negative correlation during summer and rainy season, respectively. Negative correlation of larval population of FAW with rainfall has been reported from other parts of India (Anandhi *et al.*, 2020; Gowda *et al.*, 2024). Ratnam *et al.* (2023) have also reported that the rainfall, temperature, and humidity significantly influenced the emergence of the FAW and its infestation on maize crop under East Coast agro-climatic conditions of Andhra Pradesh. Likewise, the weather parameters had a profound impact on the infestation of FAW in Pakistan (Ahmad and Ibrahim, 2021). By a simulated study, Prasad *et al.* (2022) also predicted a greater number of generations and potential threat of FAW under the changing climate.

Highly variable trap catches (number of males per trap per night) have been recorded across countries such as Argentina (Bratovich *et al.*, 2019), Mexico (Malo *et al.*, 2001), the USA (Meagher *et al.*, 2013), Costa Rica (Andrade *et al.*, 2000), and India (Bhanu *et al.*, 2020). Such variation may be attributed to ecological factors associated with the maize ecosystem, e.g., crop acreage, abundance of predatory birds, light sources in and around the cropped area, and the history of FAW invasion in the locality. The role insectivorous predatory bird in suppressing the infestation of FAW has been emphasised in Assam (Sarma *et al.*, 2023) and Central America (FAO, 2018).

The mean weekly catches varied within 1.2- 6.6 in Udalguri, 0-5.6 in Sonitpur, and 0-3.2 in Biswanath Chariali. Such inter-location variations may be attributed to the variation in ecological factors associated with the maize ecosystem, e.g., crop acreage, non-crop vegetation around the crop, abundance of predatory birds, light sources in and

around the cropped area, and the history of FAW invasion in the locality. Such variations have also been recorded in India (Bhanu *et al.*, 2020) and other countries (Andrade *et al.*, 2000; Malo *et al.*, 2001; Meagher *et al.*, 2013; Bratovich *et al.*, 2019). The dispersal of FAW mostly occurs through winged adults, although at smaller scales, dispersal to surrounding plants can occur via larvae (Garcia *et al.*, 2018). The present study highlights the importance of temperature, particularly minimum temperature, as a major driver of pest population build-up, while rainfall plays a comparatively minor role. The proposed models may help in predicting the pests and disseminating the model-based information to the farmers.

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Determination of selected minerals and antioxidant properties of six edible aquatic insect species of Bajali region, Assam, India

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ABSTRACT: This work aims to fill the gap by determining the mineral composition and antioxidant properties of selected six commonly available edible aquatic insect species. *Lethocerus indicus* (Lepeletier and Serville, 1825), *Diplonychus rusticus* (Fabricius, 1781), *Laccotrephes ruber* (Linnaeus, 1764), *Cybister tripunctatus* (Olivier, 1795), *Hydrophilus olivaceus* (Fabricius, 1781) and *Sympetrum* sp. (Newman, 1833) were sampled from different freshwater bodies located within the Bajali region of Assam, India. Quantitative analysis of selected minerals (Ca, Mg, Fe, Mn, Zn and Cu) alongside with the evaluation of antioxidant properties (total phenol and total flavonoid content) were assessed. The results showed that among all the selected insect species, *L. ruber* showed the maximum amount of Ca content (58.23 mg 100g⁻¹). *H. olivaceus* showed the maximum amount of Mg (80.25 mg 100g⁻¹), while *L. indicus* showed the highest Fe content (98.36 mg 100g⁻¹), *H. olivaceus* showed highest Zn (mg 100g⁻¹) and *C. tripunctatus* showed highest amount of Cu content (5.28 mg 100g⁻¹). Total phenolic content ranged 102 to 152.30 mg 100g⁻¹ and total flavonoid content ranged from 28.79 to 53.52 mg 100g⁻¹. The current findings highlight that the selected aquatic insects are promising resource for food and feed, contributing to improved nutrition, food security and livelihood generation in this region. © 2026 Association for Advancement of Entomology

KEY WORDS: Biochemical, entomophagy, nutrition, food and feed

INTRODUCTION

Entomophagy practice in the world has been passing through since ancient times. From delicacy to lack of other food availability, entomophagy practices have always been supporting the people. Globally, people mostly consume insect species of orders Isoptera, Lepidoptera, Orthoptera, and Hymenoptera (Van Huis, 2013). These insects are consumed as a delicacy or substitute for food, used during periods of droughts, scarcity, or floods

(Sutton, 1988; De Foliart, 1992; Adedire and Aiyesanmi, 1999; Vantomme *et al.*, 2013). However, some societies recognize entomophagy as culturally inappropriate or as disgusting, especially in urban areas (Van Huis *et al.*, 2013). Despite some societal perception, insects are gaining recognition as a food source of high nutritional value. The Entomophagy practice has been proposed to fight with the deficiencies of minerals in developing countries (Christensen *et al.*, 2006). For instance, the approximately 3.5 billion

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global populations are at risk of Calcium deficiency (Mohammed and Wimalawansa, 2025), along with that, 23.2 per cent suffer from iron deficiency, 15.3 per cent suffer from zinc deficiency, 8.7 per cent suffer from copper deficiency and 0.4 per cent suffer from magnesium deficiency (Albalawi *et al.*, 2022).

Minerals are usually divided into two classes based on amount to meet dietary requirements. The first class is macro-minerals such as calcium, magnesium etc. and the second class is micro or trace minerals such as zinc, copper, iron, manganese, etc. (Oonincx and Finke, 2021). It is identified that insects potentially provide a large amount of micro and macro minerals such as Ca, Mg, Zn, Cu, Fe and Mn (Latunde-Dada *et al.*, 2016). Edible insects are also rich in anti-oxidant properties such as phenol and flavonoid (Shantibala *et al.*, 2014). Recent development shows an increase of use of the natural and synthetic antioxidants in nutritional supplements as a functional food (Rietjens *et al.*, 2002). Antioxidants are compounds which protect cells from the oxidative stress produced by free radicals, which are some unstable molecules and can harm cells that lead to development of chronic diseases and aging (Erhirhie and Paul, 2019). In the present study, we tried to record a good amount of minerals in some commonly available edible aquatic insect species consumed by the Bodo community of Bajali region of Assam, India. The basic mineral analysis along with their anti-oxidant properties were carried out to explore their potential role in enhancing the food security of this region. By doing so, the study aims to fill up the knowledge gap related to nutritional value of edible insects of this region.

MATERIALS AND METHODS

The current study was carried out from August 2024 to September 2025. Six edible aquatic insect species, viz., *Lethocerus indicus* (Lepeletier and Serville, 1825), *Diplonychus rusticus* (Fabricius, 1781), *Laccotrephes ruber* (Linnaeus, 1764), *Cybister tripunctatus* (Olivier, 1795), *Hydrophilus olivaceus* (Fabricius, 1781) and *Sympetrum* sp. (nymph) (Newman, 1833) respectively were

chosen for the present study. These insects were selected based on their availability and consumption by the local communities of this region. The sampling sites for this aquatic insect's collection were randomly selected from various freshwater bodies of Bajali district (26.5439°N; 91.1870°E). These insects were collected using the "1 minute kick net method" (Brittian, 1974) where the vegetation was disturbed and the net was dragged through the system for a unit of time (Macan and Maudsley, 1968). Three such drags constitute a sample. The aquatic insect species were identified with the help of standard taxonomic keys and further killed and processed following standard methods (Singh and Sachan, 2006). For biochemical analysis, the insect powders were preserved in sealed plastic containers.

Analysis of mineral composition: Ca, Mg, Fe, Mn, Zn and Cu were analyzed by following the methodology of Perkin Elmer (1996). At room temperature, the samples were digested with nitric acid (HNO₃) and perchloric acid (HClO₄) in the 2:1 ratio, filtered with distilled water, and made up to a final volume of 50ml, and then the filtrate was analyzed by ZEE nit700P Atomic Absorption Spectroscopy (AAS).

Analysis of antioxidant properties: Flavonoid content of the methanolic extract of the sample (1 mg ml⁻¹) was analysed spectrophotometrically following the modified protocol (Nooman *et al.*, 2008; Stankovic *et al.*, 2014; Martínez-Morales *et al.*, 2020).

All the experiments were conducted in triplicate. The statistical data was obtained using MS Excel. The values were expressed as mean ± standard deviation of three replicates. One- way ANOVA was performed using SPSS software version 25 to check the significant differences in their mineral and antioxidant content between the insect samples at 5 per cent level.

RESULTS AND DISCUSSION

Mineral assessment: Among the aquatic edible insects analyzed for six minerals, *L. ruber* had the highest amount of Ca content (58.23 mg100g⁻¹)

followed by *L. indicus* (49.33 mg100g⁻¹), *D. rusticus* (41.82 mg100g⁻¹), *C. tripunctatus* (40.45 mg100g⁻¹), *H. olivaceus* (38.71 mg100g⁻¹) and *Sympetrum* sp. (30.75 mg100g⁻¹). While *H. olivaceus* showed the maximum amount of Mg (80.25 mg100g⁻¹), Fe (101.96 mg100g⁻¹) and Zn (11 mg100g⁻¹). In contrast *D. rusticus* exhibited the highest Mn (5.45 mg100g⁻¹) content among all the species. *C. Tripunctatus* showed the highest content in Cu (5.28 mg100g⁻¹) among all. Studies have shown that all the mineral composition in the aquatic edible insect species were found to display considerable variation from one another at the 5% level of one-way ANOVA.

Analyzing edible insect's mineral content is crucial, since it informs consumers about the quality and quantity of nutrients they are consuming. The body cannot synthesize minerals; thus, a daily intake is necessary to sustain a number of physiological and biochemical functions (Soetan *et al.*, 2010). These trends are compatible with earlier work showing that insect mineral content varies widely by species, developmental stage, and ecological conditions (Oonincx and Finke, 2021; Sarmah *et al.*, 2022). The particularly high Fe and Zn levels in *H. olivaceus* are of nutritional relevance given that iron and zinc deficiencies remain global public health challenges (Hess *et al.*, 2023; Latunde-Dada, 2024). Likewise, the elevated Ca in *L. ruber* highlights its potential role in supporting bone health,

though the bioavailability of insects-derived Ca may be influenced by the chitinous exoskeleton (Ojha *et al.*, 2021). The amount of Ca content in the present study (30.74 to 58.23 mg 100g⁻¹) is relevant with the results of Shantibala *et al.* (2014) and Sarmah *et al.* (2022), which ranged from 24.3 to 96 and 32.09 to 56.15 mg 100g⁻¹, respectively. In comparison to terrestrial insects, aquatic insects have a significantly higher Ca content (Adeduntan, 2005). As Ca is important for bone health, aquatic insects can serve as a great source. The current study's Mg content ranged from 38.30 to 80.25 mg 100g⁻¹, which is comparable to the range of 33.6 to 99 mg 100g⁻¹ found in Manipur's aquatic insects (Shantibala *et al.*, 2014). Banjo *et al.* (2006) reported lower Mg levels (0.09 to 8.2 mg 100g⁻¹) compared to the present investigation. Mg is associated with the regulation of numerous vital processes such as blood pressure, glycemic control and neuromuscular function (Bertinato *et al.*, 2015; Grober *et al.*, 2015). The Fe content of the present analysis is compatible with the aquatic insects ranging from 25.30 to 112.10 mg 100g⁻¹ (Sarmah *et al.*, 2022). Identical results were detected in Manipur (Shantibala *et al.*, 2014). In comparison to red meats, insects are superior source of iron (Williams, 2007). From the current analysis the amount of Mn, Zn and Cu were found to be present in a lesser amount than the other minerals. The concentration of Mn identified in the present study is very low. Higher Mn contents were detected in

Table 1. Mineral composition of six edible aquatic insect species (mg 100 g⁻¹).

Insect species	Ca	Mg	Fe	Mn	Zn	Cu
<i>Lethocerus indicus</i>	49.33 ± 0.95	65.34 ± 2.63	98.36 ± 0.96	2.97 ± 0.36	10.77 ± 0.78	2.18 ± 0.15
<i>Diplonychus rusticus</i>	41.82 ± 0.92	40.74 ± 0.56	76.29 ± 2.45	5.46 ± 0.38	8.73 ± 0.52	3.32 ± 0.74
<i>Laccotrephes ruber</i>	58.23 ± 0.32	51.18 ± 3.55	89.74 ± 2.37	3.06 ± 0.73	7.82 ± 0.56	2.67 ± 0.29
<i>Cybister tripunctatus</i>	40.45 ± 0.22	38.30 ± 1.50	33.24 ± 0.24	4.26 ± 0.28	6.57 ± 0.48	5.28 ± 1.00
<i>Hydrophilus olivaceus</i>	38.71 ± 1.78	80.25 ± 0.11	101.96 ± 1.14	3.42 ± 0.21	11.00 ± 0.67	1.71 ± 0.99
<i>Sympetrum</i> sp. (Nymph)	30.75 ± 0.75	40.31 ± 1.74	19.74 ± 0.26	2.37 ± 0.36	1.08 ± 0.15	1.90 ± 0.10

Data represents mean ± SD of three replicates; p < 0.05 significant difference present among species

Table 2. Phenol and flavonoid content of six edible insect species (mg 100 g⁻¹).

Species	Phenol	Flavonoid
<i>Lethocerus indicus</i>	125.23±2.36	53.52±1.18
<i>Diplonychus rusticus</i>	152.30±2.18	40.36±0.80
<i>Laccotrephes ruber</i>	108.20±1.35	33.46±0.63
<i>Cybister tripunctatus</i>	208.06±0.98	45.21±0.76
<i>Hydrophilus olivaceus</i>	118.60±1.42	28.79±3.02
<i>Sympetrum</i> sp. (Nymph)	102.20±1.00	30.13±2.29

Data represents mean ± S.D of three replicates; $p < 0.05$ significant difference present among species

sundried edible black ants (Bhulaidok *et al.*, 2010). Zn is instrumental in preserving the stability of genetic material and regulating gene activity, as it participates in processes such as DNA transcription, chromatin organization, replication and repair (Dreosti, 2001). The Zn concentration levels in this investigation fall comfortably within the FAO (2001) mean range of 0.22-2.10 mg 100g⁻¹. The Zn content from the present study ranged from 1.08 to 11mg/100 g is uniform with the earlier findings, 4.98 to 7.22 mg 100g⁻¹ (Sarmah *et al.*, 2022). A higher amount for Zn was detected in Manipur (Shantibala *et al.*, 2014). A higher Zn content was reported in previous researches on terrestrial edible insects that ranged from 11.9 to 22.7 mg 100g⁻¹ (Bhulaidok *et al.*, 2010).

Antioxidant properties: In the current analysis, the *C. tripunctatus* displayed the highest phenol content (208.06 mg 100g⁻¹). The highest amount of flavonoid content (53.52 mg 100g⁻¹) was noted in *L. indicus*. In all the aquatic insect species, the antioxidant properties have demonstrated a notable variation. A high amount of phenol can have critical health effects, including decreased fertility, decreased young survival rate, growth inhibition (Babich and Davis, 1981). According to ATSDR (Agency for Toxic Substances and Disease Registry), 2008, adults require a minimal lethal oral dose of phenol of about 70 mg kg⁻¹. The phenol content from the current study is less than the lethal

dose. So, consumption of these edible aquatic insects is safe. Flavonoids play a significant role in regulating cell cycle, inducing apoptosis, and inhibiting the growth of cancer cells (Rue *et al.*, 2018). The current results are pertinent with the earlier findings, with phenol content ranging 117.39 to 363.80 mg 100g⁻¹ and flavonoid ranging from 17.70 to 50.82 mg100g⁻¹ (Sarmah *et al.*, 2022). Recent reports revealed differences in the content of phenolics and flavonoids in insects and were associated with diet, habitat, processing technology and are progressively being acknowledged for their bioactive and antioxidant characterization (Torres-Castillo and Olazarán-Santibáñez, 2023; Ruiz *et al.*, 2025). Additionally, insect-derived edible items have been found to retain phenolics bio-accessibility during digestion, contributing measurable antioxidant activity (Rocchetti *et al.*, 2024). These edible aquatic insects exhibit significant mineral and antioxidant activity, highlighting their potential as valuable food sources. In order to promote both environmental balance and nutritional well-being, it is imperative that these insects be conserved and used sustainably as alternate food sources. The inclusion of these insects in wider dietary and health-related applications is supported by this study, which also lays the groundwork for more thorough biochemical studies. Ensuring their conservation and sustainable management is essential to maximizing their micronutrient potential. The results also serve as valuable information for the local communities of Bajali region of Assam enhancing awareness of the dietary and health benefits associated with insect consumption. Future studies should concentrate on establishing standardized processing methods to maintain nutritional quality, evaluating environmental sustainability, and evaluating nutrient bioaccessibility using in vitro and in vivo digestion models. Food security and resilience could be significantly enhanced by raising public awareness and incorporating edible aquatic insects into nutritional policies.

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Field evaluation of various insecticides against bollworms and sucking pests in colour cotton

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ABSTRACT: A field trial on the comparative performance of insecticides against bollworms and sucking pests of coloured cotton (variety: Vaidehi-95) conducted, revealed significant reductions compared with the control. Flonicamid 50WG proved highly effective in controlling sucking pests, recording lowest mean populations of leafhopper (2.79 per 3 leaves), thrips (7.33 per 3 leaves), aphids (9.46 per 3 leaves) and whitefly (3.89 per 3 leaves), and was statistically on par with Buprofezin 25 EC for most pests. Spinosad 45 SC, Chlorantraniliprole 18.5 SC, Flubendiamide 39.35 SC, and Emamectin benzoate 5 SG demonstrated high efficacy against bollworms. Spinosad 45 SC recorded the lowest American bollworm (2.89 larvae per 10 plants) and pink bollworm (2.24 larvae per 20 bolls) incidence, followed closely by Chlorantraniliprole 18.5 SC. In terms of green boll damage, Spinosad 45 SC (26.08%) and Chlorantraniliprole 18.5 SC (27.74%) were most effective, with Flubendiamide 39.35 SC and Emamectin benzoate 5 SG providing moderate protection. *Beauveria bassiana* was consistently the least effective treatment across all pest groups.

