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Note from the Chief Editor

Dear Entomologists,

The Association for Advancement of Entomology (AAE) has been successful in the publication of all issues of ENTOMON as per schedule. The March, 2022 issue (Volume 46, issue 1) is placed before you.

The National Academy of Agricultural Sciences (NAAS) rating of ENTOMON is 4.69 in 2021. The University Grants Commission, New Delhi has recognized ENTOMON by including the journal in the official list of scientific journals (UGC-CARE List Group I). ENTOMON is included in CABI's full text repository. By including the scientific papers in the repository, it is ensured that the research documents can be easily located by scientists and researchers throughout the world, both now and in the future. This would also be a valuable contribution for global users of CAB Direct and other related databases. ENTOMON is partnering with EBSCO for dissemination of papers published in the journal.

ENTOMON is a member of the Committee on Publication Ethics (COPE) and the journal follows the guidelines of COPE.

SCOPUS, Elsevier's abstract and citation database, has included ENTOMON for indexing. SCOPUS will be indexing all the articles published in ENTOMON with effect from 2012. Scopus Preview Cite score tracker 2021 for ENTOMON is 0.4.

ENTOMON has gained wide acceptance among the members, readers, peer reviewers and other stakeholders.

ENTOMON is grateful to Professor K. Madhavan Nair, the AAE web site Manager for his unstinting support and selfless services. ENTOMON owes allegiance to the late Professor M. Koshy, M/s SB Press, Thiruvananthapuram and the management in bringing out the journal.

We are thankful to the National Academy of Agricultural Sciences (NAAS), UGC-CARE, CABI, the Committee on Publication Ethics, EBSCO and Scopus for their support to ENTOMON.

ENTOMON profoundly acknowledges the peer reviewers for the critical evaluation of the manuscripts submitted to the Journal.

We acknowledge the continuous support of authors and their institutions as well as the subscribers.

Regards

Dr M.S. Palaniswami
Chief Editor

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Short Communication

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Species composition of dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) in the coffee plantation of Nilgiri Biosphere Reserve of the Western Ghats, India

Subha Babu Jayaprakash* and Sabu K. Thomas

Post graduate and research department of zoology, St. Joseph's College, Devagiri, Kozhikode, 673008, Kerala, India.
Email: subhababucm@gmail.com; sabukthomas@gmail.com

ABSTRACT: Analysis of species composition of dung beetles in the shaded coffee plantations of Nilgiri Biosphere Reserve of south Western Ghats revealed a checklist of 38 species. The presence of many endemic species, very primitive rare old world tribe *Canthonini* (represented by the genus *Ochicanthon*), the first report of *Onthophagus lilliputanus* and the presence of two species (*O. truncaticornis* and *O. discedens),* which were deemed as extinct from the natural forests of the Western Ghats make the dung beetle assemblage in the coffee plantation unusual. The study showed species composition of dung beetles in the shaded coffee plantation with a comparatively smaller area had no major differences with the nearby natural forests in the Nilgiri Biosphere Reserve. Higher species richness and presence of some unique species in the coffee plantation belt compared to other agricultural habitats highlights the significance of shaded coffee plantations as an important nested habitat in the forest-agriculture land matrix of the moist Western Ghats.

KEY WORDS: Species check list, rare old world tribe, first report, nested habitat

INTRODUCTION

The dung beetles are a highly specialized trophic group (Scarabaeinae: Scarabaeidae: Coleoptera), mainly adapted to dung and organic debris consumption at both the adult and larval stages (Hanski and Cambefort, 1991). They play a key role in the forest and agricultural ecosystem as they recycle faecal material, fertilize and aerate the soil, recycle nitrogen, organic carbon and other nutrients, protect seeds from predation, aid in seed dispersal, parasite suppression, serve as a food source for birds and mammals (Hanski and Cambefort, 1991; Nichols *et al.,* 2008). Scarabaeid dung beetles belong to three distinct taxonomic groups: Scarabaeinae, Geotrupinae and Aphodiinae (Baraud, 1985). Among these subfamilies, Scarabaeinae is the only group that is predominantly coprophagous (faeces eating), while the majority of Aphodiinae and Geotrupinae are saprophagous (eaters of decaying organic matter) and not true dung beetles (Halffter and Mathews, 1966).

Tropical rain forests are the most species-rich and functionally significant terrestrial ecosystems supporting more than half of global biodiversity (Myers *et al.,* 2000). The Western Ghats, a biodiversity hotspot in southern India is scattered

* Author for correspondence

with plantations that were once tropical rain forests (Dolia *et al.,* 2008). Conversion of original forests to smaller fragments of variable size and agricultural farmlands and plantations of tea, coffee, rubber and cardamom in the south Western Ghats peaked in the 1980s (Daniels, 1992; Daniels *et al.,* 1990) and led to considerable biodiversity loss in the south Western Ghats (Nair, 1991). One of the most important plantation crop is coffee and it is predominantly grown at high altitudes regions of southern states of India (Velmourougane, 2016). Indian coffee industry which had plantation coverage of 270,821 ha in 1990-1991 has increased to 340,306 ha by 1999-2000 (25.7%), almost entirely in the Western Ghats region of southern India (Coffee Board, 2001; Raman, 2006).

Studies on the biodiversity in coffee plantations of the Western Ghats have recorded high biodiversity for birds, mammals, butterflies, amphibians and bats (Bali *et al.,* 2007; Dolia *et al.,* 2008; Anand *et al.,* 2008; Rathod and Rathod, 2013; Wordley *et al*., 2017). But there is no study towards assessment of dung beetles diversity in the shaded coffee plantations of the Western Ghats region. Many studies from the Neotropical region have demonstrated that coffee agroecosystems with complex forest-like vegetation structures (shaded) have significantly high biodiversity (Perfecto *et al.,* 1996; Greenberg *et al.,* 1997; Moguel and Toledo, 1999; Johnson, 2000; Perfecto *et al.,* 2003; Perfecto and Armbrecht, 2003; Somarriba *et al.,* 2004), particularly dung beetle diversity (Moron, 1987; Pineda *et al.,* 2005; Horgan, 2005, 2009). Studies from cocoa agroforestry also showed similar results of higher species richness of dung beetles and provided suitable habitat for forest-dependent species (Harvey *et al*., 2006; Shahabuddin *et al.,* 2010). However, no data exists on the status of the dung beetles from the shaded coffee plantations of the Western Ghats. Also no records on how far the coffee habitat modification might have lead to the decline and disappearance of many rare and endemic dung beetle species reported earlier by Arrow (1931) in the Western Ghats. Hence the present study was undertaken to assess the dung beetle species composition in the coffee plantation belts of south Wayanad in the Nilgiri Biosphere Reserve (NBR) of the south Western Ghats.

MATERIALS AND METHODS

Specimens were collected from a coffee plantation belt of NBR of the south Western Ghats (Fig. 1) using pitfall traps from January to December 2015. Specimens were identified with the aid of keys available in Arrow (1931) and Balthasar (1963a, b) and by comparing with type specimens available in the research centre and Zoological Survey of India, Western Ghats regional station, Calicut. Verified specimens were curated in the insect collections of Tamil Nadu Agricultural University, Coimbatore and in the national insect collections of Zoological Survey of India, Western Ghats regional station, Kozhikode. Images were captured using microscope Leica M205C Stereo zoom and measured with Leica LAS V4.5 software. Abbreviations and markings used**:** ORR - Oriental Region; PAR - Palaearctic Region; IAR - Indo-Australian Region

RESULTS

Order: Coleoptera: Family: Scarabaeidae: Subfamily: Scarabaeinae

Tribe- Sisyphini

Genus 1. *Sisyphus* Latreille, 1807

Sisyphus Latreille, 1807; Gory, 1833; Lacordaire, 1856; Reitter, 1892, 1893; Péringuey, 1901; Arrow, 1927, 1931; Balthasar, 1935a, 1963a; Haaf, 1955.

1. *Sisyphus* (s.str.) *longipes* Olivier, 1789 Fig. 2 (1)

Sisyphus (s.str.) *longipes* Olivier, 1789; Arrow, 1927, 1931; Haaf, 1955; Balthasar, 1963a.

Distribution: ORR- India (West Bengal; Maharashtra; Odisha; Karnataka; Tamil Nadu: Ooty, Nilgiri Hills; Kerala: Wayanad, Thekkady), Myanmar, Sri Lanka, Thailand.

Tribe- Canthonini

Genus 2. ***Ochicanthon* Vaz-de-Mello, 2003**

Ochicanthon Vaz-de-Mello, 2003; Boucomont, 1914a; Arrow, 1931; Paulian, 1945; Balthasar, 1963a.

2. *Ochicanthon laetus* Arrow, 1931 Fig. 2 (2)

Ochicanthon laetus Arrow, 1931; Balthasar, 1963a; Vaz-de-Mello, 2003; Latha *et al.,* 2011.

Distribution: ORR-India (Kerala: Nilgiri hills, Wayanad, Malampuzha), Thailand.

3. *Ochicanthon tristis* Arrow, 1931 Fig. 2 (3)

Ochicanthon tristis Arrow, 1931; Balthasar, 1963a; Vaz-de-Mello, 2003; Latha *et al.,* 2011.

Distribution*:* ORR-India (Tamil Nadu; Kerala: Nilgiri Hills, Silent valley, Thirunelli).

Tribe- Coprini

Genus 3. ***Catharsius* Hope, 1837**

Catharsius Hope, 1837; Burmeister, 1846; Péringuey, 1901; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963a; Paulian, 1945.

4. *Catharsius molossus* Linnaeus, 1758 Fig. 2 (4)

Catharsius molossus Linnaéus, 1758; Harold, 1877; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963a; Paulian, 1945.

Distribution: ORR-Afghanistan, Cambodia, India (Andaman Islands; Assam; Bihar; Odisha; West Bengal; Karnataka; Kerala: Wayanad, Nelliyampathy, Thekkady), Laos, Malaysia, Sri Lanka, Sunda Islands, Thailand, Vietnam. PAR-India (Sikkim; Uttarakhand), China, Nepal, Taiwan.

5. *Catharsius sagax* Quenstedt, 1806 Fig. 2 (5)

Catharsius (s.str.) *sagax* Quenstedt, 1806; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963a.

Distribution: ORR-Bangladesh, India (West Bengal; Bihar; Punjab; Mumbai; Madhya Pradesh; Tamil Nadu: Nilgiri Hills, Palani Hills; Kerala: Peerumedu, Travancore, Wayanad).

Genus 4. *Copris* Geoffroy, 1762

Copris Geoffroy, 1762; Burmeister, 1846; Reitter, 1892, 1893; Péringuey, 1901; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1933, 1935a, 1936a; Janssens, 1939; Paulian, 1945.

6. *Copris* (s.str.) *repertus* Walker, 1858 Fig. 2 (6)

Copris (s.str.) *repertus* Walker, 1858; Gillet, 1911; Arrow, 1931; Balthasar, 1933, 1935a, 1963a.

Distribution: ORR-India (Bihar; Maharashtra: Mumbai; Madhya Pradesh; Chattisgarh; Karnataka; Tamil Nadu: Nilgiri Hills, Anamalai Hills; Kerala: Malabar, Nelliyampathy, Palghat, Thekkady, Wayanad), Sri Lanka, Thailand PAR-China.

Genus 5. *Paracopris* Balthasar, 1939

Paracopris Balthasar, 1939, 1963a; Paulian, 1945; Löbl and Smetana, 2006; Sabu *et al.,* 2011.

7. *Paracopris davisoni* Waterhouse, 1891 Fig. 2 (7)

Paracopris davisoni Waterhouse, 1891; Arrow, 1931; Balthasar, 1963a; Löbl and Smetana, 2006; Sabu *et al.,* 2011.

Distribution: ORR-India (Karnataka; Tamil Nadu: Nilgiri Hills, Palani Hills; Kerala: Nelliyampathy, North Malabar, Peerumedu, Travancore, Thekkady, Wayanad).

Tribe- ONTHOPHAGINI

Genus 6. *Caccobius* Thomson, 1863

Caccobius Thomson, 1863; Harold, 1867; Jekel, 1872; Waterhouse, 1875; Reitter, 1892, 1893; D'Orbigny, 1898, 1913; Péringuey, 1901, 1908; Boucomont and Gillet, 1921; Arrow, 1931; Portevin, 1931; Porta, 1932; Matsumura, 1936; Paulian, 1945; Balthasar, 1949, 1963a.

-subg. *Caccophilus* Jekel, 1872; D'Orbigny, 1898, 1913; Balthasar, 1935c, 1949.

8. *Caccobius* (*Caccophilus*) *meridionalis* Boucomont, 1914 Fig. 2 (8)

Caccobius (*Caccophilus*) *meridionalis* Boucomont, 1914a; Arrow, 1931; Balthasar, 1949, 1963a.

Distribution: ORR-India (Maharashtra; Karnataka; Tamil Nadu: Anamalai Hills, Nilgiri Hills; Kerala: Mahe, Nelliyampathy, Thekkady, Wayanad), Sri Lanka.

9. *Caccobius* (*Caccophilus*) *ultor* Sharp, 1875 Fig. 2 (9)

Caccobius (*Caccophilus*) *ultor* Sharp, 1875; Balthasar, 1963a.

Distribution: ORR-India (Maharashtra: Mumbai, Khandesh; Punjab, Rajasthan, Uttar Pradesh, Haryana: Kanneri; Karnataka: Budipadaga; Kerala: Nelliampathi, Ranipuram).

10. *Caccobius* (*Caccophilus*) *unicornis* Fabricius, 1798 Fig. 2 (10)

Caccobius (*Caccophilus*) *unicornis* Fabricius, 1798; Boucomont, 1914a; Arrow, 1931; Balthasar, 1933, 1949, 1963a; Paulian, 1945.

-*nitidiceps* Fairmaire, 1893; Boucomont, 1914a; Boucumont and Gillet, 1921.

-*yamauchii* Matsumura, 1936.

Distribution: ORR-India (Tripura; Assam; West Bengal; Madhya Pradesh; Kerala: Silent valley, Wayanad), Indonesia (Borneo, Java, Sumatra), Malay Peninsula, Myanmar, Sri Lanka, PAR-Taiwan, China. IAR - Philippines.

Genus 7. *Onthophagus* Latreille, 1802

Onthophagus Latreille, 1802; Mulsant, 1842; Erichson, 1848; Lacordaire, 1856; Mulsant and Rey, 1871; Reitter, 1892, 1893; D'Oribgny, 1898, 1913; Peringuey, 1901, 1908; Reitter, 1909; Bedel, 1911; Boucomont, 1914b; Boucomont and Gillet, 1921; Boucomont, 1924; Arrow, 1931; Portevin, 1931; Porta, 1932; Balthasar, 1935b, 1963a; Savchenko, 1938; Paulian, 1941, 1945; Endrödi, 1956; Tesar, 1957.

-subg. *Proagoderus* Lansberge, 1883; D'Oribgny, 1913; Boucomont, 1914a; Marcus, 1917; Balthasar, 1963a.

-*Tauronthophagus* Shipp, 1895.

-subg. *Serrophorus* Balthasar, 1935b; Paulian, 1945; Balthasar, 1963a.

-subg. *Micronthophagus* Balthasar, 1935b; Paulian, 1945.

-subg. *Colobonthophagus* Balthasar, 1935b; Paulian, 1945; Balthasar, 1963a.

-subg. *Paraphanaeomorphus* Balthasar, 1959, 1963a.

-subg. *Matashia* Matsumura, 1938.

-subg. *Macronthophagus* Ochi, 2003.

11. *Onthophagus* (s.str.) *amphicoma* Boucomont, 1914 Fig. 2 (11)

Onthophagus (s.str.) *amphicoma* Boucomont, 1914a; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Tamil Nadu: Nilgiri Hills; Kerala: Mahe, Nelliampathi, Travancore, Thekkady)

12. *Onthophagus* (s.str.) *andrewesi* Arrow, 1931 Fig. 2 (12)

Onthophagus (s.str.) *andrewesi* Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Karnataka; Tamil Nadu: Anamalai Hills, Nilgiri Hills; Kerala: Nelliyampathy, Thekkady, Wayanad).

13. *Onthophagus* (*Paraphanaeomorphus*) *bifasciatus* Fabricius, 1781 Fig. 2 (13)

Onthophagus (*Paraphanaeomorphus*) *bifasciatus* Fabricius, 1781; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Assam; Bihar; West Bengal; Sikkim; Tamil Nadu: Nilgiri Hills; Kerala: Wayanad, Thekkady), Myanmar.

14. *Onthophagus* (s.str.) *bronzeus* Arrow, 1907 Fig. 2 (14)

Onthophagus (s.str.) *bronzeus* Arrow, 1907; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Maharashtra: Mumbai; Karnataka; Tamil Nadu: Nilgiri Hills; Kerala: Nelliyampathy, Thekkady, Wayanad).

15. *Onthophagus* (s.str.) *cervus* Fabricius, 1798 Fig. 2 (15)

Onthophagus (s.str.) *cervus* Fabricius, 1798; D'Orbigny, 1898; Boucomont, 1914b; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Madhya Pradesh; Maharashtra; West Bengal; Karnataka; Tamil Nadu: Coimbatore; Puducherry; Nilgiri Hills; Kerala: Calicut, Wayanad, Thekkady), Sri Lanka PAR- India (Uttarakhand).

16. *Onthophagus* (*Colobonthophagus*) *dama* Fabricius, 1798 Fig. 2 (16)

Onthophagus (*Colobonthophagus*) *dama* Fabricius, 1798; D'Oribgny, 1898; Arrow, 1931; Balthasar, 1963a; Löbl & Smetana, 2006.

-*Onthophagus cervicornis* Kirby, 1825; Rossini *et al.,* 2014: 111–115

Distribution: ORR-India (Maharashtra; Sikkim; Bihar; West Bengal, Karnataka; Tamil Nadu: Anamalai Hills, Nilgiri Hills; Kerala: Nilambur, Wayanad, Thekkady), Sri Lanka. PAR-India (Uttarakhand), Nepal, Bhutan.

17. *Onthophagus* (s.str.) *devagiriensis* Schoolmeesters and Thomas, 2006 Fig. 2 (17)

Onthophagus (s.str.) *devagiriensis* Schoolmeesters and Thomas, 2006.

Distribution: ORR-India (Kerala: Wayanad, Ranipuram).

18. *Onthophagus* (*Parascatonomus*) *discedens* Sharp, 1875 Fig. 2 (18)

Onthophagus (*Parascatonomus*) *discedens* Sharp, 1875; Boucomont, 1914a; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935b, 1963a; Paulian, 1945.

-*ssp. laotianus* Boucomont, 1919; Boucomont and Gillet, 1921; Balthasar, 1935b.

Distribution: ORR-Myanmar, Siam, Indo-China, Malay Peninsula, India (Bengal; Uttar Pradesh; Tamil Nadu: Nilgiri hills; Sikkim).

19. *Onthophagus* (*Gibbonthophagus*) *duporti* Boucomont, 1914 Fig. 2 (19)

Onthophagus (*Gibbonthophagus*) *duporti* Boucomont, 1914a; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935b, 1963a; Paulian, 1945; Löbl and Smetana, 2006*;* Kabakov and Shokhin, 2014.

Distribution: ORR-India (Arunachal Pradesh; Bihar; West Bengal; Karnataka; Tamil Nadu: Nilgiri Hills; Kerala: Thekkady), Laos, Myanmar, Vietnam: Tonkin.

20. *Onthophagus* (s.str.) *fasciatus* Boucomont, 1914 Fig. 2 (20)

Onthophagus (s.str.) *fasciatus* Boucomont, 1914a; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Madhya Pradesh; Maharashtra: Mumbai; Karnataka; Kerala: Nelliyampathy, Thekkady, Wayanad; Tamil Nadu: Anaimalai Hills, Madhura, Nilgiri Hills; West Bengal) PAR-India (Uttarakhand).

21. *Onthophagus* (s.str.) *faveri* Boucomont, 1914 Fig. 3 (2)

Onthophagus (s.str.) *faveri* Boucomont, 1914a; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Karnataka; Kerala: Nelliyampathy, Thekkady, Wayanad; Tamil Nadu: Coimbatore, Nilgiri Hills), Sri Lanka.

22. *Onthophagus* (s.str.) *furcillifer* Bates, 1891 Fig. 3 (22)

Onthophagus (s.str.) *furcillifer* Bates, 1891; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Assam; Punjab, Kerala: Nelliyampathy, Thekkady, Wayanad). PAR-India (Kashmir; Uttarakhand).

23. *Onthophagus* (s.str.) *insignicollis* Frey, 1954 Fig. 3 (23)

Onthophagus (s.str.) *insignicollis* Frey, 1954; Balthasar, 1963a.

Distribution: ORR-India (Bihar; Kerala: Wayanad, Nelliampathi, Ranipuram).

24. *Onthophagus* (s.str.) *kchatriya* Boucomont, 1914 Fig. 3 (24)

Onthophagus (s.str.) *kchatriya* Boucomont, 1914a; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Karnataka; Kerala: Nilambur, Thekkady; Tamil Nadu: Anamalai Hills, Nilgiri Hills, Yercaud).

25. *Onthophagus* (s.str.) *lilliputanus* Lansberge, 1883 Fig. 3 (25)

Onthophagus lilliputanus Lansberge, 1883; Boucomont, 1921a; Arrow, 1931.

Distribution: ORR-India (West Bengal; Maharashtra: Mumbai; Tamil Nadu: Madras; Coimbatore), Myanmar; Indonesia: Java, Borneo; PAR-India (Kashmir; Punjab); IAR-Philippines.

***26. Onthophagus (s.str.) ludio* Boucomont, 1914 Fig. 3 (26)**

Onthophagus (s.str.) *ludio* Boucomont, 1914a; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-India (Maharashtra: Belgaum, Mumbai, Nagpur; Kerala: Nilgiri hills), Sri Lanka.

27. *Onthophagus* (s.str.) *pacificus* Lansberge, 1885 Fig. 3 (27)

Onthophagus (s.str.) *pacificus* Lansberge, 1885; Boucomont, 1914a; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-Bangladesh, India (Assam; West Bengal; Karnataka; Tamil Nadu: Nilgiri Hills; Kerala: Nelliyampathy, Thekkady, Wayanad), Indonesia: Borneo, Java, Sumatra; Myanmar, Malaysia, Sunda Islands, Thailand, Laos, Vietnam. PAR-India (Uttarakhand), China.

28. *Onthophagus* (s.str.) *socialis* Arrow, 1931 Fig. 3 (28)

Onthophagus (s.str.) *socialis* Arrow, 1931.

Distribution: ORR-India (Maharashtra: Mumbai; Karnataka: Belgaum, Coorg; Tamil Nadu: Nilgiri hills)

29. *Onthophagus* (s.str.) *tnai* Nithya and Sabu, 2012 Fig. 3 (29)

Onthophagus (s.str.) *tnai* Nithya and Sabu, 2012.

Distribution: ORR-India (Kerala: Silent valley, Panathady).

30. *Onthophagus* (s.str.) *truncaticornis* Schaller, 1783 Fig. 3 (30)

Onthophagus (s.str.) *truncaticornis* Schaller, 1783; Harold, 1870, 1880; Arrow, 1931; Balthasar, 1963a.

-*forcipatus* Harold, 1873; Arrow, 1931.

Distribution: ORR-India (Maharashtra: Mumbai; Tamil Nadu: Nilgiri hills; Karnataka: Mangalore).

31. *Onthophagus* (s.str.) *turbatus* Walker, 1858 Fig. 3 (31)

Onthophagus (s.str.) *turbatus* Walker, 1858; Boucomont, 1914b; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1963b.

Distribution: ORR-India (Maharashtra; Karnataka; Tamil Nadu: Puducherry, Nilgiri Hills; Kerala: Mahe, Malabar, Nelliyampathy, Thekkady, Wayanad), Sri Lanka.

32. *Onthophagus* (s.str.) *unifasciatus* Schaller, 1783 Fig. 3 (32)

Onthophagus (s.str.) *unifasciatus* Schaller, 1783; Fabricius, 1792; Arrow, 1931; Balthasar, 1963a.

-*prolixus* Walker, 1858; Harold, 1869.

Distribution: ORR-India (Maharashtra: Mumbai; Bengal; Bihar; Tamil Nadu: Coimbatore, Madras; Kerala: Nilgiri Hills), Sri Lanka (Colombo, Kandy).

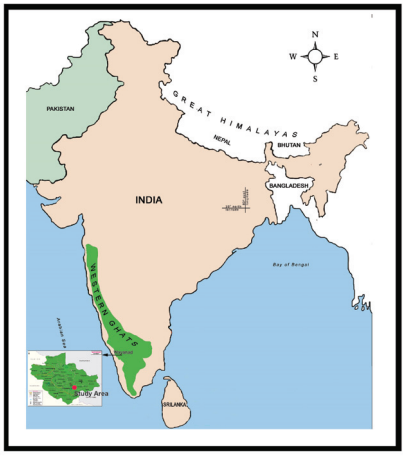

Fig. 1 Coffee plantation belt of Nlgiri Biosphere Reserve of south Western Ghats

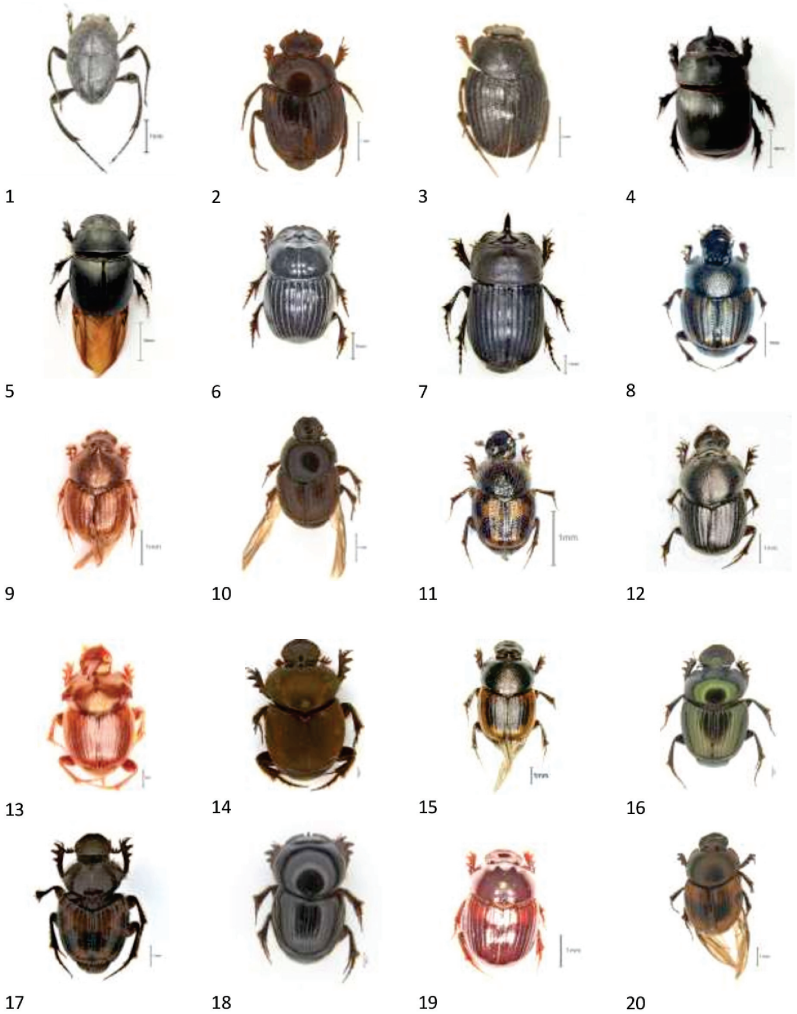

Fig. 2 (1) *Sisyphus longipes* (2) *Ochicanthon laetus* (3) *Ochicanthon tristis* (4) *Catharsius molossus* (5) *Catharsius sagax* (6) *Copris repertus* (7) *Paracopris davisoni* (8) *Caccobius meridionalis* (9) *C. ultor* (10) *C. unicornis* (11) *Onthophagus amphicoma* (12) *O. andrewesi* (13) *O. bifasciatus* (14) *O. bronzeus* (15) *O. cervus* (16) *O. dama* (17) *O. devagiriensis* (18) *O. discedens* (19) *O. duporti* (20) *O. fasciatus*

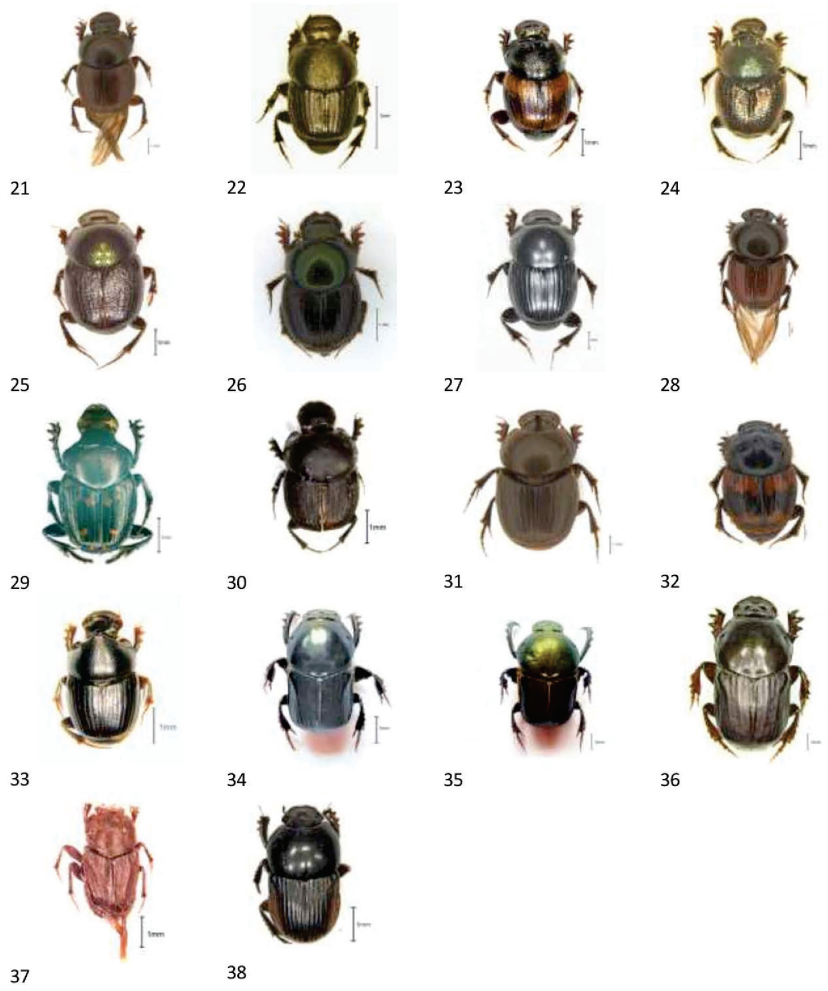

Fig. 3 (21) *Onthophagus faveri* (22) *O. furcilifur* (23) *O. insignicollis* (24) *O. kchatriya* (25) *O. lliputanus* (26) *O. ludio* (27) *O. pacificus* (28) *O. socialis* (29) *O. tnai* (30) *O. truncaticornis* (31) *O. turbatus* (32) *O. unifasciatus* (33) *O. urellus* (34) *Onitis falcatus* (35) *O. subopacus* (36) *O. virens* (37) *Tibiodrepanus setosus* (38) *Oniticellus cinctus*

33. *Onthophagus* (*Coloboniphagus*) *urellus* Boucomont, 1919 Fig. 3 (33)

Onthophagus (*Coloboniphagus*) *urellus* Boucomont, 1919; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1963a.

