Uttar Pradesh Journal of Zoology

Volume 45, Issue 23, Page 39-47, 2024; Article no.UPJOZ.4367
ISSN: 0256-971X (P)

Feeding Behavior and Ovipositional Preference of the Tea Mosquito Bug, *Helopeltis theivora* (Hemiptera: Miridae) on Tea, *Camellia sinensis* (Theales: Theaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i234684

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4367

Original Research Article

Received: 04/10/2024
Accepted: 08/12/2024
Published: 14/12/2024

ABSTRACT

The main objective of the present study is to investigate the feeding behavior and ovipositional preference of the tea mosquito bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) on tea plants, *Camellia sinensis* (Theales: Theaceae). The feeding behavior studies revealed that the number of feeding punctures per day, feeding puncture diameter and total leaf area damaged by feeding gradually increased from the early nymphal stage to the adult stage. The maximum feeding

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Cite as: *Bharathi, N. Santhana, Annet Babu, A. Prabhu, S. Rishi Karan, M. L. Ajith, and T.P. Rabeesh. 2024. "Feeding Behavior and Ovipositional Preference of the Tea Mosquito Bug, Helopeltis Theivora (Hemiptera: Miridae) on Tea, Camellia Sinensis (Theales: Theaceae)". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (23):39-47. https://doi.org/10.56557/upjoz/2024/v45i234684.*

punctures were produced by females (155.4 ± 3.29), while the minimum was produced by 1st instar nymphs (87.0 ± 3.74). All stages of tea mosquitoes exhibited significant feeding behavior in all time intervals, with the maximum feeding behavior observed between 12:01 and 15:00. The ovipositional site preference study showed that female TMBs mostly prefer sites between the 1st and 2nd leaf for egg laying, while sites between the 1st and bud, and below the 3rd leaf, are least preferred. These study results may lead to the development of new cultural practices for the better management of the tea mosquito bug in tea ecosystems.

Keywords: Bio ecology; pest; tea ecosystem; oviposition.

1. INTRODUCTION

Tea plantations are a crucial component of the agricultural landscape, making a significant contribution to global tea markets. However, the productivity and quality of tea plantations in South India are often threatened by various pests (Ahuja et al., 2013). Among the insects that inhabit these environments, the tea mosquito bug, *Helopeltis theivora* stands out as a significant pest, capable of causing considerable damage to tea crops (Roy et al., 2015). This small, sap-sucking insect shows a distinct preference for tender shoots and young leaves of the tea plant, *Camellia sinensis* (Ahmed and Mamun, 2014). These delicate plant parts provide the insect with a rich source of nutrients and by using piercing-sucking mouthparts they puncture the plant tissues and feed on the sap (Thube et al., 2020). By understanding their feeding preferences, patterns and ovipositional behaviour, growers can implement targeted management strategies to minimize damage and protect the integrity of the tea crop. This, in turn, ensures the long-term viability and profitability of the tea plantations (Roy et al., 2009). This study focuses on comparing the number of feeding punctures, puncture diameters, feeding duration, and feeding site preferences of all stages of the tea mosquito bug including nymphs and adults. It also examines ovipositional site preferences.

2. MATERIALS AND METHODS

2.1 Experimental Conditions

All laboratory experiments were conducted at the Division of Entomology, UPASI TRF Tea Research Institute (10°16'09.4"N; 76°58'02.7"E), Valparai, Tamil Nadu, India. The laboratory experiments were conducted from October 2023 to January 2024 under the following conditions: temperatures ranged between 18°C and 23°C, with relative humidity between 70% and 90%.

2.2 Mass Rearing of Tea Mosquito Bug

Various life stages of TMB were collected from the UPASI experimental farm, Valparai and transported to the laboratory for mass rearing. The mass rearing process followed the method established by Sudhakaran (2000) with some minor adjustments. Mass rearing continued until the completion of the study. The necessary quantity of each TMB was obtained from the mass rearing unit and used for the current study.

2.3 Studies on Feeding Behavior

To study the feeding time of the tea mosquito bug in laboratory conditions, adults (male and female) and nymphs (1st to 5th instar) of the tea mosquito bug were released individually to feed on tea shoots kept inside a plastic jar with the upper end closed with muslin cloth. The insects were allowed to feed on the shoots, which were changed every 3 hours (00:00-03:00, 03:01-06:00, 06:01-09:00, 09:01-12:00, 12:01-15:00, 15:01-18:00, 18:01-21:00, 21:01-24:00). The study consisted of eight treatments (time intervals) and was replicated five times. After removing the shoots at specific times, the total feeding marks and punctures produced by each life stage of the TMB were counted, and the diameter of the feeding punctures was recorded. For the feeding site preference study, the feeding punctures from all three leaves and buds were counted and compared to identify which site on the tea shoot was most preferred by each stage of the tea mosquito bugs.