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KEY WORDS: Green boll damage, larval incidence, aphids, leaf hopper, whitefly, bioefficacy

INTRODUCTION

Cotton stands as the most significant natural fibre crop, widely cultivated to fulfil domestic textile needs and support exports. It holds a crucial place in India's economy, offering direct and indirect employment to more than 60 million individuals. With the greatest extent of land under cotton cultivation globally approximately 130.61 lakh ha India also leads in production, yielding around 343.47 lakh bales annually (COCPC, 2023). Notably, it is the sole country cultivating all four domesticated cotton

species: *Gossypium hirsutum*, *G. barbadense*, *G. arboreum*, and *G. herbaceum*, valued for their lint, oil, and feed by-products. The crop is mainly grown across ten major states, Karnataka, Telangana, Andhra Pradesh, Maharashtra, Madhya Pradesh, Tamil Nadu, Gujarat, Punjab, Haryana, Rajasthan (Waghmare, 2022). In recent times, coloured cotton, which produces naturally pigmented fibres in shades like brown, green, and red is gaining growing attention for its ecological and sustainable value. Cotton is highly vulnerable to insect pest infestation, with approximately 1,326 arthropod species that

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affect its cultivation (Hargreaves, 1948). In India around 166 insect and mite species infest cotton crops (Khan and Rao, 1960), causing substantial yield loss, estimated at 50–60 percent in severe cases (Puri *et al.*, 1999). The pest complex can be broadly classified into two main groups. The bollworm complex includes key species such as the American bollworm (*Helicoverpa armigera* (Hübner, [1808])), spotted bollworms (*Earias insulana* (Boisduval, 1833) and *E. vittella* (Fabricius, 1794)), and the pink bollworm (*Pectinophora gossypiella*) (Saunders, 1844). The sucking pest complex comprises the leafhopper (*Amrasca biguttula biguttula* (Ishida, 1913)), aphid (*Aphis gossypii* Glover, 1877), thrips (*Thrips tabaci* Lindeman, 1889), and whitefly (*Bemisia tabaci* (Gennadius, 1889) (Nagrare *et al.*, 2023). India continues to experience low average productivity, with lint yields around 451 kg per ha, mainly due to persistent damage inflicted by arthropod pests (CCI, 2021). Naturally coloured cotton, reintroduced in India through research at the ICAR - Central Institute for Cotton Research (CICR), Nagpur, draws from indigenous ‘Desi’ germplasm like Yerrapatti varieties, with renewed focus post-1995 Satyam Committee recommendations to curb dye pollution. Uses span niche eco-textiles for no-fade apparel, upholstery, and handloom products, prized for dye-free processing, breathability, and flavonoid pigments enhancing UV resistance, though wax content aids ginning (Deepak *et al.*, 2022). Recent studies reveal no confirmed correlation lowering pest occurrence compared to white cotton; bollworms, aphids, and whiteflies infest similarly (Naik *et al.*, 2025). The study aims to evaluate the efficacy of various types of chemical insecticides against these pests, in colour cotton pest management.

MATERIALS AND METHODS

A field study was undertaken during 2022–23 at the Entomology Block, ICAR - CICR, Nagpur, to assess the bioefficacy of different insecticides against key pests of cotton. The coloured cotton variety ‘Vaidehi-95 (non-Bt)’ was sown at a spacing of 90 × 60cm, following all recommended agronomic practices. Insecticidal treatments (Table

2) were applied whenever any of the major sucking pests (thrips, leafhoppers, aphids, or whiteflies) or bollworms crossed the economic threshold level (ETL) (Table 1). Two sprays of the respective insecticides were administered at 15-day intervals. Data on sucking pests were collected from the uppermost three fully developed leaves of 10 randomly chosen plants in each treatment, one day prior to spraying and again at 7- and 14-days post-application. American bollworm incidence was assessed on 10 plants per treatment, whereas pink bollworm infestation and green boll damage were determined through destructive sampling of 20 bolls per treatment. The per cent reduction in pest population over the control was calculated using the standard formula.

Reduction over control (%) =

$$\frac{\text{No. of insects in control} - \text{No. of insects in treatment}}{\text{No. of insects in control}} \times 100$$

$$\text{Green boll damage (\%)} = \frac{\text{No. of damaged green bolls}}{\text{Total No. of green bolls}} \times 100$$

The experimental data collected for were analysed using ANOVA under a randomized complete block design, applying appropriate statistical transformations (arc sine or square root) with the aid of R software (R Core Team, 2016).

RESULTS AND DISCUSSION

Bioefficacy of insecticides on sucking pest incidence: Application of insecticides resulted in a marked decline on the incidence of key sucking pests relative to the untreated control. Flonicamid 50WG consistently emerged as the most effective molecule, recording the lowest mean populations of leafhopper (2.79/3 leaves), thrips (7.33/3 leaves), aphids (9.46/3 leaves), and whiteflies (3.89/3 leaves) and was statistically at par with Buprofezin 25 EC for leafhopper (3.13/3 leaves), aphids (10.20/3 leaves), and whiteflies (4.31/3 leaves) and thrips (7.81/3 leaves). Spinosad 45 SC also maintained moderate suppression across pests, particularly against leafhopper (4.40/3 leaves), aphids (13.36/3 leaves), whiteflies (9.03/3 leaves) and thrips (11.04/3 leaves). By contrast, *B. bassiana* consistently recorded the highest pest populations, with mean counts of 8.08, 18.17, 20.49, and 15.32 per 3 leaves

for leafhopper, thrips, aphids, and whiteflies, respectively, showing the least efficacy among all treatments, while the untreated control recorded the maximum infestations by all insect pests (Table 2). This findings are supported by Nemade *et al.* (2017) who reported that Flonicamid 50WG was highly effective against major sucking pests (Akola, Maharashtra), resulting in the highest cotton yield (1681.02 kg ha⁻¹). Similarly, Gaurkhede *et al.* (2015) further validated the efficacy of Flonicamid 50WG in controlling sucking pests of Bt cotton (Akola, Maharashtra), which subsequently resulted in increased seed cotton yield. Further, Sasikumar *et al.* (2018) demonstrated that spraying of Flonicamid 50WG markedly lowered the occurrence of sucking pests in Srivilliputhur (Tamil Nadu), achieving the lowest average populations of leafhopper (1.12 and 1.18/3 leaves), thrips (1.48 and 0.81/3 leaves), and whitefly (0.90 and 0.97/3 leaves). These reports clearly indicated the superior performance of Flonicamid 50WG in managing multiple sucking pests simultaneously. Likewise, Sontakke *et al.* (2013) reported that Buprofezin 25 EC at 150 g a.i. ha⁻¹ was highly effective against thrips, leaf hopper, aphids and whiteflies (Bolangir district, Odisha). Sasikumar *et al.* (2018) further confirmed that Flonicamid 50WG at 100 g a.i. ha⁻¹ consistently reduced pest populations, while Buprofezin 25 SC at 250 g a.i. ha⁻¹ was the next best treatment, recording comparatively higher counts of leafhopper (1.83 and 2.40/ 3 leaves), thrips (0.98 and 0.89/ 3 leaves) and whitefly (1.03 and 1.04/3 leaves). Neem oil effectively controls whiteflies by disrupting

their molting and feeding through azadirachtin, while *B. bassiana* spores readily infect their soft cuticles, resulting in synergistic population reduction. In contrast, aphids resist these treatments due to thicker waxy cuticles that block *Beauveria* penetration, combined with rapid parthenogenetic reproduction that outpaces neem's effects.

Comparable results were reported by Kumar and Dhawan (2011), who found both Dinotefuran 20 SG and Flonicamid 50WG effective in controlling cotton leafhopper. Similar pattern was evident by Sreekanth and Reddy (2011) (Guntur, Andhra Pradesh); Zala *et al.* (2014) (Anand, Gujarat); Bajya *et al.* (2016) (Gurugram, Haryana); Nemade *et al.* (2015) (Akola, Maharashtra); Sreenivas *et al.* (2015) (Raichur, Karnataka) and Navi *et al.* (2021) (Chamarajanagar, Karnataka), who consistently highlighted the effectiveness of Flonicamid 50WG at 150 g ha⁻¹ and related insecticides in reducing sucking pest populations in cotton. Overall, Flonicamid 50 WG was shown to be the most cost-effective treatment, highlighting its potential as a key option to control sucking pests in Bt cotton. Flonicamid is a systemic insecticide that belongs to the pyridinecarboxamide group and represents a new class of chemistry known as chordotonal organ modulators (IRAC Group 29). Its unique mode of action involves disrupting the function of chordotonal organs sensory structures responsible for balance, movement, and coordination resulting in starvation through inhibition of stylet penetration into plant tissues. This effectively

Table 1. Economic threshold for major insect pests in cotton

No	Pest	Economic threshold level (ETL)
1	<i>Amrasca biguttula biguttula</i>	2 jassids per leaf or appearance of yellowing/cupping symptoms
2	<i>Aphis gossypii</i>	10–15 aphids per leaf or 50 aphids per 10cm terminal shoot
3	<i>Thrips tabaci</i>	8–10 thrips per leaf
4	<i>Bemisia tabaci</i>	6–8 adults per leaf (middle canopy)
6	<i>Pectinophora gossypiella</i>	10% green boll/flower damage or 8–10 moths/trap/night
7	<i>Helicoverpa armigera</i>	5 larvae per 100 plants or 10% boll damage

Table 2. Efficacy of insecticides against sucking pests in coloured cotton

Treatments/Dose ha ⁻¹	Leafhopper		Thrips		Aphids		Whiteflies	
	Mean*	(%) [#]	Mean*	(%) [#]	Mean*	(%) [#]	Mean*	(%) [#]
Neem oil 3000ppm/ 7.5L	5.70 (2.49) ^c	61.75	11.61 (3.48) ^b	64.14	16.75 (3.86) ^c	58.85	11.07 (3.40) ^c	55.88
Flonicamid 50 WG/ 150g	2.79 (1.81) ^a	81.26	7.33 (2.80) ^a	77.37	9.455 (2.80) ^a	76.76	3.89 (2.09) ^a	84.51
Chlorantraniliprole 18.5 SC/150mL	6.87 (2.71) ^d	53.90	16.65 (4.14) ^d	48.58	18.69 (4.11) ^d	54.06	13.44 (3.73) ^d	46.44
Flubendiamide 39.35 SC/250mL	6.98 (2.73) ^d	53.16	16.38 (4.11) ^d	49.41	18.97 (4.14) ^d	53.39	13.37 (3.72) ^d	46.70
Spinosad 45 SC/ 220mL	4.40 (2.21) ^b	70.48	11.04 (3.40) ^b	65.90	13.36 (3.40) ^b	67.18	9.03 (3.09) ^b	64.01
Emamectin benzoate 5 SG/200g	4.54 (2.24) ^b	69.54	14.43 (3.86) ^c	55.44	13.93 (3.48) ^b	65.78	9.48 (3.16) ^b	62.24
Buprofezin 25 EC/ 1000mL	3.13 (1.90) ^a	79.01	7.81 (2.88) ^a	75.87	10.20 (2.88) ^a	74.93	4.31 (2.19) ^a	82.84
Fenvalerate 20 EC/ 500mL	5.80 (2.51) ^c	61.08	14.70 (3.90) ^c	54.60	17.02 (3.90) ^c	58.18	11.43 (3.45) ^c	54.46
<i>Beauveria bassiana</i> 10 ⁸ /2500g	8.08 (2.93) ^e	45.77	18.17 (4.32) ^e	43.89	20.49 (4.32) ^e	49.66	15.32 (3.95) ^e	38.93
Control	14.89 (3.92) ^f	0.00	32.38 (5.73) ^f	0.00	40.69 (5.73) ^f	0	25.09 (5.06) ^f	0.00
CD at 5%	0.12		0.15		0.16		0.18	
CV (%)	5.72		7.19		5.86		4.46	

* Mean incidence per 3 leaves and mean of two sprays at 7 and 14 DAS; (%)[#]- Reduction (%) over control; In a column means followed by different letters are significantly different by DMRT (p=0.05) ; Values in parentheses are transformed values

prevents pests such as thrips, leafhoppers, aphids, and whiteflies from feeding. Upon exposure, Flonicamid 50WG rapidly induces cessation of feeding by both contact and ingestion, ultimately leading to pest mortality. For example, aphids treated with Flonicamid 50WG show marked inhibition of salivation and sap uptake even after attaching to leaf surfaces (Morita *et al.*, 2007). Unlike widely used insecticides including neonicotinoids, pymetrozine, or pyrifluquinazon, Flonicamid operates through a distinct mechanism

and importantly, so far, no instances of cross-resistance with conventional insecticides have been documented.

Bioefficacy of insecticides bollworms incidence: All insecticidal treatments significantly lowered bollworm incidence in coloured cotton compared to the untreated control. Against American bollworm, Spinosad 45 SC showed the lowest larval population (2.89 larvae/10 plants) with 81.69 percent reduction over control, and its efficacy was statistically on par with Chlorantraniliprole 18.5

SC (3.24 larvae/10 plants). This was trailed by Flubendiamide 39.35 SC (4.36 larvae/10 plants) and Emamectin benzoate 5 SG (4.50 larvae/10 plants). Less suppression was evident with Flonicamid 50 WG (5.66 larvae/10 plants) and Fenvalerate 20 EC (5.76 larvae/10 plants). In contrast, *B. bassiana* recorded the highest larval incidence (8.85 larvae/10 plants) with only 43.93 percent reduction, while the untreated control registered 15.78 larvae/10 plants (Table 3). A similar trend was observed for pink bollworm. Spinosad 45 SC again proved most effective (2.24 larvae/20 bolls; 84.82% reduction), and its performance was at par with Chlorantraniliprole 18.5 SC (2.59 larvae/20 bolls). These were followed by Flubendiamide 39.35 SC (3.71 larvae/20 bolls) and Emamectin benzoate 5 SG (3.85 larvae/20 bolls). Conversely, *B. bassiana* showed the least effectiveness (8.20 larvae/20 bolls), whereas the control recorded the maximum infestation (14.76 larvae/20 bolls) (Table 3). These results are in line with the findings of Divya *et al.*

(2020), who reported that Chlorantraniliprole 18.5 SC recorded the lowest larval population with the highest reduction over control (71.00%) from Chamarajanagar (Karnataka). Likewise, the current findings align with the reports of Bhute *et al.* (2023) and Pathan *et al.* (2021) as they recorded lowest larval incidence of 3.67 and 3.09/ 20 bolls, respectively in Chlorantraniliprole 18.5 SC treated plots as compared to all other treatments (Rahuri, Maharashtra). Rakhesh *et al.* (2024) in Raichur (Karnataka) observed that Chlorantraniliprole 18.5 SC resulted in the lowest average incidence of pink bollworm (3.26 larvae/10 bolls; 74.32% reduction over control), which was statistically on par with Spinosad 45 SC (3.45 larvae/10 bolls) and Flubendiamide 39.35 SC (3.53 larvae/10 bolls). Similarly, Prasad and Rao (2010) found that Chlorantraniliprole at 40 g a.i. ha⁻¹ and Spinosad at 75 g a.i. ha⁻¹ minimized square and boll damage by *H. armigera*. The present results are in agreement with the findings of Bhute *et al.* (2023), Pathan *et al.*

Table 3. Efficacy of various insecticides against larval incidence of bollworm in cotton

Treatments	American bollworm		Pink bollworm		Green boll damage	
	Mean*	(%) [#]	Mean*	(%) [#]	Mean*	(%) [#]
Neem oil 3000ppm	6.10 ^c (2.57)	61.34	5.45 ^c (2.44)	63.08	41.29 ^c (39.98)	41.12
Flonicamid 50 WG	5.66 ^c (2.48)	64.13	5.01 ^c (2.35)	66.06	40.26 ^c (39.38)	42.59
Chlorantraniliprole 18.5 SC	3.24 ^a (1.93)	79.47	2.59 ^a (1.76)	82.45	27.74 ^a (31.78)	60.45
Flubendiamide 39.35 SC	4.36 ^b (2.20)	72.37	3.71 ^b (2.05)	74.86	33.89 ^b (35.60)	51.68
Spinosad 45 SC	2.89 ^a (1.84)	81.69	2.24 ^a (1.66)	84.82	26.08 ^a (30.71)	62.82
Emamectin benzoate 5 SG	4.50 ^b (2.24)	71.48	3.85 ^b (2.09)	73.92	34.55 ^b (36.00)	50.73
Buprofezin 25 EC	7.34 ^d (2.80)	53.49	6.69 ^d (2.63)	54.67	46.65 ^d (43.08)	33.48
Fenvalerate 20 EC	5.76 ^c (2.50)	63.50	5.11 ^c (2.37)	65.38	40.86 ^c (39.73)	41.74
<i>Beauveria bassiana</i> 10 ⁸	8.85 ^c (3.06)	43.93	8.20 ^c (2.75)	44.46	52.20 ^c (46.26)	25.57
control	15.78 ^f (4.03)	0.00	14.76 ^f (3.91)	0.00	70.13 ^f (56.87)	-
CD at 5%	0.21		0.11		1.52	
CV (%)	5.22		3.61		6.28	

* Mean of two sprays at 7 and 14 DAS; (%)[#]- Reduction (%) over control; In a column means followed by different letters are significantly different by DMRT (p=0.05) ; Values in parentheses are transformed values

al. (2021), and Sarode *et al.* (2019), who also observed the lowest green boll damage 11.33, 8.78 and 3.50 percent, respectively—in Bt cotton plots treated with chlorantraniliprole 18.5 SC compared with other treatments.

Bioefficacy of insecticides on green boll damage: All insecticidal treatments markedly reduced green boll damage compared to the untreated control. Among them, Spinosad 45 SC recorded the lowest damage (26.08%), achieving a 62.82 per cent reduction over the control, and was statistically at par with Chlorantraniliprole 18.5 SC (27.74%), followed by Flubendiamide 39.35 SC and Emamectin benzoate 5 SG (with 33.89 and 34.55 % damage respectively). A moderate level of protection was obtained with Flonicamid 50 WG (40.26 %), Neem oil 3000 ppm (41.29 %) and Fenvalerate 20 EC (40.86 %). Buprofezin 25 EC was relatively less effective, recording 46.65 per cent boll damage with only 33.48 per cent reduction over control (Table 3). *B. bassiana* was the least effective treatment, resulting in the highest boll damage and the lowest damage reduction (52.20 and 25.57%, respectively). In comparison, the untreated control exhibited maximum boll damage of 70.13 per cent. These findings are in agreement with the observations reported by Rakesh *et al.* (2024), who observed the lowest pink bollworm damage in plots treated with Chlorantraniliprole 18.5 SC (16.5% boll damage; 77.34% reduction over control), Spinosad 45 SC (16.62%) and Flubendiamide 39.35 SC (17.54%). Similarly, present results corroborate the findings of Bhute *et al.* (2023), Pathan *et al.* (2021), and Sarode *et al.* (2019), who observed minimum green boll damage of 11.33, 8.78 and 3.50 percent, respectively, in Chlorantraniliprole 18.5 SC at 150 ml ha⁻¹ treated Bt cotton plots compared with other treatments.

Spinosad targets the insect nervous system by binding to nicotinic acetylcholine receptors at unique sites (different from neonicotinoids) and partly affecting GABA-gated chloride channels. This dual action causes neural hyperexcitation, leading to tremors, paralysis, rapid feeding cessation, and eventual death within hours to a few days. It is

highly effective against *H. armigera*, *P. gossypiella*, and other lepidopterans, while remaining relatively safe to beneficial arthropods. Whereas, Chlorantraniliprole and Flubendiamide are anthranilic diamides, acts on ryanodine receptors in muscle cells, triggering uncontrolled calcium release, depletion of calcium stores, and disruption of muscle function. Larvae quickly stop feeding, become lethargic, and die from paralysis and exhaustion. Its long residual activity, systemic/translaminar movement, and high selectivity make it effective against lepidopterans with minimal impact on non-target organisms.

Flonicamid 50 WG excels in suppressing sucking pests like leafhoppers, thrips, aphids, and whiteflies in coloured cotton, outperforming others with the lowest mean populations and matching with the efficacy of Buprofezin. Whereas, Spinosad 45 SC and Chlorantraniliprole 18.5 SC dominate bollworm control and green boll damage reduction. Integrated use of Flonicamid for sucking pests alongside Spinosad and Chlorantraniliprole for bollworms offers optimal, cost-effective IPM in coloured cotton, boosting yields as per regional studies.