Distribution: ORR-Myanmar, India (Tamil Nadu: Nilgiri Hills; Kerala: Wayanad).

Tribe- Onitini

Genus 8. *Onitis* Fabricius, 1798

Onitis Fabricius, 1798, 1801; Castelnau, 1840; Lacordaire, 1856; Lansberge, 1875; Bedel, 1892; Reitter, 1892, 1893; Peringuey, 1901; Arrow, 1931; Balthasar, 1935a, 1963a; Janssens, 1937; Paulian, 1945.

34. *Onitis falcatus* Wulfen, 1786 Fig. 3 (34)

Onitis falcatus Wulfen, 1786; Lansberge, 1875; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963a; Janssens, 1937; Paulian, 1945.

-*hymalajicus* Redtenbacher, 1848.

-*sphinx* Herbst (nec Fabricius), 1789.

Distribution: ORR-Vietnam: Tonkin, Laos, Myanmar, Thailand, India (West Bengal; Karnataka;

Kerala: Mahe, Malabar; Wayanad), PAR-India (Uttarakhand), China, Taiwan, IAR-Philippines.

35. *Onitis subopacus* Arrow, 1931 Fig. 3 (35)

Onitis subopacus Arrow, 1931; Balthasar, 1935a, 1963a; Janssens, 1937.

-*philemon* Lansberge (nec Fabricius), 1875; Boucomont, 1914a; Boucomont and Gillet, 1921.

Distribution: ORR- India (Madhya Pradesh; West Bengal; Assam; Bihar; Tamil Nadu: Anamalai Hills; Kerala: Nelliampathi, Wayanad), Myanmar, Sri Lanka, Sunda Islands, Thailand, Vietnam. PAR-India (Kashmir; Uttarakhand), Afghanistan, Nepal, China.

36. *Onitis virens* Lansberge, 1875 Fig. 3 (36)

Onitis virens Lansberge, 1975, 1875; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963a; Paulian, 1945.

-*amplectens* Lansberge, 1875.

Distribution: ORR - Myanmar, North Vietnam, Laos, Thailand, India (Bihar; West Bengal; Maharashtra; Karnataka; Tamil Nadu: Nilgiri Hills, Anamalai Hills; Kerala: Peerumedu, Travancore, Wayanad). PAR-India (Uttarakhand), China.

Tribe- Oniticellini

Genus 9. *Tibiodrepanus* Krikken, 2009

Tibiodrepanus Krikken, 2009; Kirby, 1828; Castelnau, 1840; Lacordaire, 1856; Péringuey, 1901; Boucomont and Gillet, 1921; Boucomont, 1921b; Arrow, 1931; Balthasar, 1935a, 1963a; Paulian, 1945; Janssens, 1953.

-*Ixodina* Roth, 1851.

-*Cyptochirus* Lesne, 1900.

-*Drepanochirus* Peringuey, 1901; Boucomont, 1921b.

37. *Tibiodrepanus setosus* Wiedemann, 1823 Fig. 3 (37)

Drepanocerus setosus Wiedemann, 1823; Arrow, 1931; Janssens, 1953; Balthasar, 1963a; Krikken, 2009.

Distribution: ORR-India (Maharashtra; Madhya Pradesh; Kerala: Nilambur, Nelliyampathy, Thekkady, Wayanad), Sri Lanka. PAR-India (Uttarakhand)

Genus 10. *Oniticellus* Dejean, 1821

Oniticellus Dejean, 1821; Lacordaire, 1856; Reitter, 1892, 1893; Péringuey, 1901; Boucomont and Gillet, 1921; Boucomont, 1921b; Arrow, 1931; Portevin, 1931; Porta, 1932; Balthasar, 1935a; Paulian, 1941, 1945; Janssens, 1953.

38. *Oniticellus cinctus* Fabricius, 1775 Fig. 3 (38)

Oniticellus (s. str.) *cinctus* Fabricius, 1775; Boucomont, 1914a; Boucomont and Gillet, 1921; Arrow, 1931; Balthasar, 1935a, 1963b; Paulian, 1945; Janssens, 1953.

-*serratipes* Drury, 1770.

Distribution: ORR - Malaysia, Java, south China, Thailand, India (Madhya Pradesh; Maharashtra; West Bengal; Karnataka; Tamil Nadu: Nilgiri Hills; Kerala: Wayanad). PAR- India (Uttarakhand).

DISCUSSION

Among the thirty-eight species of dung beetles recorded from the shaded coffee plantation of south Wayanad, eight species, (*Ochicanthon laetus, O. tristis, Onthophagus andrewesi, O. amphicoma, O. bronzeus, O. devagiriensis, O. tnai*, *Paracopris davisoni*) were endemic to the Western Ghats and two species, *Onthophagus truncaticornis* and *O. discedens* were recorded as extinct species in the checklist of dung beetles from the moist south Western Ghats (Sabu *et al.,* 2011). The record of two species of the genus *Ochicanthon* [*O. tristis* (Arrow, 1931) and *O. laetus* (Arrow, 1931)] from the study site is significant, since *Ochicanthon* belonged to the very primitive and rare old world tribe Canthonini and all known *Ochicanthon* species are moist forest dwellers of the Indo-Pacific bioregion and in the Indian subcontinent, they are confined to the moist forests of south-

western and north-eastern India and absent from the vast intervening stretches of central India (Krikken and Huijbregts, 2007; Latha *et al.,* 2011). The presence of *Ochicanthon* species in the coffee plantation belts indicates that the recent habitat modifications in the Western Ghats have not wiped out the relict old world dung beetles (primitive groups) from the coffee plantations. The first report of *Onthophagus lilliputanus* from the moist south Western Ghats indicates that further studies from vaster areas of coffee plantations of the Western Ghats highlight the chance of revealing new additions to the species list of the Nilgiri Biosphere Reserve of the South Western Ghats.

The current study recorded many rare and endemic dung beetle species reported earlier by Arrow (1931) from the Western Ghats region. This supports the findings of Nearctic and Neotropical studies that shaded coffee plantations show higher abundance of coprophagous dung beetles similar to native forests (Moron, 1987; Estrada *et al.,* 1998; Arenallo *et al.,* 2005; Horgon, 2005, 2009; Pineda *et al.,* 2005; Halffter *et al.,* 2007; Sarges *et al.,* 2012) and also serve as refuges for many forest dung beetle species (Perfecto *et al.,* 1996; Moguel and Toledo, 1999; Arellano *et al.*, 2005).

The present study showed that species composition of dung beetles in the shaded coffee plantation with a comparatively smaller area had no major differences with that of the nearby natural forests of Thirunelli (North Wayanad Forest Division) and Thariode (South Wayanad Forest Division) in Wayanad (Vinod, 2009), which is an integral part of the Nilgiri Biosphere Reserve. Further similar studies in other coffee plantation belts of the South Western Ghats (Anamalais, Baba Budan giri, Chikmagalur, Coorg) are needed to understand the trends at much broader scale in the Western Ghats. Non-record of the genus *Liantogus* belonging to the dweller functional guild from the coffee plantations and abundant in the regional forests is attributed to the requirement for undisturbed large dung pads of megaherbivores like elephants and gaur for dwellers in general (Vinod and Sabu, 2007; Vinod, 2009). Thirty eight species collected from the coffee plantation of a small size is not very low in comparison with the 46 species recorded from a larger forest region in Wayanad (Vinod, 2009); Twelve out of 38 species were exclusively found in the coffee plantation, namely, *Caccobius ultor, C. unicornis, Onthophagus amphicoma, O. discedens, O. duporti, O. kchatriya, O. lilliputanus, O. ludio, O. socialis, O. tnai, O. truncaticornis, and O.unifasciatus*. Twenty one out of 46 species reported from the forest region (Vinod, 2009) were not recorded from the coffee site.

Higher species richness and presence of some unique species in the coffee plantation belt compared to other agricultural habitats with 28 species recorded from the agriculture belt of Wayanad (Vinod, 2009), 26 species from the semiurban agricultural belt in the Malabar Coast (Simi *et al.,* 2012), 25 species from the agriculture belt of Nelliampathi (Latha, 2011), and 31 species recorded from the agriculture fields of North Malabar (Simi, 2014) highlights the significance of shaded coffee plantations as an important nested habitat in the forest-agriculture land matrix of the moist western Ghats.

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Screening of cotton germplasm for their reaction against leafhopper, *Amrasca biguttula biguttula* Ishida (Homoptera: Cicadellidae)

K. Senguttuvan*, M. Murugan and N. Sathiah

Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India.
Email: senguttuvan.k@tnau.ac.in

ABSTRACT: Preliminary screening of twenty-nine cotton genotypes (*Gossypium hirsutum*) against the leafhopper *Amrasca biguttula biguttula* Ishida undertaken, during August 2019 to February 2020, revealed fifteen genotypes viz., TCH 357, TCH 1809, TCH 1828, TCH 1895, TCH 1897, TCH 1941, TSH 383, TVH 002, TVH 003, TKH 0762, TKH 1225, SVPR 6, CO 15, KC3 and Suraj, as moderately resistant. Selected preliminary screening entries subjected to advanced screening revealed TCH 357, TCH 1809, TCH 1895, TCH 1897, TCH 1941, TCH 1828, TSH 383, TVH 002, TVH 003, TKH 0762, SVPR 6 and CO 15 as moderately resistant with population range of 2.75 to 4.42 numbers per three leaves and KC3 resistant. In the artificial screening, the resistant cultivar KC3 had least leafhoppers (2.67 per plant) and it had 56 trichomes per 300 μm^2, which was higher than resistant check NDLH 1938. The resistant genotype KC3 had the maximum phenol (4.3 mg g^{-1}), amino acid (136 mg g^{-1}) and tannin (167 mg g^{-1}), while the susceptible genotype DCH 32 had the lowest amount of total phenol (1.2 mg g^{-1}), amino acids (18 mg g^{-1}) and tannin (40 mg g^{-1}).

KEYWORD: Genotypes, resistant cultivars, trichomes, phenols, tannin

INTRODUCTION

Amrasca biguttula biguttula Ishida (Homoptera: Cicadellidae), has been abundant in cotton in recent years, from the vegetative through reproductive stages of crop growth. Pesticides used to manage pests in the cotton ecosystem in India account for 45 per cent of all pesticides used. India, which has one third of the world's cotton farmers, account for 54 per cent of all pesticides used annually in cotton, despite occupying just 5 per cent of land under crops (Environmental Justice Foundation, 2007; Aktar *et al.,* 2009). As a result, developing a resistant/ tolerant cultivar is critical to reduce the three "R's" in the environment: resistance, resurgence, and residues. With this background, studies on refinement of screening methodology, identification of host plant phenotypic and genotypic features that contributes to resistance to leafhopper, using scanning electron microscope and biochemical characterisation were undertaken.

MATERIALS AND METHODS

Preliminary screening under natural condition

Twenty-nine cotton genotypes including NDLH – 1938 (Resistant check) and DCH 32 (Susceptible check), were included in the screening experiment (Table 1). Standard check was sown in the middle and ends of two sides of the genotypes with a spacing of 75cm row x 45 cm plant, in the 20 m^2 plots.

* Author for correspondence

Genotypes were sown in four rows in a randomised block design with three replications. Between each genotype, bhendi was sown as an infester crop and the bhendi plants in the susceptible check were clipped after a substantial increase of leafhopper population (>2 nos./3 leaf) at 30 days after sowing (DAS). If the adult leafhopper population in susceptible check (>8 nos./3 leaf) was not reached even after 75 days, the experiment was repeated until the population was sufficient in natural conditions. From sowing to harvest, no pesticides were used. Standard agronomic cultivation practices were followed. Screening experiment was undertaken in the fields of the Department of Cotton in Coimbatore during August 2019 – February 2020.

Population assessment of leafhopper under natural condition

Nymphs population of leafhoppers was recorded on ten randomly selected plants in each replication for all the genotypes, including standard check at 30, 45 and 60 days after sowing. In each plant three leaves from top, middle and bottom were observed and mean population per three leaves was recorded.

Damage assessment of leafhopper under natural condition

Hopper burn injury was assessed as per the methodology enumerated by Indian Central Cotton Committee (1960). A visual rating of hopper injury on each genotype was recorded on 30, 45 and 60 days after sowing and leafhopper injury grade index was calculated.

Grade	Symptoms
1 -	Leaves free from crinkling or with no yellowing, bronzing and drying
2 -	Few leaves on lower portions of the plant curling, crinkling and slight yellowing
3-	Crinkling and curling all over, yellowing, bronzing and browning in the middle and lower portion, plant growth hampered
4 -	Extreme curling, yellowing, bronzing and browning, drying of leaves and defoliation, and stunted growth

Leafhopper injury grade Index (LIGI)

A leafhopper injury grade index was calculated as proposed by Nageswara Rao (1973),

$$LHRI = \frac{G1 \times P1 + G2 \times P2 + G3 \times P3 + G4 \times P4}{P1 + P2 + P3 + P4}$$

Where G represented the number of the grade of ICCC (now ICAR-CICR) and P represented the number of leafhopper population of same plant under the each entry. Grade index with ≤ 1.0 grouped as resistant, $1.0 > \leq 2.0$ as moderately resistant, $2.0 > \leq 3.0$ as susceptible and $3.0 > \leq 4.0$ as highly susceptible.

Advanced screening under artificial condition

Artificial screening for leafhopper resistance was conducted at the net house, Department of Cotton, TNAU, Coimbatore. Cotton seeds of genotypes along with standard check for resistant and susceptible to leafhopper were sown during September 2020 in pots and for each entry three plants were maintained. The genotypes in the potted plants were placed at random but equidistantly apart in a circle inside the hopper net. Each pot represented a replication. Three replications were maintained for each genotype. Three hundred field collected adults were released in the middle and the top of the set up. Caging was used for screening. The leafhopper was released on 15 day old plants and counts of population and hopper burn injury were taken up to 60 days after sowing.

Trichome density by Scanning Electron Microscope

A fresh leaf sample was taken from a 6-week-old plant in a pot culture. Fixation was done using glutaraldehyde and formaldehyde, followed by osmium tetroxide postfixation. By critical point drying, the fixed tissue was dehydrated by liquid carbon dioxide. For observation under the microscope, the dried specimen was mounted on a specimen stub using an adhesive such as epoxy glue and sputter-coated with gold alloy. A microtome was used to segment the samples. Images taken with a scanning electron microscope (SEM) at 1000x magnification were captured. In a 300μm^2 region, SEM images of trichome density were gathered.

Table 1. Preliminary screening of genotypes under natural condition against leaf hopper

No.	Genotype	No./ 3 leaves	grade index	Rating	SCY/pl (g)
1	TCH 357	2.24(1.94)^{d}	1.82	MR	375(13.62)^{n}
2	TCH 1764	3.46(1.32)^{i}	2.35	S	320(13.44)^{ij}
3	TCH 1772	4.87(1.80)^{lm}	2.57	S	355(18.32)^{l}
4	TCH 1807	4.25(1.66)^{k}	2.85	S	380(18.18)^{n}
5	TCH 1809	2.76(1.66)^{f}	1.46	MR	300(18.40)^{gh}
6	TCH 1811	3.73(1.41)^{j}	2.28	S	350(16.45)^{kl}
7	TCH 1828	3.47(2.40)^{i}	1.34	MR	300(12.06)^{gh}
8	TCH 1895	1.75(0.71)^{c}	1.49	MR	255(0.71)^{d}
9	TCH 1897	3.42(1.56)^{i}	1.38	MR	435(3.76)^{o}
10	TCH13/22	4.97(1.35)^{m}	3.42	HS	385(3.73)^{n}
11	TCH13/24	4.72(1.52)^{l}	2.67	S	475(4.34)^{p}
12	TCH 1941	2.50(1.47)^{e}	1.56	MR	325(4.32)^{hi}
13	TSH 383	1.75(1.47)^{c}	1.37	MR	310(4.31)^{i}
14	TSH 387	3.00(1.38)^{g}	2.86	S	355(4.12)^{l}
15	TVH 002	1.25(1.70)^{a}	1.27	MR	285(3.54)^{fg}
16	TVH 003	2.25(1.10)^{d}	1.32	MR	280(1.10)^{df}
17	TVH 007	3.25(1.44)^{h}	2.67	S	260(2.06)^{de}
18	TKH 0762	2.25(1.36)^{d}	1.33	MR	365(2.06)^{l}
19	TKH 1225	1.25(1.42)^{a}	1.21	MR	265(2.20)^{e}
20	SVPR 6	1.75(1.40)^{c}	1.23	MR	215(2.20)^{c}
21	CO 14	3.25(1.40)^{h}	2.42	S	310(2.19)^{hi}
22	CO 15	1.75(1.40)^{c}	1.24	MR	385(2.15)^{n}
23	CO 17	3.25(1.48)^{h}	2.45	S	185(2.01)^{b}
24	KC3	1.25(1.26)^{a}	1.12	MR	180(1.26)^{b}
25	RCH 659	2.75(1.39)^{f}	2.34	S	335(1.60)^{jk}
26	BGDS 1063	2.25(1.36)^{d}	2.46	S	330(1.60)^{j}
27	Suraj (check)	2.25(1.39)^{d}	1.38	MR	325(1.64)^{ij}
28	NDLH – 1938 (RC)	1.50(1.38)^{b}	0.84	R	270(1.64)^{ef}
29	DCH 32 (SC)	5.25(1.38)^{n}	3.29	HS	145(1.64)^{a}
	SE	0.0175		0.2258	
	CD	0.0350		0.4523	

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Table 2. Advanced screening of genotypes under artificial condition

No.	Genotype	No. /3 leaves	Grade Index	Rating
1	TCH 357	4.00(1.94)[h]	1.9	MR
2	TCH 1809	2.75(2.04)[b]	2.0	MR
3	TCH 1828	3.83(2.00)[g]	1.9	MR
4	TCH 1895	3.50(1.93)[e]	1.8	MR
5	TCH 1897	3.67(1.78)[f]	2.0	MR
6	TCH 1941	3.75(1.58)[fg]	1.8	MR
7	TSH 383	4.42(2.71)[i]	1.8	MR
8	TVH 002	2.92(0.71)[c]	1.8	MR
9	TVH 003	3.17(1.56)[d]	1.9	MR
10	TKH 0762	3.67(1.59)[f]	1.7	MR
11	TKH 1225	3.27(1.58)[d]	2.5	S
12	SVPR 6	3.67(1.56)[fg]	2.0	MR
13	CO 15	3.50(1.51)[e]	2.0	MR
14	Suraj	3.24(1.44)[d]	2.6	S
15	KC3	2.67(1.79)[b]	1.0	R
16	NDLH – 1938 (RC)	2.00(1.10)[a]	1.0	R
17	DCH 32 (SC)	6.83(1.44)[j]	3.1	HS
	SE	0.0204		
	CD	0.0415		

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Estimation of biochemical parameters

Estimation of total phenol: Fresh leaf sample (200 mg) was extracted with 10 ml absolute methanol. One ml of Folin Ciocalteau reagent diluted with equal volume of distilled water before use was added to one ml of alcohol extract in a test tube followed by 2 ml of 20 per cent sodium carbonate. The mixture was heated on a boiling water bath for one minute. The blue colour was measured at 660 nm. Reagent blank was maintained with 80 per cent ethanol. Total phenols were calculated from catechol standard (Bray and Thorpe, 1954).

Estimation of tannin: A 200 mg sample of fresh cotton leaf was extracted with 10 ml absolute methanol. The extracted material was rotavated for 20 minutes with a screw cap culture tube. The mixture was centrifuged at 3000 rpm for 10 minutes, and the supernatant was utilised to conduct the analysis. Each extract is poured in one ml aliquots into a culture tube (each sample maintains with blank). Five ml of vanillin reagent (sample) and five ml of 4 percent HCL solution (blank) were added at 1.0 minute intervals, then maintained in a water bath for exactly 20 minutes. The absorbance at 500 nm was measured using a Spectrophotometer.

Table 3. Leaf trichome density of advanced genotypes

SNo.	Genotypes	No./ 3 leaves	Rating	Trichomes(300 µm²)		
				No.	Branch	Total
1	TCH 1897	3.67(1.92)^{f}	MR	4	4	16
2	TVH 002	2.92(2.04)^{c}	MR	2	2	4
3	TCH 1828	3.83(1.80)^{g}	MR	7	2	14
4	SVPR 6	3.67(2.00)^{f}	MR	9	2	18
5	TCH 1941	3.75(1.78)^{fg}	MR	5	2	10
6	TSH 383	4.42(1.58)^{i}	MR	6	2	12
7	TCH 1895	3.50(2.24)^{e}	MR	2	1	2
8	TCH 357	4.00(0.71)^{h}	MR	3	2	6
9	TVH 003	3.17(1.55)^{d}	MR	5	4	20
10	TKH 0762	3.67(1.59)^{f}	MR	3	8	24
11	TCH 1809	2.75(1.52)^{b}	MR	5	4	20
12	CO 15	3.50(1.58)^{e}	MR	6	3	18
13	KC3	2.67(1.51)^{b}	R	7	8	56
14	NDLH – 1938 (RC)	2.00(1.44)^{a}	R	4	4	16
15	DCH 32 (SC)	4.50(1.65)^{i}	HS	1	1	1
	SE	0.0187				
	CD	0.0382				

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Estimation of total free amino acids: Phosphate buffer pH 7.0 was used to extract 200 mg of cotton leaves. In test tubes, 1.0 ml of extract was collected. The leaf extract was then combined with 1.0 ml of 10% pyridine and 1.0 ml of 2.0% ninhydrine solution in each tube. The tubes were then heated in a boiling water bath for half an hour, and the volume was increased to 15.0 ml with distilled water to dilute the solution. The coloured solutions' absorbance was measured at 570 nm (Hamilton and Van Slyke, 1943).

Statistical analysis

The total number of insects in the population was transformed to get square root values. The results of the laboratory biochemical analysis were translated into arcsine transformed numbers. Duncan's Multiple Range Test was used to differentiate the mean values of the treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Preliminary screening

Mean leafhopper incidence ranged from 1.25 (KC 3) to 5.25/3 leaves (DCH 32). Based on the resistance index, the twenty nine genotypes were grouped into four categories *viz*., resistant, moderately resistant, susceptible and highly susceptible. Among these genotypes, fifteen genotypes were identified as moderately resistant *viz*., TCH 357, TCH 1809, TCH 1828, TCH 1895, TCH 1897, TCH 1941, TSH 383, TVH 002, TVH 003, TKH 0762, TKH 1225, SVPR 6, CO 15, KC3 and Suraj. Leafhopper population was comparatively low in these entries with leafhopper injury grade II which was on par with standard check NDLH 1938. The remaining genotypes were recorded as susceptible (11 genotypes) and TCH13/22 was highly susceptible to leafhopper which was on par with

Table 4. Biochemical analysis of genotypes

No	Genotypes	Rating	Total phenol (µg/g)	Amino acid (µg/g)	Tannin (µg/g)
1	TCH 357	MR	3.3(1.94)^{e}	81(9.92)^{d}	152(12.55)^{h}
2	TCH 1809	MR	3.7(2.00)^{g}	84(8.22)^{de}	116(11.73)^{c}
3	TCH 1828	MR	2.8(1.82)^{bc}	86(9.72)^{ef}	99(11.98)^{b}
4	TCH 1895	MR	3.1(2.07)^{d}	92(8.69)^{i}	127(9.92)^{d}
5	TCH 1897	MR	2.7(2.19)^{b}	76(11.68)^{c}	135(12.94)^{e}
6	TCH 1941	MR	2.9(2.12)^{c}	83(9.51)^{de}	140(12.27)^{ef}
7	TSH 383	MR	2.9(1.30)^{c}	95(4.30)^{ij}	138(6.36)^{ef}
8	TVH 002	MR	2.7(0.37)^{b}	87(0.71)^{fg}	138(0.71)^{ef}
9	TVH 003	MR	3.4(1.57)^{ef}	98(3.23)^{j}	157(3.61)^{h}
10	TKH 0762	MR	3.5(1.58)^{f}	67(2.95)^{b}	137(3.50)^{ef}
11	SVPR 6	MR	2.8(1.52)^{bc}	94(3.20)^{i}	143(3.53)^{fg}
12	CO 15	MR	3.8(1.60)^{g}	75(3.03)^{c}	98(3.23)^{b}
13	KC3	R	4.3(1.64)^{i}	136(3.49)^{k}	167(3.67)^{i}
14	NDLH 1938 (RC)	R	4.0(1.62)^{h}	90(3.16)^{g}	150(3.57)^{gh}
15	DCH 32 (SC)	HS	1.2(1.34)^{a}	18(2.19)^{a}	40(2.62)^{a}
	SE		0.0164	0.0827	0.1549
	CD		0.0336	0.1693	0.3174

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

the susceptible check, DCH 32 (Table 1). Insect resistant crop varieties have the unique advantage of providing inherent insect control which is compatible with other methods of insect control and provides more practical approach in leafhopper management. Several workers reported on the varietal susceptibility in cotton to *Amrasca devastans* and accessions resistant to leafhopper (Balasubramanian *et al.,* 1978; Ambekar and Kalbhor, 1981; Chandramani *et al*., 2004). Manivannan *et al.,* 2017).