2.4 Studies on Ovipositional Site Preference

In the study on ovipositional site preference, newly mated females (>5 days old; < 10 days old) were used to study ovipositional site preference. Mated females were released into a plastic container consisting of tea shoots (three leaves and a bud) in a glass vial. Females were

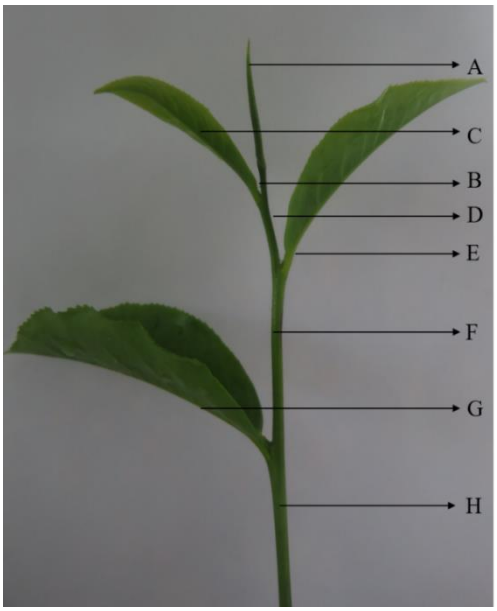

Fig. 1. A typical tea shoot with three leaves and a bud. A=Auxilary bud; B=Stalk between the first leaf and a bud; C=Lower side of the mid rib; D=Stalk between the first and second leaf; E=Lower side of the mid rib of the second leaf; F=Stalk between the second and third leaf; G=Lower side of the mid rib of the third leaf; H=Below the third leaf

allowed to lay eggs in the tea shoots for one day. A total of 35 females were evaluated, with each one considered a replication. After 24 hours, the tea shoots were removed, and the number of eggs in eight different places on a typical tea shoot (auxiliary bud, stalk between 1st and bud, lower side of 1st leaf mid rib, stalk between 1st and 2nd leaf, lower side of 2nd leaf mid rib, stalk between 2nd and 3rd leaf, lower side of 3rd leaf midrib and below 3rd leaf) were carefully counted and recorded. A detailed structure of a typical tea shoot is elucidated in Fig. 1.

2.5 Statistical Analysis

Data obtained from the feeding behavior and the number of feeding punctures made by various life stages of the tea mosquito bug during different time intervals were pooled and subjected to a One-way ANOVA. The means were separated using the protocols outlined by Gomez and Gomez (1984) and Panse and Sukhatme (1985).

3. RESULTS AND DISCUSSION

3.1 Feeding Punctures and Feeding Durations

An adult TMB takes approximately 3.5 to 4.2 minutes to complete a single feeding. The feeding durations of each life stage of TMB gradually increased from the nymph to the adult stage (Table 1). During adult feeding, a circular mark appears as soon as the tea mosquito's proboscis enters the leaf tissue. Initially, the feeding mark is transparent for up to 3 hours, after which an outer ring begins to form. For 48 hours, the outer circular ring gradually moves towards the centre of the feeding mark, eventually sealing it completely with a black colour within 4-5 days. The various stages of the feeding mark at different time intervals are depicted in Fig. 2.

The various life stages of the TMB exhibit different numbers of feeding punctures during their respective stages of development: first instar (87.0), second instar (90.6), third instar (104.8), fourth instar (160.6), fifth instar (121.6), adult male (163.4) and adult female (155.4) (Fig. 3). The number of feeding punctures gradually increased from the early nymphal stages to the adult stage. Additionally, the feeding puncture diameter and feeding durations of TMB varied and gradually increased in the different life stages of TMB (Table 1). The feeding puncture diameter of the first, second, third, fourth, fifth, adult male and adult female were 0.132mm, 0.166mm, 0.198mm, 0.176mm, 0.228mm, 0.254mm and 0.294mm, respectively. The total leaf area damaged by feeding per individual per

day was 11.4mm, 14.94mm, 20.39mm, 23.16mm, 27.59mm, 42.42mm and 45.96mm, respectively.