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Ecology and conservation of ant communities in the Alappuzha coast, Kerala, India

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ABSTRACT: The diversity, composition, and ecological significance of ant communities across three distinct coastal habitats - vegetated dunes, coconut groves, and disturbed sandy patches along the Alappuzha coast of Kerala were studied. A total of 21 ant species from 16 genera and 5 subfamilies were identified. Species richness and Shannon diversity were highest in vegetated dunes (17 species; $H' = 2.97$; $1-D = 0.91$), followed by coconut groves (14 species; $H' = 2.54$; $1-D = 0.84$) and disturbed sandy patches (11 species; $H' = 1.98$; $1-D = 0.74$). Soil organic matter and moisture content showed strong positive correlations with ant abundance and diversity. Field observations and seed removal experiments revealed that ants contribute significantly to seed dispersal, soil turnover, and nutrient cycling. The findings highlight the sensitivity of coastal ant assemblages to habitat conditions and their potential as bioindicators for monitoring and conserving coastal ecosystems.

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KEY WORDS: Bioindicators, seed dispersal, soil parameters. diversity indices, species richness

INTRODUCTION

Ants (Hymenoptera, Formicidae) are among the most ecologically dominant and functionally diverse insect groups on Earth, performing as ecosystem engineers (Bagyaraj *et al.*, 2016) influencing soil aeration, nutrient cycling, seed dispersal, and regulation of arthropod populations and act as bioindicators (Philpott *et al.*, 2010; Chowdhury *et al.*, 2023). Despite their ecological importance, studies on coastal ant communities in India are scarce. Most research has focused on forest and agricultural ecosystems (Bharti and Sharma, 2009). Coastal and insular ant communities have been examined in Australia (Lach and Thomas, 2008), Brazil (Fetiosa *et al.*, 2022; Chen *et al.*, 2015),

and the Gulf of Mexico. However, studies on coastal ant fauna in India are limited, and little is known about species-habitat associations, diversity patterns, and interactions with soil and vegetation in these coastal ecosystems. The coastal region of Alappuzha, Kerala, with its sandy beaches, dunes, lagoons, and estuarine backwaters, presents mosaic microhabitats shaped by tidal influences, monsoon climate, and human activities (Praveen and Kunnampalli, 2024). The varied landforms of Alappuzha produce diverse soil and vegetation conditions that influence ant community composition and nesting patterns. This microhabitat diversity often affects species distribution and functional roles, as ants tend to associate with specific substrates and vegetation types (Wills, 2018). This

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study addresses the gap by assessing species composition, habitat-linked diversity, and soil-faunal associations of ants across three contrasting habitats: vegetated dunes, coconut groves, and disturbed sandy patches along the Alappuzha coast of Kerala, India.

MATERIALS AND METHODS

The study was conducted along the Alappuzha coast (9.49.4927° N; 76.3178° E). Sampling was conducted between March and June 2024. Three representative habitat type - vegetated dunes, coconut groves, and disturbed sandy patches located approximately 100m within the seashore were selected for ant sampling. Ants were collected using a combination of standardized pitfall traps, bait traps, and hand collection methods (Bestelmeyer, 2000) to ensure comprehensive sampling of both ground-dwelling and foraging species. Collected specimens were preserved in 70 per cent ethanol and identified to species level using standard taxonomic keys (Bingham, 1903; Bolton, 1994; Bharti, 2016) and regional keys and the identifications were subsequently confirmed by Dr. Kalesh Sadasivasan, Research Associate, Travancore Nature History Society. Field observations and experimental approaches were employed to assess the ecological roles of ants in the coastal habitats. Standardized seed-removal experiments were conducted to evaluate ants' contributions to seed dispersal and soil turnover. Experimental plots (1m X 1m) were established within each habitat type, and marked seeds were placed at fixed intervals. The rate and pattern of seed removal were recorded at 2-h intervals over a 24-h period, following protocols adapted from (Gómez *et al.*, 2003; Andersen and Majer, 2004).

Soil samples were collected from each trap location for physicochemical analysis. Organic matter was estimated using Walkley and Black method (Walkley and Black, 1934). Soil moisture was determined by the gravimetric method; and pH was measured in a soil–water suspension using a calibrated digital pH meter. Diversity indices such as Shannon–Wiener (H') and Simpson's ($1-D$) were calculated to assess species richness and evenness across habitats (Magurran, 2013).

Statistical analyses, including one-way ANOVA, were performed to test differences in ant diversity among habitats, and Pearson's correlation was used to evaluate the relationships between soil parameters and ant diversity (Zar, 1999). Direct field observations were made to record behavioral interactions of ants with other invertebrates, focusing on trophic associations, predatory activities, and mutualistic relationships such as tending of Hemipterans or association with aphids. Opportunistic observations were also included to capture rare or infrequent behavioral events (Hölldobler and Wilson, 1990). For conservation assessment, potential threats to ant populations, including habitat disturbance, vegetation clearance, litter removal, and anthropogenic pressures such as tourism and waste accumulation, were identified through systematic field inspection and informal interviews with local residents. Each species was evaluated according to the IUCN Red List Criteria (2012) to determine its conservation status and potential vulnerability within the coastal ecosystem. Inter-specific interactions between ants and other animals (birds, reptiles, and arthropods) were observed to elucidate their ecological relationships and the role of ants in maintaining coastal trophic networks. Data from these observations provided qualitative insight into the functional importance of ants across the studied habitats.

RESULTS AND DISCUSSION

A total of 1,950 samples representing 21 species, 16 genera, and five subfamilies were recorded across the three coastal habitats of Alappuzha (Table 1). Species richness was highest in vegetated dunes (17 species), followed by coconut groves (14) and disturbed sandy patches (11). The dominant genera *Camponotus* (28%), *Paratrechina* (24%), and *Solenopsis* (18%) together accounted for over 70 per cent of the total samples. A survey conducted on the ant diversity in the University of Kerala Campus also revealed a total of 64 species across six subfamilies. Species belonging to the Myrmicinae dominated (51.5%) followed by Formicinae (20.6%), Ponerinae (13.2%), Dolichoderinae (4.4%), Pseudomyrmicinae (4.4%) and Dorylinae (1.5%) (Antony *et al.*, 2021).

Diversity indices revealed significant habitat variation, with the Shannon–Wiener index (H') being highest in vegetated dunes (2.97) and lowest in disturbed patches (1.98). Similarly, Simpson's index ($1-D$) indicated greater evenness in vegetated dunes (0.91) than in disturbed patches (0.74), signifying reduced dominance in more stable habitats. The ponerine ant *Diacamma rugosum* (Le Guillou, 1842) was confined to vegetated dunes, whereas *Anoplolepis gracilipes* (Smith, 1857) and *P. longicornis* (Latreille, 1802) were restricted to disturbed habitats, indicating their tolerance to habitat modification. Anas *et al.* (2021) conducted a study on Macrohymenopteran diversity in Thrissur (Thommana Kole wetland), identifying Formicinae as the species-rich subfamily of ants.

Soil parameters also varied significantly among habitats. Soil organic matter and moisture were highest in vegetated dunes (0.8 and 12%) and lowest in disturbed patches (0.3 and 6%). A strong positive correlation ($r = 0.74$) existed between soil organic matter and species richness (Fig. 1), whereas soil pH (6.9–7.1) showed no significant effect ($r = 0.12$, $p > 0.05$). Ant abundance differed significantly among habitats (ANOVA, $F_{2,27} = 7.11$, $p < 0.01$), being greatest in vegetated dunes (60 ± 8 individuals/trap) and least in disturbed patches (30 ± 5 individuals/trap), implying higher heterogeneity and resource availability in stable habitats.

Functional observations and seed removal experiments highlighted the ecological significance of ants in coastal systems. Seed removal rates (Table 2) were highest in vegetated dunes (68%), moderate in coconut groves (52%), and lowest in disturbed patches (35%). The principal seed-transporting species *Meranoplus bicolor* (Guerin-Meneville, 1884) and *S. geminata* (Fabricius, 1804) facilitated secondary seed dispersal and soil turnover, enhancing nutrient cycling and microhabitat formation.

Trophic interactions were diverse (Table 3). Predatory activity by *Odontomachus similimus* and *Tetramorium smithi* Mayr, 1879 regulated invertebrate populations, while scavenging by *Camponotus parius* Emery, 1889, *C. rufoglaucus*

Table 1. Ant species recorded in the Alappuzha coastal habitats

No.	Subfamily/ Species
Formicinae	
1	<i>Oecophylla smaragdina</i> (Fabricius, 1793)
2	<i>Paratrechina longicornis</i> (Latreille, 1802)
3	<i>Camponotus parius</i> Emery, 1889
4	<i>Camponotus rufoglaucus</i> (Jerdon, 1851)
5	<i>Anoplolepis gracilipes</i> (Smith, 1857)
Ponerinae	
6	<i>Diacamma rugosum</i> (Le Guillou, 1842)
7	<i>Diacamma ceylonense</i> Emery, 1897
8	<i>Odontomachus similimus</i> Smith, 1858
Myrmicinae	
9	<i>Crematogaster hodgsoni</i> Forel, 1902
10	<i>Crematogaster subnuda</i> Mayr, 1879
11	<i>Tetramorium smithi</i> Mayr, 1879
12	<i>Tetramorium indicum</i> (Forel, 1902)
13	<i>Tetramorium salvatum</i> Forel, 1902
14	<i>Meranoplus bicolor</i> (Guerin-Meneville, 1884)
15	<i>Trichomyrmex destructor</i> (Jerdon, 1851)
16	<i>Pheidole sharpi</i> Forel, 1902
17	<i>Solenopsis geminata</i> (Fabricius, 1804)
Dolichoderinae	
18	<i>Tapinoma melanocephalum</i> (Fabricius, 1793)
19	<i>Technomyrmex albipes</i> (Smith, 1861)
20	<i>Dolichoderus taprobanae</i> (Smith, 1858)
Pseudomyrmicinae	
21	<i>Tetraponera allaborans</i> (Walker, 1859)

(Jerdon, 1851) and *P. longicornis* accelerated organic matter decomposition. Mutualistic associations involving *Technomyrmex albipes* (Smith, 1861) and *C. rufoglaucus* with

Table 2. Seed removal rates (SRR) and seed – transporting ants across coastal habitats

Habitat	SRR (%)	Dominant species	Note on seed fate
Vegetated dunes	68	<i>Meranoplus bicolor</i> , <i>Camponotus compressus</i> , <i>Solenopsis geminata</i>	Transported to nests or buried in soil
Coconut groves	52	<i>Meranoplus bicolor</i> , <i>S. geminata</i>	Partial burial and redistribution
Disturbed sandy patches	35	Opportunistic species (<i>S. geminata</i> , others)	Limited seed dispersal and soil incorporation

[Seed removal rates (SRR) were calculated as the percentage of seeds removed from the experimental plots within 24 hours of deployment]

Table 3. Functional roles and observed behaviors of key ant species, across Alappuzha coastal habitats

Ant species	Functional role	Behavior/interaction	Habitat preference
<i>Meranoplus bicolor</i>	Seed disperser, soil aerator	Transporting seeds, soil-nesting, organic matter redistribution	Dunes, groves
<i>Solenopsis geminata</i>	Seed disperser, scavenger	Seed burial, scavenging, soil turnover	All habitats
<i>Pheidole sharpi</i>	Soil aerator, seed disperser	Soil nesting, seed movement, nutrient cycling	Dunes, dead wood remaining's
<i>Odontomachus similimus</i>	Predator	Preying on termites and dipteran larvae	Dunes
<i>Tetramorium smithi</i>	Predator	Termite and larval predation	Dunes
<i>Camponotus parius</i>	Scavenger, pest regulator	Removing dead insects and plant debris	Groves, dunes
<i>C. rufoglaucus</i>	Mutualist	Protects Hemipterans for honeydew	Dunes, Groves
<i>Technomyrmex albipes</i>	Mutualist	Tending sap-sucking Hemipterans	Dense plant cover
<i>Paratrechina longicornis</i>	Scavenger	Accelerates decomposition	Disturbed sandy patches

hemipterans on *Spinifex littoreus* (Burm.f.) Merr. and *Cocos nucifera* L. reflected complex ant–plant–insect networks.

Habitat-specific behavioral adaptations indicated that vegetated dunes supported complex nesting and multi-species coexistence, whereas disturbed sandy patches were dominated by opportunistic and invasive taxa, underscoring the sensitivity of ant assemblages to anthropogenic disturbance. Local knowledge corroborated these patterns. 82 per cent of respondents recognised ants as beneficial for soil fertility and pest regulation, while 18 per cent reported nuisance species (*S. geminata* and *Anoplolepis gracilipes*).

Interactions between ants and vertebrates, such as the Indian Common Myna *Acridotheres tristis*

(Linnaeus 1766), the Greater Racket-tailed Drongo *Dicrurus paradiseus* (Linnaeus, 1766), and the skink *Lankascincus fallax* (Peters, 1860), revealed the integration of ants into the broader coastal trophic web. Nest mounds and litter turnover further supported detritivorous and edaphic fauna, enhancing overall biodiversity.

These findings indicate that ant diversity and ecological functioning are closely linked to habitat stability and soil quality. The dense vegetation and higher organic matter in vegetated dunes provided favourable nesting sites and microclimatic buffering, consistent with earlier reports from tropical systems (Parr and Bishop, 2022). In contrast, the simplified communities in disturbed patches, dominated by *P. longicornis* and *A. gracilipes*, mirror patterns from other degraded landscapes (Bharti and Sharma,

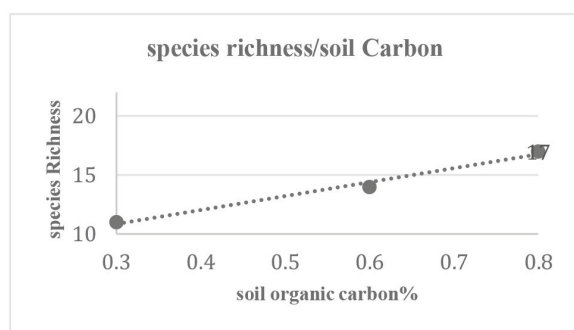


Fig. 1 Correlation between soil organic carbon content and species richness

2009; Wetterer, 2005). The observed positive association between organic matter and species richness supports previous evidence that nutrient-rich soils enhance foraging opportunities and colony establishment (Culliney, 2013).

The exclusive occurrence of *Diacamma rugosum* in vegetated dunes suggests sensitivity to disturbance and dependence on stable microhabitats (Gadagkar *et al.*, 1989). Such species serve as indicators of habitat quality and restoration potential. Overall, the study confirms that ants act as ecosystem engineers in coastal dune systems, facilitating processes such as seed dispersal, soil modification, predation, and mutualism that maintain ecosystem stability (Hölldobler and Wilson, 1990).

Comparative studies from tropical coasts (Floren, 2014; Antony *et al.*, 2021) similarly report that structurally intact dunes harbour more resilient ant assemblages. The decline in diversity along the disturbance gradient observed in Alappuzha aligns with global trends, reaffirming the value of ants as bioindicators of ecosystem integrity. Protecting the structural and ecological stability of vegetated dunes is essential for sustaining native ant populations and the key ecosystem functions they regulate. Conservation strategies should prioritise minimising anthropogenic disturbances and restoring degraded dune habitats to enhance the resilience and ecological stability of the Alappuzha coast.

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First report of *Tabanus (Tabanus) rubidus* Wiedemann, 1821 (Diptera, Tabanidae) from Assam, India, with detection of multidrug-resistant *Mammaliicoccus sciuri* and *Staphylococcus kloosii*

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ABSTRACT: A survey of *Staphylococcus* and *Mammaliicoccus* bacteria in *Tabanus (Tabanus) rubidus* Wiedemann, 1821 (Diptera, Tabanidae) was conducted in three habitats in Assam, India — Lokhrah, Ghar Chuk, and the Royal Global University campus — during peak fly activity in 2024. This constitutes the first record of *T. rubidus* from Assam. A total of 120 adult flies were collected and their morphological identity confirmed under a stereomicroscope using established taxonomic keys. Bacterial identification using the VITEK® 2 Compact system confirmed 168 staphylococcal isolates across six species. *Mammaliicoccus sciuri* was the most prevalent (82 isolates, 48.8%), followed by *Staphylococcus kloosii* (44 isolates, 26.2%). Antimicrobial susceptibility testing against 13 drugs revealed that 51.2 per cent of *M. sciuri* isolates were multidrug-resistant (MDR), with high resistance to methicillin (47.8%), erythromycin (65.4%), and tetracycline (40.5%). Among *S. kloosii*, 31.8 per cent were MDR, with notable resistance to penicillin (72.7%) and clindamycin (38.2%). All isolates remained susceptible to vancomycin and linezolid. These findings document the carriage of multidrug-resistant staphylococci in *T. rubidus* in Assam, suggesting that horse flies may serve as environmental reservoirs of antimicrobial resistance.

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KEY WORDS: Record, survey, staphylococcal isolates, reservoir, antimicrobial resistance

INTRODUCTION

Horse flies (Diptera, Tabanidae) are widespread blood-feeding insects reported as mechanical carriers of various bacterial pathogens (Mullens, 2024). *Tabanus (Tabanus) rubidus* Wiedemann,

1821, a large tabanid species with type locality in Bengal, has been recorded from West Bengal, Meghalaya, Orissa, Sikkim, and Arunachal Pradesh in India, and from China, Indonesia, Pakistan, and the Philippines (Banerjee *et al.*, 2020). The species is predominantly active between May–July and

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November–February and feeds on cattle, buffalo, horse, and wild herbivorous animals. It is closely related to *T. triceps* Thunberg, 1827, but is distinguished by the presence of a median stripe of pale tomentum on abdominal tergites I–VI appearing as a series of connected narrow triangles, and by characteristic step-like lateral abdominal stripes (Ricardo, 1911; Banerjee *et al.*, 2017). Despite its wide distribution in adjacent states, no published record of *T. rubidus* from Assam exists. This study reports *T. rubidus* for the first time from Assam and examines the staphylococcal bacteria harboured by this fly species.

Antimicrobial resistance (AMR) is a major global public health concern, and environmental reservoirs of resistant bacteria are increasingly recognized (Ferri *et al.*, 2017). *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri*) is an emerging opportunistic pathogen with high genetic adaptability and frequent multidrug resistance, recovered from livestock, wildlife, and clinical specimens (Boonchuay *et al.*, 2023). *Staphylococcus kloosii*, a coagulase-negative species, also carries resistance genes and has been found in diverse environmental contexts (Ocloo *et al.*, 2023). The occurrence of such bacteria in haematophagous insects is of public health interest because flies may acquire bacteria from their environment or from hosts during blood feeding. This study reports the presence and antimicrobial susceptibility profiles of staphylococci detected in *T. rubidus* from Assam both of which are first from the state.