Advanced screening

Seventeen entries including standard checks *viz*., NDLH 1938 and DCH 32 were selected from the preliminary screening for advanced screening experiment under protected net house. Advanced screening experiment revealed 12 entries *viz.,* TCH 357, TCH 1809, TCH 1895, TCH 1897, TCH 1941, TCH 1828, TSH 383, TVH 002, TVH 003, TKH 0762, SVPR 6 and CO 15 as moderately resistant with population ranging from 2.75 to 4.42 numbers per three leaves (Table 2). KC3 was reordered as resistant entry to the leafhopper which was on par with the resistant standard check NDLH 1938. The remaining two genotypes (TKH 1225 & Suraj) were recorded as susceptible and DCH 32 highly susceptible (Table 2). Adult leafhoppers were seen settling in more numbers on highly susceptible and susceptible plants indicating more sustained feeding on susceptible plants. Mohankumar (1996) and Manish (1998) reported similar results. Barroga and Bernardo (1993) observed more preferential settling of *A. devastans* on susceptible cotton. Considering the morphological mechanism of leafhopper resistance, Uthamasamy (1985) reported that morphological characters such

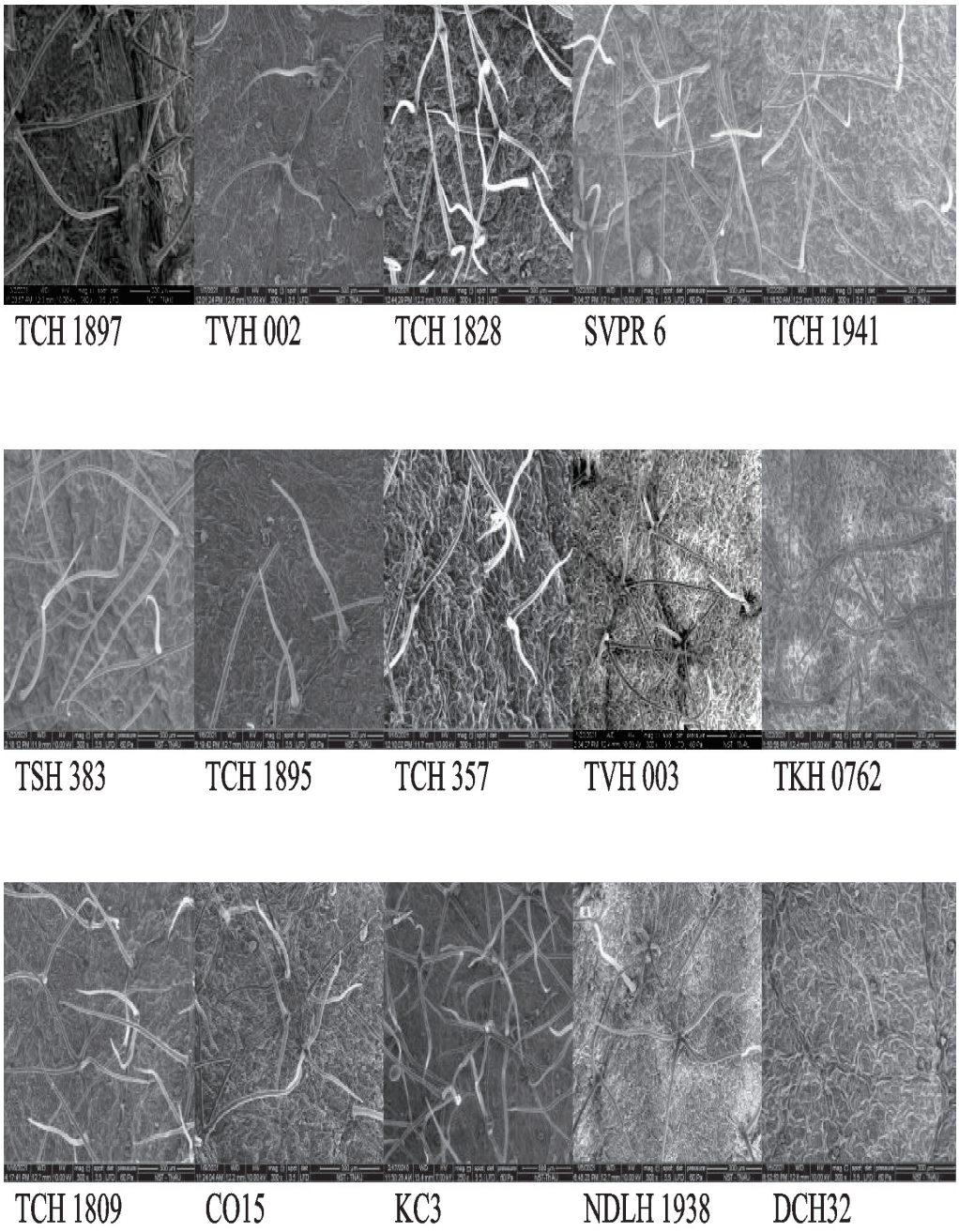

Fig 1. Trichomes density photograph by Scanning Electron Microscope

as hairiness of leaves, toughness of leaf veins, thickness of leaf lamina, length of the hair and angle of insertion are associated with the resistance to leafhoppers. H-1316, GISV-216 and CPD 1019 were reported as resistant (Rao *et al.,* 2011). Nine entries were categorized as highly resistant to leaf hopper with injury grade 1 with a population range of 0.57 to 3.57 per 3 leaves per plant (Sasikumar and Rathika, 2020).

Morphological parameters

Among the fifteen entries, nine genotypes were observed with maximum number of trichomes (12 to 56/ 300μm^2). It is concluded that trichome density play a major role in resistance and found to have significant negative correlation with the leafhopper population and damage (Fig. 1 & Table 3). The plant leaf characters, *i.e.,* leaf length, trichome density and trichome size are essential features to minimize jassid populations in cotton crop (Kanher *et al.,* 2016). The incidence of leafhoppers was also reported to be lower in high hairy varieties and higher in low hairy genotypes, indicating that trichome density plays a substantial role in sucking pest resistance (Manivannan *et al*., 2017).

There are reports that leafhopper injury grade having negative association with plant height, inter nodal length, leaf hair density, hair length, hair density on mid vein, total chlorophyll, chlorophyll a and chlorophyll b and positive association with the moisture content (Ambekar and Kalbhor, 1981, Uthamasamy, 1985; Sivasubramanian *et al.,* 1991; Mohankumar, 1996; Murugesan and Kavitha, 2010; Venkatesha, 2014; Bhatti *et al.,* 2015; Amin *et al.,* 2017; Khalil *et al.,* 2017) of which the contribution of leaf hair density to the leafhopper resistance was made vivid in the present study.

Biochemical parameters

Phenol (4.3 μg g^{-1}), amino acid (136 μg g^{-1}) and tannin (167 μg g^{-1}) was maximum in the resistant variety (KC3) which was higher than the standard check (NDLH 1938). Remaining eleven moderately resistance entries showed total phenol content ranging between 2.7 and 3.8 μg g^{-1}. The susceptible culture DCH 32 recorded the lowest phenol (1.2 μg g^{-1}), amino acids (18 μg g^{-1}) and tannin (40 μg g^{-1}). All three biochemical parameters were on the higher side in the resistant varieties but lower side in the susceptible culture (Table 4). Lower leafhopper damage injury index was reported with higher quantity of biochemical components like tannins, phenols and gossypol (Rohini *et al.,* 2011; Shinde *et al.,* 2014; Venkatesha, 2014; Harijan *et al.,* 2017). Biochemical profiles revealed that higher level of chlorophyll, nitrogen, protein, amino acids and reducing sugars favors the leafhopper infestation. In contrast, phenol compound act as feeding deterrent, as most of the resistant genotypes showed higher level of phenol (Venkatesha, 2014; Manivannan *et al.,* 2021). Total phenols showed significant and negative correlation with jassid incidence in genotypes of cotton. Highly susceptible entries are preferred for settling and feeding whereas varieties less preferred for settling are less preferred for oviposition (Bhatti *et al.,* 2015; Bhoge *et al.,* 2019).

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Ascertaining foraging rate of pollinators in *Ricinus communis* L. in Haryana, India

Sudhanshu Bala Nayak, Manish K Yadav*, R. Nihal, V. Ramalakshmi, Lipsa Dash and Deepayan Padhy

Centurion University of Technology and Management, Paralakhemundi, Odisha, India.
Email: manish.yadav@cutm.ac.in

ABSTRACT: Foraging rate of different pollinator species under the genus *Apis* and non-*Apis* studied on two castor (*Ricinus communis*) hybrid under agro-ecological conditions of Haryana, revealed that irrespective of interspecific variation, the maximum foraging rate was found associated with *Apis florea* followed by *A. dorsata*, *A. cerana* and *A. mellifera.* Further, *Eristalnus* sp. visits of flowers were the lowest. Adding to the visiting frequency, honey producing bees (*Apis* sp.) visited significantly more flower per unit time compared to other pollinators (non-*Apis*) species. The study gave an idea of different foraging rates of different pollinators on castor flower.

KEYWORDS: Castor, *Apis*, non-*Apis* pollinators

INTRODUCTION

Ricinus communis L. commonly known as castor, belonging to the family Euphorbiaceae is grown throughout the India, for its oil content (Nayak *et al.,* 2020). Primarily it is native to Ethiopian region of tropical East Africa (Ladda and Kamthane, 2014). Crop yield mainly depends on insect mediated pollination. Foraging behaviour that impact gene flow can guide the design of pollinator strategies to mitigate gene flow (Brunet and Van Etten, 2019). The measurement of pollinator visitation frequency can clarify the relationships between pollinators, pollinated crops and the seed set (He *et al.,* 2019). Studies were carried out on the foraging behaviour of pollinating insects, honey bees and other pollinators, in castor flowers in 2018 and 2019.

MATERIALS AND METHODS

Experimental Area: Investigations were carried out to determine the foraging rates of different castor pollinators, on two castor hybrids GCH-7 and DCH-177 at Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana (29°10'N, 75°46'E, 215.2 m AMSL). All sampling was conducted in the year 2018 and 2019 on the castor cultivars (@1000m^2 area per each cultivar) and crops were grown at a temperature of 33 ± 2^{0}C. Depending up on the cultivar and the weather condition, flowering took place 65-75 days after sowing for. For raising a healthy crop, all the recommended crop production practices including fertilizer application, irrigation, weeding and other cultural operations were followed as per CCSHAU package of practices.

* Author for correspondence

Foraging rate: Foraging rate of different castor pollinators were recorded in terms of number of flowers visited per minute. Observations were recorded during peak flowering period of crop in the month of August and September, at two-hour interval starting from morning 06 00 to evening 18 00 h. For each major pollinator, fifteen observations were recorded at flowering stage of crop. Accordingly, the average number of flowers visited per minute was calculated for each species. After a flower was visited once, this specific flower was labelled; the type of bee that visited the flower was recorded. Pollinator's specimens were identified in the field by the authors using the identification key. The recorded data was analyzed by using three-way ANOVA in Randomized Block Design using SPSS software with Duncan Multiple range Test ranking.

RESULTS AND DISCUSSION

Foraging rate of pollinators on *R. communis* cv. GCH-7

The foraging rate *i.e.,* the average number of flowers visited by an individual forager varied irrespective of time and species. When considering the pooled mean data of two years (2018 and 2019) at different time intervals, the foraging rate ranged 1.324 to 11.694 flowers/ minute. Peak foraging rate was recorded at 10 00-12 00 h (11.694 flowers/ minute) followed by 12 00-14 00 h (8.509 flowers/ minute), while the lowest rate was found at 14 00-16 00 h (1.324 flowers/minute). During the peak period of activity the maximum number of flowers visited was by *Apis florea* (11.694 flowers/ minute), followed by *Apis dorsata* (9.595), *Apis cerana* (7.946) and *Apis mellifera* (7.626). Whereas lowest was recorded for *Eristalinus* sp. (2.876), followed by *Melipona bivolor* (3.423) and *Polistes* sp. (3.875). For *Megachile lanata*, *Polistes* sp. and *M. bicolor* foraging activity was not observed during 06 00-08 00 morning hour. No foraging activity was found for *Polistes* sp. and *Eristalinus* sp. during 16 00-18 00 h (Table 1).

Foraging rate of pollinators on *R. communis* cv. DCH-177

The forage rate (number of flowers visited per min) of different insect pollinators on *R. communis* cv. DCH-177 during 2018 and 2019 was pooled and observed that as in GCH-7, the cultivar DCH-177 also similar trend of forage rate (Table 2).. While considering the mean data at different time intervals, the foraging rate ranged between 1.156 to 8.944 flowers/minute where the peak foraging rate was recorded at 10 00-12 00 h (8.944 flowers/minute) followed by 08 00-10 00 h (6.374 flowers/minute) while the lowest rate was found at 14 00-16 00 h (1.156 flowers/minute) and 16 00-18 00 h (1.749 flowers/minute). During the peak period of activity the maximum number of flowers was visited by *A. florea* (8.944) followed by *A. dorsata* (8.037), *A. cerana* (7.463) and *A. mellifera* (6.631), whereas the low foraging rate were recorded for *Eristalinus* sp. (2.884), *M. bivolor* (3.334) and *Polistes* sp. (3.841). Like GCH-7 hybrid, for *M. lanata*, *Polistes* sp. and *Melipona bicolor* no foraging activity was observed during 06 00-08 00 morning hour. The highest foraging rate for all pollinators was recorded during 10 00-12 00 h respectively. No foraging activity was found for *Polistes* sp. and *Eristalinus* sp. during 16:00-18:00 h.

However, the pollinators were selective in choosing the castor hybrids and there were significant differences found regarding the foraging rates between two hybrids. Among the pollinators, *A. florea* had the highest foraging rate while the *Eristalinus* sp. was having with lowest foraging rate. Interaction of pollinator and cultivar was also differed significantly.

Foraging rate (number of flowers visited per min) are mainly governed by host factor (type of resources), flower anthesis, floret structure and environmental factors play an important role governing in field activity of social bees as well as solitary bees. The foraging rate of pollinators, determines the comparative tripping efficiency eventually (Martiniello *et al.,* 2003). Similar results observed by Jat *et al.* (2014), where they reported the mean foraging rate of *A. dorsata, A. mellifera*, and *A. florea* as 6.2, 6.4 and 6.6 (flower/min) on *T. alexandrinum* respectively. The observations at different hours of the days also showed the peak foraging rate between 10 00-12 00 h and it was followed by 12 00-14 00 h, while the lowest rate

Table 1. Foraging rate of pollinators on Castor cv. GCH-7 during 2018 & 2019 (data pooled)

Pollinator	06 00-08 00 h	08 00-10 00 h	10 00-12 00 h	12 00-14 00 h	14 00-16 00 h	16 00-18 00 h
Apis dorsata F.	6.929^{e}	7.735^{b}	9.595^{e}	8.509^{e}	7.376^{g}	5.895^{f}
Apis mellifera L.	6.255^{d}	7.525^{b}	7.626^{d}	7.620^{b}	6.181^{f}	4.628^{e}
Apis cerana F.	6.070^{d}	6.982^{b}	7.946^{d}	5.088^{c}	4.263^{e}	2.391^{bc}
Apis florea F.	3.928^{cd}	6.856^{b}	11.694^{f}	4.361^{c}	3.549^{d}	3.050^{d}
Vespa sp.	2.665^{b}	3.986^{a}	5.555^{c}	2.993^{b}	3.614^{d}	2.396^{bc}
Tetragonula iridipennis (Smith)	2.460^{b}	3.875^{a}	5.745^{c}	3.296^{b}	3.141^{c}	2.885^{cd}
Megachile lanata F.	0.000^{a}	3.769^{a}	4.691^{bc}	2.248^{a}	2.511^{b}	2.351^{b}
Meliopona bicolor Lepeletier	0.000^{a}	3.553^{a}	3.423^{a}	3.079^{b}	2.561^{b}	1.934^{b}
Polistes sp.	0.000^{a}	3.186^{a}	3.875^{ab}	2.190^{a}	2.286^{b}	0.000^{a}
Eristalinus sp.	2.026^{b}	3.168^{a}	2.876^{a}	2.171^{a}	1.324^{a}	0.000^{a}

Means followed by same letter in the column do not differ significantly by DMRT (P=0.01)

Table 2. Foraging rate of pollinators on *R. communis* cv. DCH-177 during 2018 & 2019 (pooled)

Pollinator	06 00-08 00 h	08 00-10 00 h	10 00-12 00 h	12 00-14 00 h	14 00-16 00 h	16 00-18 00 h
Apis dorsata F.	6.075^{d}	6.374^{c}	8.037^{ef}	6.021^{e}	4.501^{d}	4.020^{e}
Apis mellifera L.	5.505^{cd}	5.410^{b}	6.631^{d}	5.245^{d}	4.205^{d}	3.678^{e}
Apis cerana F.	6.070^{d}	5.970^{bc}	7.463^{de}	4.963^{d}	4.400^{d}	2.404^{c}
Apis florea F.	4.484^{c}	6.231^{bc}	8.944^{f}	3.736^{c}	3.736^{d}	2.925^{d}
Vespa sp.	1.773^{b}	3.959^{a}	5.257^{c}	2.429^{ab}	2.989^{c}	1.889^{bc}
Tetragonula iridipennis (Smith)	2.530^{b}	3.793^{a}	5.200^{c}	3.258^{c}	2.641^{bc}	2.010^{bc}
Megachile lanata	0.000^{a}	3.677^{a}	4.340^{bc}	2.241^{a}	2.253^{bc}	2.183^{bc}
Meliopona bicolor Lepeletier	0.000^{a}	3.553^{a}	3.334^{ab}	3.153^{bc}	2.061^{b}	1.749^{b}
Polistes sp.	0.000^{a}	3.186^{a}	3.841^{ab}	2.413^{ab}	2.036^{b}	0.000^{a}
Eristalinus sp.	2.788^{b}	3.189^{a}	2.884^{a}	2.044^{a}	1.156^{a}	0.000^{a}

Means followed by same letter in the column do not differ significantly by DMRT (P=0.01)

was found at 16 00-18 00 h. Similar activity had been documented in coriander by Shivashankara *et al.* (2016) who reported the daily activity of pollinators showed that foraging activity begins in the morning (its variable depending upon insect) and reached to maximum between 12 00 and 15 00 h, then decreased till sunset. Similarily Rizzardo *et al.* (2012) also observed that in early morning, the number of bees collecting pollen and nectar increasing slowly from 7 00 to 13 00 h and decreasing towards 17:00 h and peak period was in between 7:00 to 9:00 h decrease towards to the hottest time of the day (13:00 h). Current study is in line with by Reddy *et al.* (2015) and Maity *et al.* (2014), reported that climatic factor plays a significant role in influencing the foraging activity and behavior of social insects especially honeybees. Dalio (2018), recorded that the Asian bee, *A. cerana* visited more numbers of flowers per minute (18.10), followed by *A. mellifera* (17.36) and *A. dorsata* (13.87) while foraging rate of *A. florea* (7.53 flowers/min) was comparatively low on *Brassica napus*. Srivastava *et al.* (2017) reported the mean foraging rate was the maximum for *A. dorsata* (5.35 ± 0.33 flowers/min) followed by that of *A. mellifera* (4.87 ± 0.34 flowers/min), *A. cerana* (1.75 ± 0.23 flowers/min) and lowest foraging rate was recorded of *A. florea* (0.11 ± 0.09 flowers/min) on *Brassica oleracea*. Present observations were in line with findings of Mohapatra and Sontakke (2012) who recorded the mean foraging rate of 6.7-7.4 flowers/minute, 9.3-11.5 flowers/minute and 5.2-5.8 flowers/minute by *A. cerana, A. dorsata* and *A. florea* on sesame, respectively. Kunjwal *et al*. (2014) also reported that the *A. mellifera* was having highest foraging rate with 11.48 flowers followed by *A. dorsata* (4.03), *A. cerana* (2.09) and *T. laeviceps* (1.93) in mustard. However, Nagpal *et al*. (2019) in *B. juncea*, observed highest foraging rate with *A. cerana i.e.,* 13.92 flowers followed by *A. mellifera* (12.86), *A. dorsata* (10.35) and *A. florea* (6.99). In the case of *A. cerana* the highest foraging rate was observed (18.20 flowers/min) followed by *A. dorsata* (17.57), *A. mellifera* (17.32) and *A. florea* was found to be visited minimum number of flowers (6.45) on mustard (Poonam, 2019). Khamhari (2013) also reported the mean foraging rate varied from 6.84 to 12.13 flowers/minute in niger. Among different bee species, *A. mellifera* visited significantly more numbers of flowers (10.28 flowers/minute) followed by *A. cerana* (9.13 flowers/minute). The maximum foraging rate 12.13 flowers/minute was observed at 12 00 to 14 00 h, while the minimum rate was 6.84 flowers/minute observed at 16 00 to 18 00 h.

The present investigations based on the behaviour of various bees in context of two genotypes of castor namely GCH-7 and DCH-177, revealed that the maximum foraging rate is associated with *A. florea* which have their benefits of locality and small body size as well, followed by *A.dorsata A. cerana* and *A. mellifera*, while the minimum was by *Eristalnus* sp. In the case of their visiting frequency, honey producing bees have been visiting significantly more flowers in per unit time compared to other pollinator.

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Feeding behaviour of pit building of antlion *Myrmeleon pseudohyalinus,* Holzel 1972 (Neuroptera: Myrmeleontidae) in different mediums, instars and hunger levels

K. Anila*, Francy K Kakkassery and Joyce Jose

Research and Postgraduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur 680001, Kerala, India.
Email: antlionanila@gmail.com, kakkassery@yahoo.com, joyceofthejungle@gmail.com

ABSTRACT: Feeding behaviour of *Myrmeleon pseudohyalinus* (Holzel, 1972) larvae was studied in sand and soil media for second and third instars, and at two hunger levels (fed/starved) in laboratory conditions. Common ant *Anoplolepis gracilipes* (Smith, 1857) was used as prey in all the experiments. From the eight experiments, feeding time, prey escape and capture success were analysed. The predatory efficiency studied indicated that the capture success was high in second instar larvae irrespective of hunger level and medium. The relationships between selected behaviour, instar, medium and condition were studied and also the relationship between selected behaviour, instar, medium, condition and time period were analysed. From the Canonical Correspondence Analysis (CCA), it is clear that the prey beating, emergence and submergence behaviours were influenced by the larval instar (second and third) and quiescence, pit clearing and jaw set behaviour patterns were influenced by their condition (fed or starved). CCA also shows an influence of time period in the feeding behaviour pattern of antlion larvae.

KEYWORDS: Predatory efficiency, Canonical Correspondence Analysis, antlion instars

INTRODUCTION

Neuropterans are mainly predators in both larval and adult stages, but in the case of Family Myrmeleontidae, the larval stages are more predaceous than adults and have interesting strategies for feeding, pit building and predation. Adult antlion's survival period is below one month when compared to the long larval period of up to two years. The larvae make conical pits in the substrate and wait for the prey to fall down in to it. Conical pits are dual purposed as it shelters the antlion from enemies and traps its unsuspecting prey too. Exceptions to pit building are the Mediterranean antlion species *Neuroleon microstenus* (McLachlan, 1898) which dig in sand backwards and wait for the prey (Devetak *et al*., 2010).

Pit building by antlion larvae in sand or dry soil is accomplished by a series of backward movements. After making the pit, they wait for the prey and once the larvae encounter a prey, many behaviours are exhibited followed by feeding. Predation helps to maintain the balance of animal populations and the predators selectively remove the young, old and diseased or injured individuals from prey populations (Southwick, 1976). Napotilano (1998) reported that

* Author for correspondence

twelve distinct behaviours were exhibited by antlions to accomplish feeding, namely attack, holding, submergence, emergence, prey beating, feeding, use pit clearing, head roll, prey clearing, grooming, quiescence and jaw set. Scharf *et al.* (2009) proved that *Myrmeleon hyalinus* larvae grow faster during the feeding phase and lost mass during starvation period. Ants are the common prey of antlion larvae. Sometimes, the ants bite antlions immediately after they have been caught and grasps the antlions mandible and dies without releasing the hold rendering the antlion unable to make its pit (Lucas and Brockmann, 1981). Eisner *et al.,* (1993) documented *Myrmeleon carolinus* (Banks, 1943) larvae sucking the body contents without puncturing the acid sac of the formic acid spraying ants (*Camponotus floridanus* Buckley, 1866*).* Studies on feeding behaviour of antlion larvae from India are negligible and the objective of the present study is to give a baseline data about the feeding behaviour of this least studied group in Kerala.

MATERIALS AND METHODS

Antlion larvae were collected by handpicking or by using a spoon (Maoge *et al*., 2014) and were transferred to a paper cup (diameter 6 cm and height 6 cm) filled with sand/soil. The collected specimens of antlion larvae were identified by using standard taxonomic keys (Stange, 2004 and Ghosh, 2000) and confirmed by molecular sequencing. *Myrmeleon pseudohyalinus* Holzel, 1972 was the species mostly found in Kerala. As it is a first report of the species from India, the sequence was deposited in NCBI with an accession number MN711710.

Eight experiments were conducted for understanding the feeding behaviour of *M. pseudohyalinus* larvae in various conditions (Table 1). For this plastic trays (23X23cm) were filled with sand or soil to a thickness of 5 cm and maintained at room temperature (28-32^{o}C). The fed larvae were given one prey per day and in the case of starved larvae, they were starved for 3 days prior to the experiment. A single larva was introduced into the centre of a tray and allowed to make its pit. The larvae were kept undisturbed for 3 days prior to experiment to get it acclimatised to that situation.

The common ant (*Anoplolepis gracilipes Smith, 1857*- worker) was used for the feeding purpose both in rearing and experiments, because it is the most abundant prey in the antlion larval pit irrespective of species (mean size 0.45±0.05 cm) and placed in the centre of the pit. The behavior of the antlion was noted by using a hand lens up to the completion of feeding without any interruption. From the observation data the capture success of the larvae were analyzed and the prey escapes were observed.

RESULTS AND DISCUSSION

Twelve distinct behaviours were identified. Seventy five percentage of the larvae took 0-35 minutes for the completion of its feeding process (attack to jaw set) and the remaining (25%) of the larvae took 0-45 minutes to complete its feeding irrespective of the conditions. Irrespective of the conditions, the second instar (95%) larvae were more successful than third instar (75 %) larvae. Predatory efficiency of *M. pseudohyalinus* larvae has been shown in Table 1. The highest prey escape was noted in the combination of third instar fed larvae in sand medium. The duration of feeding was lengthy in fed second instar larvae in sand medium and starved second instar larvae in sand. The capture success of the prey by antlion larvae were high in the medium soil compared with sand media.

CCA was performed to visualize the relationships between different behaviour and conditions. The Eigen values for Axis1 (87.61%) and 2 (12.38%) added upto 99.99 percent which indicates that 99 percent of the variance has been covered. Therefore robustness of the test is very high and can be used for interpretation. The prey beating, emergence and submergence behaviours are influenced by the larval instar (second and third). The following behaviour: quiescence, pit clearing and jaw set behaviour patterns were influenced by their condition of being fed or starved. Head roll behaviour was only related to the medium of the substrate in which the larva was inhabiting and it was more in soil media (Fig. 1).