The present study found that the feeding punctures and their diameters gradually increased and showed a direct correlation with the age of the tea mosquitoes. In this study, females produced the maximum feeding punctures per day followed by males, 4th instar, 5th instar, 3rd instar, 2nd instar and 1st instar. Among the nymphal instars, the fifth instar exhibited a higher feeding puncture diameter and feeding duration. Similarly, among adults, the adult female showed a higher feeding puncture diameter and feeding duration. Sudhakaran (2000) and Roy et al. (2009) reported a similar observation that the number of feeding punctures is directly correlated with the age of TMB. The total area consumed by the tea mosquitoes also gradually increased with age, which may be due to the increased gut size and feeding rate during the growth of insects from early stages to later stages (Slansky and Scriber, 1985).

3.2 Feeding Site Preference

All developmental stages feed on all parts of a typical tea shoot (bud, first leaf, second leaf and third leaf). However, the number of feeding punctures varied significantly between the different parts of a typical tea shoot. The feeding site preference of each developmental stage of TMB was determined based on the number of feeding punctures in each respective site of a typical tea shoot. The results showed that the first leaf of a tea shoot was highly preferred by the first instars, second instars, third instars, fifth instars and adult females, with mean numbers of feeding punctures per day of 65.6, 41.6, 49, 45.4, and 59.2, respectively. On the other hand, the fourth instars and adult males showed mean numbers of feeding punctures of 82 and 72.6, respectively on the second leaf of a typical tea shoot. The feeding preference of TMB on a typical tea shoot was categorized as follows: first leaf > second leaf > bud > third leaf.

The feeding site preferences of different life stages of TMB during various time intervals are illustrated in Figs. 4 and 5. The first instar significantly fed on the first leaf of a tea shoot in all time intervals. The second instar mostly fed on both the first and second leaf of a shoot, with relatively low feeding observed between 06:01 and 09:00. The third instar also fed mostly on the first leaf of tea shoots. The fourth instars fed mostly on the first and second leaf of tea shoots. The fifth instars, adult males and adult females fed on all parts of tea shoots except the third leaf.

The feeding site preference of different life stages of the TMB naturally depends on various factors, including leaf surface morphology, surface texture, and surface wax composition (Chakraborty et al., 1978; Schoonhven et al., 1998; Roy et al., 2009). Additionally, the biochemical compositions (polyphenols, catechins, total carbohydrates proteins) and metabolites (flavonoids, phenolic acid, terpenoids and soluble sugar content) of various parts of the tea shoot also play a vital role in feeding site preference (Sudhakaran, 2000; Borthakur and Bora, 2023).

3.3 Feeding Time Interval

The various life stages of the tea mosquito bug including first instar, second instar, third instar, fourth instar, fifth instar, adult male and adult female exhibited highly significant feeding behavior across different time intervals (Table 2). The first instars showed higher feeding punctures between 12:01 and 15:00, with comparable results in the time interval between 18:01 and 21:00. They also exhibited lower feeding punctures during the time intervals of 06:01 - 09:00 and 21:01 - 24:00. Second instars displayed higher feeding punctures during the time interval of 12:01 - 15:00 and lower feeding punctures during 06:01 - 09:00. Third instars exhibited higher feeding punctures between 12:01 and 15:00, and lower feeding punctures during the intervals of 03:01 - 06:00, 09:01 - 12:00 and 00:00 - 03:00. Fourth instars had higher feeding punctures during the time intervals of 12:01 - 15:00, comparable to the interval 18:01 - 21:00, and lower feeding during the time interval of 15:01 - 18:00. Fifth instars showed higher and lower feeding punctures during the time intervals of 00:00 - 03:00 and 15:01 - 18:00, respectively. Male TMBs exhibited higher and lower feeding punctures during the time intervals of 06:01 - 09:00 and 09:01 - 12:00, respectively. Female TMBs showed higher feeding punctures between 12:01 and 15:00, with comparable results to the time intervals of 00:00 - 03:00, 03:01 - 06:00 and 15:01 - 18:00. From the study, all life stages of TMB expressed feeding behavior on all time intervals. However, most TMB life stages showed relatively high feeding punctures during the time intervals between 12.01 and 15:00 (Table 2).