MATERIALS AND METHODS

Adult *Tabanus* flies were collected from three locations in Assam, northeastern India, during June to September 2024 using sweep nets between 09:00 and 16:00 hours: Lokhrah (26.1944° N; 91.7697° E), an agricultural area with water bodies and livestock; Ghar Chuk (26.2156° N; 91.7845° E), a mixed farmland and residential zone; and the Royal Global University campus (26.2571° N; 91.5973° E), a semi-urban setting. A total of 120 adult flies were captured. Each specimen was placed individually in a sterile labelled 15mL tube, transported to the laboratory at 4°C, and identified

morphologically under a stereomicroscope using standard taxonomic keys for Tabanidae (Banerjee *et al.*, 2017).

All the 120 specimens collected in the survey were identified as *T. (T.) rubidus* on the basis of the following diagnostic characters: face covered with grey tomentum and white hairs; palpi stout and large, light yellow with black pubescence; frontal callus oblong with short linear extension; thorax dark reddish with grey tomentum and indistinct stripes; abdomen lilac to reddish brown, with median stripes composed of greyish-white tomentose triangular spots appearing as a series of connected narrow triangles on tergites I to VI; lateral stripes composed of greyish-white irregular tomentose spots appearing step-like; sides of abdomen serrated; femora dark; fore tibiae light yellowish but dark at apices; wings hyaline with a slight yellowish tinge in the middle. These characters distinguish *T. rubidus* from the related *T. triceps*, which lacks the series of connected triangular pale spots on the abdomen (Ricardo, 1911; Banerjee *et al.*, 2017).

For bacterial isolation, each fly was surface-sterilised by sequential immersion in 70 per cent ethanol (30 sec) and one per cent sodium hypochlorite (30 sec), followed by three one-minute rinses in sterile distilled water (Sharma *et al.*, 2020). Each sterilised fly was homogenised in 1mL phosphate-buffered saline (PBS, pH 7.4) and serially diluted. Aliquots were plated on, Nutrient Agar and incubated aerobically at 37°C for 24–48 hours. Gram-positive, catalase-positive cocci were presumptively classified as staphylococci and subjected to definitive species identification using the VITEK® 2 Compact system with GP identification cards (Funke and Funke-Kissling, 2005).

Antimicrobial susceptibility testing (AST) followed for 13 antibiotics across eight drug classes: penicillin G, oxacillin, and cefoxitin (beta-lactams); erythromycin and clindamycin (macrolides); gentamicin (aminoglycoside); tetracycline and doxycycline (tetracyclines); ciprofloxacin and levofloxacin (fluoroquinolones); trimethoprim-sulfamethoxazole (sulfonamide); vancomycin

(glycopeptide); and linezolid (oxazolidinone) (Jorgensen *et al.*, 2007). Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes (Magiorakos *et al.*, 2012). Quality control was maintained using *S. aureus* and *S. epidermidis*.

RESULTS AND DISCUSSION

A total of 120 specimens were morphologically confirmed as *T. (T.) rubidus* Wiedemann, 1821. This constitutes the first confirmed record of this species from Assam, India, extending its known distributional range in northeastern India beyond West Bengal, Meghalaya, Orissa, Sikkim, and Arunachal Pradesh (Maity *et al.*, 2020). The collected specimens exhibited body morphology, aligning with standard morphotaxonomic descriptions. The morphological identification was supported by the characteristic abdominal pattern of connected narrow triangular pale tomentose spots and step-like lateral stripes, which distinguishes it from *T. triceps*. The female-biased

collection reflects the haematophagous habit required for oogenesis in tabanids.

Microbiological processing of the flies yielded 168 staphylococcal isolates belonging to six species, confirmed via the VITEK® 2 Compact system (Table 1). This diversity suggests that *T. rubidus* harbours a complex bacterial community, mirroring findings from tabanid studies in other regions, such as northeastern China (Qiu *et al.*, 2024). *Mammaliicoccus sciuri* was the most prevalent species (48.8%), followed by *Staphylococcus kloosii* (26.2%). The high prevalence of *M. sciuri* (formerly *S. sciuri*) is significant, as it is an emerging opportunistic pathogen frequently associated with livestock and clinical environments (Boonchuay *et al.*, 2023). Other isolates included *S. xylosus* (11.9%), *S. epidermidis* (7.1%), *S. haemolyticus* (3.6%), and *S. saprophyticus* (2.4%).

Antimicrobial resistance (AMR) profiles revealed a high burden of resistance in the dominant species

Tabanus (Tabanus) rubidus as an Environmental Reservoir of MDR Bacteria in Assam, India

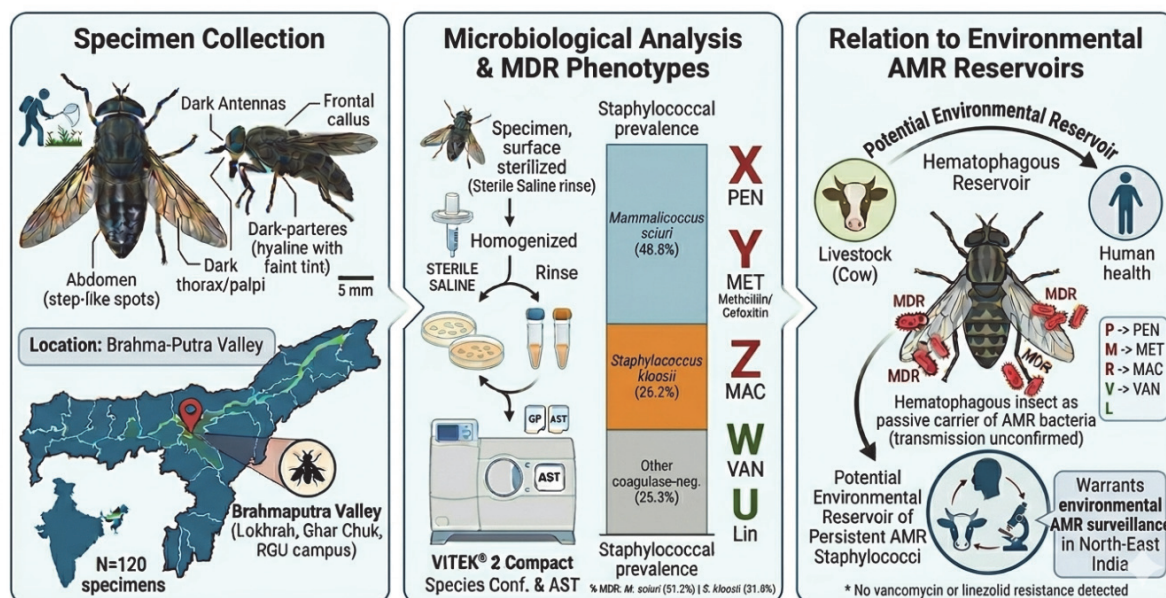


Fig. 1. Graphical summary of *Tabanus (Tabanus) rubidus* as a carrier of multidrug-resistant (MDR) bacteria in Assam, India, showing specimen collection sites in the Brahma-putra Valley, microbiological analysis pipeline (VITEK® 2 Compact), staphylococcal prevalence, key resistance phenotypes, and the potential role of the fly as an environmental reservoir of AMR staphylococci

Table 1. Distribution of *Staphylococcus* and *Mammaliicoccus* species isolated from *Tabanus (Tabanus) rubidus* (n = 168 isolates from 120 flies)

Species	No. of isolates (%)	Lokhrah	Ghar Chuk	RGU campus
<i>Mammaliicoccus sciuri</i>	82 (48.8)	34	24	24
<i>Staphylococcus kloosii</i>	44 (26.2)	16	14	14
<i>S. xylosus</i>	20 (11.9)	9	6	5
<i>S. epidermidis</i>	12 (7.1)	5	4	3
<i>S. haemolyticus</i>	6 (3.6)	3	2	1
<i>S. saprophyticus</i>	4 (2.4)	2	1	1
Total	168 (100)	69	51	48

Table 2. Antimicrobial resistance (% resistant) of *Mammaliicoccus sciuri* (n = 82) and *Staphylococcus kloosii* (n = 44).

Antibiotic	<i>M. sciuri</i> (n=82) %R	<i>S. kloosii</i> (n=44) %R
Penicillin G	68.3	72.7
Oxacillin (methicillin)	47.8	10.9
Cefoxitin	47.8	15.5
Erythromycin	65.4	15.5
Clindamycin	15.1	38.2
Gentamicin	18.0	20.0
Tetracycline	40.5	18.2
Doxycycline	38.5	16.4
Ciprofloxacin	22.9	12.7
Levofloxacin	20.0	10.9
TMP-SMX	25.4	15.5
Vancomycin	0.0	0.0
Linezolid	0.0	0.0

(Table 2). Among *M. sciuri* isolates, 47.8 per cent exhibited methicillin resistance (concurrent oxacillin and cefoxitin resistance). Resistance to penicillin G was 68.3 per cent, followed by erythromycin (65.4%) and tetracycline (40.5%). This high rate of methicillin resistance is a public health concern,

as it often confers co-resistance to most beta-lactam antibiotics. In *S. kloosii*, penicillin G resistance was the highest (72.7%), while methicillin resistance was lower at 15.5%. Reassuringly, all isolates remained susceptible to vancomycin and linezolid, consistent with their status as last-resort clinical treatments.

Multidrug resistance (MDR) was detected in 40.5 per cent of the total isolates, with a significantly higher prevalence in *M. sciuri* (51.2%), compared to *S. kloosii* (31.8%) (Table 3). These findings indicate that coagulase-negative staphylococci in haematophagous insects serve as important environmental reservoirs within the resistome (Ocloo *et al.*, 2023). While this study documents *T. rubidus* as a potential carrier of AMR

Table 3. Multidrug resistance (MDR) summary by species

Species	Total isolates	MDR isolates	MDR %
<i>Mammaliicoccus sciuri</i>	82	42	51.2
<i>Staphylococcus kloosii</i>	44	14	31.8
<i>S. xylosus</i>	20	7	35.0
<i>S. epidermidis</i>	12	3	25.0
<i>S. haemolyticus</i>	6	2	33.3
<i>S. saprophyticus</i>	4	0	0.0
Total	168	68	40.5

staphylococci, but it does not assess vector competence or demonstrate transmission nor provide evidence of vector competence or transmission to vertebrate hosts. The flies likely acquire these bacteria from soil, dung, or host skin during feeding (Greenberg, 1965). Given that northeastern India is a critical region for AMR monitoring (Malik *et al.*, 2024), these results highlight the necessity of incorporating insect-associated reservoirs into future surveillance and genotypic characterization studies.

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An investigation on behavioural, morphological and developmental changes induced by eucalyptus oil nanohybrid emulsions in larvae of *Aedes aegypti* (Linnaeus)

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ABSTRACT: Zinc and copper sulphide-based hybrid nanoemulsions were synthesized using eucalyptus oil and lethal dosage of nanohybrids exposed to larvae of *Aedes aegypti*, to assess their effects on its development, behaviour and morphological features. The larvae showed retardation in development and late emergence of adult after treatment with metal sulphide hybrid nanoemulsions. Morphologically, intense damage to cuticle, pigmentation of gut, contraction and shrunken body was noted visually under light microscope. The larvae showed modified behaviour such as crankiness, restlessness, agitation and aggressiveness. These behavioural and developmental studies are of great significance in management of *Aedes* mosquito. © 2026 Association for Advancement of Entomology

KEY WORDS: Mosquito, metal sulfides, essential oil, nanoemulsions, vectors

INTRODUCTION

Mosquitoes (Diptera, Culicidae) cause a serious threat to humans throughout the world as they play a role of vectors which carry a number of devastating pathogens and parasites (Mishra *et al.*, 2017). *Aedes aegypti* (Linnaeus), commonly known as yellow fever mosquito, is most common vector that transmits several diseases such as Zika, Yellow fever, dengue and chikungunya (Sandhu *et al.*, 2023a). The prevalence of dengue has amplified greater than eight-fold during the last twenty years. In 2000, a total of 505,430 reported cases of dengue; this number increased to 2.4 million approximately by 2010 and reached 52 lacs by 2019 (WHO, 2023). Application of various synthetic insecticides such as Organophosphates (OPs),

Pyrethroids and treated nets etc. is the primary mosquito control method nowadays and is the only way to combat with mosquito-borne diseases (Dulacha *et al.*, 2022). These chemical measures posed mosquito resistance, resurgence, ecological problems as well as severe impacts on survival of non-target organisms (Sandhu *et al.*, 2023a). In context to this, plethora of scientific reports showed that there is a wide competence in botanical pesticides against mosquito vectors (Pavela, 2015). Plants contain aromatic and volatile components called essential oils and can be easily extracted. Moreover, due to their less toxic nature to non-target organisms make them an efficient control measure against broad range of insect pests (Elango *et al.*, 2009). *Eucalyptus* spp. belongs to family Myrtaceae had shown tremendous repellent effects to larvae

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of *Aedes* spp. (Cheng *et al.*, 2009). To improve the efficacy and stability of essential oils, Research in developing nations is mostly concentrated on green nanotechnologies (Kojom Foko *et al.*, 2021). Researchers also had shown a great deal of interest in metallic nanoparticles made using various metals that exhibit uncommon and wider chemical and physical properties in recent years (Sabri *et al.*, 2016). Therefore, in order to create metallic nanoparticles, several metals including iron, zinc, silver, gold, platinum, and titanium have been used. Out of these, silver nanoparticles have been studied more thoroughly and shown to have the ability to be more lethal to mosquito larvae, fungus, and bacteria (Ga'al *et al.*, 2018). Copper and zinc oxide nanoparticles prepared through green synthesis methods exhibited larvicidal efficacy against *Ae. aegypti* and also showed toxicity in cotton leafworm, *Spodoptera littorals* larvae and pupae (Shaker *et al.*, 2016; Selvan *et al.*, 2018). Apart from metal oxide nanoparticles, metal sulfides are considered as non-toxic types of metals (Wang *et al.*, 2013). Larvicidal potential of Copper sulfide (NCuS) and Zinc sulfide nanoparticles (NZnS) was evaluated against *Ae. aegypti*, with LC_{50} of 4.42 and 4.49ppm after 24 hours (Sandhu *et al.*, 2022; 2023a). To improve the efficacy and synergistic toxicity against *Ae. aegypti*, larvicidal potential of copper and zinc sulfide nanohybrids has also been prepared and revealed respective LC_{50} values of 5.00 and 7.63ppm after 24 hours of treatment (Sandhu *et al.*, 2023a, c). A study was undertaken with the synthesized eucalyptus oil emulsions and for their larvicidal efficiency (Sandhu and Vashishat, 2022). The effect of these nano composites on the behaviour of *Ae. aegypti* 4th instar was also studied.

MATERIALS AND METHODS

The research was conducted using effective copper and zinc sulfide-based hybrid nanoemulsions prepared from copper sulphide (CuS) and zinc sulphide (ZnS) nanoparticles in combination with eucalyptus oil nanoemulsion against *Ae. aegypti* larvae. The larvae were exposed to effective median lethal dosages of hybrid nanocomposite for twenty-four hours. The toxic impact of nanohybrids

was thoroughly studied. The larvae of *Ae. aegypti* were collected from different residential water collections from urban region of the District Ludhiana.

Aqueous nanoemulsions with non-polar oil, Tween and water were obtained after ultrasonication for 30 minutes. The emulsions having six percent non-polar eucalyptus oil and Tween 20 in different ratios (1:3, 1:2 and 1:1) prepared and checked for thermodynamic stability tests and larvicidal toxicity (Sandhu and Vashishat 2022). Stock solutions of metal sulfide nanoparticles i.e. NCuS and NZnS were prepared in 35.8 and 48.69ppm concentrations (Sandhu *et al.*, 2022; Sandhu *et al.*, 2023b). Afterwards, effective Copper sulphide nanoparticles (CuSNPs) and Zinc sulphide nanoparticles (ZnSNPs) were mixed with most stable eucalyptus oil nanoemulsion and whole formulation was sonicated to make metal nanohybrid or hybrid nanoemulsions (Sandhu *et al.*, 2023a, c). Different fractions of eucalyptus oil nanoemulsions and Zinc and Copper sulphide nanoparticles (1:1, 1:2, 1:3, 1:4, 1:5) were prepared from stock solutions and tested for stability tests.

The previous research has revealed the LC_{50} of aqueous eucalyptus oil nanoemulsion formulated with copper sulfide (NCuS) and zinc sulfide nanoparticles (NZnS) known as hybrid nanoemulsions (Sandhu *et al.*, 2023a, c). The calculated LC_{50} and LC_{90} values were obtained after treating with CuS based hybrid nanoemulsions in *Ae. aegypti* larvae were recorded as 5.00 and 5.81ppm, and with ZnS based hybrid nanoemulsions exposure were computed to be 7.63 and 9.22ppm. An experiment was conducted with 25 larvae and effective concentrations of nanohybrids for 24 hours. The larvae which survived were monitored until adult emergence. The developmental period and adult emergence were evaluated the underlying outcomes of prepared hybrid nanoemulsions. The experiment was performed in three replicates with each concentration. Simultaneously, larvae were also treated to median lethal dosages of CuS and ZnS based hybrid nanoemulsions for 24 hours in three replicates having 25 larvae each. The dead larvae were observed vigilantly under light

microscope for various morphological aberrations. Notable changes in head, thorax, abdomen and in siphon, antennae, eyes and mouth brushes were observed and compared with control larvae. The photomicrographs were taken using digital camera (Nikon DS-R11) attached to the microscope.

Effect of eucalyptus oil-based hybrid nanoemulsions on larval behavioral response:

The larvae were carefully monitored at specific time intervals followed by final observations at 24 hours. The changes in behavioural pattern i.e. wriggling speed, horizontal and vertical movements, aggression, aggregation, excitation, inability to rise up to the water surface, abnormal positions, convulsions and self-biting activity were noted. The larval behaviour symptoms were photographed with Nikon DSLR Digital Camera (Nikon D5200 with 18-55mm lens). In the same way, control larvae were also observed and compared with treated larvae.

RESULTS AND DISCUSSION

The prepared nanoemulsions were screened initially on the basis of certain parameters and various stability characteristics (Tables 1, 2). The most stable nanoemulsions were selected and larvicidal assays were performed visual appearance of synthesized oil nanoemulsions and hybrid nanoemulsions. The larvae exposed to LC_{50} and LC_{90} concentrations of CuS and ZnS based hybrid nanoemulsions, exhibited delayed development and reduced emergence of adult and it was dose-dependent. The development period was 5 days which increased significantly by 8.00 and 10.61 percent, 7.00 and 9.12 percent due to exposure of LC_{50} and LC_{90} dosages of CuS and ZnS nanoemulsions respectively. The adult emergence was 96-98 per cent in control, but it was only 10.21-12.66 percent at exposure of LC_{90} of CuS and ZnS nanoemulsions. Moreover, other increasing dosages affected the larval development significantly but more affect was noted in CuS based hybrid nanoemulsions.

Morphological observations of the larvae exposed with lethal concentrations of CuS and ZnS nanohybrids revealed ruptures in cuticle,

pigmentation, darkening or blackening of alimentary canal (Fig. 1d, 40X). However, in control larvae there was no alterations in larval external organs like head, eyes, antennae, thorax (Fig. 1a, b, 400X), abdominal scales, siphon with subventral or hair tuft (Fig. 1c, 400X) when observed under light microscope.