CCA was performed to visualize the relationships between different behaviours, instars, medium,

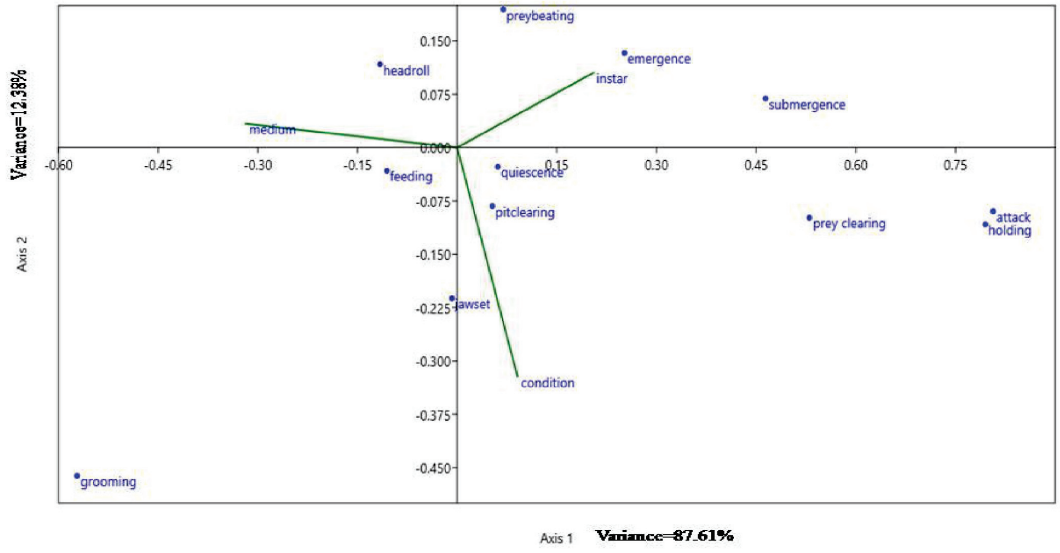

Fig. 1. CCA map showing relationships between selected behaviour and instar, medium and condition

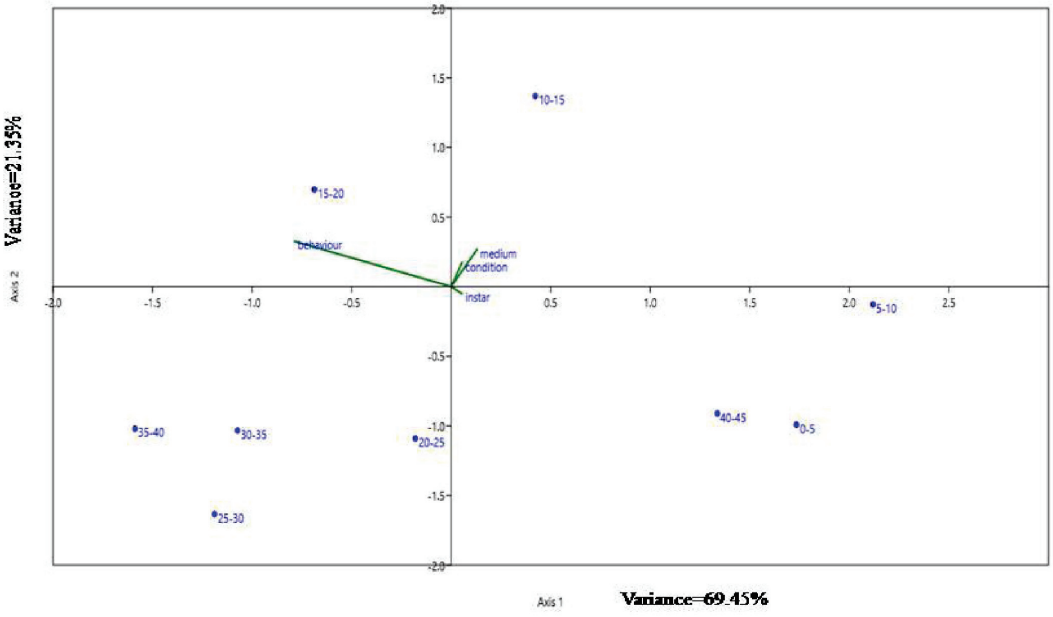

Fig. 2. CCA map showing relationships between selected behaviour and instar, medium and condition & time period

Table 1. Predatory behaviour of *M. pseudohyalinus* antlion larvae in different medium, instars and at hunger levels

Medium	Instars	Fed/ Starved	Feeding time (Min)	Prey escape (%)	Capture success (%)
Sand	Second	Fed	0-45	20	80
Sand	Third	Fed	0-35	50	50
Dry soil	Second	Fed	0-35	0	100
Dry soil	Third	Fed	0-35	0	100
Sand	Second	Starved	0-45	0	100
Sand	Third	Starved	0-35	33	67
Dry soil	Second	Starved	0-35	0	100
Dry soil	Third	Starved	0-35	18	82

(n=15 to 17 replications)

condition and time period. The Eigen values for Axis 1 and 2 per cent added up to 90.8 perceent which indicates that 90 percent of the variance has been covered. Medium (sand and soil), condition (fed and starved) or instar (second and third) did not seem to play much of a role in the behaviour but, time period seemed to affect behaviour. The behaviour in the first five and last five minutes of observation seemed very similar (mainly inactivity). Behaviour of 5-10 minutes duration also showed similarity to this group. Behaviour during and after 10-15 minutes was very unique and therefore lay in different quadrats. Rest of the five minute windows showed similar behaviour (Fig. 2).

In all the conditions, the larvae show similar behaviour in the first five minutes. Attack, holding, submergence, emergence, prey beating and feeding are the six behaviour patterns shown in this time period. In addition to this, head roll behaviour pattern is present in 5-10 minutes period of feeding except in the combination starved second instar larvae in sand medium. Starved second instar larvae in soil medium shows pit clearing behaviour in addition to this head roll.

The 10-15 minute period of fed second instar larvae, third instar larvae and starved third instar larvae shows similarity in behaviour patterns and the starved second instar larvae in sand media, pit clearing and prey clearing were found in addition to the common behaviour (prey beating, feeding and head roll) in this time period. In soil media, the second instar fed larvae shows quiescence and jaw set behaviour pattern in this period and the third instar fed larvae shows similar behaviour pattern of second and third instar fed larvae in sand media.

During 15-20, 20-25 and 25-30 minutes *M. pseudohyalinus* larvae showed prey beating, feeding, pit clearing, head roll, prey clearing, grooming, quiescence and jaw set activities. During 30-35 minutes, the jaw set and quiescence are the main patterns and considered as the end point of feeding behaviour. The behaviour patterns of fed second instar larvae in sand medium (Fig. 3) and fed third instar larvae in soil medium (Fig. 6) showed that the maximum feeding activity was present in 15 to 30 minutes. In the fed third instar larvae in sand medium (Fig. 4) and starved third instar larvae in sand medium (Fig. 4) two peaks were present in the activity patterns (0-15 minutes and 15-30 minutes). The remaining experiments such as starved second instar larvae in sand medium (Fig. 3), fed second instar larvae in soil medium, starved second instar larvae in soil medium (Fig. 5) and starved third instar larvae in soil medium (Fig. 6) did not show a prominent pattern of feeding activity with respect to time period.

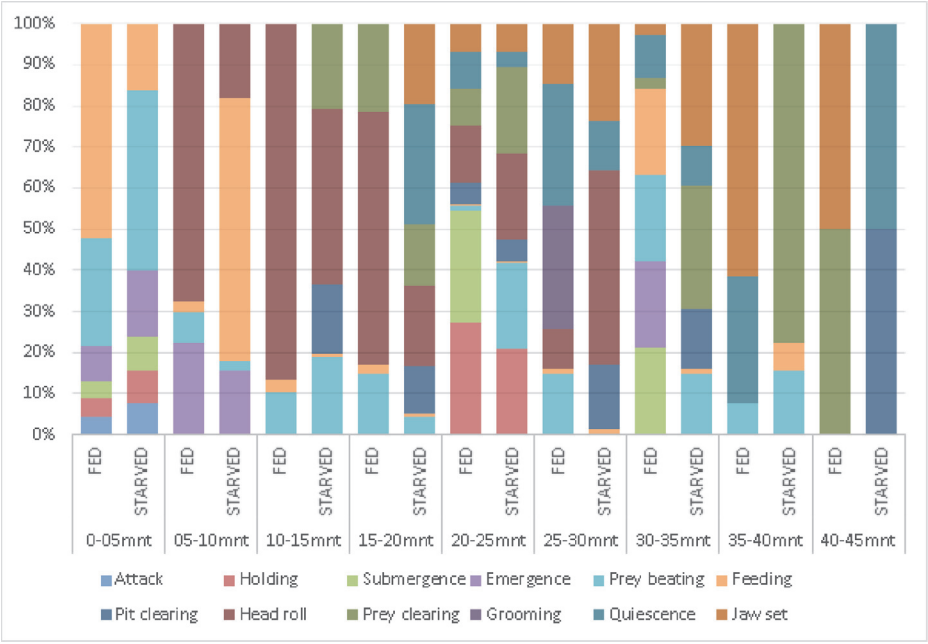

Fig 3. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed and starved second instar larvae in each time interval in sand medium (y axis shows % occurrence of behaviour patterns)

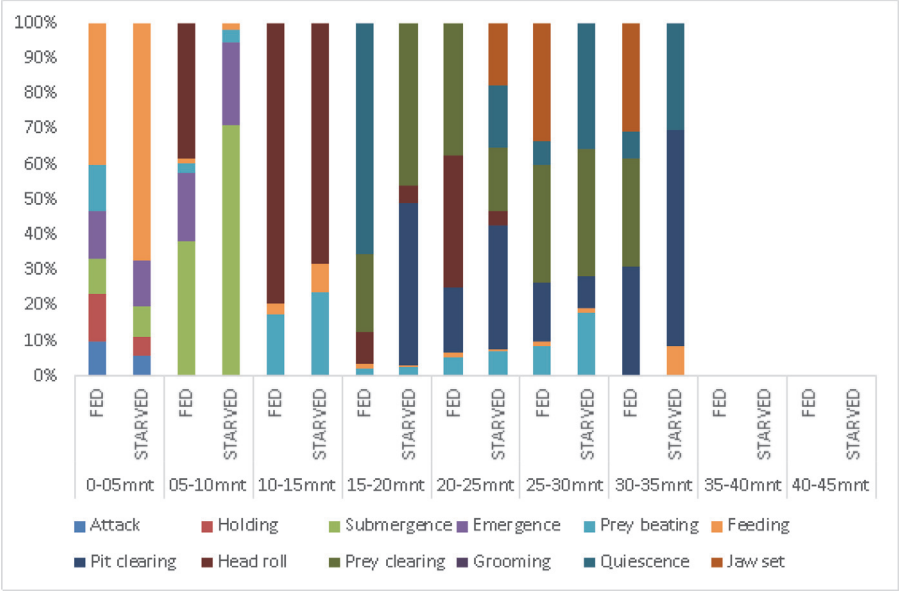

Fig 4. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed and starved third instar larvae in each time interval in sand medium (y axis shows % occurrence of behaviour patterns)

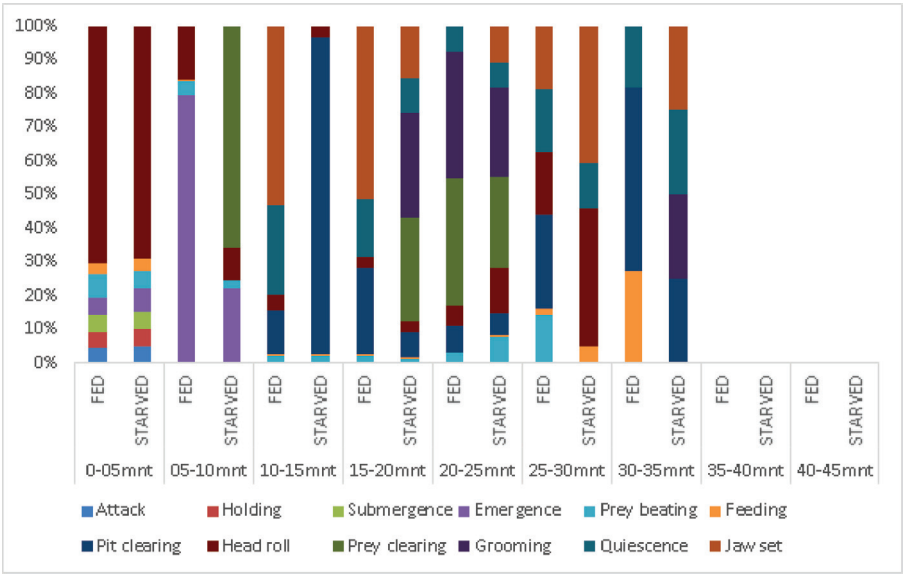

Fig 5. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed and starved second instar larvae in each time interval in soil medium (y axis shows % occurrence of behaviour patterns)

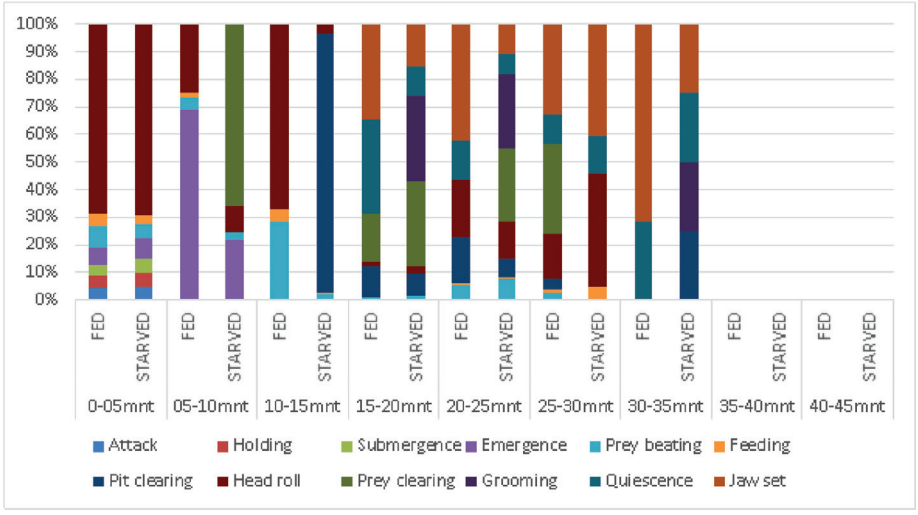

Fig 6. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed and starved third instar larvae in each time interval in soil medium (y axis shows % occurrence of behaviour patterns)

Antlion larvae build its pit for predation and shelter. The predatory efficiency was described by examining the predatory behaviour patterns. From these experiments, the prey escape and capture success were calculated. Capture success of prey irrespective of different conditions, hunger level and instars are 50-100 per cent. Prey escape was noticed in fed second instar larvae in sand medium, fed third instar larvae in sand medium, starved third instar larvae in sand medium and starved third instar larvae in soil medium. The highest percentage of prey escape was found in fed third instar larvae in sand medium. It may be because of the lowest hunger level decreased its need and the final instar caused the larvae to pupate and emerge as adult. The prey escape was highest in sand medium due to the larger particle size which may help the prey to fill the pit by disturbing the medium easily, hence the highest capture success was observed in soil medium. The larval pit in soil medium has more stability due to its small particle size compared to sand medium.

Myrmeleon pseudohyalinus larvae took 35 to 45 minutes to feed its prey in laboratory conditions. The capture success noted from the study was 50-100 per cent and the capture success rate (95%) was higher in second instar larvae than third instar larvae (75%). This is different from Nonato and Lima (2011) who noted that the third instar (96.96%) larvae are more successful than second instar larvae (69.70%).

Drosophila melanogaster Meigen, 1830 (Lima, 2016), *Formica cinerea* Mayr, 1853 (Turza *et al*., 2020), *Tribolium castaneum* Herbst, 1797 (Bakoidi *et al*., 2019) were used for feeding antlion larvae in previous studies. Cain (1987) studied the prey capture behaviour of *Brachynemurus* larvae of Florida. *Brachynemurus* larvae lying in the pit by exposing only its mandibles above sand. It took 15-50 minutes to feed and throw away the prey in laboratory conditions. Kross and Pilgrim (2012) studied the predation rate of *M. brasiliensis* larvae by offering leaf cut ant and the third instar larvae with a predation rate of 96.96 per cent, second instar larvae with 69.7 per cent and first instar larvae with 14.28 per cent. In all the experiments the dominant behaviour pattern observed was feeding (47.8 - 77.8%). Prey beating was the behaviour noticed followed by feeding except in starved third instar larvae in sand medium. Here the attack and holding were the two dominant patterns followed by feeding. The feeding behaviour defines the success of an organism in a habitat. This study gave a base line data about feeding behaviour and predatory efficiency of *M. pseudohyalinus* larvae and the influencing components like time period, different conditions, and instars from India.

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Diversity and foraging activity of flower visitors/ pollinators of *Momordica charantia* L., in Tamil Nadu, India

A. Yogapriya*, B. Usharani# and K. Suresh

Department of Entomology, Agricultural College and Research Institute,
Tamil Nadu Agricultural University, Madurai 625104, Tamil Nadu, India;
#SMS, ICAR-KVK, Tamil Nadu Agricultural University, Madurai 625104, Tamil Nadu, India.
Email: yogaento96@gmail.com; ushateja@yahoo.com, sureshento2009@gmail.com

ABSTRACT: Periodical field surveys were conducted in bitter gourd fields at weekly intervals in two locations at N. Poolampatti village, Vaiyampatti block, Tiruchirapalli district to study the diversity of insects visiting bitter gourd flowers. During the study 51 species of flower visitors/ pollinators were recorded which include 19 species of Hymenoptera, 15 species of Lepidoptera, seven species of Diptera and six species of Coleoptera. *Tetragonula iridipennis* was the most abundant pollinator followed by *Apis florea, Halictus* sp. and *Apis cerana indica;* while *Syrphus ribesii* was the dominating Dipteran pollinator. Among the Lepidopterans, *Pachliopta hector* was the major pollinator followed by *Danaus chrysippus*, *Tirumala limniace* and *Delias eucharis.* Species richness (S) was at its maximum (26 species) at 0800 – 1000 hours and minimum (07 species) at 1600-1800 hours. Shannon's and Simpson's diversity indices were maximum at 1000-1200 hours with 2.52 and 9.13 respectively. Shannon's evenness ranged from 0.44 – 0.86 and Simpson's evenness was 0.28 – 0.54 with maximum at 1000-1200 hours.

KEY WORDS: Abundance**,** indices, species diversity, species richness

INTRODUCTION

Bitter gourd, *Momordica charantia* L. is an internationally known plant consumed for its beneficiary health effects. It is also known as bitter gourd, bitter melon, bitter cucumber, balsam pear and African cucumber. Bitter gourd can be consumed in vegetable form, juice, or can be made in smoothie. It has many culinary uses and in folk medicine (Heiser, 1979). India, China, Latin America and Africa have been using bitter gourd in their ancient traditional medicines. The extract of bitter gourd possess antioxidant, antimicrobial, antiviral, anticarcinogenic and antihepatotoxic properties and also lowers blood sugar (Welihinda *et al.,* 1986; Raman and Lau, 1996). Ethano-medical reports indicate use of bitter gourd in folkloric medicine treatment of various ulcers, diabetes and several other infections (Gürbüz *et al.,* 2000; Scartezzini and Speroni, 2000; Beloin *et al.,* 2005). Among the 20 cucurbits cultivated in India, bitter gourd is one of the most important vegetable crops belonging to the family Ccurbitaceae. It is widely distributed in China, Malaysia and tropical Africa (Bailey, 1949). It is cultivated throughout India and is also found growing as a crop in many parts of the country.

* Author for correspondence

The fruits are relatively inexpensive source of proteins, (1.6 g/100 g of fruit), minerals (0.08 g), rich in Vitamin C and yields 25 k Cal/100 g of fruit. It is supposed to be laxative, wormicide, blood purifier and said to be curative for rheumatism, diabetes and asthma.

Flowering phenology of cucurbits ensures cross-pollination for better fruit set and yield. The flowers of cucurbits are usually monoceious *i.e.* they produce male and female flowers separately on the same plant but at different internodes. The flowering ratio of male to female is 15:1. The pistillate and the staminate flowers open on the same day. But the male flowers are borne first, a fortnight earlier than the female flower. Flowers arise singly from different internodes. Insects are required for pollen transfer because of the large sized pollen grains, their stickiness, and the way they are released from the anthers (Lauria and Fred, 1995). The female flower borne on ovary i.e., inferior ovary and the stigma is receptive throughout the day. In male flowers, anthers dehisce when the corolla expands but the pollens remains on the anther as a sticky mass. The maximum pollination occurs in the forenoon. In the afternoon, the female flower closes but never reopens whether or not pollination has taken place. Highest per cent of fruit set resulted from deposition of pollen on the stigma between 0900 to 1200 hours of the day (Bailey, 1949). Cucurbits flowers are visited by a wide range of insect pollinators. Species of bees, wasps, ants, butterflies, flies, and beetles have been reported to provide pollination services to cucurbit flowers (Delaplane *et al.,* 2000, Free, 1993; McGregor, 1976). Pollinators are simultaneously vital to supporting both natural ecosystems and human food security, which is a unique position for such a diverse group of organisms. The past two decades have seen unprecedented interest in pollinators and pollination ecology, stimulated in part by concerns about the decline of pollinator abundance and diversity in some parts of the world (Ollerton, 2017). Bees are the most studied and utilized pollinators for cucurbit crops throughout the world and provide the greatest contribution to the pollination of cucurbits (Delaplane *et al.,* 2000; Garibaldi *et al.,* 2013). Hence the present study was undertaken to record the diversity of insects (floral visitors/ pollinators) visiting bitter gourd flowers.

MATERIALS AND METHODS

Periodical field surveys were conducted in bitter gourd fields at weekly intervals in two locations at N. Poolampatti (10.6344^{o} N and 78.3110^{o} E) village, Vaiyampatti block, Tiruchirapalli district during *Kharif*, 2018 with two replications to study the diversity of insects visiting bitter gourd flowers. The experimental plot was kept free from chemical sprays during the flowering period. The diversity of floral visitors/ pollinators of bitter gourd were recorded in five randomly selected one square meter area during flowering period at 0600 - 0800, 0800 - 1000, 1000 - 1200, 1200 - 1400, 1400 - 1600 and 1600 - 1800 hours for five minutes in each randomly selected one square meter area using an insect net in 5 spots. These observations were started at 10 per cent bloom and continued at weekly intervals for twenty two weeks. The data were later averaged time-wise and group-wise to infer the pollinator fauna as well as the dominance of particular group. Insects foraging on pollen and nectar from the flower have also been recorded by observing the foraging activity of the insect visually to know the role of the flower visitors in pollination service. Honeybees with their activity of extending their proboscis into the flowers are considered as nectar collectors and bees carrying pollen on their hind legs were determined as pollen collectors (Balachandra *et al*., 2014).

Relative abundance, species richness and Shannon's and Simpson's diversity indices were also calculated as a mathematical measure of species diversity and evenness of species as mentioned below. Insect specimens were collected from the field with aerial nets and preserved in 70 per cent ethanol and photographed in stereo-zoom microscope (AXIOCAM) at the Centre of Innovation, Agricultural College and Research Institute, Madurai.

Species Richness (S): Number of different species present in a community; number of pollinators visiting the flowers in a time period.

Diversity indices were calculated to understand the rarity and commonness of species in a community which is an important tool for understanding community structure.

(a) Shannon's diversity index (H): The proportion of species *i* relative to the total number of species (p*i*) was calculated and was summed across species and multiplied by -1 (Davila *et al.,* 2012).

$$H = - \sum^{s}_{i=0} pi \ln pi$$

The total number of species in the community known as species richness (S) was found out. Shannon's equality (EH) was calculated by dividing H by Hmax (here Hmax = lnS). Equality assumes a value between 0 and 1 being complete evenness.

$$EH = H/H\max = H / \ln (S)$$

(b) Simpson's diversity index (D): The proportion of species I relative to the number of species (*pi*) was calculated and squared. The squared proportions for all the species were summed and the reciprocal was taken.

$$D = 1/\sum^{s}_{i=1} pi^2$$

Equitability (ED) was calculated by taking Simpson's index (D) and it as a proportion of maximum value D could assume if individuals in the community were completely evenly distributed (Dmax) which equals S.

$$ED = D/D \max$$

(c) Species composition: Number of species belonging to each category was calculated as species composition (abundance). Species composition percentage was calculated by dividing number of species in each group by total number of species and multiplied with 100.

(d) Relative Abundance (RA): Relative abundance of a species or a group of species in the community (floral visitors/ pollinators) was calculated by dividing total number of species present by total number of individuals in a group/ community. RA(%) calculated to find how a particular group of species is common or rare among the total pollinators available/ recorded. Thus, can find the efficiency of a particular pollinator among all the other pollinators.

$$\text{Relative abundance (\%)} = \frac{\text{No. of species present}}{\text{Total no. of species}} \times 100$$

Statistical analysis: The mean values were square root transformed and compared by Least Significant Difference (LSD) at 5 per cent probability with AGRES software to assess the effective pollinator and peak hours of pollination services provided by the pollinators.