Table 1. Feeding behavior of tea mosquito bug

Life stages	Feeding punctures / individual / day	Feeding puncture diameter (mm)	Feeding duration (in min.) / feeding spot	Total leaf area (in mm) damaged by feeding / individual / day
Instars				
First	87.0 ± 3.74 (46.90)^{a}	0.132 ± 0.013 (5.32)^{a}	2.62 ± 0.08 (9.51)^{a}	11.4
Second	90.6 ± 4.93 (47.84)^{ab}	0.166 ± 0.011 (5.40)^{b}	2.94 ± 0.11 (9.92)^{b}	14.94
Third	104.8 ± 6.53 (51.41)^{ab}	0.198 ± 0.008 (5.47)^{c}	3.46 ± 0.18 (10.56)^{d}	20.39
Fourth	160.6 ± 4.51 (63.56)^{c}	0.176 ± 0.005 (5.42)^{d}	3.22 ± 0.13 (10.27)^{c}	23.16
Fifth	121.6 ± 2.07 (55.36)^{abc}	0.228 ± 0.008 (5.54)^{e}	3.86 ± 0.19 (11.02)^{f}	27.59
Adults				
Male	163.4 ± 5.06 (64.06)^{c}	0.254 ± 0.008 (5.60)^{f}	3.62 ± 0.15 (10.75)^{e}	42.42
Female	155.4 ± 3.29 (62.53)^{c}	0.294 ± 0.006 (5.69)^{g}	3.9 ± 0.27 (11.06)^{f}	45.96
CD @ 5%	(9.18)	(0.01)	(0.18)	-
SEM	4.40	0.00	0.09	-
CV %	0.79	0.52	0.57	-
F value	115.48	271.90	62.04	-

Values are mean ± SD (n=5); Figures in parentheses are transformed values of √(x+1); Figures followed by the same alphabets in a vertical column are not significantly different at five per cent level.

Table 2. Number of feeding punctures made by various life stages of the tea mosquito bug during different time intervals.

T. nos.	Time	Mean no. of feeding punctures (*n*=5)						
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Male	Female
T1	00:00 - 03:00	10.6 (17.0)^{bc}	8.6 (15.49)^{b}	4.6 (11.76)^{a}	26.8 (26.25)^{cd}	29 (27.31)^{e}	18.6 (22.13)^{b}	31.6 (28.54)^{d}
T2	03:01 - 06:00	8.6 (15.48)^{ab}	15.4 (20.22)^{cd}	1.4 (7.71)^{a}	13.6 (19.08)^{b}	12.8 (18.56)^{bc}	17.4 (21.38)^{b}	29.2 (27.45)^{d}
T3	06:01 - 09:00	6.8 (13.87)^{a}	3.6 (10.63)^{a}	10 (16.53)^{b}	8.6 (15.46)^{ab}	14.8 (19.84)^{cd}	31.4 (28.33)^{c}	14.8 (19.85)^{c}
T4	09:01 - 12:00	12.8 (18.56)^{cd}	11.8 (17.89)^{bc}	4.6 (11.74)^{a}	33 (29.11)^{de}	14.2 (19.47)^{bcd}	3.6 (9.34)^{a}	5.4 (12.56)^{b}
T5	12:01 - 15:00	14 (19.35)^{d}	17.2 (21.30)^{d}	35.2 (30.02)^{d}	37.6 (31.02)^{e}	16.6 (20.94)^{cd}	28.4 (27.11)^{bc}	31.8 (28.62)^{d}
T6	15:01 - 18:00	12.6 (18.44)^{cd}	12.4 (18.24)^{bcd}	12.6 (18.44)^{bc}	5.8 (12.92)^{a}	6.6 (13.67)^{a}	19.2 (22.21)^{b}	27.6 (26.73)^{d}
T7	18:01 - 21:00	13.4 (18.95)^{d}	12 (17.80)^{bc}	16.8 (21.06)^{c}	24.2 (25.10)^{e}	18 (21.79)^{d}	26.2 (26.00)^{bc}	0 (5.00)^{a}
T8	21:01 - 24:00	8.2 (15.14)^{a}	9.6 (16.21)^{b}	19.6 (22.43)^{c}	11 (17.15)^{ab}	9.6 (16.25)^{ab}	18.6 (22.10)^{b}	15 (19.84)^{c}
CD @ 5%		(1.73)	(3.15)	(4.50)	(4.70)	(3.34)	(5.81)	(3.37)
SEM		0.83	1.51	2.16	2.25	1.60	2.79	1.62
CV %		1.51	2.63	3.26	2.22	2.08	2.70	1.64
F value		21.51	15.43	51.22	55.99	34.75	19.06	118.15

Figures in parentheses are transformed values of √(x+1); Figures followed by the same alphabets in a vertical column are not significantly different at five per cent level.