Essential oils extracted from various plants in combination with metallic nanoforms are considered to be safe and effective against mosquito larvae (Benelli and Mehlhorn, 2016). The previous findings showed that killing potential of ZnS and CuS-based hybrid nanoemulsions against *Ae. aegypti* larvae with a $LC_{50, 90}$ of 7.63, 9.22ppm and 5.00 and 5.81ppm respectively (Sandhu *et al.*, 2023a, c). The present investigation demonstrated the latent effects of ZnS and CuS nanohybrids of eucalyptus oil exposure resulted in dose-dependent delayed larval development and lessened adult emergence of treated larvae. Comparing CuS and ZnS based nanohybrids, the former had an enhanced and stronger effect. When *Ae. vagus* and *Ae. albopictus* larvae are treated with zinc oxide nanoparticles, resulting in slowed larval growth, indicating the prolonged effects of the nanoparticles (Gunathilaka *et al.*, 2021) and they also observed an increased duration of 13 days (at LC_{25}), 14.67 days (at LC_{50}), 16.67 days (at LC_{75}), and 17 days (at LC_{100}) in comparison to the control trial of developmental study (8 days), demonstrating the concentration-dependent impacts in present study (Gunathilaka *et al.*, 2021). In other studies, by Amrutha and John (2025) reported the effect of basil and turmeric essential oil nanoemulsions was quite toxic against eggs and adults of pests of rice.

ZnS and CuS-based hybrid nanoemulsions treated larvae showed behavioural changes as compared to the control sets. The control larvae displayed normal behaviour (Fig. 2a) and within one hour of hybrid nanoemulsion exposure, the larvae showed aggressive and restless activity with increased exposure times, i.e., an altered, excitable and abnormal movements, biting of own anal papillae, mouth parts preceding to the rounded or circular structures (Fig. 2b). It was found that these persistent actions and symptoms of the treated

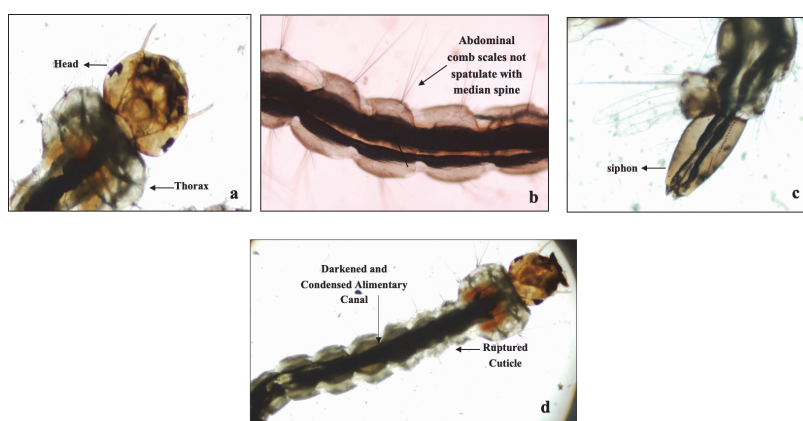


Fig. 1 Effect of copper and zinc sulphide nano hybrids on morphology of *Ae. aegypti* larvae

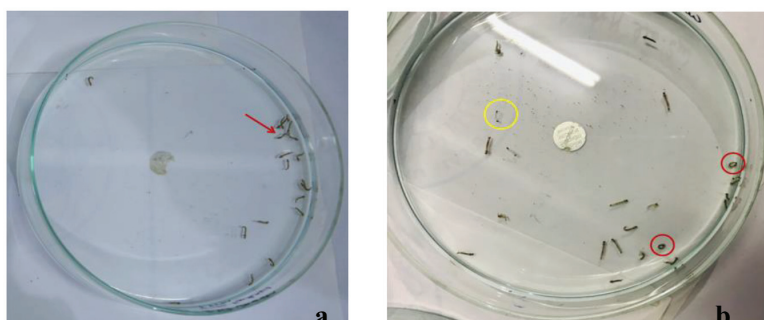


Fig. 2 Effect of copper and zinc sulfide nano hybrids on behaviour of *Ae. aegypti* larvae – a) Arrow showing control larvae showing normal movement and behaviour; b) Treated larvae - the red and yellow circles indicate aggressive and gill-biting behaviour and disappearance of the gut

larvae became more agitated and observed their twisting motions and various larvae showed vibration and paralytic movements. Moreover, cuticle layer of the copper-based hybrid nanoemulsion treated larvae got completely damaged as compared to zinc sulphide based nano hybrid. Despite the fact that several studies have shown that exposure to plant extracts causes behavioural changes in mosquito larvae, including agitated and excited behaviour, circular movements making ring shape structure, the impact of metal sulphide-based hybrid nanoemulsions as behaviour modifiers has not yet been evaluated. *Aedes aegypti* and *Cx. quinquefasciatus* larvae showed similar vibrating and paralytic symptoms after treatment of fungal isolates and appeared as neurobehavioral toxicant (Ragavendran *et al.*, 2019) which is similar with current study. The results are in contrast to previous reports on the larval behaviour of *Ae. aegypti* after treatment with *Achyranthes aspera* silver nanocomposites which reported that there were no noticeable behavioural changes in larvae except the induced mortality after

24 hours (Sharma *et al.*, 2022). The present study was in consistent with earlier findings of how extracts from *A. aspera* leaves and stems affect the behaviour of *Ae. aegypti* larvae (Sharma *et al.*, 2016), causing paralysis, convulsions, and aggression.

The structural analysis of *Ae. aegypti* larvae treated with CuS and ZnS nano hybrids formulated with eucalyptus oil nanoemulsions, revealed apparent effects, including gut darkening and disorganization, body shrinkage, ruptures in cuticle, contraction of anal papillae and a slight contraction of the internal membrane of anal papillae. These effects represented contact and systemic toxicity of nano hybrids affecting both larval cuticle and gut. Some studies have demonstrated that the toxic effect of green nanoparticles on the survival of *Ae. aegypti* larvae which is similar to the present study and previous reports (Sharma *et al.*, 2017, 2020). The coloured or pigmented gut content brought on by CuS and ZnS based nano hybrids exposure supports the theory that a broken gut may hinder

Table 1. Parameters for testing prepared aqua nanoemulsions of non-polar eucalyptus oil

Oil: Tween 20	Thermodynamic Stability	Optical transparency	Appearance	Phase separation
1:1	-	Poor	Milky	Yes
1:2	Most stable	Highest	Clear	No
1:3	-	Moderate	Turbid	Yes

Table 2. Parameters for testing prepared eucalyptus oil and copper sulphide and zinc sulphide based hybrid nanoemulsions

CuSNPs: Oil ZnS NPs: Oil	Thermodynamic Stability	Optical transparency	Appearance*	Phase separation
1:1	-	Moderate	Dense	No
1:2	-	Moderate	Slight dense	No
1:3	-	Moderate	Slight dense	No
1:4	-	Moderate	Slight dense	No
1:5	Most stable	Highest	Clear	No

*Appearance of zinc sulfide based nanohybrids was dense to slight dense (1:1 to 1:4)

the survival of larvae. Some researchers have documented similar impacts of ZnO nanocomposites made by *Lobelia leschenaultiana* on the exterior structure of *Aedes aegypti* larvae (Banumathi *et al.*, 2017). While comparing with the current study, they also noted the larvae's significantly diminished abdomen area, as well as the removal of their mouth brushes and antennae. These behavioural and developmental related investigations are quite significant for controlling in management of *Aedes* mosquito. These hybrid nanoemulsions can be employed as a safe as well as efficient measure in controlling mosquitoes.

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Morpho-molecular aspects of two deathly mosquitoes from Manipur, India

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ABSTRACT: The morphometrics along with the molecular approach of two species *Anopheles* species and *Aedes aegypti* (L), was taken up. Live mosquitoes and their larvae were gathered from various locations throughout Manipur, including Thoubal, Imphal East, and Imphal West. Using the appropriate keys and comparing them with the data at hand, the morphometric identification was completed. After extracting DNA from adults and sequencing it using Cytb primers, a phylogenetic tree was created using identical sequences from the NCBI Genbank. *Anopheles* in present studies reports two species, *Anopheles longirostris* and *An. jamesii* but the molecular work is based only on *An. longirostris*. Though their niche is overlapping, both *Anopheles* are quite distinct from one another. While *An. longirostris* is a mosquito species that is a significant malaria vector than *An. jamesii*. *Aedes aegypti* was more prevalent in valley districts. © 2026 Association for Advancement of Entomology

KEY WORDS: Vectors, *Anopheles longirostris*, *An. jamesii*, *Aedes aegypti*, phylogenetic tree

INTRODUCTION

Certain mosquitoes are responsible for dreaded human diseases like malaria, dengue, yellow fevers, Japanese encephalitis etc. as they can spread pathogens like harmful viruses, nematodes, and protozoa (Soghigian *et al.*, 2020). With the synergic effect of urbanization and climate change, the gravity of the disease burden due to mosquito as vector multiplied many folds. The life cycles of mosquitoes are accelerated by higher temperatures, which results in faster reproduction and larger populations. The mosquito vectors exhibit quite different resting, biting, vectorial capacity and habitat to show different epidemiology of respective species (Sriwichai *et al.*, 2016; Chaiphongpachara and Laojun, 2020). This necessitates for true

identification of the vector species for formulating species specific vector controls after accurate vector information for effective control (Payakkapol *et al.*, 2016; Sumruayphoet *et al.*, 2020). But absence of certain morphological key features due to hasty handling of the specimens in the process of capturing them in the fields, leading to misidentification (Chaiphongpachara *et al.*, 2022). Furthermore, there are several *Anopheles* mosquito species form complex groups, comprising morphologically indistinguishable sibling species (Muller *et al.*, 2013). The knowledge in molecular biology has undergone significant progress, leading to the development of techniques useful for species identification (Walton *et al.*, 1999). Therefore, the use of modern molecular biology techniques for the species-based identification of mosquito vectors is

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an extremely alternative to standard morphological methods (Samung *et al.*, 2016). The morphological approach is still an important approach for species identification. Molecular approaches that efficiently differentiate conspecific mosquitoes such as the barcode region (Hebert *et al.*, 2003) improve identification accuracy considerably, but are time consuming, expensive in terms of post-polymerase chain reaction processing and depend heavily on DNA sequencing (Ajamma *et al.*, 2016). The species of mosquito from the state was dissected through the morphometric and molecular approaches for the first time. In the present studies two species *Anopheles longirostris*, *An. jamesii* and *Aedes aegypti* was undertaken but the molecular work was done only on *An. longirostris*.

MATERIALS AND METHODS

Manipur lies in the North eastern state of India between 23.83° N to 25.68° N Latitudes and 93.03° E to 94.78° E Longitudes. The valley lies at an elevation of approximately 790m above the sea level with the area covered of 22,327 km². The state belongs to subtropical to temperate climate with an annual rainfall of about 913mm (June to September) which made the favourable ecosystem for mosquito diversity. Present study begins with the collection of specimens using aerial nets from the foot hill sides and around the Loushi pat (Lake) of Tekcham Maning Leikai, Thoubal district Manipur, India with GPS Tag of Lat 24.553479°; Long 93.99151° and few adults and larvae from other parts of Manipur valley (Table 1). Some of the collected specimens were preserved as dry stuff to preserve for long term preservation and also to retain the morphological integrity. While few were fixed using fixative (1 glacial acetic acid: 3 alcohol) for cytological studies. Fifteen of the selected specimens were kept in absolute alcohol for molecular analysis.

For morphological identification and descriptions pictorial keys were taken as references. To identify from adult mosquito species, the insects collected by hand swiping of net were sorted into mosquito and non-mosquito species. Only the mosquitoes of the concerned were collected in collection chambers while the rest were released on spot. The collected

mosquitoes were differentiated and group into male and female then morphological characteristics were observed to differentiate up to Genera and sub-genus. Lastly base on the all-morphological features, and following keys of Narayan *et al.* (2024), Walter Reed Biosystematics Unit (WRBU), Rueda (2004), WHO, Huang (1977, 1980), the mosquitoes were identified to group, complex or species level.

For molecular analysis four groups 15 specimens each which were morphologically identified were kept in separate centrifuge tube containing absolute alcohol with different serial number marked on it by permanent marker. DNA samples were extracted from 15 adult mosquitoes of each species by the standard phenol/chloroform method (Sambrook *et al.*, 1989) and stored at the Dhanamanjuri University, Imphal. PCR was performed using Taq DNA polymerase (GeNei Merck, Mumbai, India) and a model 2720 Thermal Cycler (Applied Biosystems). The complete mitochondrial Cytb coding sequence (470 bp) was amplified using the universal primers CYT BF and CYT BR (L20934) (Lyman, 1999). The amplified fragments were used as templates for sequence determination with the above universal primers and two internal primers (BioKart Bangalore). Both DNA strands of the PCR products were sequenced using an automated method with a BigDye terminator Cycle Sequence Kit (ver. 3.1; Applied Biosystems) and an automated sequencer (model 3130; Applied Biosystems). Sequence fragments obtained with different primers were assembled using MEGA5 software (Tamura *et al.*, 2011), and the sequences were aligned by Clustal W.

Constructed neighbour-joining (NJ) tree using MEGA5. In NJ analyses, the substitution model that better described the substitution patterns of the Cytb(HKY+G; Hasegawa *et al.*, 1985) data was determined by MEGA5. In ML analysis, the tree topology search was evaluated using simultaneous nearest-neighbour interchange (NNI). In NJ analysis, the Kimura two-parameter model (K2) was used. The reliability of nodes was assessed using 1000 bootstrap replicates.

Determined Cytb gene sequences (470 bp) in two (13 *Aedes* Manipur; 14 *Anopheles* Manipur)

mosquitoes from Manipur. Constructed NJ trees using the Cytb sequences, together with database sequences, to assess the phylogenetic relationships within and between species.

RESULTS AND DISCUSSION

The characteristics of adult *Ae. aegypti* areas follows-scutum has lyre-shaped silvery- white scales. Proboscis is dark in colour. Wing scales are

dark. Legs have white basal bands. In larva strong black hooks on sides of thorax is present. One row of comb scales with pitchfork- shaped is present. Pupa: paddles have stubble. Palps have silvery-white scales at the tips. Clypeus has white scales. All these features are in accordance with the other authors (Figs. 2, 3). The adults are predominantly found during daytime and very agile. The larvae are usually procured from unused utensil or storage water tank of any material with rain water deposits.



Fig1. Different sites of species collection from different habitats of valley districts



Fig. 2 *Aedes aegypti*, (A) dorsal view of male mosquito scutum with lyre-shaped silvery-white scales; (B) female adult with white basal bands on tarsomers; (C) wing with dark scales

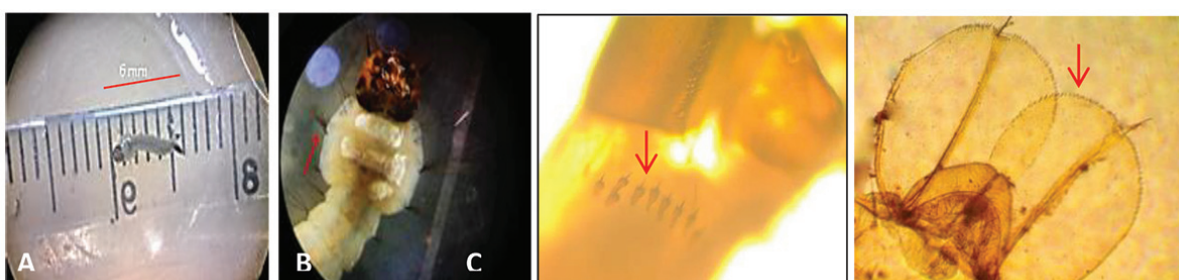


Fig. 3 Larva (*Ae. aegypti*) - (A) 4th instar larva is about 6mm in length; (B) larva with a strong black hook-on lateral side of thorax; (C) single row of comb scales with pitchfork-shaped; (D) Pupal paddles with stubble structure on the curve margin

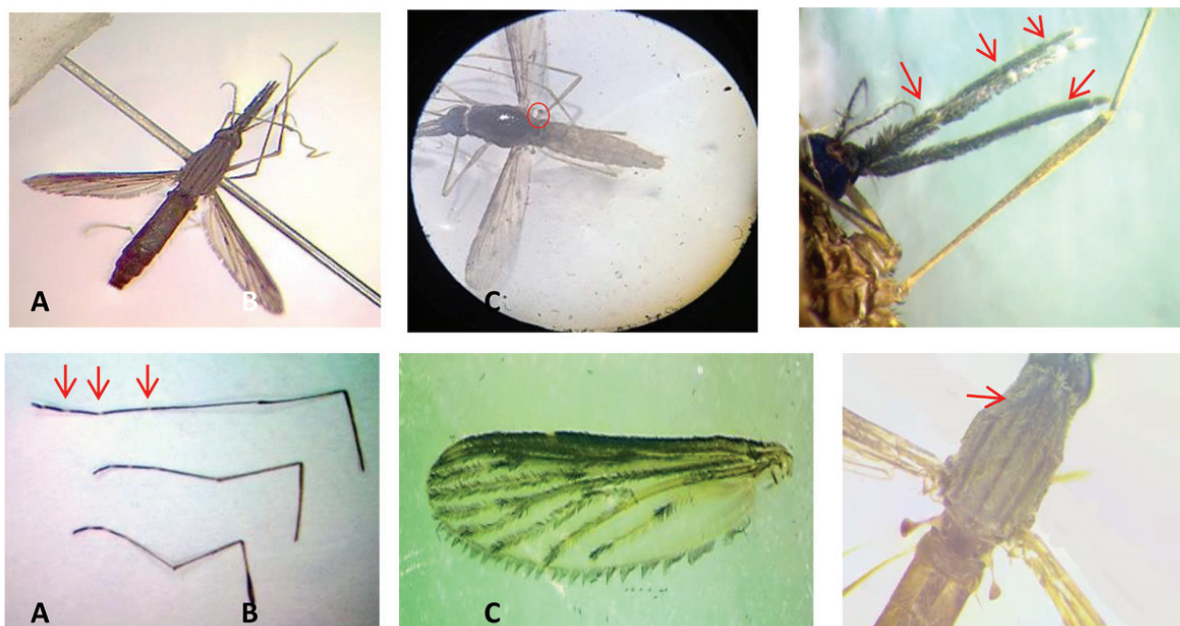


Fig. 4 (A) *Anopheles longirostris* (Adult); (B) Halter capitellum black-scaled dorsally; (C) Palps with distinctive pair of dark bands are separated by narrow pale band while Proboscis completely dark; (D) Legs with tarsi white spotted or bands; (E) Dark and pale spots on the wings; (F) Scutum with broad decumbent pale scales throughout