RESULTS AND DISCUSSION

Floral visitors/ pollinators collected were from five different orders *viz.,* Hymenoptera (37.26%), Diptera (13.72%), Lepidoptera (29.41%), Coleoptera (11.76%) and Hemiptera (3.93%). Order Araneae contributed two species *Oxyopes javanus* and *Neoscona* sp. (3.93%). In general the activity of the pollinating insects like honey bees, other solitary bees and butterflies were higher in the morning hours from 0600 hours to1200 hours. Diversity of floral visitors *i.e.* number of species was higher during the mid-hours (1200 to 1400 hours). In the order Hymenoptera, family Apidae dominated in pollinating bitter gourd flowers with six species, where three species *viz., Apis cerana indica, Apis dorsata* and *Apis florea* contributed maximum. Other pollinators under the family were *Tetragonula iridipennis*, *Amegilla zonata* and an unidentified species. It is followed by Halicitidae family with three species *viz., Halictus* sp*., Lasioglossum* sp. and *Nomia* sp. Xylocopidae, Megachilidae, Chrysididae, Bombicidae, Vespidae and Formicidae were the other families noted as the pollinators/ visitors. Under Lepidoptera, Nymphalidae and Papilionidae dominated with five species each. This was followed by Pieridae with two species. Hesperidae, Noctuidae and Lycaeidae contributed one species each in the pollination services. Among Dipterans, Syrphidae (4 species), Culicidae, Tabanidae and Caliophorodae families (one species each) were recorded. Miridae and Lygaeidae family of the order Hemiptera contributed one species each. Order Coleoptera contributed one species each from Chrysomelidae

Table 1. List of floral visitors/ pollinators in bitter gourd ecosystem

No.	Pollinators	Collecting*	
		Pollen	Nectar
	Order - Hymenoptera		
	Family - Apidae		
1.	*Apis cerana indica* Fab.	yes	yes
2.	*Apis dorsata* Fab.	yes	yes
3.	*Apis florea* Fab.	yes	yes
4.	*Tetragonula iridipennis* Smith	yes	yes
5.	*Amegilla zonata* L.	yes	yes
6.	Unidentified species	yes	yes
	Family - Xylocopidae		
7.	Xylocopa violacea L.	yes	yes
	Family - Halicitidae		
8.	Halictus sp	yes	yes
9.	Lasioglossum sp.	yes	yes
10.	Nomia sp.	yes	yes
	Family - Megachilidae		
11.	Megachile sp.	yes	yes
	Family - Chrysididae		
12.	Chrysis sp.	yes	yes
	Family - Vespidae		
13.	*Polistes gallicus* L.	yes	yes
	Family - Bombicidae		
14.	*Bombus* sp.	yes	yes
	Family - Formicidae		
15.	*Camponotus pensylvanicus*		
	De Geer	No	yes
16.	*Monomorium minimum* Buckley	No	yes
17.	*Dorymyrmex* sp.	No	yes
18.	*Technomyrmex albipes* Smith	No	yes
	Family Trichogrammatidae	No	yes
19.	*Trichogramma pretiosum* Riley	No	yes
	Order - Diptera		
	Family - Syrphidae		
20.	*Syrphus ribesii*	No	yes
21.	*Episyrphus* sp.	No	yes
22.	*Eristalis* sp.	No	yes
23.	*Ischiodon scutellaris* F.	No	yes
	Family Calliophoridae		
24.	*Licilia* sp.	No	yes
	Family Tabanidae		
25.	*Tabanus* sp.	No	yes
	Family Culicidae		
26.	*Culex quinquefasciatus* Say	No	yes

No.	Pollinators	Collecting*	
		Pollen	Nectar
	Order Lepidoptera		
	Family - Nymphalidae		
27.	*Danaius chrysippus* L.	No	yes
28.	*Danaus genutia* Cramer	No	yes
29.	*Eurema hecabe* L.	No	yes
30.	*Eurema blanda* Boisduval	No	yes
31.	*Tirumala limniace* Cramer	No	yes
	Family - Papilionidae		
32.	*Delias euchrasis* Drury	No	yes
33.	*Papilio demoleus* L.	No	yes
34.	*Pachliopta hector* L.	No	yes
35.	*Pachliopta aristolochiae* L.	No	yes
36.	*Pachliopta pandiyana* L.	No	yes
	Family - Pieridae		
37.	*Pieris sp.*	No	yes
	Family - Pieridae		
38.	*Hypolimnas bolina* L	No	yes
	Familiy - Lycaenidae		
39.	*Lampedes boeticus* L.	No	yes
	Family - Hesperiidae		
40.	*Hasora chromus* Cramer	No	yes
	Family - Noctuidae		
41.	*Spodoptera litura* Fab.	yes	yes
	Order - Coleoptera		
	Family - Chrysomelidae		
42.	*Aulacophora fovecolis* Dejean	yes	yes
	Family - Meloidae		
43.	*Hycleus pustulata* Thunberg	No	yes
	Family - Coccinellidae		
44.	*Chilomenes sexmaculata* F.	No	yes
45.	*Illies cincta* F.	yes	yes
46.	*Menochilus sexmaculatus* F.	yes	yes
47.	*Henosepilachna septima* Dieke	No	No
	Order - Hemiptera		
	Family - Miridae		
48.	Unidentified species	No	yes
	Family - Lygaeidae		
49.	*Graptostethus servus* F.	No	yes
	Order - Araneae		
	Family - Oxyopidae		
50.	*Oxyopes javanus*	No	No
51.	*Noescona* sp.	No	No

* Collecting pollen/nectar from the flowers

Table 2. Foraging activity of floral visitors/ pollinators of bitter gourd at different hours of the day

Pollinators	No. of individuals/5min/m²						
	0600 – 0800	0800 – 1000	1000 – 1200	1200 – 1400	1400 – 1600	1600 – 1800	*Mean
***Apis* hymenopterans**							
A. cerana indica	2.53 (1.74)^{b}	2.38 (1.70)^{b}	0.73 (1.11)^{fg}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.94
A. florea	0.00 (0.71)^{m}	0.28 (0.88)^{hj}	5.02 (2.35)^{a}	2.86 (1.83)^{b}	0.42 (0.95)^{e}	0.00 (0.71)^{g}	1.43
A. dorsata	0.69 (1.09)^{c}	0.82 (1.15)^{dl}	0.11 (0.78)^{lmn}	0.00 (0.71)n	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.27
Non *Apis* hymenopterans							
T. iridipennis	5.31 (2.41)^{a}	6.22 (2.59)^{a}	3.01 (1.87)^{b}	1.46 (1.4)^{e}	0.24 (0.86)^{g}	0.00 (0.71)^{g}	2.71
A. zonata	0.00 (0.71)^{m}	1.06 (1.25)^{c}	1.2 (1.30)^{de}	0.62 (1.06)^{i}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.48
Unidentified apidae	0.00 (0.71)^{m}	0.24 (0.86)^{ijk}	0.29 (0.89)^{jk}	0.02 (0.72)^{mn}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.09
Halictus sp.	0.00 (0.71)^{m}	0.65 (1.07)^{e}	2.28 (1.67)^{c}	1.82 (1.52)^{d}	0.28 (0.88)^{f}	0.00 (0.71)^{g}	0.84
X. violacea	0.49 (0.99)^{e}	0.56 (1.03)^{f}	0.02 (0.72)^{mn}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.18
P. gallicus	0.00 (0.71)^{m}	0.00 (0.71)^{o}	1.02 (1.23)^{e}	0.96 (1.21)^{g}	0.18 (0.82)^{h}	0.00 (0.71)^{g}	0.36
V. orientalis	0.00 (0.71)^{m}	0.22 (0.85)^{ijkl}	0.42 (0.96)^{ij}	0.8 (1.14)^{h}	0.2 (0.84)^{h}	0.00 (0.71)^{g}	0.27
Megachile sp.	0.00 (0.71)^{m}	0.65 (1.07)^{e}	1.24 (1.32)^{dj}	1.08 (1.26)^{f}	0.02 (0.72)^{i}	0.00 (0.71)^{g}	0.50
C. pensylvanicus	0.00 (0.71)^{m}	0.00 (0.71)^{o}	1.28 (1.33)^{d}	0.48 (0.99)^{j}	0.6 (1.05)^{d}	0.2 (0.84)^{d}	0.43
M. mnimum	0.04 (0.73)^{l}	0.42 (0.96)^{g}	2.86 (1.83)^{b}	4.65 (2.26)^{a}	3.04 (1.88)^{a}	1.68 (1.48)^{a}	2.12
Dorymyrmex sp.	0.08 (0.76)^{k}	0.00 (0.71)^{o}	0.00 (0.71)^{n}	0.3 (0.89)^{k}	1.02 (1.23)^{e}	0.16 (0.81)^{e}	0.26
Unidentified	0.04 (0.73)^{l}	0.00 (0.71)^{o}	0.00 (0.71)^{n}	0.02 (0.72)^{m}	0.2 (0.84)^{h}	0.18 (0.82)^{d}	0.07
Dipterans							
C. quinque-fasciatus	0.00 (0.71)^{m}	0.20 (0.84)^{jklm}	0.42 (0.96)^{ij}	0.24 (0.86)^{l}	0.00 (0.71)^{i}	0.02 (0.72)^{f}	0.15
S. ribesii	0.00 (0.71)^{m}	0.28 (0.88)^{hi}	0.48 (0.99)^{hi}	0.01 (0.71)^{mn}	0.24 (0.86)^{g}	0.00 (0.71)^{g}	0.17
I. scutellaris	0.00 (0.71)^{m}	0.00 (0.71)^{o}	0.82 (1.15)^{f}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.14
Lepidopterans							
D. chrysippus	0.48 (0.99)^{e}	0.20 (0.84)^{jklm}	0.02 (0.72)^{mn}	0.22 (0.85)^{l}	0.28 (0.88)^{f}	0.00 (0.71)^{g}	0.20
D. genutia	0.22 (0.85)^{gh}	0.00 (0.71)^{o}	0.20 (0.84)^{kl}	0.02 (0.72)^{mn}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.07

E. hecabe	0.24 (0.86)^{g}	0.20 (0.84)^{jklm}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.07
E. blanda	0.26 (0.87)^{f}	0.16 (0.81)^{m}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.07
T. limniace	0.20 (0.84)^{ij}	0.24 (0.86)^{ij}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.42 (0.96)^{e}	0.22 (0.85)^{e}	0.18
D. euchrasis	0.24 (0.86)^{gh}	0.32 (0.91)^{h}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.02 (0.72)^{i}	0.00 (0.71)^{g}	0.10
P. demoleus	0.00 (0.71)^{m}	0.18 (0.82)^{klm}	0.03 (0.73)^{mn}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.04
P. hector	0.67 (1.08)^{d}	0.84 (1.16)^{d}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.25
P. aristolochiae	0.22 (0.85)^{h}	0.20 (0.84)^{jklm}	0.02 (0.72)^{mn}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.07
P. pandiyana	0.18 (0.82)^{j}	0.16 (0.81)^{lm}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.06
Pieris sp.	0.04 (0.73)^{l}	0.02 (0.72)^{o}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.01
H. chromus	0.21 (0.84)^{i}	0.08 (0.76)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.06
H. bolina 0.	22 (0.85)^{h}	0.02 (0.72)^{o}	0.12 (0.79)^{lm}	0.00 (0.71)^{n}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.08
Coleopterans							
Aulacophora sp.	0.00 (0.71)^{g}	0.20 (0.84)^{jklm}	0.42 (0.96)^{ij}	0.22 (0.85)^{l}	0.00 (0.71)^{i}	0.00 (0.71)^{g}	0.14
Coccinellids	0.00 (0.71)^{m}	0.00 (0.71)^{o}	0.62 (1.06)^{gh}	2.02 (1.59)^{c}	1.82 (1.52)^{b}	0.26 (0.87)^{b}	0.79
S.Ed	0.01	0.02	0.04	0.0133	0.01	0.003	
CD	(0.05)	0.02	0.04	0.08	0.0665	0.02	0.007

*Each value is a mean of twenty two observations;
Figures in parenthesis are square root transformed values;
In a column, means followed by same letter(s) are on par by LSD (p= 0.05)

Table 3. Species richness and diversity indices of floral visitors/ pollinators

	Time (hours)					
Parameters	**0600 – 0800**	**0800 – 1000**	**1000 – 1200**	**1200 - 1400**	**1400 – 1600**	**1600 – 1800**
Species richness (S)	19	26	23	18	15	7
Shannon's H index	2.01	2.41	2.52	2.27	2.09	1.30
Shannon's E index	0.68	0.74	0.80	0.79	0.77	0.67
Simpson's D index	4.19	5.77	9.18	7.38	5.49	2.44
Simpson's E index	0.22	0.22	0.40	0.41	0.37	0.35

Table 4. Foraging activity of major bitter gourd pollinators

Pollinator	Foraging hours	Time spent (seconds)*
T. iridipennis	0600 - 1200	48.2(6.94)[a]
A. florea	1000 - 1400	12.18(3.48) [b]
A. cerana indica	0600 - 1000	7.12(2.66)[bc]
A. zonata	1000 - 1400	3.60(1.89)[c]
Halictus sp.	1000 - 1400	8.20(2.86)[b]
Megachile sp.	1000 - 1400	6.32(2.51)[bc]
S.Ed		0.63
CD (0.05)		1.319

and Meloidae and four species from Coccinellidae (Table 1).

The insects visit the flowers for nectar, pollen and extrafloral nectars (*Trichogramma pretiosum*) from flowers (Tian *et al.*, 2016), while some predatory species visits the flowers for their prey. Spiders prey on small pollinating bees that visit the flowers very often for pollen and nectar source *viz., Neoscona* sp. on stingless bees, *T. iridipennis* (Telle *et al.,* 2018). Hemipteran, *Graptostethus servus* F. feed on the moisture present in flowers, shoots, stems and leaves (Chin *et al.*, 2009). Hence all the flower visitors cannot be considered as pollinators.

Foraging activity of flower visitors/ pollinators

Apis florea was the major pollinator followed by *A. cerana indica* and *A. dorsata.* The mean population of *A. florea* was maximum at 1000 - 1200h (5.02/m^2/5 min) followed by 1200 - 1400 hours (2.86/m^2/5 min). At 0600 – 0800, 0800 - 1000h and 1000 - 1200h the total population of *Apis* that visited bitter gourd was 1.07, 1.16 and 1.95/m^2/5min respectively (Table 2). The time spent by *A. florea* and *A. cerana indica* was 12.18 and 7.12 seconds per flower respectively (Table 4).

In non *Apis* hymenopterans, the major pollinator was *T. iridipennis* followed by *Megachile* sp., *Halictus* sp., *A. zonata*, *P. gallicus* and *X. violacea*. The mean population of *T. iridipennis* was found to be maximum (6.22/m^2/5 min) at 0800 to 1000h followed by (5.31/m^2/5 min) at 0600 to 0800h. The total population of non-*Apis* hymenopterans was maximum at 1000-1200h with (1.14/m^2/5 min) and minimum at 1600-1800 hours with (0.19/m^2/5 min). *T. iridipennis* spent on an average 48.20 seconds/ flower while *Halictus* sp. spent 8.20 seconds/ flower. Among Dipterans, *S. ribesii* was the major pollinator followed by

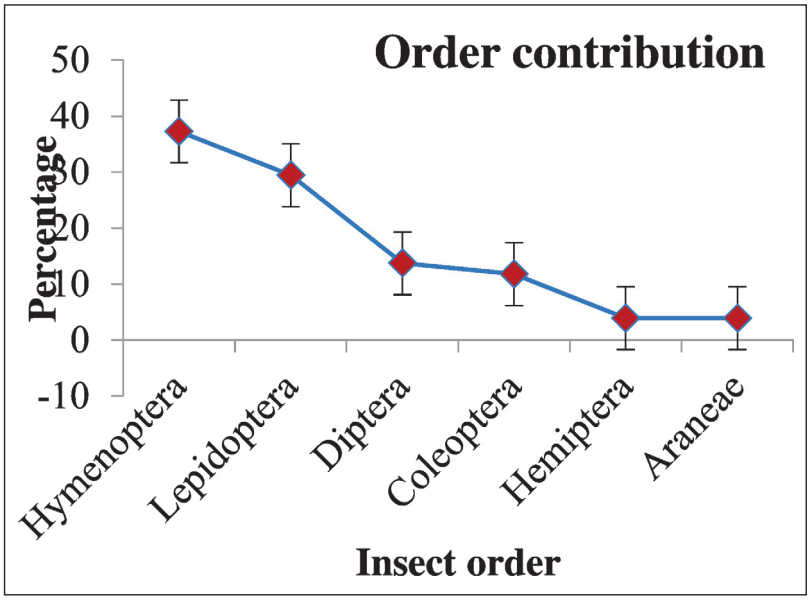

Fig. 1 Flower visitors of bitter gourd ecosystem

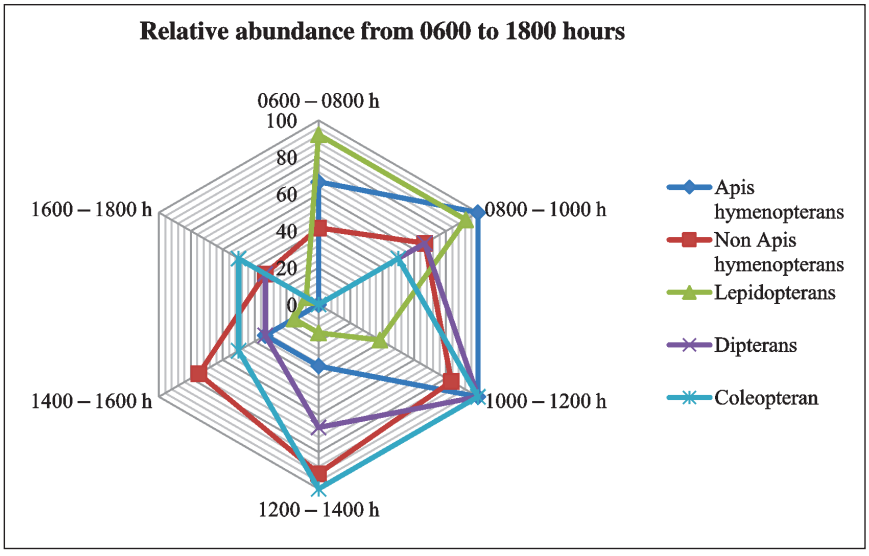

Fig. 2 Relative abundance of floral visitors/ pollinators at different hours in bitter gourd ecosystem

I. scutellaris. The population of Dipterans visiting bitter gourd was maximum at 1000-1200 hours (0.57/m^2/5 min). Amid Lepidopterans, *P. hector* was the major pollinator followed by *D. chrysippus*, *T. limniace, D. euchrasis, H. bolina, D. genutia*, *E. hecabe*, *E. blanda, P. aristolochiae, P. pandiyana, Pieris* sp.,and *P. demoleus.* Lepidopterans were at their maximum at 0600 -0800 and 0800-1000 hours with 0.24 and 0.20/m^2/ 5min and minimum during 1200 – 1400 and 1600 – 1800 hours with 0.02/m^2/ 5 min. Lepidopterans were found during all hours of the day (Table 2).

During 0600-1000 hours, *T. iridipennis, A. cerana indica, A. dorsata, X. violacea* and *P. hector* were the major pollinators along with *Halictus* sp. from 0800 – 1000 hours. While *A. florea, T. iridipennis, Halictus* sp. and *A. zonata* were dominating pollinators during 1000 – 1200 hours. Major bee pollinators and their peak foraging hours in bitter gourd indicate *T. iridipennis, A. florea, Halictus* sp. and *A. cerana indica* are effective pollinators. In general, Apis hymenopterans activity was high during the forenoon while non-Apis hymenopterans and Dipterans were slightly higher during the afternoon. Lepidopterans were found at all times of the day with maximum activity in the morning hours (Table 2, 4).

Species richness and Diversity indices

Species richness and relative abundance were calculated to insist on the commonness of a species and its importance and effectiveness as a pollinator. Shannon's and Simpson's diversity indices were calculated as a mathematical measure of diversity and abundance. Shannon's diversity (H) and Shannon's evenness (E) indices deals with diversity i.e. Shannon's H and E increases as diversity of species increases while, Simpson's diversity D and Simpson's evenness E increases in relation with the abundance of the species. Shannon's index will be at the maximum when the diversity is higher and Simpson's index will be higher when the abundance of the species is highest. Both Shannon's and Simpson's diversity indices were calculated to compare the diversity and abundance of floral visitors/ pollinator species at different hours of the day and the results obtained were proportional.

Species richness (S) was at its maximum with 26 species at 0800-1000h and minimum at 1600-1800 h (Table 3). Relative abundance of the pollinators was also proportional with Species richness. The results of Shannon's and Simpson's diversity indices depicts that both diversity and abundance of floral

visitors/ pollinator species was maximum at 1000 – 1200h with 2.52 and 9.18 and minimum at 1600 – 1800 hours with 1.30 and 2.44 respectively. Shannon's evenness ranged from 0.67 – 0.80 and Simpson's evenness was from 0.22 – 0.40. The evenness calculated namely Simpson's E and Shannon's E were not much variable during the day with high evenness in periods when the diversity and abundance were high i.e. 1000 – 1200h with 0.40 and 0.80 respectively. It is cognizable that the activity and abundance of pollinators is high from 0600 to 1400 hours of the day (Table 2, 3).

Species composition

Total number of floral visitors/ pollinators recorded in the bitter gourd ecosystem have been divided order-wise to calculate the contribution of each order in the pollination service of bitter gourd. Order Hymenoptera has been divided into two groups namely, *Apis* hymenopterans and Non *Apis* hymenopterans, since the genus *Apis* includes the major pollinators of all crops, the honey bees. Order hymenoptera holds the maximum share of pollinators of 45.46 per cent (*Apis* hymenopterans 9.09% and Non *Apis* hymenopterans (36.36%)) followed by Lepidopterans (39.40%), Dipterans (9.09%) and Coleopterans (6.07%) (Fig. 1).

Relative abundance

Relative abundance (RA%) of *Apis* hymenopterans were in the peak from 0600h to 1000 hours which decreased thereafter. While the RA of non *Apis* hymenopterans were maximum during 0800 – 1000 hours chased by 1200 – 1400, 1400 – 1600 and 0800 – 1000 hours respectively, whereas 0600 – 0800 and 1600 – 1800 hours had shown the least activity. Abundance of Lepidopterans was ultimate during 0800 – 1000 hours, whilst the Dipterans showed interest from 1000 – 1400h (Fig. 2).

The present results are in accordance with reports of Subhakar *et al.* (2013), who reported that *T. iridipennnis*, *H. guttuorosus* and *A. florae* were the major pollinators in bitter gourd among the 14 species of pollinators recorded. Further, the findings are also in line with the findings of Bodlah and Waqar (2013) who recorded eight species of pollinators visiting ridge gourd, bitter gourd and brinjal where hymenopterans dominated with six species. Balina *et al.* (2012) studied the diversity, abundance and pollination efficiency of native bee pollinators of bitter gourd in India and recorded nine bee species from three families (Apidae, Halictidae and Megachilidae). Abundance of *Halictus* sp. was maximum followed by *Megachile* sp. and *A. dorsata,* which coincides with the present findings. *T. iridipennis* and *A. cerana indica* were the most abundant species that visit the bitter gourd flowers. Hence, they can be effectively used for supplementing pollination in bitter gourd to get a higher yield in bitter gourd. Since, the diversity and abundance of floral visitors/ pollinators is high during 1000 – 1200, 0800 – 1000, 1200 – 1400 and 0600 - 0800 hours, spraying insecticides during the morning hours should be avoided to conserve the population and activity of pollinators in bitter gourd.

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Larvicidal potential of rhizome extracts of *Elettaria cardamomum* (L.) Maton against filarial vector, *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae)

Subrata Mallick

Department of Zoology, Maharajadhiraj Uday Chand Women's College, Burdwan 713104, West Bengal, India.
Email: mallick.subrata60@gmail.com

ABSTRACT: Investigation on the larvicidal potential of *Elettaria cardamomum* (L.) Maton rhizome extracts against filarial vector, *Culex quinquefasciatus* Say, was undertaken with crude concentrations 0.1-0.5 per cent and 40, 50, and 60 ppm of each of petroleum ether, hexane and ethyl acetate rhizome extracts revealed that first instar larvae were most susceptible to crude rhizome extract with 100 per cent mortality at 0.5% after 24 hrs of exposure. Among three solvent extracts, ethyl acetate extract showed maximum mortality (96.66±3.33%) at 60 ppm after 72 hrs of exposure. LC_{50} values of larvicidal bioassays by crude rhizome extract were 0.1002, 0.0794, 0.1275 and 0.6334 ppm for 1st, 2nd, 3rd and 4th instars larvae after 72 hrs of exposure, respectively and LC_{50} values for larvicidal bioassays by petroleum ether, hexane, and ethyl acetate rhizome extracts were 48.3629, 40.9613 and 37.0282 ppm against 3rd instar larvae after 72 hrs of exposure, respectively. Preliminary phytochemical analyses of the rhizome extracts showed presence of secondary metabolites. Non target organisms, tadpoles of frog and 4th instar larvae of *Chironomus circumdatus,* were not affected by the crude as well as ethyl acetate rhizome extracts. Larvicidal efficacy of the rhizome extracts of *E. Cardamomum* against *Cx. quinquefasciatus* mosquito species has been reported first time.

KEY WORDS: Crude extract, solvent extracts, LC_{50} values, phytochemicals, regression

INTRODUCTION

Out of 300 different species of mosquitoes, 100 species act as vectors of several diseases (Rozendaal, 1997; Abou-Enaga, 2014). Mosquitoes transmit of many diseases, viz. dengue, dengue hemorrhagic fever, filariasis, malaria, yellow fever, chikungunia and Japanese Encephalitis (Ghosh *et al*., 2012; Sogan *et al*., 2018). *Culex quinquefasciatus* Say1823 (Diptera: Culicidae) is the vector of lymphatic filariasis disease (Mallick and Chandra, 2015a). In 50 countries of the world, 859 million people become threatened by lymphatic filariasis (WHO, 2021). Synthetic chemical insecticides are used to control mosquito and these insecticides cause the development of resistance in mosquitoes. Natural products of botanical origin have insecticidal efficacy and are bio degradable, safe to the environment, less harm to beneficial insects and are effective to control targeted species (Maharaj *et al*., 2011; Hwang *et al*., 2017; Mdoe *et al*., 2014; Shoukat *et al*., 2016). *Elettaria cardamomum* (L.) Maton, also known as true or green cardamom, is a perennial herbaceous plant.

* Author for correspondence

It belongs to Zingiberaceae family and is a commercially significant spice. It has anticancer, antioxidant, anti-inflammatory and antimicrobial properties (Alam *et al.*, 2021; Cárdenas Garza *et al.*, 2021). Many researchers experimented on different plants against several species of mosquitoes and unfolded their larvicidal potential (Rawani *et al*., 2010; Hossain *et al*., 2011; Mallick *et al*., 2014; Mallick *et al*., 2015a; Mallick *et al* 2015b; Mallick and Chandra, 2015b; Mallick and Chandra, 2016; Mallick, 2021). The study aims to investigate the larvicidal potential of rhizome extracts of *E. cardamomum* against the larvae of *Cx quinquefasciatus*.

MATERIALS AND METHODS

Rhizomes of *E. cardamomum* were collected from the Medicinal Plant Garden of M.U.C. Women's College, Purba Bardhaman, West Bengal, India ($23^0 16'$ N, $87^0 54'$ E) during the month of August (the monsoon season) and cleaned and subsequently dried on paper towel. Fresh cleaned rhizomes were cut into very small pieces, crushed by the electrical grinder and the juice of rhizomes was filtered through muslin cloth and the filtrate was used as stock test solution (filtrate of rhizome served as 100% concentrated solution). Extracts @ 0.1, 0.2, 0.3, 0.4, and 0.5 per cent concentrations were prepared for bioassays.

For the preparation of different solvent extracts of rhizome, maceration procedure was used (Sharma *et al.,* 2016). Collected cleaned rhizomes were cut into small bits and dried in shade for 14-15 days. Dried small pieces of rhizomes were ground in electric blender and thereafter sieved for obtaining powder material. Petroleum ether, hexane and ethyl acetate, were used to obtain solvent extracts. 50 g of powder material of rhizomes was socked first in 500 ml of petroleum ether, kept in a bottle and thereafter closed the mouth of the bottle tightly for a period of near about 15 days with frequent agitation daily. Final petroleum ether extract of rhizomes was filtered by What Man 42 no. filter paper and the filtrate extract was concentrated by evaporation, to obtain the semi-solid extract and kept in a refrigerator at 4^0 C for further bioassays. After obtaining petroleum ether extractive of rhizome, the same plant material of rhizomes was soaked successively in hexane and thereafter in ethyl acetate for a period of near about 15 days each, with frequent agitation daily. After filtering each of the hexane and ethyl acetate rhizome extracts was concentrated by evaporation and the semi-solid extract of hexane and ethyl acetate kept in a refrigerator at 4^0 C for bioassay experiments.

Preparation of graded concentrations of different semi-solid solvent extracts: After preliminary trialing, 40, 50, and 60 ppm graded concentrations of each of petroleum ether, hexane and ethyl acetate extract of rhizome, were prepared for larvicidal bioassay experiments. From each of semi-solid aforesaid solvent extracts of rhizomes, stock solutions were prepared on 5 per cent ethanol, separately. Petroleum ether, hexane and ethyl acetate rhizome extracts @ 0.1 g of each dissolved separately in 1 ml of ethanol and thereafter added 19 ml distilled water to get stock test solutions (5000 ppm) of the said different solvent extracts. From stock test solutions, graded concentrations of each of different solvent extracts of rhizome were prepared, by taking required volume of stock test solution of each solvent extract and mixing by required volume of water, to get different concentrations test solutions for larvicidal bioassays.

Test mosquito species: Larvae of *Cx. quinquefasciatus* mosquito were collected. Larvae of different instars were kept in a plastic tray with water. Larvae were provided powdered mixture of dog biscuits and dried yeast powder (ratio 3:1). Larval colonies were maintained at 27 ± 2^0 C temperature and 80-85 per cent relative humidity. Larvae transformed into pupae, and near about 200 pupae were transferred to two separate plastic bowls (225 ml capacity) containing water, and thereafter two plastic bowls with pupae were kept in a mosquito cage (30×30×30 cm) where adult mosquitoes emerged. Ten per cent glucose solution in a plastic bowl with a cotton wick was kept in the cage for adult mosquitoes feeding. On day five adults were provided with blood meal from restrained pigeon. Two plastic bowls with 100 ml water were kept in the cage for oviposition. F1 generation larvae were used for larvicidal bioassays.

Larvicidal bioassays were conducted according to standard protocol of WHO with suitable modifications (WHO, 2005). All instars larvae were used during bioassays with crude extract of rhizomes. Twenty larvae were put in different plastic bowls (225 ml capacity), each containing 100 ml of test solution of different doses of crude extract (0.1-0.5%), to investigate the percent mortalities of larvae. Negative control experiments were set on 100 ml of tap water only. Only 3rd instar larvae of *Cx. quinquefasciatus* were used for larvicidal bioassays with the different solvent extracts. Twenty larvae were put in plastic bowls, each containing 100 ml of test solution of different doses (viz., 40, 50, and 60 ppm) of each solvent extract. Ethanol treated control experiments (ethanol treated) were set on 100 ml of water with 0.5 ml of ethanol. Each set of experiment for crude as well as different solvent extracts was replicated three times, including three replicates of control experiments (for crude as well as solvent extracts) on separate three days. The percent mortalities were noted after 24, 48 and 72 hrs of post exposure cumulatively. Larvae were detected dead when the larvae were unable to move after touching their body with a fine brush.