Table 3. Oviposition site preference of the tea mosquito bug

Oviposition site	Egg distribution %
Auxilary bud	04.74 (11)
Stalk between 1st and bud	02.59 (6)
Lower side of 1st leaf mid rib	11.64 (27)
Stalk between 1st and 2nd leaf	37.07 (86)
Lower side of 2nd leaf mid rib	17.67 (41)
Stalk between 2nd and 3rd leaf	17.24 (40)
Lower side of 3rd leaf midrib	06.03 (14)
Below third leaf	03.02 (7)
The value in the parenthesis are number of eggs.	

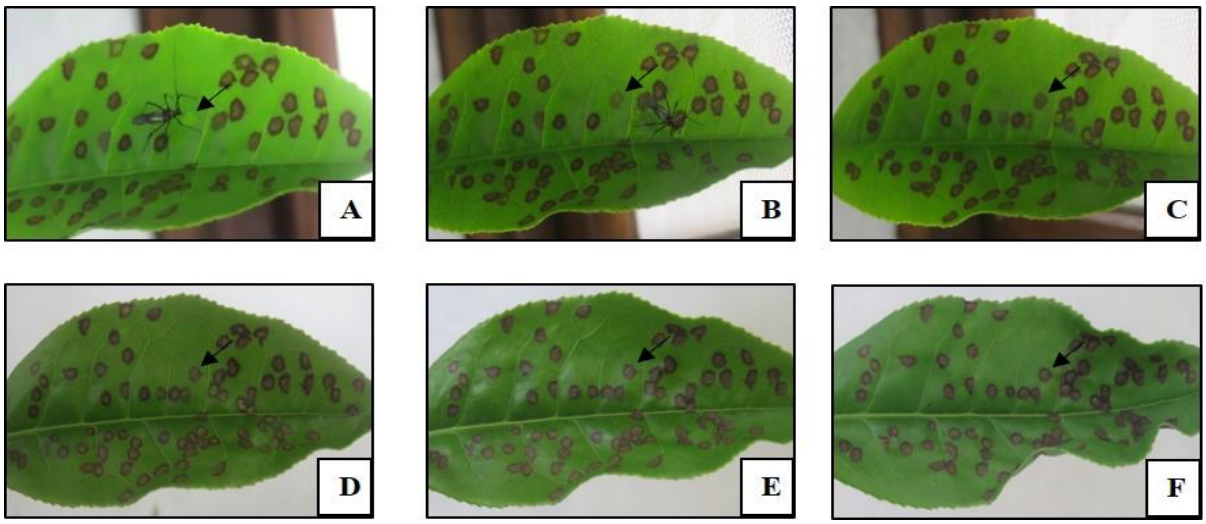

Fig. 2. Adult tea mosquito bugs feeding on tea leaves were observed at six different tine points: Immediately while feeding (A), I hour after feeding (B), 3 hours after feeding (C), 6 hours after feeding (D), 24 hours after feeding (E), and 48 hours after feeding (F)

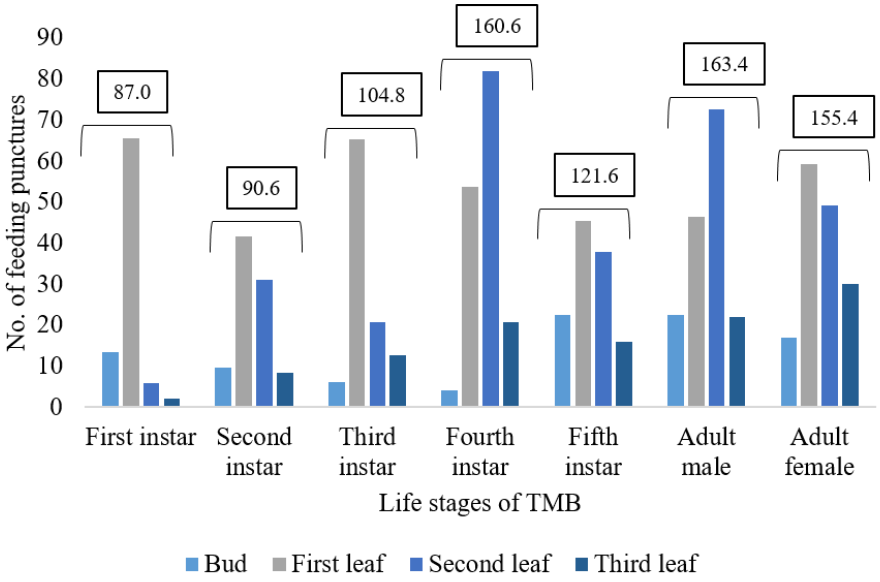

Fig. 3. Number of feeding punctures produced by each life stage of TMBs per day (The values in the box represent the total number of feeding punctures)