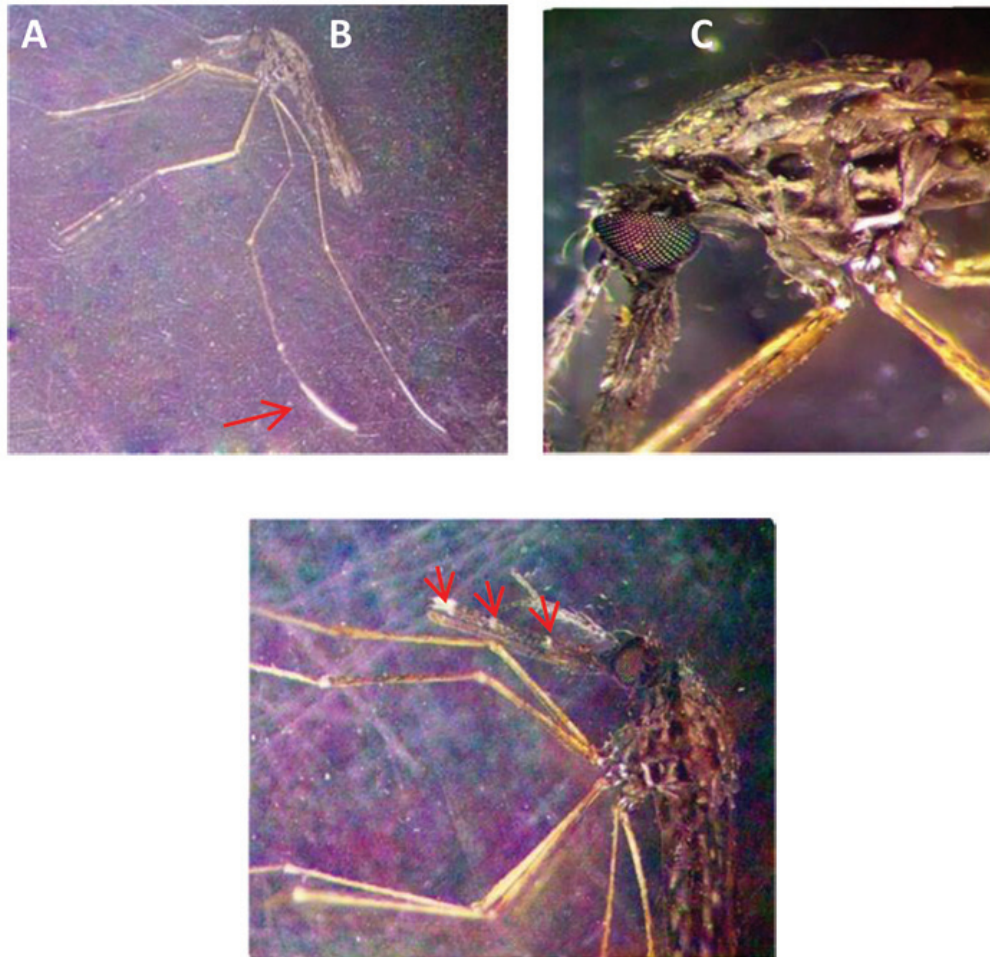


Fig. 5 *Anopheles jamesii*: A- adult female with tarsi 3, 4, 5 of hind limbs completely white; B- Lateral view of thorax with three distinct black patches below the scutum on post pronotal, pre alar and upper mesepimeral; C- Apical pale band nearly equal to the pre apical dark band on palps

Two *Anopheles* species are found in the same habitats. The habitat is the goat shed in the Tekcham, Thoubal district. First one is *An. jamesii* whose characteristics are: Pale area on costa and subcostal including vein 1, pale area greater than or equal to 4, Present of specking in the legs, Apical pale band nearly equal to the pre apical dark band, Hind tarsomeres 5, 4, 3 completely pale (Fig. 5A-C). The morphometrical features of this species are quite in accordance and confirmative keys. But other second features are blending of two species: *An. longirostris* and *An. frauti*. The characteristic features pointing towards *An. longirostris* are: Palpi and probosis are approximately equal in length, Palpi

are dark with pale bands, Apical pale band is about the same length as the pre-apical dark band, Wings have four dark spots on the costa, Dark and pale scales are arranged in a distinctive pattern with a pale fringe spots at vein tips, Covered in narrow dark scales, No distinct pale lines/stripes on the scutum, Dorsal side of the abdominal tergite without pale marking, Ventral side often with lighter bands. And characteristics that point towards *An. frauti* are Apical half of palp dorsal view has mostly 4 white and 3 black bands, Distinct black bands, separated by narrow pale band, 4th white band is reduced or completely dark, proboscis mostly dark but small apical pale band present (Fig. 4). The

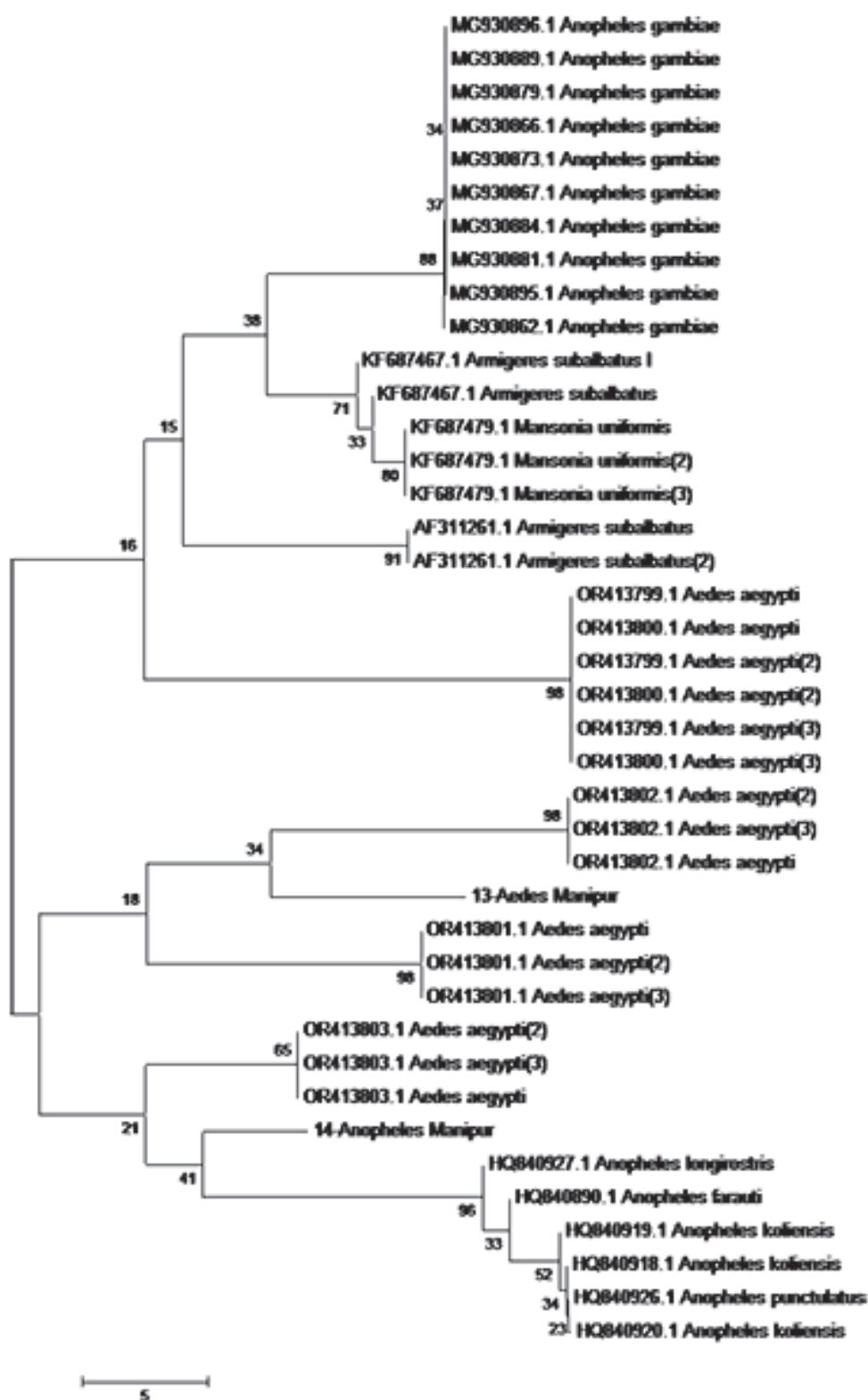


Fig. 6 Phylogenetic tree constructed by neighbour joining technique using the Cytb sequences, together with database sequences, to assess the phylogenetic relationships within and between species

molecular analysis details of *Ae. aegypti* and *An. longirostris* were ascertained using the cytochrome b genes (Cyt b). Though the characteristics are mixing and also in the phylogenetic tree also but the nearest being the *An. longirostris* (Fig. 6).

Dengue cases in different districts of Manipur had experienced a sudden surge from 2022/2023. The species collected from infected areas is *Ae. aegypti*. The larvae of *Ae. Aegypti* are collected from water tank that store rain water but hard to collect from drain/sewage water meaning the species is more adapted in the natural water source rather than the tap water or brackish water though reports come from brackish water (Ramasamy *et al.*, 2011). This might be the reason behind the surge of the disease monsoon. Female mosquitoes *Ae. aegypti* rested on cloth hangings and curtains, followed by furniture as reported by Dalpadado *et al.* (2022). So, after rain it is advisable to chlorinate the water tank or any other place where the larvae could harbour like unused tyre, utensils, plastic bottles or any other rain water accumulating materials as the female will lay eggs for spreading the disease in the crowded urban areas etc. Dengue is an arboviral disease. It is one of the eight neglected tropical disease (WHO, 2016). The South Asian (SEA) and the western Pacific represent 44

75% of the current global burden of dengue (Aguiar *et al.*, 2015). In the early 2000s, dengue was endemic in a few southern states of India (Maharashtra, Karnataka, Tamil Nadu and Pondicherry) and northern states (Delhi, Rajasthan, Haryana, Punjab and Chandigarh). It has recently spread too many states, including the union territories (Chakravarti, 2012). In Manipur, the disease assumed to be almost absent before 2020 though some cases of exotic origin like from Delhi through students. The cases of the disease are rising tremendously in last four years (Sanathoi *et al.*, 2024). This disease is causing health alert in valley areas particularly in Imphal East and West. As the vector is confirmed through morphology and molecular methods the future action plan is to study the biology and life cycle for effective control of this disease.

Another emerging vector is the *Anopheles* species which was assumed to be absent in the valley district though their presence from Moreh (Tengnoupal District) (Dutta *et al.*, 2005), and Jiribam district was evident from the climatic condition like hot and humid condition in these areas as reported by Ayekum *et al.* (2021). But it is very surprised to collect two species of *Anopheles* from Tekcham, Thoubal district. *Anopheles jamesii* is

Table 1. Sites of species collection from different habitats of valley districts alongwith coordinates

No.	Site	District	GPS Tag.	Specimen	Habitat
1.	NgakrabamLeirak	Imphalwest	Lat 24.786753°; Long 93.937369°	Adult <i>Aedes</i> sp.	Water tank
2.	KhuraiKonsamLeikai	Imphal east	Lat 24.834592°; Long 93.973789°	<i>Aedes aegypti</i>	Flower vase with watercontainer
3.	Ekop pat	thoubal	Lat 24.58777972°; Long 93.95153314°	Adult <i>Mansonia uniformis</i>	Bushes near water bodies
4	TekchamManingleikai	Thoubal	Lat 24.553117°; Long 93.991191	Adult <i>Mansonia uniformis</i>	Foot hills
5	D.M.U campus	Imphal	Lat 24.822097°; Long 93.941272°	Adult <i>Armigeres</i>	Foot of wall
6.	Loushipat	Thoubal (Lake)Tekcham	Lat 24.553479°; Long 93.99151°	<i>Anopheles longirostris</i>	Goat shed
7	Loushipat	Thoubal (Lake)Tekcham	Lat 24.553479°	<i>Anopheles jamesii</i> Long 93.99151°	Goat shed

quite distinct from the other on the basis of morphology. The species was reported from Meghalaya (Malaria, J., 2021) through molecular identification technique, from Coastal Karnataka (Gowthami *et al.*, 2024) the study highlighted its prevalence in the area during the declining phase of Malaria; Goa (Ajeetkumar *et al.*, 2024) study found it to be positive carrier of *Plasmodium* during longitudinal mosquito surveys. It was also reported from Nagaland (Knight and Stone, 1977, Reinert, 1975, 2001). The molecular approach not only identifies the species it also enables us to determine the age, time of diversification, more precisely the point of speciation. For instance, the two most important disease vectors in the *Ae.* subgenus *Stegomyia* that have become closely associated with humans in behavior, habitat preferences, and invasiveness, *Ae. (Stegomyia) aegypti* and *Ae. (Stegomyia) albopictus*, diverged from one another 31 MYA (Moureu *et al.*, 2015). In the subfamily Anophelinae, the lineages confined to what is now the Americas diverged first in the form of the genus *Chagasia* during the early Cretaceous, 142 MYA. Approximately 40 million years later, the separation of Gondwana is reflected in the divergence times of *Anopheles* lineages: the *Anopheles* subgenera associated exclusively with the Americas diverged from all other *Anopheles* 103 MYA (Soghigian *et al.*, 2023). The other species of *Anopheles* is assumed to be *An. longirostris* through molecular approach though the blending features with *An. farauti*. Out of the 17 species of *Anopheles* reported by Dutta *et al.* (2005) either *An. longirostris* or *An. farauti* is reported from Manipur. So, intense work is required in future to confirm and validate the occurrence of the species. Till date the occurrence of malaria from the Thoubal particularly Tekcham is not detected from the said area or not inspected thoroughly. If the vector is present sooner or later the disease might show up in near future so area should be informed about the potentiality of the occurrence of the disease in the area. The two fatal species viz., *Ae. aegypti* and *An. longirostris* are confirmed through morphologically and molecular methods in the present study. *Aedes aegypti* is responsible for dengue in the valley district while *An. longirostris* is assumed to be the vector for malaria. Till date

the occurrence of malaria from the Thoubal particularly Tekcham is not detected from the said area or not inspected thoroughly.

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Survey of the moth fauna (Lepidoptera, Heterocera) in Christian College, Chengannur campus, Kerala, India

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ABSTRACT: In a survey on the moth fauna of the Christian College, Chengannur campus, 84 species belonging to 15 families were recorded from diverse habitats ranging from planted forest, butterfly garden, botanical garden and rubber plantations. The family Erebidae showed maximum dominance, followed by Crambidae, Geometridae and Pyralidae. © 2026 Association for Advancement of Entomology

KEY WORDS: Lepidoptera, biodiversity, Erebidae, Crambidae, fauna, suburban

Kerala, located in the heart of the Western Ghats, is a focal point for moth diversity due to its varied ecosystems. Numerous surveys highlight this richness. Alex *et al.* (2021) recorded 503 moth species under 42 families in Kavvai River basin; Mathew (1994) reported 245 species of moths from the Silent valley national park; Mahesh *et al.* (2024) recorded 483 moth species across 44 families in central Kerala; Sondhi *et al.* (2021) added new records from Shendurney and Ponmudi; Mathew (2018) reported 675 moth species from Vagamon; and Swafvan and Sureshan (2022) reported 34 new records agroecosystems of Kerala (2022). Campus-based studies have widely documented moth diversity, providing excellent opportunities for biodiversity research while serving educational and conservation purposes (Metilda and Livingstone, 2022; Nayak and Ghosh, 2020). The present study investigates the moth diversity of Christian College, Chengannur campus, aiming to contribute to the

lepidopteran fauna of Alappuzha district and to enhance understanding of moth communities in educational landscapes of central Kerala.

The study area, Christian College Chengannur, is a suburban campus covering six hectares and located on a hilltop at 9°18'23.3"N; 76°37'34.0"E, in the extreme eastern part of Alappuzha district, Kerala, India, on the banks of the Pamba river. The region experiences a tropical monsoon climate characterised by high humidity, abundant rainfall, and relatively stable temperatures year-round. The survey was conducted from February to July 2023 (pre-monsoon to monsoon), when the temperature ranged from 23 to 35 °C. The semi-urban campus (50-60% vegetated) comprises diverse habitats: a planted mini-forest (Shanthisthal, 15-16 cents) with around 200 trees and 116 species of plants, including rare, endangered, and threatened species; Miyawaki forest (Vidyavanam); botanical garden

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Table 1. List of moth fauna recorded in Christian College, Chengannur campus

No.	Family/ Species
	Bombycidae
1	<i>Trilocha varians</i> (Walker, 1855)
	Crambidae
2	<i>Agrioglypta itysalis</i> (Walker, 1859)
3	<i>Bocchoris inpersalis</i> (Zeller, 1852)
4	<i>Bradina</i> sp.
5	<i>Cnaphalocrocis patnalis</i> (Bradley, 1981)
6	<i>Diaphania indica</i> (Saunders, 1851)
7	<i>Eurrhyarodes bracteolalis</i> (Zeller, 1852)
8	<i>Filodes fulvidorsalis</i> (Hübner, 1832)
9	<i>Glyphodes bicolor</i> (Swainson, 1821)
10	<i>Haritalodes derogate</i> (Fabricius, 1775)
11	<i>Herpetogramma</i> sp.
12	<i>Nistra coelatalis</i> (Walker, 1859)
13	<i>Notarcha aurolinalis</i> (Walker, 1859)
14	<i>Omiodes</i> sp.
15	<i>Omiodes diemenalis</i> (Guenée, 1854)
16	<i>Paliga</i> sp.
17	<i>Palpita annunifer complex</i> (Inoue, 1996)
18	<i>Parotis</i> sp.
19	<i>Parapoynx stagnalis</i> (Zeller, 1852)
20	<i>Patania</i> sp..
21	<i>Pycnarmon cribata</i> (Fabricius, 1794)
22	<i>Ravanoa xiphialis</i> (Walker, 1859)
23	<i>Sameodes cancellalis</i> (Zeller, 1852)
24	<i>Spoladea recurvalis</i> (Fabricius, 1775)
25	<i>Sufetula</i> sp.
26	<i>Tatobotys biannualis</i> (Walker, 1866)
	Erebidae
27	<i>Aemene cf spotoptera</i>
28	<i>Arctornis</i> sp.
29	<i>Artaxa</i> sp.
30	<i>Ammatho cf cuneonotatus</i>
31	<i>Bertula abjudicalis</i> (Walker, 1859)
32	<i>Bastilla crameri</i> (Moore, 1885)
33	<i>Bastilla joviana</i> (Stoll, 1782)
34	<i>Cretonotos gangis interrupta complex</i> (Linnaeus, 1763)
35	<i>Cretonotos transiens</i> (Walker, 1855)
36	<i>Cyclosiella dulcicula</i> (Swinhoe, 1890)
37	<i>Enispa elataria</i> (Walker, 1861)
38	<i>Erebus ephesperis</i> (Hübner, 1823)
39	<i>Erebus hieroglyphica</i> (Drury, 1773)
40	<i>Ericeia</i> sp.
41	<i>Eressa</i> sp.
42	<i>Erygia spissa</i> (Guenée, 1852)
43	<i>Eublemma</i> sp.
44	<i>Hipoepa biasalis</i> (Walker, 1859)
45	<i>Lygniodes vampyrus</i> (Fabricius, 1794)