Phytochemicals: Ethanol and water extracts of dried rhizomes were used for phytochemical analyses through standard protocols (Trease and Evans, 1989; Sofowara, 1993; Harborne, 1984) with modifications.

a) Alkaloids test (Mayer's test) **-** Five ml of ethanol extract was taken in a test tube to which 2 drops of 2N HCL and thereafter 2 drops of Mayer's reagent were added. The resultant pale yellow color precipitation indicated the presence of alkaloids.

b) Terpenoids test (Salkowski test) - 5 ml of ethanol extract was taken in a test tube and thereafter 5 ml of chloroform and 1 ml of concentrated H_2SO_4 were added carefully to it. The reddish brown coloration at the interface indicated the terpenoids.

c) Steroids test - 5 ml of ethanol extract was taken in a test tube and thereafter 2 ml of concentrated H_2SO_4 was gently added to it. The resultant brown color ring indicated steroids.

d) Flavonoids test **-** 5 ml of water extract was taken in a test tube and treated with 1 ml of NaOH solution. Intense color was observed and it became colorless when dilute HCL to it added, indicating the presence of flavonoids.

e) Tannins and phenol compounds test **-** 5 ml of water extract was taken in a test tube and 4-5 drops of ferric chloride were added to it. The occurrence of blue green coloration indicated tannins and phenol compounds.

f) Test of saponins (frothing test) - 8 ml of aqueous extract was taken in a test tube and was shaking vigorously. The persistence of frothing indicated the presence of saponin.

Effect of crude and ethyl acetate rhizomes extracts on non-target organisms: Tadpoles of frog and 4th instar larvae of *Chironomus circumdatus* were chosen as they live in the same habitat of mosquito larvae and were tested with appropriate lethal concentrations i.e. LC_{50} value (dose) of crude as well as ethyl acetate rhizome extracts, as per protocol, described by Mallick *et al.* (2016a) with modification. Twenty tadpoles of frog and twenty 4th instar *C. circumdatus* larvae were released in each of two beakers (each beaker of 500 ml contained 200 ml of pond water of aforesaid doses of crude rhizome extract), and the similar experiments for ethyl acetate rhizome extract was conducted. Each experiment for crude as well as ethyl acetate extracts was folded thrice on separate three days. Concurrently, negative control experiments with 200 ml of pond water only and ethanol treated control experiments with 199.5 ml of pond water with 0.5 ml of ethanol were run parallel. Mortality of non-target creatures was noted for a period of 24, 48, and 72 hrs of exposures, cumulatively.

Computer software, 'STAT PLUS-2009' – trial version and MS EXCEL-2007, was used to calculate the LC_{50} and LC_{90} values through Log probit analyses, as well as for R^2 (coefficient of determination), regression equation, mean per cent mortality, standard error, and ANOVA analysis.

Table 1. Mortality of different instars of *Culex quinquefasciatus* exposed to different concentrations of crude rhizome extract of *Elettaria cardamomum*

Instars	Conc. (%)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 h	48 h	72 h
1st	0.1	36.66±3.33	43.33±3.33	53.33±3.33
	0.2	60.00±5.77	70.00±5.77	76.66±3.33
	0.3	73.33±6.67	86.66±3.33	96.66±3.33
	0.4	86.66±6.67	93.33±3.33	96.66±3.33
	0.5	100.00±0.0	100.00±0.0	100.00±0.0
2nd	0.1	26.66±3.33	46.66±3.33	56.67±3.33
	0.2	40.00±5.77	50.00±0.00	63.33±3.33
	0.3	50.00±5.77	63.66±3.33	73.33±3.33
	0.4	56.66±3.33	66.67±3.33	76.66±8.82
	0.5	70.00±5.77	76.66±3.33	83.33±3.33
3rd	0.1	26.66±8.82	43.33±3.33	50.00±5.77
	0.2	30.00±5.77	46.67±3.33	53.33±3.33
	0.3	33.33±6.67	50.00±5.77	56.66±3.33
	0.4	43.33±8.82	56.67±3.33	63.33±3.33
	0.5	46.66±8.82	60.00±5.77	73.33±3.33
4th	0.1	10.00±0.00	16.66±3.33	23.33±3.33
	0.2	13.33±3.33	23.33±3.33	33.33±3.33
	0.3	16.66±3.33	30.00±0.00	36.66±3.33
	0.4	23.33±3.33	36.66±3.33	43.33±3.33
	0.5	26.66±3.33	40.00±5.77	46.66±3.33

No mortality in control (for all instars)

RESULTS AND DISCUSSION

In the larvicidal activity of crude rhizome extract, mortality increased with the increase in concentration and time of exposure in all the instars. First instar larvae were most susceptible to crude rhizome extract and showed cent percent mortality at 0.5 per cent after 24 hrs of exposure. No larval mortality was observed on the negative control experiments (Table 1). Crude rhizome extract experiments showed, gradually decreasing LC_{50} and LC_{90} values in time for different larval instars. R^2 values in almost all cases showed close to one which denotes that mortality percent was strongly correlated with the dose of the crude extract (Table 2). In the larvicidal efficacy experiments of petroleum ether, hexane and ethyl acetate rhizome extracts, the mortality increased with the increase in concentration and time of exposure. Among the three solvent rhizome extracts, ethyl acetate extract showed maximum mortality after 72 hrs of exposure. Ethanol treated control experiments did not show any mortality (Table 3). LC_{50} and LC_{90} values gradually decreased in time against 3rd instar larvae in the different solvent extracts. R^2 values are close to one in almost all cases. Among three solvent extracts, ethyl acetate extract showed low LC_{50} and LC_{90} values after 24, 48 and 72 hrs of exposure. Among three solvent extracts, the ethyl acetate extract showed as the most potent larvicide (Table 4). Crude rhizome extract of *E. cardamomum* showed great efficacy in larval mortality against filarial vector, *Cx. quinquefasciatus*. First instar

Table 2. Log probit and regression analyses of larvicidal activity of crude rhizome extract of *Elettaria cardamomum* against different larval instars of *Culex quinquefasciatus*

Instars	Periods(h)	LC_{50}(%)	LC_{90}(%)	Regression equations	R^2- values
1st	24	0.1483	0.4265	Y = 25.3280+153.3400 X	0.9916
	48	0.1215	0.3204	Y =37.6630+136.6700X	0.9527
	72	0.1002	0.2483	Y = 50.6600+113.3400X	0.9049
2nd	24	0.2767	1.8879	Y =17.6620+103.3400X	0.9943
	48	0.1428	2.2635	Y =36.9930+76.6700X	0.9237
	72	0.0794	1.1905	Y =50.6610+66.6700X	0.9901
3rd	24	0.7531	31.0841	Y =19.9970+53.3300X	0.9774
	48	0.2289	31.7545	Y =37.9940+46.6800X	0.9615
	72	0.1275	5.7803	Y = 42.3320+56.6600X	0.9686
4th	24	2.3693	47.7411	Y =5.0000+43.3200X	0.9912
	48	1.0482	28.9825	Y = 11.3270+60.0100X	0.9939
	72	0.6334	16.3633	Y =19.6640+56.6600X	0.9815

R^2= Coefficient of determination; LC= Lethal Concentration
Y= Mortality; X= Concentration

Table 3. Mortality of third instars of *Culex quinquefasciatus* exposed to different concentrations of different solvent extracts of rhizomes of *Elettaria cardamomum*

Solvent extracts	Conc. (ppm)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 h	48 h	72 h
Petroleum ether	40	23.33 ±3.33	33.33±3.33	40.00±5.77
	50	33.33±3.33	43.33±3.33	50.00±5.77
	60	46.66±.6.67	56.66±6.67	63.33±8.82
Hexane	40	30.00±0.00	40.00±.5.77	50.00±5.77
	50	43.33±3.33	53.33±3.33	60.00±5.77
	60	66.66±3.33	70.00±5.77	76.66±8.28
Ethyl acetate	40	46.66±3.33	53.33±3.33	63.33±3.33
	50	60.00±3.33	70.00±0.00	73.33±3.33
	60	83.33±3.33	90.00±0.00	96.66±3.33

No mortality in control

Table 4. Log probit and regression analyses of different solvent extracts of rhizome of *Elettaria cardamomum* on third instar larvae of *Culex quinquefasciatus*

Solvent extracts	Periods (h)	LC_{50}(ppm)	LC_{90}(ppm)	Regression equations	R^2- values
Petroleum ether	24	64.0288	143.4722	Y=23.8850+1.1665X	0.9966
	48	54.4058	130.2267	Y= 13.8850+1.1665X	0.9966
	72	48.3629	116.9358	Y=7.2150+1.1665X	0.9966
Hexane	24	51.2659	88.8038	Y=44.9867+1.8330X	0.9878
	48	46.3743	91.1377	Y=20.5567+1.5000X	0.9979
	72	40.9613	85.3110	Y=4.4300+1.3330X	0.9898
Ethyl acetate	24	42.4847	71.2085	Y=28.3450+1.8335X	0.9879
	48	39.5602	62.5172	Y= 20.565+1.8335X	0.9986
	72	37.0282	56.6899	Y=5.5517+1.6665 X	0.9744

R^2= Coefficient of determination; LC= Lethal Concentration

Y= Mortality; X= Concentration

larvae were most susceptible to crude rhizome extract of the plant. First instar larvae showed cent per cent mortality only at 0.5 per cent dose after 24 hrs of exposure. Second, third, and fourth instars larvae showed maximum mortality, 83.33±3.33, 73.33±3.33, and 46.66±3.33 per cent respectively after 72 hrs of exposure. First, second, third, and fourth instars larvae showed LC_{90} values 0.4265, 1.8879, 31.0841and 47.7411 per cent respectively after 24 hrs of exposure.

Patel *et al.* (2018) worked with crude leaf as well as berry extracts of *Solanum nigrum* to investigate the larvicidal activity against dengue vector, *Aedes aegypti* and observed that fourth instar larvae showed 100 per cent mortality at 5 per cent dose after 48 hrs of exposure and fourth instar larvae showed maximum percent mortality (52.33 and 99.33% at 5 % dose by the effect of crude green berry and crude black berry extracts, respectively, after 72 hrs of exposure). Rawani *et al.* (2013) reported larvicidal activity of crude *S. niagram* crude berry extract against *Cx. quinquefasciatus* at 3 per cent dose. Mallick Halder *et al.* (2011) reported cent per cent mortality of first instar larvae of *Cx. quinquefasciatus* with crude and methanol leaf extracts of *Typhonium trilobatum* at 0.4 per cent after 72 hrs of exposure.

In the present study, ethyl acetate extract showed the highest percent mortality among three solvent rhizomes extracts having LC_{50} value 37.0282 ppm against 3rd instar larvae after 72 hrs of exposure followed by petroleum ether (LC_{50} value 40.9613 ppm) and hexane (LC_{50} value 48.3629 ppm) rhizome extracts of the plant. Many researchers experimented with ethyl acetate extract of the different plants against larvae of *Cx. quinquefasciatus* mosquito species to observe their larvicidal activity. Kamaraj *et al.* (2010) worked with hexane, ethyl acetate and methanol extracts of *Zingiber zerumbet* L., *Gymnema sylvestre* (Retz) Schult, *Cassia angustifolia* Vahl, *Mimosa pudica* L., *Aristolochia indica* L., *Diospyros melanoxylon* Roxb., *Dolichos biflorus* L., and *Justicia procumbens* L. against early 4th instar larvae of *Cx. quinquefasciatus* Say and *Cx. gelidus* Theobald and reported highest larval mortality in ethyl acetate extract of *D. biflorus* against *Cx. quinquefasciatus* Say having LC_{50} and LC_{90} values 34.76 and 172.78 ppm, respectively, after 24 hrs of exposure. Kumar *et al.* (2012) observed cent per cent mortality of late third or early fourth instar of *Cx. quinquefasiciatus* at 250 ppm and 300 ppm doses of petroleum ether and ethyl acetate extracts of dried whole plant, *Tephrosia purpurea* (L) Pers. Mallick and Chandra

(2015c) reported 100 per cent mortality at 5 ppm with ethyl acetate leaf extract of *Annona reticulata* L. against 3rd instar larvae of *Cx. quinquefasciatus*, having LC_{50} and LC_{90} values 1.4556 and 6.6383 ppm after 48 hrs of exposure. Jayaraman *et al.* (2015) investigated hexane, choloroform, ethyl acetate, acetone and methanol extracts of seven aromatic plants against *Cx. quinquefasciatus, Ae. aegypti, and Anopheles stephensi* for 12 and 24 hrs of exposure periods and showed various levels of larvicidal activity but ethyl acetate extract of *Chloroxylon swietenia* showed the remarkable larvicidal activity; Larvae of *Cx. quinquefasciatus* showed LC_{50} and LC_{90} values 194.22 and 458.83 ppm, respectively, after 12 hrs of exposure. Bagavan *et al.* (2018) examined on hexane, ethyl acetate, chloroform, methanol and acetone extracts of leaves of the medicinal plants viz. *Leucas aspera*, *Acalypha indica*, *Ocimum sanctum, Achyranthes aspera*, and *Morinda tinctoria* against the early fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* for a period of 24 hrs of exposure, and they observed that ethyl acetate leaf extract of *Achyranthes aspera*s showed the highest larvicidal potential against both species. Other extracts showed moderate larvicidal efficacy. Saponin compound was isolated from ethyl acetate leaf extract of *A. aspera*s which showed LC_{50} values 18.20 and 27.24 ppm against *Ae. aegypti* and *Cx. quinquefasciatus* respectively.

In the completely randomized three way ANOVA analyses, using instars, hours and concentrations as independent variables and mortality percentage as dependent variable, showed statistical significance in larval mortality (F value = 6.1551 $p<0.05$) in terms of doses of crude rhizome extract, instars of *Cx. quinquefasciatus* and of time of exposure.

Preliminary phytochemical analyses of rhizome extracts of *E. cardamomum* revealed the presence of alkaloids, terpenoids, steroids, flavonoids, tannins, phenols and saponins in the of rhizome extracts. Among three solvent extracts, ethyl acetate extract was most potent in larval mortality. Petroleum ether and hexane are highly non-polar solvents and only non-polar phytocompounds may be present in petroleum ether and hexane rhizome extracts which may cause larval mortality, but the polarity of ethyl acetate is medium. Due to its medium polarity nature both polar and non-polar phytocompounds may be present in ethyl acetate rhizome extract which may cause larval mortality. Due to this reason, ethyl acetate rhizome extract showed larval mortality among the solvent extracts.

The test on the effect of crude and ethyl acetate rhizome extracts on non-target organisms indicated that there were no mortality and abnormal behaviour on frog tad poles and 4th instar larvae of *C. circumdatus* up to 72 hrs of exposure. Non-target organisms were non responsive to crude as well as ethyl acetate rhizome extracts, so their uses for controlling mosquito population will be safer.

There are preliminary phytochemical investigation of several plant extracts and mosquito larvicidal activity along with the observation of the effects on non-target organisms (Singha *et al*., 2011; Singha *et al*., 2012; Mallick *et al*., 2016a; Mallick et al., 2016b). In recent times, the insecticides of plant origin have been given more importance for their bio-degradable, nontoxic, and ecofriendly nature. Mosquito larvicidal property of *E. cardamomum* rhizome (ethyl acetate and crude extracts) has been unfolded for the first time and the findings of the present study revealed that crude as well as ethyl acetate rhizome extracts of *E. cardamomum* showed larvicidal potential against *Cx. quinquefasciatus*.

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Phylogeny of Indian Himalayan population of *Bombus haemorrhoidalis* Smith 1852 (Hymenoptera: Apidae) inferred from mitochondrial DNA sequences

Sunaullah Bhat[1], A. R. N. S. Subbanna[2], Amit Paschapur[2*], J. Stanley[2], Sandeep Kumar[1] and Jai Prakash Gupta[2]

[1]Department of Zoology, Kumaun University- SSJ Campus, Almora, Uttarakhand 263601, India.
[2]ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand 263601, India.
Email: amitp3929@gmail.com; bhatsunaullahijt@gmail.com; Avupati.Subbanna@icar.gov.in; mjstanley1980@gmail.com; sandeepento@gmail.com; guptajp80@gmail.com

ABSTRACT: Molecular variation and phylogenetic relationship of *Bombus haemorrhoidalis* Smith 1852 was studied using partial cytochrome oxidase I (COI) and cytochrome *b* (cyt *b*) sequences of mitochondrial genome. The COI and cyt *b* sequences were compared with available sequences of *B. haemorrhoidalis* and other *Bombus* species belonging to different subgenera to avail divergence within and between species. The BLASTn analysis of obtained COI sequence had cent percent identity to a hymenopteran species BOLD deposit AAC6447 (MAHYM005-10.COI-5P) which is a *Bombus* species from Pakistan. Both these species formed a separate cluster amongst the tested *B. haemorrhoidalis* species in phylogeny with pair wise genetic distance of 0.003. Moreover, the minimum evolution tree between the species revealed *B. haemorrhoidalis* is phylogenetically close with *B. funerarius* and the pair wise genetic distance between these two species was 0.102. Interestingly, *B. haemorrhoidalis* and *B. funerarius* formed separate minor cluster amongst the *Bombus* species tested for phylogeny. Additionally, both COI and cyt *b* genes were A+T biased and showed single nucleotide polymorphisms between and within species. The phylogenetic relationship of COI sequences also revealed single species status of *B. haemorrhoidalis* in the Indian Himalayan region and was evolutionarily associated with *B. funerarius* which is an another species in subgenera, Orientalibombus. The phylogeny of cyt *b* sequences showed that the *B. haemorrhoidalis* is evolutionarily close to pennsylvanicus group species. The study illustrates a complex genetic variation coupled with highly structured evolutionary divergences between and within species and provides the first report of cyt *b* sequence of *B. haemorrhoidalis*.

KEYWORDS: *Bombus* species, phylogenetic analysis, genetic variation, evolutionary divergence, COI sequences

INTRODUCTION

The members of family Apidae (Hymenoptera) are a highly divergent group of social, eusocial and semi social insects including bumble bees, euglossines (orcid bees), honeybees, stingless bees (*Melipona*) contributing to majority of worlds pollination services. Bumble bees are considered as versatile pollinators due to their more working hours and buzz pollination of crops (Chauhan *et al.,* 2016). Other

* Author for correspondence

important attributes like short flight range, cold hardiness and high elevation adaptation make them important pollinators of wild and agricultural flowering plants and crops with both ecological and economic importance (Streinzer *et al.,* 2019). In natural plant–pollinator interaction, bumble bees are often considered as keystone species due to generalist pollination services, thereby assisting plant community diversity by visiting both rare and abundant plant species (Memmott *et al.,* 2004; Goulson *et al.,* 2008; Burkle *et al.,* 2013; Cusser and Goodell, 2013). In some instances, bumble bees are the only efficient pollinators and the crop productivity is completely dependent on the ecological services provided by these insects (Sinu *et al.,* 2011; Streinzer *et al.,* 2019).

Bumble bees are highly conspicuous and abundant in cold and temperate regions. Due to their important ecosystem services, the group has become an interesting target for early naturalists and entomologists. This has led to identification of around 260 species (Williams, 1998; updated online at http://www.nhm.ac.uk/researchcuration/ research/ projects/bombus/ index.html). The current global efforts on bumble bee taxonomy are targeted towards the species distribution and subgeneric variations (Streinzer *et al.,* 2019). Decline in the population of bumble bees (Cameron *et al.*, 2011; Bartomeus *et al.,* 2013) might be due to climate change, heat stress, spill-over of pathogens, changes in agricultural practices and deforestation (Hoiss *et al.,* 2012; Kerr *et al.,* 2015; Rasmont *et al.,* 2015; Jacobson *et al.,* 2018).

Among the different agroecological regions of India, the north western Himalayan region has its own diverse fauna of bumble bees. In the mid hills of Uttarakhand Himalayas, *B. haemorrhoidalis* is one of the key bumble bee species visiting many of the cultivated crop plants and wild plantations. Moreover, this is the only species of pollinator found during low temperature regimes in the region due to their thermoregulatory ability (Corbet *et al.,* 1993) and actively visits the flowering plants throughout the day.

Amongst different markers, partial sequences of mitochondrial genes especially cytochrome oxidase I (cox 1) and cytochrome *b* (cyt *b*) are well suited to resolve the phylogenetic issues of a wide range of hierarchical levels in insects (Simmons and Weller, 2001; Bertsch *et al.,* 2010). High mutation rate, maternal inherence and evolutionary memory made these two genes particularly important in any phylogeny related studies (Simmons and Weller, 2001). Besides, DNA sequence based approaches are independent of insect developmental stages but within species variation is the only ambiguous issue (Ahrens *et al.,* 2007) which can also be well documented using these mitochondrial markers. In the present study, both cox 1 and cyt *b* genes from the population of *B. haemorrhoidalis* native to Uttarakhand Himalayas, India were amplified. The sequence variation in these two genes was used to establish the genetic divergence and phylogenetic relationship with other *Bombus* species.

MATERIALS AND METHODS

Test insects: Specimens of *B. haemorrhoidalis* for a phylogenetic study were collected during March to December 2020 from toad flax (*Linaria vulgaris*) and ornamental poppy (*Papaver orientale*) in the premises of agricultural fields of the Vivekananda Hill Agriculture Institute, farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora located at 29°372 N, 79°402 E with an altitude of 1310 msl in Uttarakhand state of North Western (NW) Himalayan Region, India

DNA extraction: Henry *et al.* (1990) method was followed for the extraction of genomic DNA. The legs of the target specimens were ground in the liquid nitrogen in micro-centrifuge tube with the help of blunted tip of the pipette. Sample obtained were washed with TENT buffer (10 mM Tris- Cl (pH 7.4), 5 mM EDTA, 10 mMNaCl, 0.5 per cent Triton X-100). Sample were centrifuged for 10 min at 10000 rpm, pellet was suspended in TEN buffer (TENT without Triton X-100) comprising 1 per cent sodium dodecyl sulphate and 1 mg/ml of proteinase K. After incubation at 37 ° C for 4 h, 1/10 th volume of 5 M NaCl were added and thoroughly mixed. The genomic DNA was extracted twice from phenol/chloroform, isopropanol was used to precipitate DNA and obtained DNA was suspended in 100 µl of TE buffer. RNA residues were removed

by incubation with DNase-free RNase A for 1 h at 37°C. To visualize intact genomic DNA 0.8 per cent agarose gel was used and the genomic DNA was diluted to get working solution of 20–25 ng/µl.

PCR protocol: PCR reaction mix was as follows: 50 ng of DNA template, 200nM of dNTPs, 1mM of each primer (Table 1), 2.5 units of Taq DNA polymerase and 5µl of PCR reaction buffer to make a final volume of 50 µl. Entire reactions were performed in the thermal cycler (Biorad) with an initial 5 min denaturation step at 92°C, accompanied by 35 amplification cycles consisting of 1 min denaturation at 92°C, 45 s annealing at 48°C and 2 min extension at 72°C with an additional final 10 min extension step at 72°C. The amplification was visualized and confirmed in the gel documentation system (Alpha Image Analyzer, Alpha Innotech Corporation) by 1 per cent agarose-EtBr gel electrophoresis of 10µl PCR product. Negative PCR controls were carried out to reduce cross-contamination.

Sequencing and data analysis: The amplified products of the particular gene were purified through gel elution columns (Sigma), sequenced directly on an automated DNA sequencer (ABI 377) with the help of the Big Dye terminator kit (Applied Biosystems) as per manufacturer's guidelines. Nucleotide sequences were aligned with the Clustal Omega (1.2.1) multiple sequence alignment (McWilliam *et al.,* 2013) and phylogenetic and molecular evolutionary analyses were done utilizing the software MEGAX (Molecular Evolutionary Genetic Analysis version 4) (Kumar *et al.,* 2018). The phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei, 1987) utilizing the distance matrix from the alignment. The nucleotide sequences were also converted into amino acid sequences with the help of invertebrate mitochondrial genetic code (Gasteiger *et al.,* 2003) and were aligned using Clustal omega software (McWilliam *et al.,* 2013). NCBI accession numbers of other sequences COI and cyt b of *B. haemorrhoidalis*, *Bombus* species were presented along with results. The obtained sequences of COI and cyt b were submitted to NCBI Gene Bank nucleotide sequence databases (accession number ON073847).

RESULTS AND DISCUSSION

The BLASTn analysis of obtained COI sequence had cent percent identity to a hymenopteran species BOLD deposit AAC6447 (MAHYM005-10.COI-5P) which is a *Bombus* species from Pakistan. Both these species formed a separate cluster amongst the tested *B. haemorrhoidalis* species for phylogeny (Fig. 1) with Pair wise genetic distance of 0.003 (Table 2). As the entire mitochondrial DNA sequence was used for the construction of phylogeny, the Cox I and cyt b region proves to be highly conserved region, through which molecular characterization of insects can be taken up with higher degrees of specificity. When the phylogenetic tree was constructed with the MEGA X 10.0.5 software (Fig. 1), it was observed that the *B. haemorrhoidalis* species native to Indian Himalayas formed separate group with hymenopteran species BOLD deposit AAC6447 (MAHYM005-10.COI-5P) in the maximum likelihood evolution tree. The node support estimated using 1000 bootstrap pseudo-replicates, showed that these species evolved together as they showed 99% node value.

However, the intra-species diversity (n=10) in 683 bp nucleotide sequence region of Cox1 was manifested in the form of 11 single nucleotide polymorphism (SNPs) by utilizing CLUSTAL Omega (1.2.4) multiple sequence alignment software (Supplementary Fig. 1 and 2). The mean number nucleotide frequency amongst the *B. haemorrhoidalis* was also examined and it was observed that the COI sequences were usually A+T biased with the concentration of A+T exceeding 76.95%, while the concentration of G+C was well below 23.05% in which the concentration of thymine was highest (42.15%). The average nucleotide frequencies in *B. haemorrhoidalis* are 34.80 (A), 42.15 (T), 12.83 (C), and 10.23 per cent (G). Besides, the transition/transversion rate ratios are k_1 = 6.526 (purines) and k_2 = 2.026 (pyrimidines). The overall transition/transversion bias is R = 1.381, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$. MCL estimate of nucleotide substitutions also showed maximum base substitution as A to G and vice versa with a value of 37.52

Table 1. Primers used in the study

Gene	Primer	Sequence (5'-3')	Reference
Cytb	cb1	5^2 -TATGTACTACCATGAGGACAAATATC-3^2	Schwarz *et al.* (2003)
	cb2	5^2 -ATTACACCTCCTAATTTATTAGGAAT-3^2	
COI	C1-J-2195	TTGATTTTTTGGTCATCCAGAAGT	Simon *et al.* (1994)
	AAMT3038R	TCCATTGCACTAATCTGCCATATTAG	Muraji *et al.* (2008)

Table 2. Pair wise genetic distance for partial COI gene sequences of *B. haemorrhoidalis* isolates with *B. funerarius*

Species	1	2	3	4	5	6	7	8	9	10
1. *B. haemorrhoidalis* Almora										
2. JF865997 Hymenoptera sp. BOLD:AAC6447	0.003									
3. MF582625 *B. haemorrhoidalis* DPK-B02	0.014	0.011								
4. MF582610 *B. haemorrhoidalis* DT-B01	0.014	0.011	0.000							
5. MF582604 *B. haemorrhoidalis* DAK-B12	0.014	0.011	0.000	0.000						
6. MF582600 *B. haemorrhoidalis* DMNg-B11	0.014	0.011	0.000	0.000	0.000					
7. MF582607 *B. haemorrhoidalis* DAK-B10	0.015	0.012	0.002	0.002	0.002	0.002				
8. MF582592 *B. haemorrhoidalis* DS1-B41	0.015	0.012	0.002	0.002	0.002	0.002	0.003			
9. KT334307 *B. haemorrhoidalis* voucher 4746F01	0.015	0.012	0.002	0.002	0.002	0.002	0.000	0.003		
10. MF582621 *B. haemorrhoidalis* DI2-B03	0.017	0.014	0.003	0.003	0.003	0.003	0.005	0.002	0.005	
11. MT906010 *B. funerarius*	0.102	0.097	0.094	0.094	0.094	0.094	0.096	0.096	0.096	0.097

(Table 3). The minimum evolution tree between the species revealed *B. haemorrhoidalis* is phylogenetically close with *B. funerarius*. The pair wise genetic distance between these two species was 0.102 (Table 2). Interestingly, *B. haemorrhoidalis* and *B. funerarius* formed separate minor cluster amongst the *Bombus* species tested for phylogeny.