46	<i>Mocis undata</i> (Fabricius, 1775)
47	<i>Naarda</i> sp.
48	<i>Neochera inops</i> (Walker, 1854)
49	<i>Nygmii</i> tribe
50	<i>Olepa ricinni complex</i> (Fabricius, 1775)
51	<i>Progonia kurosawaii</i> (Owada, 1987)
52	<i>Thyas coronata</i> (Fabricius, 1775)
	Eutellidae
53	<i>Chlumetia transversa</i> (Walker, 1863)
	Geometridae
54	<i>Agathia</i> sp.
55	<i>Antimimistis</i> sp.
56	<i>Aporandria specularia</i> (Guenée, 1858)
57	<i>Comibaena</i> sp.
58	<i>Comostola laesaria</i> (Walker, 1861)
59	<i>Eumelia ludovicata</i> (Guenée, 1858)
60	<i>Gymnoscelis</i> sp.
61	<i>Idaea costiguttata</i> (Warren, 1896)
62	<i>Idaea</i> sp.
63	<i>Scopula</i> sp.
	Hyblaeidae
64	<i>Hyblaea puera</i> (Cramer, 1777)
	Limacodidae
65	<i>Miresa argentifera</i> (Walker, 1855)
66	<i>Parasa lepida</i> (Cramer, 1799)
	Noctuidae
67	<i>Callopstria</i> sp.
68	<i>Condica</i> sp.
69	<i>Mythimna</i> sp.
70	<i>Spodoptera litura</i> (Fabricius, 1775)
	Nolidae
71	<i>Dyrzela plagiata</i> (Walker, 1858)
	Notodontidae
72	<i>Antheua servula</i> (Drury, 1773)
73	<i>Clostera</i> sp.
	Pyalidae
74	<i>Arippara</i> sp.
75	<i>Doloessa viridis</i> (Zeller, 1848)
76	<i>Endotricha</i> sp.
77	<i>Epicrocis</i> sp.
78	<i>Pyralis manihotalis</i> (Guenée, 1854)
	Psychidae
79	<i>Degia</i> sp.
	Sphingidae
80	<i>Pergesa acteus</i> (Cramer, 1779)
81	<i>Theretra silhetensis</i> (Walker, 1856)
	Thyrididae
82	<i>Banisia</i> sp.
83	<i>Epaena</i> sp.
	Uraniidae
84	<i>Micronia aculeata</i> (Guenée, 1857)

comprising 21 species of trees, 96 medicinal plants, 40 other species, and a few exotics; butterfly garden enriched with nectar and larval host plants, including *Citrus* sp., *Murraya koenigii*, *Glycosmis arborea*, *Crotalaria*, *Morus*, *Nerium indicum*, and *Clerodendron*; rubber plantations; patches of dense vegetation comprising several shrubs, herbs, climbers, bushes and trees like Mahagony, Acacia and Asoka and grasslands. These vegetated areas are protected from trespassing and human disturbances. Moth survey was carried out using light traps (Muirhead-Thomson, 1991), deployed in the evenings from 7 to 10 pm, twice weekly. Light traps consisted of a mercury vapour lamp suspended 1.5 meters above a white banner spread on the ground, with locations changed periodically. Moths were also recorded by direct observation, opportunistic late-evening visits to campus and hostel light sources (Prajapat *et al.*, 2024) and casual sightings near LED and tube lights (Nayak and Ghosh, 2020). Photographs were captured using smart phone (Vivo v21) or DSLR camera (Canon 200D). Some were captured using net, collected in jar, photographed and released unharmed. The moths were identified based on their physical features from relevant literature (Hampson, 1892, 1894, 1895, 1896; Gurule *et al.*, 2013; Sondhi *et al.*, 2022), Moths of India (<https://www.mothsofindia.org>) and iNaturalist (<https://www.inaturalist.org>).

A total of 84 species of moths belonging to 79 genera and 15 families were documented. Erebiidae was the dominant family with 26 species. Crambidae also had a high species richness with 25 species followed by Geometridae with 10 species, Pyralidae with 5 species, Noctuidae with 4 species, Limacodidae, Notodontidae, Sphingidae, and Thyrididae with 2 species each. The least observed moth families were Bombycidae, Eutellidae, Hyblaeidae, Nolidae, Psychidae, and Uranidae, each with one species. *Parapoynx stagnalis* and *Cnaphalocrocis patnalis* of the Crambidae family and *Endotricha* sp. of the Pyralidae family were the frequently observed species. The dominance of Erebiidae in the present study aligns with findings from numerous regions across India, including the Aravalli hill range of Rajasthan (Dar *et al.*, 2021);

Western Ghats region of Karnataka (Ravindrakumar, 2021); Madras Christian College campus, Chennai (Metilda and Livingstone, 2022); south Goa (Rao *et al.*, 2022); Bengaluru urban district (Ravishankar *et al.*, 2026); Nandhaur Wildlife Sanctuary, Uttarakhand (Arya *et al.*, 2021) and Chemancheri panchayath, Calicut, Kerala (Samynathan *et al.*, 2023). This widespread dominance reflects the adaptability of Erebiidae to diverse environmental conditions and ecological niches. Kuniyil and Peter (2022) compiled a list of about 250 Erebiinae moth species in India highlighting the extensive diversity within Erebiidae. Similar patterns of family dominance of Erebiidae, Crambidae and Geometridae have also been reported from Ranjit Sagar Conservation Reserve, Punjab (Paunikar *et al.*, 2022).

The present survey conducted during the summer recorded reasonably high moth diversity despite the limited duration. Seasonal trends reported by Praveen (2017) in paddy fields of Palakkad, indicated higher moth abundance during summer and post-monsoon. Besides temperature and humidity, vegetation structure also influences the moth species richness (Vanlalnghaka and Beihrosa, 2025). Better diversity of moth families observed indicates a stable, healthy and well-functioning ecosystem. As a preliminary checklist, for the first time, the moth diversity at the Christian College, Chengannur campus provides insights into the species composition of the moth fauna contributing to Alappuzha's biodiversity records.

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First record of *Bibio bicolor* Walker, 1848 (Diptera, Bibionidae) from India with an updated checklist of *Bibio* (Geoffroy, 1762) species from the Oriental region

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ABSTRACT: This is the first record of *Bibio bicolor* Walker, 1848 (Diptera, Bibionidae) from India. Five females of the species were recorded from roughly 2500m above sea level, ending a 175-year hiatus with no records since the original description from Java. In addition, a checklist of 48 oriental species for the genus *Bibio* Geoffroy, 1762, is also provided. © 2026 Association for Advancement of Entomology

KEY WORDS: Love bug, new record, inventory, March flies, West Bengal

Bibionidae, also known as March flies, are a family of small to medium-sized flies with pronounced sexual dimorphism. They have more than one palpal segment easily distinguishes them costal vein and R_{4+5} ending closely at the wing tip; short antennae with 4-12 compact flagellomeres; the insertion of the antenna below the complex eye; where the robust forefemur is a typical character of Bibioninae (Skartveit, 2017). Around 760 species have been described from around the world (Haarto, 2014), with their phytosaprophagous larvae working as potential nutrient recyclers, occasional pests of various crops, and adults at times serving as pollinators (Hardy, 1945, 1960, 1981; Freeman and Lane, 1985; D'Arcy-Burt and Blackshaw, 1991; Lewis and Smith, 1969; Johnson and Steiner, 1994). In the Oriental realm, the genus *Bibio* is the second most species rich genus (39 species) behind *Plecia* (65 species) (Jikon and Ke, 1988). In 1904, Meijere's description of *B. obscuripennis* from

Darjeeling, West Bengal, launched Bibionid research in India (Meijere, 1904). Brunetti described 13 species from 1911 to 1925, and Hardy was the last person to describe a *Bibio* species from India, *B. brunetti* in 1973 (Brunetti, 1911, 1912, 1913, 1925; Hardy, 1973).

Bibio bicolor Walker, 1848, has long been documented, primarily from Java with Walker describing its type locality as "East Indies" from Archdeacon Clerk's collection, but its existence in the Indian subcontinent has been unknown until now (Walker, 1848, 1849). This paper discusses the first record of *B. bicolor* from India, which will broaden its known geographical range, with its first photographic record, and provides a checklist of *Bibio* species from the Oriental realm.

The study is based on materials from the Zoological Survey of India, Kolkata (ZSI). The specimens were collected during a survey to assess dipteran

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Fig. 1 *Bibio bicolor* Walkcr, 1848, dorsal habitus; **Fig. 2** lateral habitus

Fig. 3 wing venation

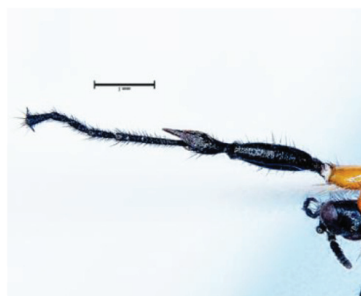


Fig. 4 Foreleg

Fig. 5 Head and forcocoxa

Fig. 6 dorsal view of thorax

fauna of Neora Valley National Park, West Bengal, India, in April 2022. Sampling was primarily done using sweep netting. Collected specimens were preserved in 70 percent ethyl alcohol for further study. A Leica EZ4 stereo microscope was used for the identification of the specimens. Images were stacked with a Leica M205 stereo - microscope coupled with a LEICA DFC 500 camera and software Leica Application Suite LAS V3.6, edited with Adobe Photoshop CC version 13.0.1 and assembled using Adobe Illustrator CS6.

***Bibio bicolor* Walker, 1848 (Figs. 1-6)**

Materials examined: 2♀♀ Rishop, Kalimpong district, 27.1112° N; 88.6526°E, 2591 Mt., 26.x.2022, coll. A. Naskar & party. 3♀♀ Neora valley National Park, Kalimpong district, 27.0824° N; 88.7007° E, 2849 Mt., 22.xi.2022, coll. A. Naskar & party.

Diagnosis: Walker in 1848 described a female from “East Indies”, present day Malay Archipelago. Entire thorax and all coxae appear red or orangish yellow (Figs. 1, 2, 5, 6); mesonotum has rather coarse golden pubescence but not particularly long

(Fig. 6). Front tibia with two spines; one spine large striated and the other much reduced (Fig. 5). Wings blackish brown (Fig. 3).

Distribution: India (West Bengal), Java.

Remarks: *B. bicolor* is similar to *B. pleciodes* (Osten Sacken, 1881) except the coxae which is entirely black and longer (not coarse) thoracic pubescence in *B. pleciodes* (refer to Hardy, 1956: 89). *Bibio imitator* (Walker, 1835) also displays similarity with *bicolor* except the lack of black abdomen which is rufous in *imitator*. Walker described *B. bicolor* from a single type specimen, later Hardy in 1956 redescribed the species using the same type specimen (Hardy, 1956). After 67 years its last description, this work extends its geographic range as well as provides first photographic evidence. Unfortunately, no males were found during the survey, which remains a major gap area in the study of this species as no male has yet been described. Furthermore, this paper provides a checklist of Oriental *Bibio* species (Table 1), which updates the species number from 39 to 48.

Table 1. Checklist of Oriental *Bibio* species

No.	Species	Distribution
1.	<i>Bibio ablusus</i> Hardy, 1965	Nepal
2.	<i>B. aequalis</i> Brunetti, 1912	China
3.	<i>B. affiniproximus</i> Hardy, 1965	Nepal
4.	<i>B. approximatus</i> Brunetti, 1911	India
5.	<i>B. aquilus</i> Hardy, 1967	Nepal
6.	<i>B. araeoceles</i> Hardy, 1967	Nepal
7.	<i>B. baishanzunus</i> Yang & Chen, 1995	China
8.	<i>B. bicolor</i> Walker, 1848	India, Indonesia
9.	<i>B. brevicealcar</i> Edwards, 1928	Malaysia
10.	<i>B. brevineurus</i> Yang & Chen, 1995	China.
11.	<i>B. brunetti</i> Hardy, 1973	India
12.	<i>B. capitaneus</i> Hardy, 1965	Nepal
13.	<i>B. collaripes</i> Brunetti, 1925	India
14.	<i>B. defectus</i> Brunetti, 1911	India
15.	<i>B. depressus</i> Meijere, 1924	Indonesia
16.	<i>B. discalis</i> Brunetti, 1911	India
17.	<i>B. flavicans</i> Edwards, 1928	Malaysia
18.	<i>B. flavissimus</i> Brunetti, 1925	India, Indonesia, Philippines
19.	<i>B. flavohirta</i> Brunetti, 1912	India
20.	<i>B. formosanus</i> Okada, 1938	Taiwan
21.	<i>B. fuscitibia</i> Brunetti, 1911	India, Nepal
22.	<i>B. gutianshanus</i> Yang, 1995	China
23.	<i>B. hainanus</i> Yang & Cheng, 2002	China
24.	<i>B. hennigi</i> Hardy, 1952	Indonesia
25.	<i>B. hortulanoides</i> Brunetti, 1911	India
26.	<i>B. hyalipterus</i> Yang & Chen, 1995	China
27.	<i>B. latifasciatus</i> Yang & Cheng, 1998	China
28.	<i>B. melanonotalus</i> Yang & Cheng, 1998	China
29.	<i>B. nepalensis</i> Hardy, 1967	Nepal
30.	<i>B. nigripennis</i> Brunetti, 1913	India
31.	<i>B. ochrostigmatus</i> Yang & Cheng, 1998	China
32.	<i>B. pallidohirtus</i> Brunetti, 1925	India
33.	<i>B. proximus</i> Brunetti, 1911	India
34.	<i>B. quadrifasciatus</i> Yang & Cheng, 2002	China

35.	<i>B. rubicundus</i> Wulp, 1884	Indonesia, Malaysia
36.	<i>B. rufifemur</i> Brunetti, 1911	India
37.	<i>B. ryukyuensis</i> Hardy & Takahashi, 1960	Japan (Ryukyu Islands)
38.	<i>B. scaber</i> Edwards, 1928	Malaysia
39.	<i>B. scaurus</i> Hardy, 1965	Nepal
40.	<i>B. sillemi</i> Duda, 1935	Pakistan
41.	<i>B. sinensis</i> Ôuchi, 1940	China
42.	<i>B. subrotundus</i> Yang, 1995	China
43.	<i>B. sumatranus</i> Meijere, 1924	Indonesia
44.	<i>B. nigerrimus</i> Duda, 1930	Nepal
45.	<i>B. tianmuanus</i> Li & Yang, 2001	China
46.	<i>B. totonigrum</i> Hardy, 1965	Nepal
47.	<i>B. trifasciatus</i> Yang & Cheng, 2002	China
48.	<i>B. zhaoi</i> Li & Yang, 2001	China

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Incidence of *Megacopta cribraria* (F.) (Hemiptera, Plataspidae) on leguminous plants from Kamrup District, Assam, India

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ABSTRACT: This is the first documentation of *Megacopta cribraria* (F.), (Hemiptera, Plataspidae) incidence on lablab bean and pigeon pea from Kamrup, Assam. The pest infestation was observed on these two crops and found increasing in the subsequent year. © 2026 Association for Advancement of Entomology

KEY WORDS: Lablab bean, pigeon pea, first report, feeding damage, crop loss

Leguminous plants are a good source of proteins, which help to improve human health (Tharanathan and Mahadevamma, 2003). These plants require less energy-intensive inputs and more importantly they do nitrogen fixing through roots (Lemke *et al.*, 2007). *Lablab purpureus* (L.) and *Cajanas cajan* (L.) Millsp (pigeon pea) are leguminous crop plant with a good source of protein, vitamins and one of the best sources of iron and lysine (Kay 1979; Bradley 1999; Pal *et al.*, 2011; Naeem *et al.*, 2016). Fifty-five insect species have been documented as pests of *L. purpureus*, among them aphids, whiteflies, *Helicoverpa armigera*, *Spilosoma obliqua*, and nearly five heteropteran insect species have been identified as some of the major insect pests (Chandra and Kushwaha, 2013; Jat *et al.*, 2017, Devashree and Patel, 2023). Nearly 150 - 250 species of insect pests infest *C. cajan* (Lateef and Reed, 1990; Srivastava and Joshi 2011), particularly pod borers and pod-sucking bugs (Shanower *et al.*, 1999; Rao and Shanower, 1999;

Bapatla *et al.*, 2020; Seni, 2021; Bandi *et al.*, 2024). *Megacopta cribraria* (F.), (Hemiptera, Plataspidae), is one such important pest on these two legumes and is a serious pest of soybean in other countries like the US, China, and Japan (Tayutivutikul and Kusigemati, 1992; Wang *et al.*, 1996; Eger *et al.*, 2010; Suiter *et al.*, 2010; Zhang *et al.*, 2012). However, from India, it was reported on lablab bean for the first time from Karnataka. Yield loss by this bug was estimated to be 9-44 per cent of lablab bean production in South India (Srinivasaperumal *et al.*, 1992; Thippeswamy and Rajagopal, 1998). Since then, it has been reported from some of the other states of India either associated with lablab plants or pigeonpea (Chandra and Kushwaha 2013; Nair *et al.*, 2017; Seni, 2021). This is the first record of *M. cribraria* incidence and infestation on these two leguminous plants from Kamrup district, Assam.

The occurrence of *M. cribraria* was first observed during insect sample collection on the leguminous

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Fig. 1 (a) *C. cajan* plantation, (b) *M. cribraria* infested *C. cajan* leaves, (c) *M. cribraria* mating adults, and (d) Egg batch on *L. purpureus* leaves

plants *C. cajan* in Horticulture Research Station, Azara (26.1055° N; 91.6099° E) and *L. purpureus* in the Department of Zoology, Cotton University, Panbazar (26.1874° N; 91.7467° E) (Kamrup, Assam, India) in November, 2022. The bug's incidence on these two plants was then closely monitored every week until the crop plants' availability. To confirm that the association of *M. cribraria* on these plants was not incidental, a close watch was kept in the subsequent year, 2023-24, from November to April, on the same host plants. The occurrence and incidence of pests on these leguminous plants were recorded weekly. The pest was identified using the suitable taxonomic keys (Schuh and Slater, 1995; Schaefer *et al.*, 2000). Voucher specimens (Registration no. I/HEM/ERS/1787) were deposited at the Zoological Survey of India (ZSI), Shillong. The incidence of the pest was determined using Wheeler's method (1969).

Considerable incidence and infestation of *M. cribraria* was found on both plants, with lablab plants experiencing slightly higher infestation ($70.05 \pm 1.67\%$) than on pigeon pea ($64.58 \pm 2.34\%$) in 2022-23. The following year, *L. purpureus* plants were again infested with the lablab bug ($79.17 \pm 2.57\%$) and in the absence of a pigeon pea plantation at Horticulture Research Station, no incidence could be recorded from that site. Infestation levels of *M. cribraria* on *L. purpureus* increased from 2022-23 to 2023-24, indicating that this bug could be a regular pest on this crop. Estimating the economic threshold level and economic injury level of a pest is of paramount importance to understand the pest's status (Pedigo *et al.*, 1986). The damage by *M. cribraria* on both plants was observed in the form of light-yellow spots and patchy leaves and poorly developed pods in the case of pigeon pea. These observations

corroborate the findings from earlier studies (Thippeswamy and Rajagopal, 1998; Chandra and Kushwaha, 2013; Seni, 2021). All the stages of the bug were observed on the plant and egg batches were also seen on the upper and lower surfaces of the leaves (Fig. 1).

Despite possessing multi-nutritional benefits (Habib *et al.*, 2017, Naeem *et al.*, 2020; Devaraj 2021), lablab bean has not achieved much attention concerning the loss it incurs to the pest infestation, particularly in the northeast region. Published reports on the lablab bean yield loss by this bug are lacking, indicating the lack of attention on such an important legume crop. While often considered a minor pest on pigeon peas (Nair *et al.*, 2017), *M. cribraria* showed significant adaptability to this crop, indicating it to be a suitable host plant for the growth and development, with performance comparable to that of its primary hosts, kudzu and soybean (Medal *et al.*, 2013). This study was a chance observation of a progressive infestation in leguminous crops.

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A preliminary survey of short-horned grasshoppers (Orthoptera, Acrididae) of Peppara Wildlife Sanctuary in Western Ghats, Kerala, India

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ABSTRACT: A preliminary survey on diversity of short-horned grasshoppers was conducted in Peppara Wildlife Sanctuary across two seasons and documented a baseline data on the grasshopper fauna of the sanctuary. Peppara Wildlife Sanctuary, an ecologically significant region characterized by diverse vegetation types and climatic conditions support rich faunal diversity. A total of 20 species belonging to 8 subfamilies were revealed during the survey, highlighting the sanctuary's notable species richness. Of the 20 species, *Dittopternis venusta* and *Phlaeoba antennata antennata* were found to be the most common species and Oedipodinae was the most dominant subfamily. A systematic checklist of the recorded diversity of short-horned grasshoppers is prepared. © 2026 Association for Advancement of Entomology

KEY WORDS: Acridids, diversity, checklist, species, richness

Grasshoppers, belonging to the order Orthoptera, one of the largest and most diverse groups of insects, play a crucial role as the dominant aerial invertebrates in natural ecosystem (Scott *et al.*, 1979; Risser *et al.*, 1981). Being primary consumers, they regulate food chains and function in the forest ecosystem's nutrient cycling. Because of their quick response to the changing environment some species are projected as an ecological indicator (Bezelet, 2011). Nowadays, these organisms provide major advances in the field of climatic change, developmental biology, ecology, evolution, genetics, bioacoustics, chemical ecology, speciation research, paleolimnology and physiology (Song *et al.*, 2015). As part of southern Western Ghats, Kerala is rich with faunal diversity with endemic species and this richness must be reflected

in the grasshopper diversity in the state also. However, these organisms are largely been ignored and received less focused attention. Latest records of orthopteran fauna indicates that Kerala has a total of 215 species belonging to 154 genera under 18 families which includes endemic and Western Ghats restricted species (Thasnim *et al.*, 2023). Bhaskar (2020) contributed significantly to the orthopteran fauna, including diversity assessments, new distributional records and habitat-based assessments; and further reported fire-induced behavioural dynamics of short-horned grasshoppers and how fire affected species abundance and composition. Acridids are diverse and comprise multiple subfamilies that differ in morphology, behaviour, and habitat preferences (Mounika *et al.*, 2024).

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Peppara Wildlife Sanctuary located in the southern Western Ghats of Kerala has an important role to play in conserving the rich biodiversity of grasshoppers. This sanctuary is relatively unexplored as far as grasshoppers are concerned. Therefore, the present study aims to document a baseline data on the short-horned grasshopper fauna of the sanctuary. The present survey was conducted in Peppara Wildlife Sanctuary (8° 34' and 8° 41' N latitude and 77° 6' and 77° 14' E longitude), which forms part of Agasthyamalai Biosphere Reserve, nestled in the southern region of the Western Ghats. The sanctuary covers 53 km² and elevation varies between 100 and 1717m. Chemmunji Mottai (1717m) is the highest mountain in this region. The temperature varies from 16 to 35° C. Forest types include west coast tropical evergreen, southern hilltop tropical evergreen, west coast semi-evergreen, southern moist mixed deciduous forest, sub-montane hill valley swamp forest etc (Kerala Forest Department, n.d). The survey was conducted for two seasons, north-east monsoon and dry winter from September 2024 to February 2025. Sampling was conducted across terrestrial habitats including, open grassland, semi evergreen forest ecosystem, forest floor substrates and moist riparian forest associated with waterfall. Grasshoppers were collected using a standard insect net, direct observation, handpicking and beating. Transect visual count method and sweep net sampling are ideal for collection. A combination of all these techniques was employed in a regular and systematic way to ensure maximum collection of grasshoppers. Collected specimens were first killed by ethyl acetate in a killing jar. After killing the specimens were preserved in 100 per cent ethyl alcohol in transparent bottles. These bottles were labelled with information regarding the collection. Both dry and wet preservations were made. Dry mounts were prepared for better understanding of certain characters like size, colour, texture etc. Live images of grasshoppers were taken in the field and at the laboratory using Nikon camera. The specimens were studied under Labomed stereomicroscope. Taxonomic literatures of Kirby (1914), Bhowmik (1986), Mandal *et al.* (2007), Srinivasan and Prabakar (2013), Cigliano *et al.* (2021) were used for the identification of short-

Table 1. Short-horned grasshoppers from different habitats of Peppara Wildlife Sanctuary in Western Ghats, Kerala, India

No.	Subfamily/ Species
Acridinae	
1.	<i>Phlaeoba antennata antennata</i> Brunner von Wattenwyl, 1893
2.	<i>P. infumata</i> Brunner von Wattenwyl, 1893
3.	<i>P. tenebrosa</i> (Walker, 1871)
Catantopinae	
4.	<i>Choroedocus illustris</i> (Walker, 1870)
5.	<i>Diabolocatantops innotabilis</i> (Walker, 1870)
6.	<i>Stenocatantops splendens</i> (Thunberg, 1815)
7.	<i>Xenocatantops humilis</i> (Serville, 1839)
Coptacrinae	
8.	<i>Eucoptacra praemorsa</i> (Stal, 1860)
Cyrtacanthacridinae	
9.	<i>Cyrtacanthacris tatarica tatarica</i> (Linnaeus, 1758)
10.	<i>Pachyacris vinosa</i> (Walker, 1870)
Eyprepocnemidinae	
11.	<i>Eyprepocnemis alacris alacris</i> (Serville, 1838)
12.	<i>Tylotropidius varicornis</i> (Walker, 1870)
Gomphocerinae	
13.	<i>Aulacobothrus luteipes luteipes</i> (Walker, 1871)
14.	<i>Gonista bicolor</i> (Haan, 1842)
Oedipodinae	
15.	<i>Dittopternis venusta</i> (Walker, 1870)
16.	<i>Gastrimargus africanus africanus</i> (Saussure, 1888)
17.	<i>G. marmoratus</i> (Thunberg, 1815)
18.	<i>Heteropternis respondens</i> (Walker, 1859)
19.	<i>Trilophidia annulata</i> (Thunberg, 1815)
Oxyinae	
20.	<i>Chitaura indica</i> Uvarov, 1929

horned grasshoppers. Collected specimens are deposited in the repository section of Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram, Kerala, India.

The study revealed the presence of 20 species of grasshoppers belonging to 8 subfamilies of family Acrididae (Table 1). The eight subfamilies documented during the survey differ mainly in head morphology, body size, wing colouration, pronotum structure, behaviour, and habitat association. Subfamily Acridinae and Gomphocerinae are distinguished by their slant-faced head morphology, while Catantopinae exhibit a more robust body form. Coptacrinae are generally smaller, cryptically coloured, and adapted to dry, scrubby habitats. Cyrtacanthacridinae include strong flying, large-bodied species with some displaying locust-like behavior. They exhibit high dispersal capacity (Song and Wenzel, 2008). Eyprepocnemidinae are usually medium sized and associated with moist grasslands and agricultural lands whereas Oedipodinae are band winged grasshoppers, having bright coloured hind wings (Otte, 1981). Oxyinae are slender, frequently green grasshoppers closely associated with wetlands and paddy fields. Many species are fully winged with high dispersal capability while some are wingless or exhibit reduced wings. They prefer stable habitat having less dispersal and are vulnerable to environmental disturbances. Generally, grasshoppers are herbivores and non-toxic, but some species exhibit aposomatism and are toxic to predators (Uvarov, 1966). Although these subfamilies differ in morphology, behavior and regional distribution, they share a common habitat (Song *et al.*, 2015).

Dittopternis venusta and *Phlaeoba antennata antennata* were found to be the most common species. Subfamily Oedipodinae was the most dominant subfamily with 5 species, followed by subfamily Catantopinae with 4 species. Among these, *Chitaura indica* belonging to subfamily Oxyinae, a brachypterous (reduced wings) species is endemic to south India and they are found in moist, grassland and forest habitats of western ghats region including Kerala.

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First record of *Neoscona jinghongensis* Yin, Wang, Xie & Peng, 1990 (Araneae, Araneidae) from India

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ABSTRACT: *Neoscona jinghongensis* Yin, Wang, Xie & Peng, 1990 is recorded for the first time from India, based on a specimen collected from Meghalaya. Detailed morphological description and photographic illustrations of the male habitus and copulatory organs are provided.

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KEY WORDS: Orb-weaver spider, new report, distribution, Meghalaya, biodiversity hotspot.

Globally, Araneidae Clerck, 1757 is the third most species-rich spider family after Salticidae Blackwall, 1841 and Linyphiidae Blackwall, 1859 (World Spider Catalog, 2025). The genus *Neoscona* was erected by Simon in 1864, with *Epeira arabesca* Walckenaer, 1841 as its type species. *Neoscona* Simon, 1864 is the fifth most species-rich genus and currently comprises 124 valid species worldwide, of which 29 are recorded from India (Caleb and Sankaran, 2025; World Spider Catalog, 2025). Yin *et al.* (1990) described *Neoscona jinghongensis* based on a female specimen, collected from Jinghong city of Yunnan Province, China. Subsequently, the male of the species was described by Mi *et al.* (2024) from Menglun Nature Reserve, Yunnan Province. In this paper, *N. jinghongensis* Yin, Wang, Xie & Peng, 1990 is reported from India for the first time based on a

male specimen collected from Meghalaya, part of the Indo-Burma biodiversity hotspot.

The specimen was collected by bush beating method and preserved (in 70% ethanol). Morphological examination of the specimen was carried out using a Leica M205A stereomicroscope. Images were captured with a Flexacam C3 camera attached to the Leica M205A stereomicroscope and processed using an extended focus montage LAS X software. The description of coloration is based on alcohol-preserved specimen. All measurements are in millimeters (mm). The distribution map was prepared with QGIS 3.34.9. Measurements of palp and leg segments are as follows: total length [femur, patella, tibia, metatarsus (excluded for palp), tarsus]. The studied specimen was deposited in the NZC-ZSI, Kolkata, India.

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Abbreviations used:

ALE - Anterior lateral eye; AME - Anterior median eye; ASL - Above sea level; C - Conductor;

E - Embolus; MA - Median apophysis; MOA - Median ocular area; NZC - ZSI National Zoological Collections of Zoological Survey of India; Pc - Paracymbium; PLE - Posterior lateral eye; PME - Posterior median eye; TA - Tegular apophysis

Systematic Account

Family Araneidae Clerck, 1757; Subfamily Araneinae Clerck, 1757;

Genus *Neoscona* Simon, 1864

***Neoscona jinghongensis* Yin, Wang, Xie & Peng, 1990 (Fig. 1)**

N. jinghongensis Yin *et al.*, 1990: 117, 165, figs. 288–290 (description of female); Yin *et al.*, 1997: 356, figs 252a–f; Song *et al.*, 1999: 299, figs. 175U–V, 176A, 180H.

N. flavescens Zhang & Zhang, 2011: 520, figs. 14–18 (male; misidentified as per Mi *et al.*, 2024: 305).

N. jinghongensis Mi *et al.*, 2024: 305, figs. 19A–J, 20A–E, 21A–E (description of male, female).

Material examined: 1♂; INDIA, Meghalaya, West Jaintia Hills, Thadlaskein; 25°30'12.4" N; 92°10'14.3" E; 139 m ASL; 11.vi.2024; Coll. P.P. Sudhin (No. NZC-ZSI-10113/18).

Diagnosis: The male copulatory organ of *N. jinghongensis* is most similar to that of *N. vigilans* (Blackwall, 1865) from which it can be easily distinguished by the terminal apophysis with a bifurcated upper layer (not bifurcated in *N. vigilans*), and conductor that is not bifurcated in prolateral view (bifurcated in *N. vigilans*). The female genitalia of *N. jinghongensis* is most similar to that of *N. bengalensis* Tikader & Bal, 1981, but differs in having a distally blunt scape (pointed in *N. bengalensis*), and sides of the scape that are almost parallel (deep constriction in *N. bengalensis*) (Mi *et al.*, 2024).

Description: Male (Fig. 1). Measurements: body length 7.47; carapace length -3.78, width 3.17; abdomen length 4.07, width 3.22; clypeus height 0.16. Eye diameters and interdistances: AME 0.18, ALE 0.14, PME 0.13, PLE 0.14, AME–AME 0.18, AME–ALE 0.28, PME–PME 0.08, PME–PLE 0.50, MOA length 0.53 with front width 0.48 and back width 0.35. Length of chelicera 1.08. Measurements of palp and legs: palp 2.52 [0.62, 0.33, 0.18, 1.39], leg I 13.91 [3.85, 1.79, 3.38, 3.42, 1.47], II 12.1 [3.54, 1.42, 2.75, 3.03, 1.36], III 6.99 [2.19, 1.04, 1.36, 1.49, 0.91], IV 10.52 [3.22, 1.36, 2.25, 2.68, 1.01]. Leg formula 1243. Carapace pear-shaped, yellowish brown with light reddish-brown lateral patches on thoracic region (Fig. 1A). Cervical groove obvious; fovea dark brown, longitudinal (Fig. 1A). Chelicerae light yellowish brown, with four promarginal and three retromarginal teeth (Fig. 1D). Endites nearly square, yellowish brown, with paler inner sides (Fig. 1B). Sternum cordiform, yellow, with interrupted paler longitudinal patches (Fig. 1B). Legs yellowish brown, with yellow annulations, covered with numerous spines and setae. Abdomen ovoid, greyish yellow, anteriorly with a pair of creamy white conspicuous patches, followed by four pairs of wedge-shaped dark brown patches; surface covered with several long scattered pale white and light brown setae and sigillae (Fig. 1A, C); venter greyish yellow with darker patches, and a pair of creamy yellow patches medially (Fig. 1B). Spinnerets yellowish brown (Fig. 1B–C).

Palp with two patellar bristles (Fig. 1F); paracymbium finger-like (Fig. 1G); median apophysis long, tapering distally with a gentle curve (Fig. 1G); embolus short, cone-shaped (Fig. 1I); conductor prominent, conical, strongly sclerotized at base but weakly sclerotized distally (Fig. 1G); terminal apophysis divided into two parts, upper part bifurcated, and lower part rounded distally (Fig. 1H).

Variation: The male specimen of *N. jinghongensis* collected from Meghalaya, India showed some variation in the abdominal pattern when compared with the specimen from China. Dorsal part of the abdomen possesses a pair of conspicuous creamy white patches anteriorly, along

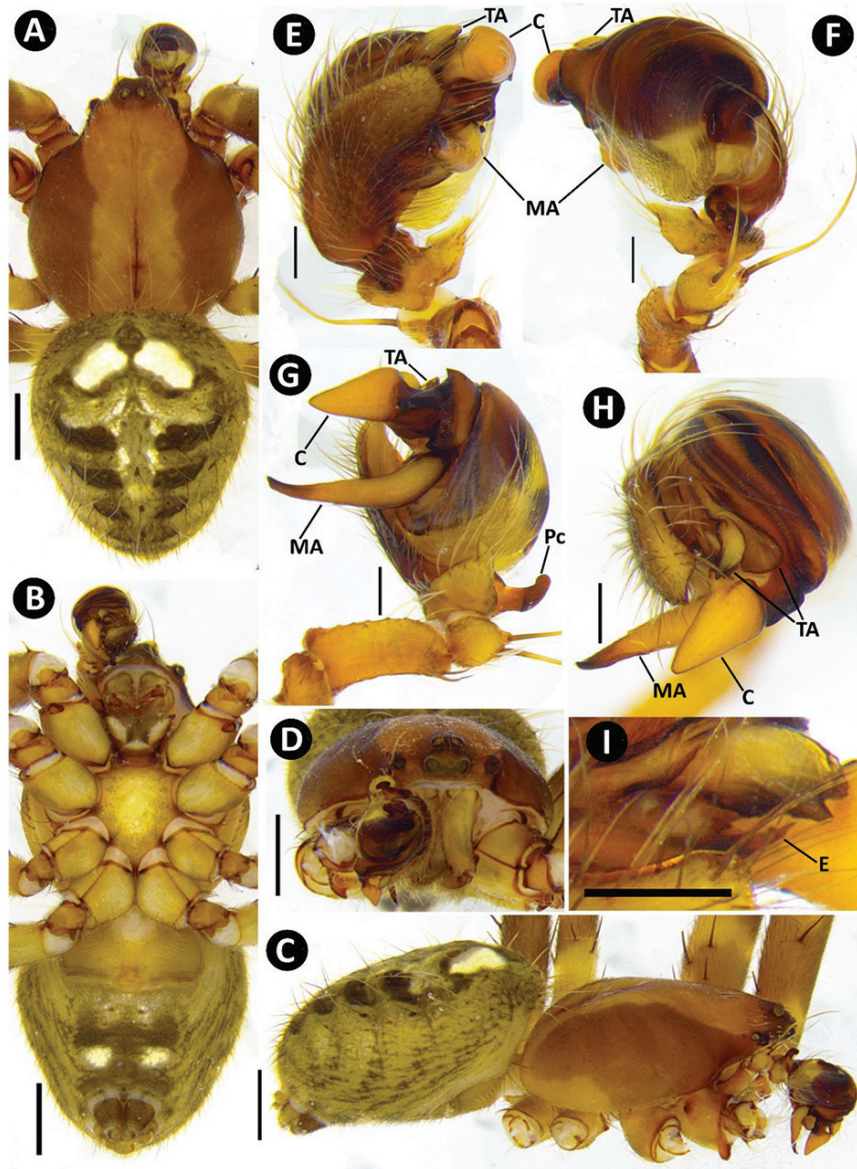


Fig. 1 Male *Neoscona jinghongensis*. A - Habitus dorsal view; B - ventral view; C - lateral view; D - frontal view; E - left palp (prolateral view); F - left palp (retrolateral view); G - left palp (ventral view); H - left palp (apical view); I - embolus. Scale bars: 1 mm for A–D; 0.25 mm for E–I

with more prominent wedge-shaped dark brown patches (Fig. 1A, C). This variation may be attributed to geographical separation.

Distribution: China, India (new record)

The genus *Neoscona* ranks fifth in terms of number of species after *Araneus* Clerck, 1757, *Mangora* O. Picard-Cambridge, 1889, *Cyclosa* Menge, 1866 *Alpaida* O. Picard-Cambridge, 1889 (World Spider

Catalog, 2025). The present study reports *N. jinghongensis* for the first time from India. With this addition, the number of species belonging to this genus increases from 29 (Caleb and Sankaran, 2025) to 30. The study also highlights the distributional range extension for the species since it was originally described from China. Additionally, the study reflects intraspecific morphological variation possibly arising from geographical isolation.

However, extensive surveys exclusively in the unexplored regions of the country are needed for further discoveries in future.

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Danaus chrysippus - a - Male with four black spots on each hindwing;
b - Female with three black spots on each hindwing



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