The sequenced 449 bp region of cyt *b* of *B. haemorrhoidalis* spans between 436 to 884 bp region of total cyt *b* sequence (1151 bp) of *A. mellifera*. The NCBI blast search of the

Table 3. Maximum composite likelihood estimate of the pattern of nucleotide substitution in COI sequences of ten (as given in phylogeny tree) *B. haemorrhoidalis* species

	A	T	C	G
A	-	6.96	2.12	**11.03**
T	5.75	-	**4.29**	1.69
C	5.75	**14.11**	-	1.69
G	37.52	6.96	2.12	-

Rates of transitional substitutions were shown in bold and those of transversional substitutions in italics

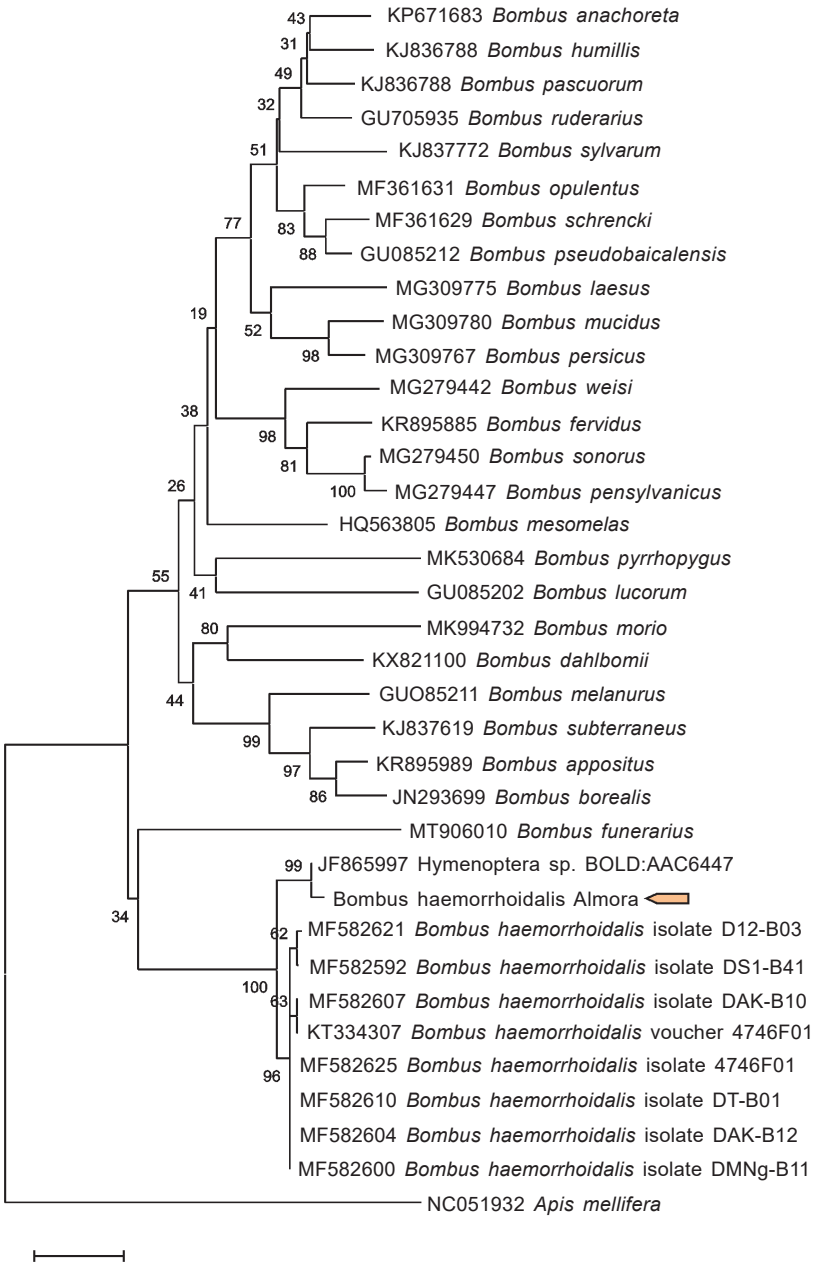

Fig. 1 ME tree with bootstrap support showing clustering of different species of *Bombus* (starts with accession numbers) constructed using partial COI sequences

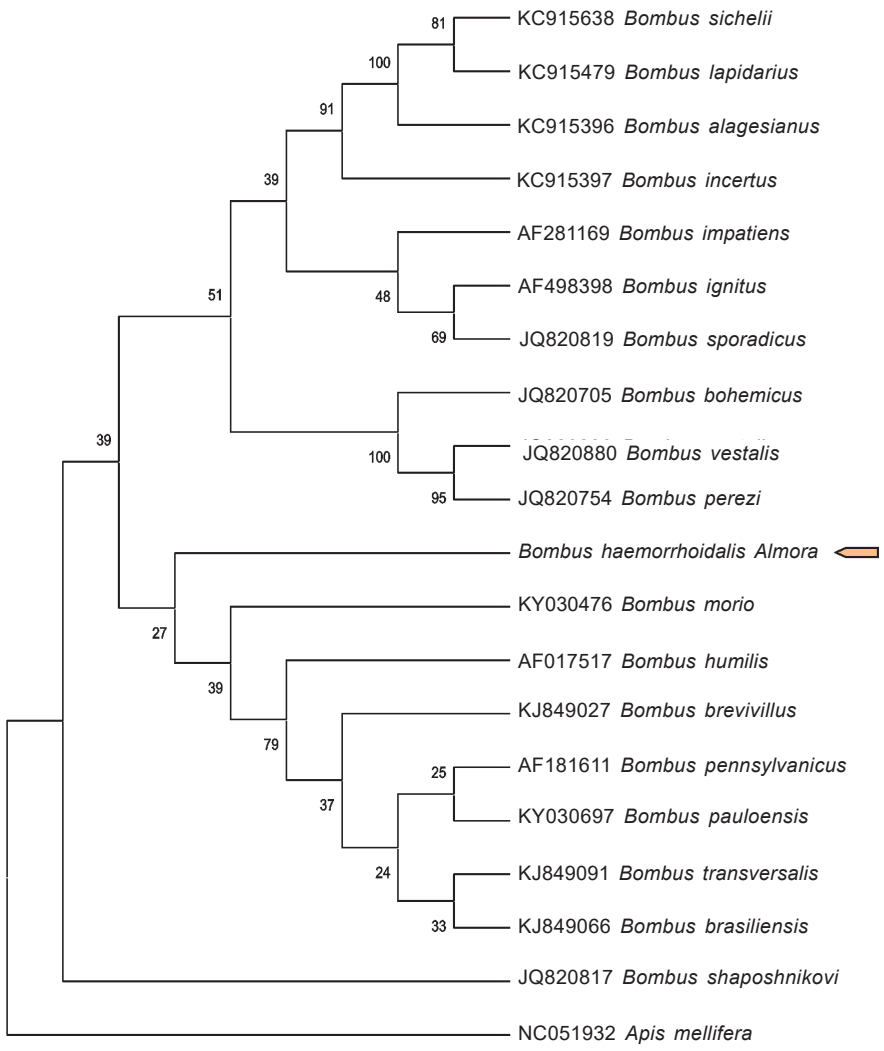

Fig. 2.ME tree with bootstrap support showing clustering of different species of *Bombus* (starts with accession numbers) constructed using partial cyt *b* sequences

sequence revealed no submissions of cyt *b* sequence for *B. haemorrhoidalis* so the study submits the first ever sequence of the species to NCBI (Accession No. ON073847). However, other *Bombus* species cyt *b* sequences are available for the sequenced region and were used in phylogeny construction and further analysis. The BLASTn search of the cyt *b* sequence revealed a proximal cyt *b*-like sequence of *B. fragrans* (88.99 percent identity) and other *Bombus* species. However, these sequences have double and triple nucleotide insertions and so are not included in phylogeny. Minimum evolution phylogeny tree constructed using 19 cyt *b* sequences of *Bombus* species clearly differentiated two sub groups where *B. haemorrhoidalis* was in evolutionary association with other seven species (Fig. 2). Interestingly, *B. shaposhnikovi* formed a separate clade. Pairwise genetic distance analysis of these eight species (including *B. haemorrhoidalis*) showed *B. haemorrhoidalis* is closely related with *B. pennsylvanicus* with 0.121 (Table 4). The mean number nucleotide frequency amongst the *B. haemorrhoidalis* was also examined and it was observed that the cyt *b* sequences were usually A+T biased with the concentration of A+T

Table 4. Pair wise genetic distance for partial cyt *b* gene sequences of *Bombus* species

S. No &Species	1	2	3	4	5	6	7	8	9
1: *B. haemorrhoidalis* Almora									
2: AF181611 *B. pennsylvanicus*	0.121								
3: KJ849091 *B. transversalis*	0.130	0.050							
4: KJ848955 *B. pauloensis*	0.139	0.050	0.057						
5: KJ849066 *B. brasiliensis*	0.142	0.047	0.052	0.062					
6: KJ849027 *B. brevivillus*	0.148	0.053	0.073	0.078	0.065				
7: JQ820817 *B. shaposhnikovi*	0.157	0.110	0.117	0.135	0.132	0.123			
8: AF017517 *B. humilis*	0.143	0.066	0.079	0.094	0.088	0.091	0.138		
9: KY030476 *B. morio*	0.172	0.118	0.114	0.121	0.130	0.127	0.132	0.139	

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Tajima-Nei model

Table 5. Maximum composite likelihood estimate of the pattern of nucleotide substitution in cyt *b* sequences of *Bombus* species

	A	T	C	G
A	-	10.93	3.1	**2.85**
T	8.91	-	**7.4**	1.78
C	8.91	**26.06**	-	1.78
G	**14.25**	10.93	3.1	-

Rates of transitional substitutions were shown in bold and those of transversional substitutions in italics

exceeding 80.24 per cent, while the concentration of G+C was well below 19.76 per cent in which the concentration of thymine/uracil was highest (44.19%). MCL estimate nucleotide substitutions of these eight sequences showed nucleotide frequencies of 36.05 per cent (A), 44.19 per cent (T/U), 12.54 per cent (C), and 7.21 per cent (G), respectively. Amongst the species maximum base substitution was observed between C to T and vice versa with 26.06 (Table 5).

The genus *Bombus* comprises 250 known species under 38 subgenera with distribution all over the world (Cameron *et al.,* 2007) which was reviewed and reduced to 15 subgenera by Williams *et al.* (2008) (https://www.nhm.ac.uk/researchcuration/research/projects/bombus/ groups.html). Besides, they are highly vulnerable species to climate change and continents like Europe, North America, and Asia are already experiencing decline in bumble bee population (Williams and Osborne 2009). India is home for around 48 species of bumble bees out of which 37 species are recorded from North West Himalayas (Saini *et al.,* 2012) and 21 species from Arunachal Pradesh (Streinzer *et al.,* 2019). The distribution of these species spanning from an altitude of 230-2990 m. Keeping in view of the ecological and economic importance of the genus *Bombus,* both biological and molecular studies are important for their conservation and successful utilization as pollinators.

In the study area, the Uttarakhand Himalayas, *B. haemorrhoidalis* is distributed bumble bee found all over the flowering crop plants. The species belongs to subgenus Orientalibombus Richards which also contains *B. braccatus* and *B. funerarius* (https://www.nhm.ac.uk/research curation/research/projects/bombus/ or.html#haemorrhoidals). The COI based phylogenetic tree clearly differentiated both *B. haemorrhoidalis* and *B. funerarius* into a separate clade. This is in agreement with the phylogeny study by Cameron *et al.* (2007) and also confirms the COI based identity of *B. haemorrhoidalis* from Uttarakhand Himalayas. The high bootstrap values also support the single

species status of the populations (Subbanna *et al.,* 2016). The number of base substitutions per site between these two species is around 0.1. Whereas for the third species under the subgenus, *B. braccatus* no COI sequence is available in NCBI data base. The phylogeny with in the species also showed significant variation with pairwise genetic variation ranging from 0.14-0.17 which are the geographic isolates from Thailand. The geographic isolate from Pakistan showed pairwise genetic variation of only 0.03. This type of geography dependent intraspecies variations (Streinzer *et al.,* 2019) and body color phenotypes are common in *Bombus* species (Hines and Williams 2012; Koch *et al.,* 2018). Streinzer *et al.* (2019) also reported *Bombus* species are one of the suitable taxa to study potential adaptations to specific climatic conditions at the individual and as well as population level. *De novo* sequencing of genomes of 17 species, representing all 15 subgenera of *Bombus* revealed dynamically evolving gene families in response to positive selection points linked to foraging, diet, detoxification, immunity to invading pathogens and adaptations to high altitudes (Sun *et al.,* 2021). Present study also shows an evidence of ecological and behavioral traits driven intraspecies variation in COI gene of *B. haemorrhoidalis*.

The high rates of nucleotide substitutions in mitochondrial genes helped molecular entomologists to estimate phylogenetic relationships among different species and populations of single species (e.g. Hebert *et al.*, 2003; Pons *et al.*, 2004; Havill *et al.*, 2007). Clustal comparison of cyt *b* sequences between the species showed variation as SNPs and unequal nucleotide frequencies. In general, all the sequences of the species are rich in AT region. Due to unavailability of cyt *b* region of different species in NCBI data, a phylogeny was prepared using available ones. The cyt *b* derived phylogenetic tree showed evolutionary association of *B. haemorrhoidalis* (belongs to Orientalibombus group) species with Morio (*B. morio*), Muscorum (*B. humilis*) and Pennsylvanicus group (*B. pennsylvanicus*, *B. pauloensis*, *B. transversalis* and *B. brasiliensis*) of *Bombus* species. The pairwise genetic distance using Tajima-Nei model also showed *B. haemorrhoidalis* was close to *pennsylvanicus* group species with an average value of 0.136. However, this grouping can't be considered as appropriate due to the use of limited available. Studies also reported that mitochondrial cyt *b* sequences also provide an accurate, rapid, and economic technique for separation of various insect pests in different orders (Jermiin and Crozier, 1994; Simmons and Weller, 2001) and found to have the same level of sequence variation and AT bias as COI (Simmons and Weller, 2001).

The phylogenetic analysis reported in the study is first of its kind with respect to *B. haemorrhoidalis* and will serve as stimulus for the *Bombus* community to initiate a more thorough evolutionary study to fill the currently existing gaps on conservation and threats of Indian bumble bees.

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Insect pests of *Ocimum sanctum* Linn. in Karnataka

K.N. Manjula, S. Renuka, R. Raja Rishi and R. Sundararaj*

Forest Protection Division, Institute of Wood Science & Technology,
18th Cross Malleswaram, Bangalore 560 003, India.
Email: rsundariwst@gmail.com

ABSTRACT: Survey conducted on *Ocimum sanctum* Linn. growing in Karnataka revealed the infestation of 16 species of insect pests. Of these, eleven are new records on *O. sanctum* in Karnataka state.

KEY WORDS: Tulsi, new pest record

Ocimum sanctum Linn. commonly known as tulsi, the queen of herbs, is one of the holiest and most cherished of many healing and healthy giving herbs of the orient. The herb is used externally as an antiseptic to treat skin infections, spots *etc.,* and juice of the plants is used to treat insect bites and ringworm and the juice is dropped into the ear to treat earaches (Priyabrata *et al.,* 2010). The main components of basil essential oil are linalool, camphor, 1, 8 cineole and germacren-D (Daneshian, 2013). The herb has the ability to lower blood sugar levels (Khan *et al.,* 2015) and the essential oils from the leaf have shown antimicrobial activity (Goyal and Kaushik, 2011). For centuries, the dried leaves of *O. sanctum* have been mixed with stored grains to repel insects (Biswas and Biswas, 2005). The leaf extract has fungicidal, insecticidal antibacterial, antifeedant and larvicidal activities (Sathe and Sathe, 2014). In spite of its medicinal importance several pests comprising of species of insects, mites and plant pathogens damage this plant in all agro-ecosystems (Gahukar, 2017).

Extensive survey was conducted to know the incidence of insect pests of tulsi during August 2017 to August 2019 which includes monthly surveys in the gardens of medicinal plants maintained at Institute of Wood Science and Technology (IWST), Bangalore; Tirumala Tirupathi Devasthanam (TTD), Malleshwaram and Kadu Malleshwara temple, Malleshwaram and biannual surveys in the gardens of Foundation for Rural Health Tradition (FRLHT), Bangalore; Indian Institute of Horticultural Research (IIHR), Bangalore, University of Agricultural Sciences, GKVK, Bangalore and Art of Living campus, Kanakpura. Also, roving survey was conducted in the medicinal plant gardens in Bagalkot, Belagavi, Dharwad, Gadag and Raichur. The nature of damage by insects found feeding on *O. sanctum* was observed. The adult and immature stages of the insects were collected and reared in the entomology laboratory of Forest Protection division at Institute of Wood Science and Technology, Bengaluru and were identified with the help of taxonomic experts.

The survey revealed the occurrence of 16 species of insects representing three orders *viz*., Hemiptera, Lepidoptera and Coleoptera infesting *O. sanctum* in Karnataka. It comprises of five species of

* Author for correspondence

defoliators and eleven species of sap suckers (Table 1). The larvae of *Hyposidra talaca* (Walker) scrape the chlorophyll content of leaves and make pin holes and often leading complete defoliation. The symptom of prominent holes in the leaves was visible by the feeding of the adults of curculionids *Blosyrus inequalis* Boh and *Leptomias* sp., while semicircular notches on the margin of leaves in 'U' shaped manner were visible by the feeding of *Myllocerus viridanus* (Fab.) and *M. undecimpustulatus* (Fab.). The infestation of sapsuckers often leads to yellowing of leaves and premature defoliation and in severe cases death of plants. The dominance of sap suckers on tulsi agrees with the reports of Sathe and Sathe (2014), Kumari and Srinivas (2018) and Triveni *et al.* (2018). Among these sucking pests, *Cochliochila bullita* (Stal.) (Plate 1A) and *Nephococcus viridis* (Green) (Plate 1B) were economically important as their infestation often leads to the death of plants (Sajap and Peng, 2010). Adults fed on tender shoots of the herb causing them to wilt and eventually die and, in many instances, nymphs and adults feed, gregariously on the leaves, leaving tiny black spots of excrement on the upper surface of the leaves (Dhiman and Jain, 2010). Nymphs and adults of aphids, *Aphis craccivora* Koch were found congregating on succulent stems and under surface of leaves and flowers of tulsi (Shelly and Singh, 2004; Sathe and Sathe 2014). Curling and crinkling of leaves and flowers which become shiny and sticky due to honey dew excreted by the aphids and growth of sooty mold are the common symptoms of infestation by aphids. But in our study *A. craccivora* (Plate 1C) were found to feed on various parts of the tulsi viz., flower bud, leaves. The adults of *Icerya aegyptica* (Douglas) (Plate 1D), a white colour giant mealy bug with tubular outgrowths all over the body were recorded on this plant. Desapping due to sap suckers, the branches dried and the plants withered (Nilamudeen and Nandakumar, 2012). The nymphs of *Clovia* sp. (Plate 1F), commonly known as spittlebugs are best known for their plant sucking nymphs which encase themselves in foam in springtime. This characteristic spittle production is associated with the unusual trait of xylem feeding but they do very little damage to plants. The symptoms of infestation by other insect pests are negligible.

Table 1: Insects infesting on *O. sanctum* in Karnataka

Defoliators
Lepidoptera: Geometridae — *Hyposidra talaca* (Walker)*
Coleoptera: Curculionidae –
Blosyrus inequalis Boh.*, *Leptomias* sp.* , *Myllocerus viridanus* (Fab.)*,
M. undecimpustulatus (Fab.)
Sucking pests - Hemiptera
Aphdidae — *Aphis craccivora* Koch
Pseudococcidae — *Nipaecoccus viridis* (Green)*
Pentatomidae — *Carbula scutellata* (Distant)*
Coreidae — *Cletus* sp.*
Cercopidae — *Clovia* sp.*
Tingidae — *Cochliochila bullita* (Stal.)
Rhyparochromidae — *Dieuches* sp.*
Monophlebidae — *Icerya aegyptiaca* (Douglas), *I. purchasi* Maskell
Ortheziidae — *Insignorthezia insignis* (Browne)*
Coccidae — *Parasaissetia nigra* (Nietner)*

* reported for the first time on *O. sanctum* in Karnataka

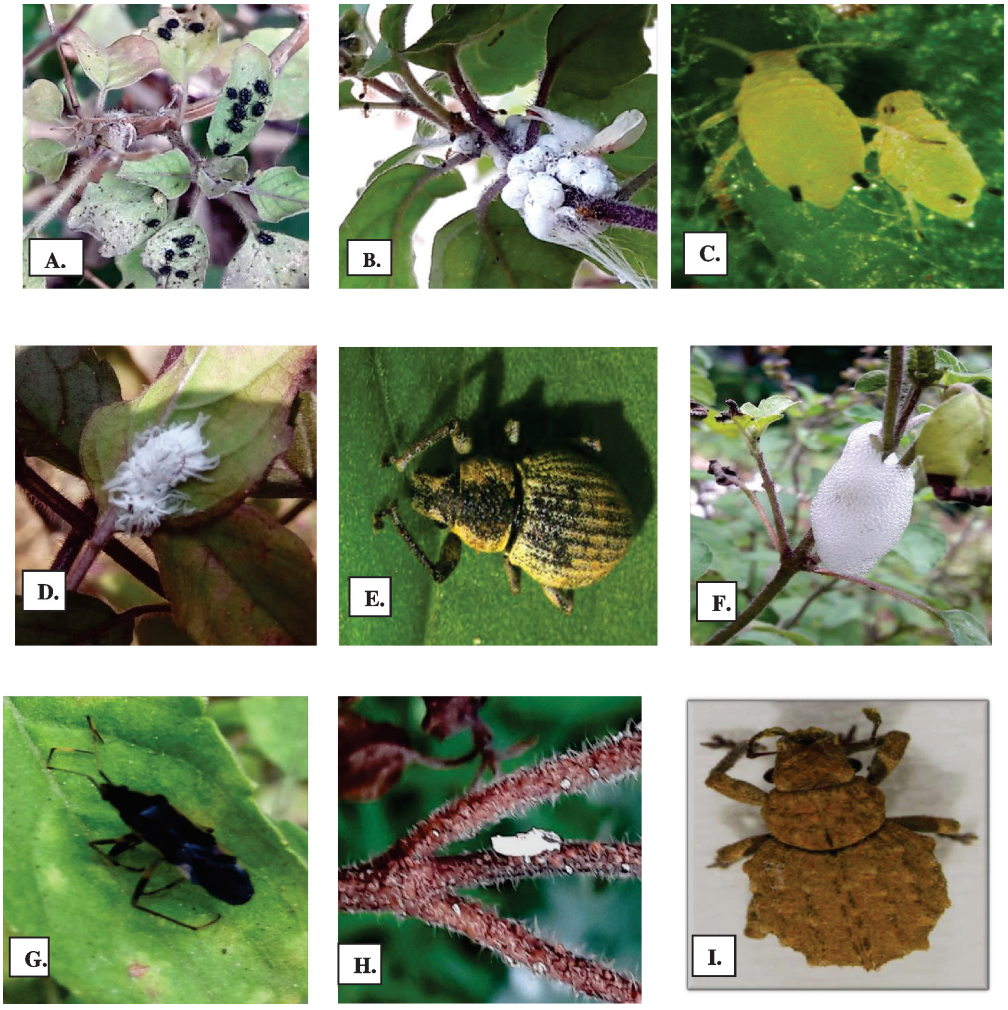

Plate 1. Insect pest of *O. sanctum*
A. *Cochlochila bullita* B. *Nipaecoccus viridis* C. *Aphis craccivora*
D. *Icerya aegyptiaca* E. *Blosyrus inequalis* F. *Clovia* sp.
G. *Dieuches* sp. H. *Insignorthezia insignis* I. *Leptomias* sp.

Among the sixteen species recorded on tulsi, eleven species *viz*., *Nipaecoccus viridis* (Green), *Blosyrus inequalis* Boh (Plate 1E.), *Clovia* sp. (Plate 1F), *C. scutellata, Cletus* sp., *Dieuches* sp. (Plate 1G.), *Insignorthezia insignis* (Browne) (Plate 1H), *Leptomias* sp. (Plate 1I.), *Myllocerus viridanus* (Fab.), *Hyposidra talaca* (Walker) and *Parasaissetia nigra* (Nietner) are first report for Karnataka. *H. talaca* is known to infest 45 host plants (Roy *et al.,* 2017), and *P. nigra* on 453 host plants (Vijay and Suresh, 2013 and Joshi *et al.,* 2017; https://scalenet.info/catalogue/). *Insignorthezia insignis* (Browne) is polyphagous (Varshney, 1992) infesting 175 plants (https://scalenet.info/catalogue/), and sandalwood (Sundararaj, 2011), and Lamiaceaeous plants like *Clerodendrum*, *Fragrant clerodendron* in Ethiopia (Fita and Wagari, 2018).

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Report of two species of spiders (Araneae: Linyphiidae: Erigoninae) from Western Ghats, Kerala, India

Anusmitha Domichan[*] and K. Sunil Jose[#]

Department of Zoology, Sacred Heart College, Thevera 682013*, Ernakulam, Kerala, India*;
[#]Department of Zoology, Deva Matha College, Kuravilangad, Kottayam 686633*, Kerala, India, Email: anusmithadomichan8@gmail.com; sunil32@gmail.com*

ABSTRACT: Two erigonine species *Oedothorax apicatus* Blackwall, 1850 and *Nasoona orissa* Tanasevitch, 2018 were reported from Central Kerala, India; *N. orissa* endemic to India, is reported after its description in 2018 and *O. apicatus* reported for the first time from India. The study contributed addition of two species to spider diversity of Kerala.

KEY WORDS: Spider diversity, erigonine species, description, addition

Erigoninae Emerton 1882, is the largest subfamily of Linyphiidae Blackwall 1859, with 411 genera. Only *Oedothorax stylus* Tanasevitch 2015, *O. cunur* Tanasevitch 2015, *O. kodaikanal* Tanasevitch 2015, *O. veloorensis* Domichan & Sunil Jose 2021*, O. cheruthoniensis* Domichan & Sunil Jose 2021, (endemic to India), and *O. retusus* Westring 1851, have been reported from Kerala so far (World spider catalog, 2022).

Linyphiid specimens were collected between 15 September and 28 December 2020 from Ernakulam and Kottayam districts, Kerala and preserved in ethyl alcohol (80%). Microphotographs were obtained with a Flexacam C1 coupled to an LEICA SAPO Automontage Microscope and processed with the Leica Application Suite X (LAS X) software. All dimensions are given in millimetres. Leg measurements are listed in the following order: total (femur, patella, tibia, metatarsus (except palp), tarsus). Female epigyne was cleared by boiling for 5 minutes in 10 per cent KOH (Domichan and Sunil Jose, 2021). The specimens are deposited in the Arachnology Lab, Deva Matha College, Kuravilangad, Kottayam, Kerala. Identity of specimens was confirmed by referring to Roberts (1987), Heimer and Nentwig (1991) and Tanasevitch (2018).

Abbreviations used in the text: ALE-anterior lateral eye; AME-anterior median eye; PLE-posterior lateral eye; PME-posterior median eye; R-receptacle; MP-median plate; P-paracymbium; PC-paraconvector; T-tegulum.

Genus: *Oedothorax* Bertkau 1883

Type species: *Oedothorax gibbosus* Blackwall 1841

Diagnosis: Males are characterized by modified carapace and by the shape of palpal tibia. The unmodified distal suprategular apophysis possess a pointed tooth in the middle. Male palp also possess an embolus with a small radix and a curved embolus

* Author for correspondence

proper, convector with a pointed distal apophysis. The epigynes are simple with: a median plate and spherical or elongated receptacles (Tanasevitch, 2015).

***Oedothorax apicatus* Blackwall 1850**

Material examined: India. 1 ♀; Kerala, Ernakulam, Kalady, Kanjoor; 10°08'39.0"N 76°25'04.4"E; 22 December 2020; leaf litter; coll. A. Domichan; DMCK LIN 032.

Diagnosis: Male carapace raised behind eyes to form a knob like projection. *O. retusus* is similar to *O. apicatus* and the central part of epigyne has a paler anterior and median area (fairly uniform in colour in *O. retusus*) and lacks a darker line anteriorly (anteriorly convex darker line present in *O. retusus*). Some epigynes of *O. apicatus* resemble those of *O. fuscus*, but differs due to relatively smaller seminal receptacles and a clear median light mark on the abdomen (Roberts, 1987).

Description: Female: Total length 2.62. Carapace yellowish with brown shades. Fovea 'U' shaped. Prosoma length 1.23, width 0.92. Ocular area slightly raised. Long, black, forward-directed hairs between eyes (Fig.1A). Enlarged white eyes, placed in black rings. Eye interdistances: AME-AME 0.01, AME-ALE 0.03, PME-PME 0.02, PME-PLE 0.03. Eye diameter: 0.02. Anterior and posterior lateral eyes juxtaposed. Anterior median eyes closely arranged. Chelicerae 0.32 long with stridulatory ridges. Sternum slightly blackish with dark borders. Heart-shaped sternum, wider near first coxa. Legs yellowish with white bands. Legs covered with short black hairs. Leg measurements: I 1.01 [0.34, 0.06, 0.13, 0.30, 0.18], II 0.92 [0.29, 0.06, 0.12, 0.28, 0.17], III 0.78 [0.23, 0.05, 0.10, 0.25, 0.15], IV 1.10 [0.38, 0.06, 0.15, 0.31, 0.20], palp [0.17, 0.05, 0.07, 0.12]. Leg formula 4123. Chaetotaxy 2-2-1-1. Trichobothrium present on metatarsi of all legs. TmI 0.26. Two long black bands, starting from the anterior side of abdomen, fuse to form a single big black patch at the posterior end. White patch on posterior end, in front of spinnerets (Fig.1B). Ventral side blackish yellow, with epigyne projecting outwards from epigastric groove. Opisthosoma length 1.39, width 0.79. Epigyne simple. Lateral lines of median plate of epigyne initially converge towards centre and then curved outwards, dorsally. Median plate wider than long. Receptacles oval-shaped and curved outwards, dorsally and ventrally (Fig. 2 A-B). Male: Unknown.

Distribution: Europe, Turkey, Caucasus, Russia (Europe to South Siberia), Kazakhstan, Iran, Central Asia, China, India (Kerala).

Genus: *Nasoona* Locket 1982

Type species: *Nasoona prominula* Locket 1982

Diagnosis: Genus *Nasoona* possess variegated abdomen and male carapace with a postocular elevation bearing a group of curved spines, stout spines or setae. The male palp is characterized by highly modified palpal tibia, poorly developed distal suprategular apophysis, reduced radix, presence of convector, presence of paraconvector. At present the paraconvector is only known in *Nasoona*. The epigyne in *Nasoona* has a epigynal cavity, divided by a septum or partially covered from above and small, spherical or bean like receptacles (Tanasevitch, 2018).

***Nasoona orissa* Tanasevitch 2018**

Material examined: India. 1 ♂; Kerala, Kottayam, Kumarakom Bird Sanctuary; 9°37'39.8"N 76°25'42.3"E; 16 September 2020; leaf litter; coll. A. Domichan; DMCK LIN 067.

Diagnosis: Distal apophysis of convector is divided into two lobes, distal process of paraconvector wide and bent. Paracymbium has an L-shaped proximal part and a distal part with a hook-shaped tip. Tibia divided into four lobes; keel-shaped prolateral part, long dorso-prolateral lobe, conical dorso-retrolateral side and a cup shaped retrolateral outgrowth. Convector is with a curved main body and distal apophasis with two uneven lobes. Paraconvector with protruded distal process (Tanasevitch, 2018).

Description: Male: Total length 2.59. Carapace golden brown. Prosoma length 1.18, width 0.72. The area behind the ocular region raised. A row of

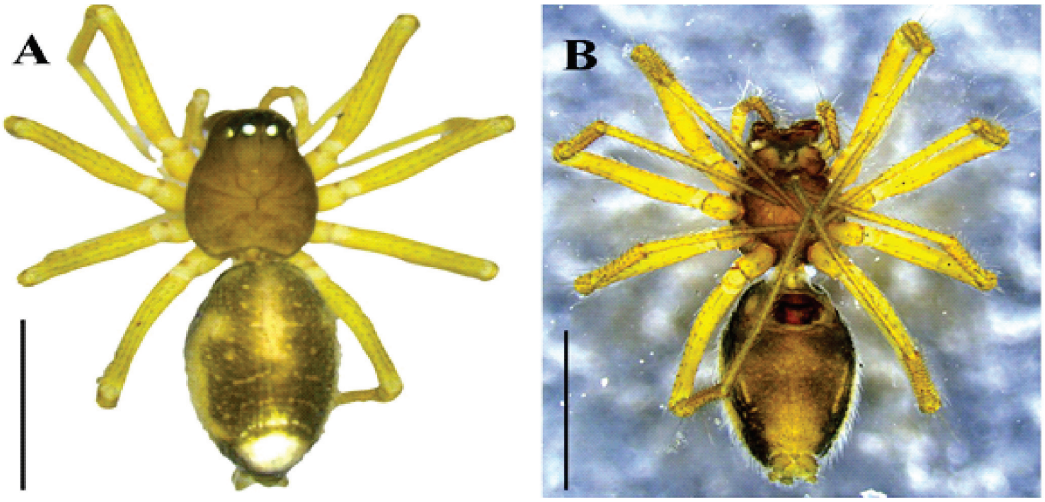

Fig.1 *Oedothorax apicatus* Blackwall, 1850. Female habitus
A. dorsal B. ventral. Scale bar: A-B 1mm.

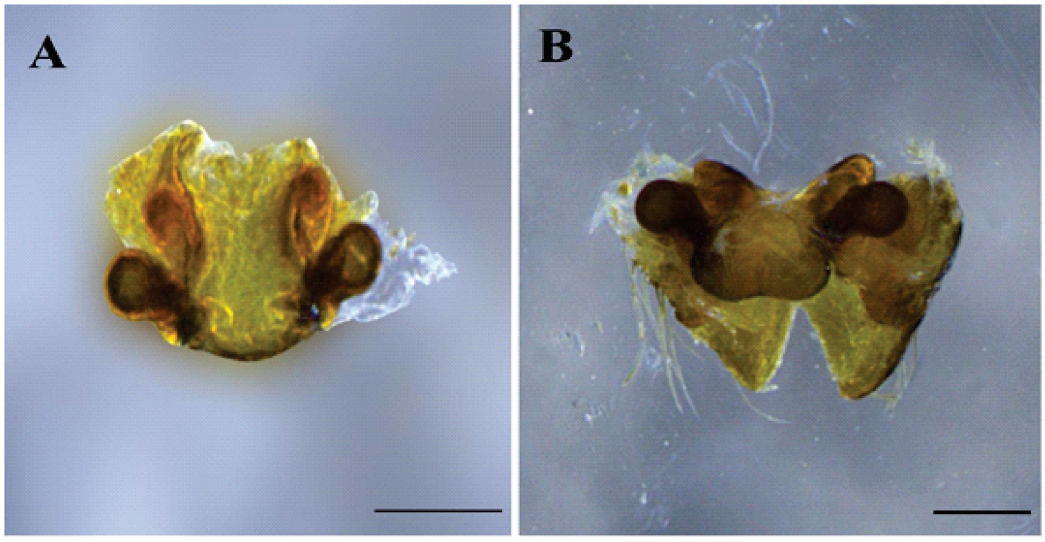

Fig.2 *Oedothorax apicatus* Blackwall, 1850. Female epigyne
A. ventral B. dorsal. Scale bar: A-B 0.1 mm.

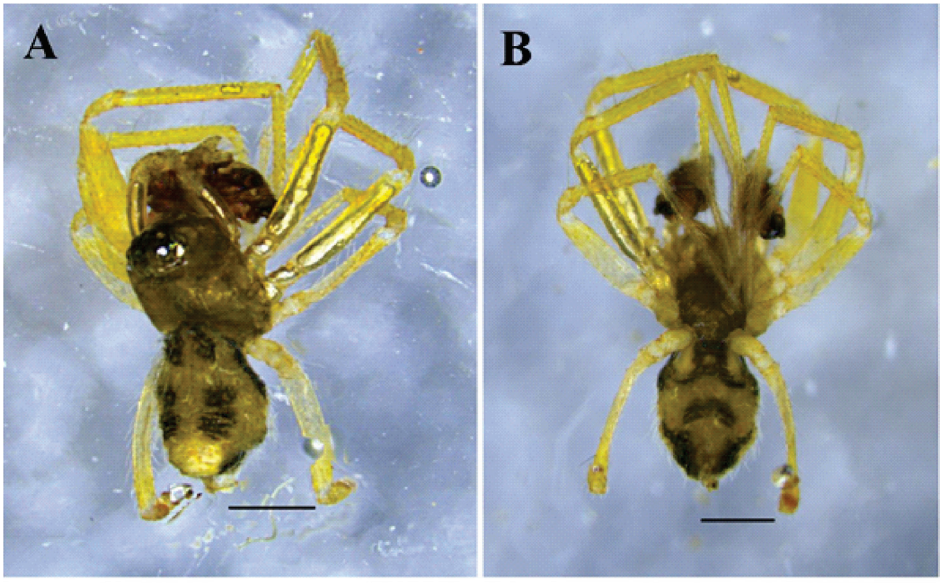

Fig.3 *Nasoona orissa* Tanasevitch, 2018 . Male habitus
A. dorsal view B. ventral view. Scale bar: A-B 1mm.

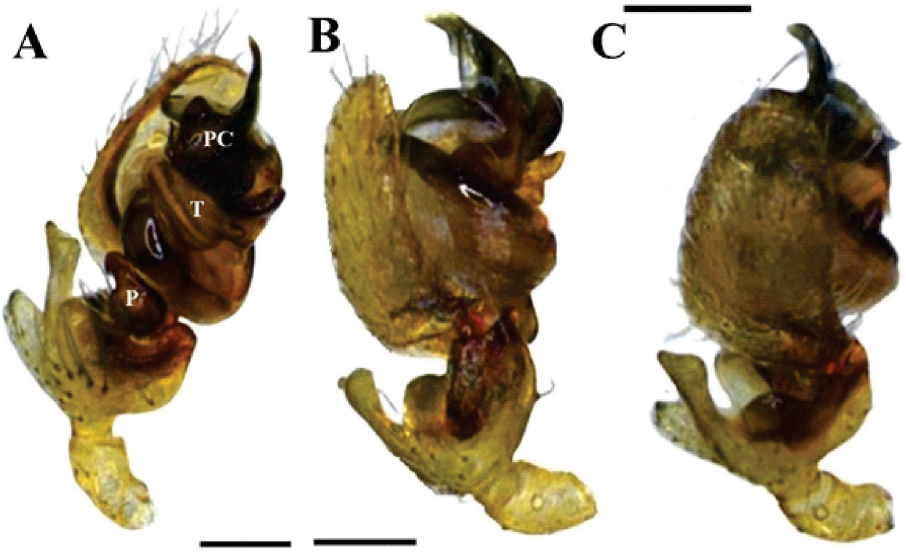

Fig.4 *Nasoona orissa* Tanasevitch, 2018. Male palp
A. ventral B. lateral C. dorso-lateral. Scale bar: A-C 0.1mm.

long, stout hairs behind the posterior median eyes. The hairs are forward-directed and slightly curved or bent (Fig.3A). Eye interdistances: AME-AME 0.01, AME-ALE 0.02, PME-PME 0.03, PME-PLE 0.04. Eye diameter: 0.02. Chelicerae 0.35 long, pale brownish. Sternum brownish, heart-shaped. Legs yellowish with black hairs and long spines. Measurements of legs and palp: I 3.48 [1.09, 0.34, 0.95, 0.89, 0.21], II 2.38 [1.01, 0.22, 0.45, 0.55, 0.15], III 1.69 [0.81, 0.20, 0.28, 0.31, 0.09], IV 3.67 [1.16, 0.37, 1.01, 0.92, 0.21], palp 1.40 [0.54, 0.14, 0.31, 0.47]. Leg formula 4123. Chaetotaxy 2-2-1-1. All legs have a trichobothrium on metatarsus. TmI 0.48. Abdomen yellowish with three pairs of black patches on dorsal side. Pair of black patches on anterior and posterior lateral sides. Pair of black patches on ventral sides (Fig. 3B). Opisthosoma length 1.41 width 0.65.

Palp: Prolateral part of tibia straight and slopes towards centre (keel shaped), dorso-prolateral part with a depression at the centre (M-shaped). Dorso-retrolateral side of tibia with a wide depression at the centre with small spines, ventro- retrolateral part cup or U shaped, dorsal prolateral outgrowth long, with a depression at the centre. Paracymbium has a narrow proximal part and wide distal part with a hook-shaped structure at the tip. Tegulum wide, narrows anteriorly, with a small projection. Subtegulum wide, almost oval shaped, anterior part narrower than posterior part. Embolus thin and long. Distal part of paraconvector long and pointed, whereas proximal part is wide. Distal apophysis of convector divided into two parts. The edges of convector and paraconvector serrated (Fig. 4 A - C). Female: Unknown

Distribution: India (Odisha, Kerala).

Oedothorax is one of the largest Linyphiid genus with 85 described species and *Nasoona* Locket 1982 has a worldwide record of 17 species (World spider catalog, 2022). The study contribute to Linyphiid diversity of Kerala by addition of two species, *O. apicatus* is reported for the first time from India and *N. crucifera* Thorell 1895 is the only representation of the genus *Nasoona* from Kerala (World spider catalog, 2022).

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Population characteristics of louse *Columbicola columbae* Linn. 1758 (Phthiraptera: Insecta) on pigeons in Uttar Pradesh, India

Satyapal Singh Rana[1], Nidhi Gupta[2*], Sanjay Kumar Bhardwaj[3] and Ghazi Khan[4]

[1]Department of Zoology, Shaheed Mangal Pandey, Govt. Girls P.G. College, Meerut, U.P. India;
[2,4] Department of Zoology, Govt. Raza P.G. College, Rampur, U.P. India;
[3] Department of Zoology, C.C.S. University, Meerut, U.P. India.
Email: dr.nidhigpta@gmail.com

ABSTRACT: Population characteristics of the pigeon louse, *Columbicola columbae* were recorded on 360 pigeons in the district Meerut, Uttar Pradesh during 2017. Parasitic infestation was 70 per cent with mean intensity of 58.5, with a range of 1-200. The louse exhibited skewed distribution on host body (variance/ mean = 44.5; index of discrepancy = 0.54; exponent of negative binomial = 0.34), however the frequency distribution pattern could not conform to negative binomial model. Sex ratio and adult nymph ratio of the louse were also skewed (M: F = 1: 1.3; A: N = 1: 1.2). Mean monthly prevalence and mean intensity of infestation exhibited significant positive correlation with the environmental temperature and photoperiod but not with relative humidity.

KEY WORDS: Ischnoceran louse, infestation, prevalence, intensity, seasonal variation

As many as nine species of Phthiraptera are known to infest the blue rock pigeon *Columba livia* (Price *et al*., 2003). Singh *et al*. (1998, 2000), Khan *et al*. (2009) and Rana *et al*. (2019) have given information on the population characteristics of a few species on Indian pigeons. Elsewhere Naz *et al*. (2010), Radfar *et al*. (2012), Copoka and Chiopehko (2013), Amaral *et al*. (2017), Boyd *et al*. (2017) and Djelmoudi *et al*. (2017) have tried to furnish information about the population levels of related phthirapteran species on pigeons belonging to different parts of world. An attempt has been made to supplement the information on the prevalence, frequency distribution pattern, population structure and the seasonal variation in the population of most common ischnoceran louse, *Columbicola columbae* Linn. 1758 (Phthiraptera: Insecta) on pigeons in Meerut region of Uttar Pradesh, India.

Thirty birds were subjected to delousing every month in the year 2017. Delousing was performed by modified fumigation method (with chloroform) adopted by Gupta *et al*. (2007). As many as 80 per cent louse load becomes recovered within 10-12 minutes. Plumage of bird was further examined under magnascope to take out the remaining louse load. The deloused pigeons were released to lead healthier life. The louse load was then separated stage wise (adults/nymphal stages) and then adult lice were further separated sex wise (under Stereozoom Trinocular Microscope). The software offered by Rozsa *et al*. (2000) was used to determine the prevalence, intensity of infestation,

* Author for correspondence

exponent of negative binomial distribution (k), index of discrepancy (D) as well as the goodness of fit between the observed and the expected frequency distribution (negative binomial). The nature of frequency distribution curve was noted by plotting the curve between the frequencies expected by the negative binomial distribution and the observed frequencies

Seventy percent of the examined pigeons (n= 360) carried the louse, *C. columbae* (Plate 1) with a mean intensity of 58.5/ pigeon; median intensity 50.0/ pigeon and sample mean abundance 40.9/ pigeon. Range of infestation was 1-200. The values of three measures of aggregation indices were - variance to mean ratio = 44.5, index of discrepancy (D) of Poulin = 0.536 and the exponent of negative binomial (k) = 0.344. The pattern of the frequency distribution was aggregated/ skewed but somehow it failed to conform the negative binomial model (Table 1).

In overall ratio, the females outnumbered the males in the natural population (M: F - 1: 1.3). The sex ratios in different months of year 2017 varied from 1: 1.2 to 1: 1.4, remained almost consistent throughout the year. The overall adult nymph ratio remained at 1: 1.2 (nymphal population slightly exceeded the adults). The adult nymph ratio varied from 1: 0.7 to 1: 1.4 in different months of the year. Interestingly, the adults dominated the nymphal population during the cooler months, January to March (adult nymph ratio (A:N) - 1: 0.7 to 1: 0.8) but thereafter the nymphal population dominated over the adults from April to December 2017 (1: 1.1 - 1: 1.4). The ratio of three nymphal instars was 1:1: 1 in April and 1: 1.4: 1.6 in November (Table 2).

The prevalence of infestation was minimum (60.0%) in January and February and gradually rose to 76.6% during July to September and returned to lower level (60.0%) in December (Fig. 1). Similarly, the mean intensity of infestation remained minimum in January (33.3/ pigeon) but gradually rose to 71.9/ pigeon in July, decreased to 56.2 in August, but reached the maximum 100.4 in September. The mean intensity of infestation decreased thereafter to 40.6 levels in December (Fig. 2). There was significant correlation between mean monthly prevalence of *C. columbae* and the mean monthly temperature ($r = 0.96$; $df = 10$; $p < 0.05$) and the photoperiod ($r = 0.845$; $df = 10$; $p < 0.05$) but not to the relative humidity ($r = 0.075$; $df = 10$; $p < 0.05$).

Table 1. Population characteristics of pigeon louse

Parameters	louse
Sample size	360
Infested	252
Range	1-200
Prevalence	70%
Mean intensity/ pigeon	58.5
Median intensity/ pigeon	50
Mean abundance/ pigeon	40.92
Variance/mean/ pigeon	44.45
Sample abundance/ pigeon	85.24
Index of discrepancy	0.536
K (negative binomial)	0.344
df	39
χ^2	324.51

Table 2. Population composition of pigeon louse during different months

Month	**M: F**	**A: N**	**I: II: III**
January	1:1.3	1:0.7	1:1.5:1.5
February	1:1.2	1:0.8	1:1.3:1.6
March	1:1.3	1:0.8	1:1.3:1.8
April	1:1.2	1:1.3	1:1:1
May	1:1.3	1:1.2	1:1.2:1.4
June	1:1.2	1:1.4	1:1.1:1.2
July	1:1.3	1:1.4	1:1.4:1.5
August	1:1.2	1:1.1	1:1.1:1.1
September	1:1.3	1:1.2	1:1.1:1.4
October	1:1.3	1:1.1	1:1.2:1.5
November	1:1.4	1:1.2	1:1.4:1.6
December	1:1.4	1:1.1	1:1.2:1.7
Over all	1:1.3	1:1.2	1:1.4:1.4

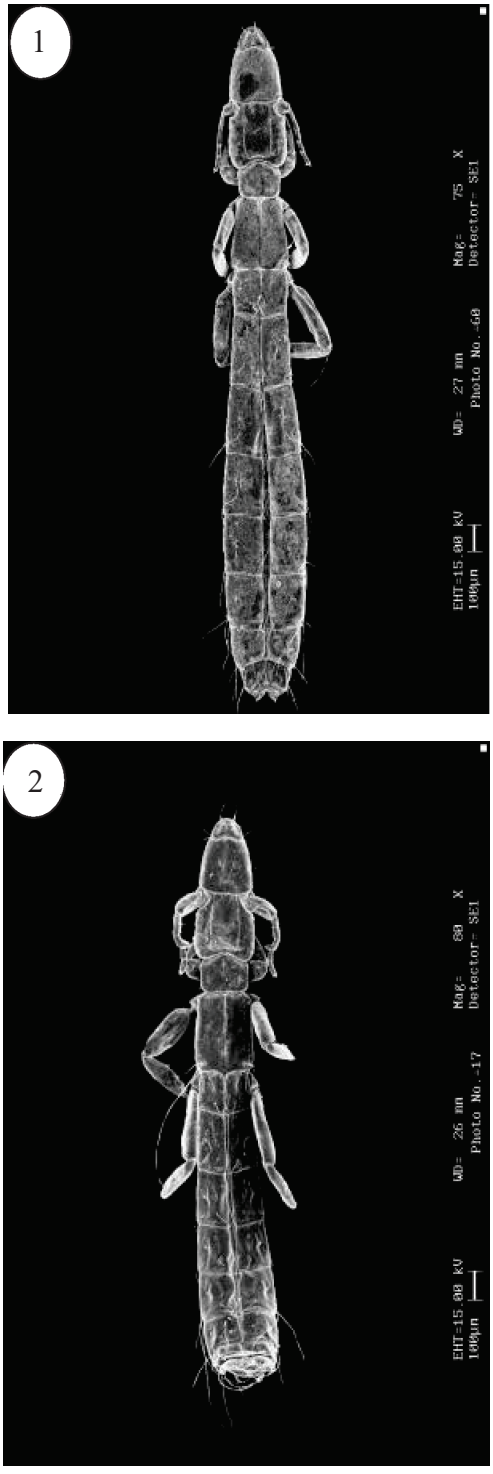

Plate I - SEM photographs of pigeon louse *C. columbae*: 1. Adult female; 2. Adult male

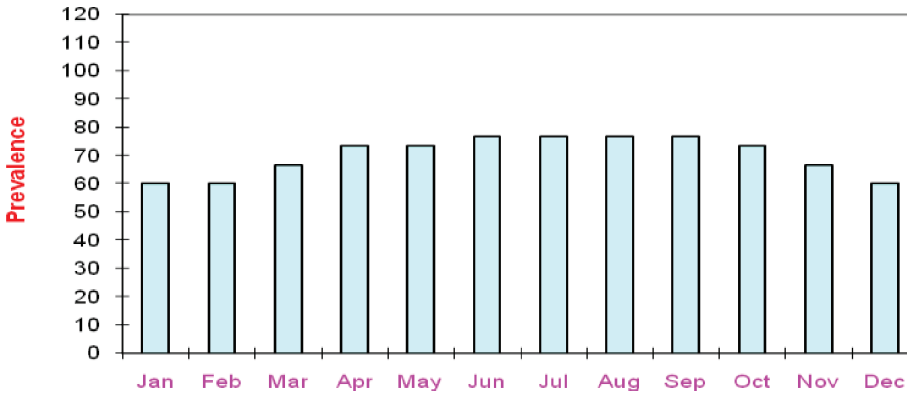

Fig.1. Mean monthly prevalence of *C. columbae* on 360 pigeons in district Meerut in the year 2017

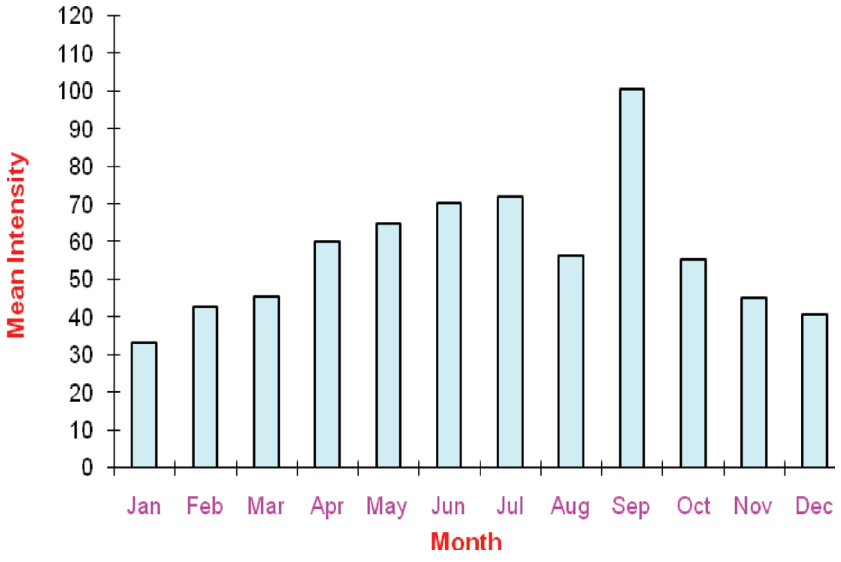

Fig. 2. Mean intensity of *C. columbae* on 360 pigeons in district Meerut in the year 2017.

In the same way, the mean monthly intensities were also significantly correlated with mean monthly temperature ($r = 0.772$; $df = 10$; $p < 0.05$) and photoperiod ($r = 0.646$; $df = 10$; $p < 0.05$) but not to relative humidity ($r = 0.44$; $df = 10$; $p < 0.05$, Table 3; Fig. 2).

Few phthirapterists (Naz *et al*., 2010; Radfar *et al*., 2012; Copoka and Chiopehko, 2013; Amaral *et al*., 2017; Boyd *et al*., 2017 and Djelmoudi *et al*., 2017) described the population characteristics of selected lice species of the pigeons from different parts of world. Singh *et al*. (1998) identified the presence of four species of pigeon lice on 50 pigeons in Dehradun. They observed the prevalence of *C. columbae* as 100 percent with mean intensity

Table 3. Correlation (r) between the mean monthly prevalence and infestation intensity of the pigeon louse with mean monthly temperature, relative humidity and photo period

Population	Temperature	R H	Photoperiod
Prevalence	$+0.957^{**}$	$+0.075^{NS}$	$+0.845^{**}$
Intensity	$+0.772^{**}$	$+0.0444^{NS}$	$+0.646^{**}$

of 141 lice/ bird. On the other hand, Khan *et al*. (2009) found the prevalence of this louse as 61.0% (mean intensity 53.4/ bird; ranges, 5-184; n = 205). During the present study, the prevalence of *C. columbae* on 360 pigeons in Meerut district of U.P. was 70.0 per cent (mean intensity - 58.5 per bird).

Bird lice are known to exhibit "skewed" clumped/ aggregated distribution on the body of their hosts (Marshall, 1981). In fact, the bird lice are not randomly distributed among their hosts (most of the infested hosts carry few lice but a few hosts harbor most of the louse load). Rekasi *et al*. (1997) have recommended the use of negative binomial distribution to describe the pattern of abundance of lice. During the present study, it was observed that the frequency distribution pattern of *C. columbae* was skewed but it could not be confirmed as negative binomial model.

Review of literature indicated that most of the phthirapteran species exhibit bias in sex ratio, with the female predominance in the population (Marshall, 1981). During the present study also the male, female ratio remained 1: 1.3, while nymphal population dominated over adult population (adult nymph ratio - 1: 1.2).

The avian lice generally peak in summers (Marshall, 1981). Several factors affect the seasonal variation in the population of avian lice. Singh *et al*. (2000) recorded the seasonal variation in the population of two pigeon lice on five birds (by *in situ* counts) and found positive correlation between the mean monthly live lice index and mean monthly temperature. Present study also indicated significant positive correlation between the mean monthly prevalence/intensity and monthly temperature, however, the correlation between the prevalence/ intensity of *C. columbae* and the relative humidity remained insignificant.

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Department of Agricultural Entomology, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695522, Kerala, India. web: www.entomon.in; Email: aae@kau.in

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