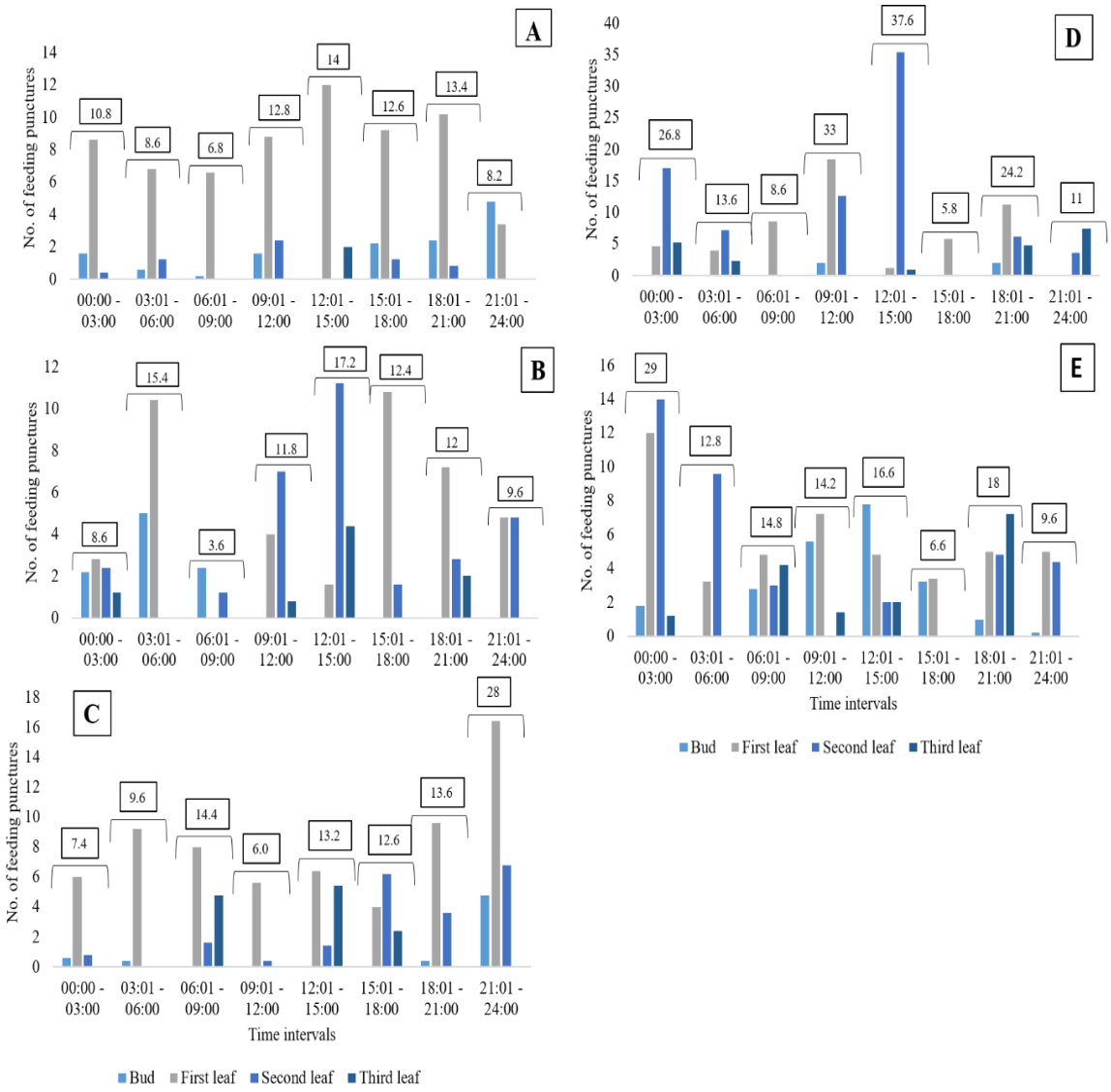

Fig. 4. Number of feeding punctures produced by 1st instar (A), 2nd instar (B), 3rd instar (C), 4th instar (D) and 5th instar (E) during different time intervals (The values in the box represent the total number of feeding punctures)

3.4 Ovipositional Site Preference

A total of 232 eggs were observed in the ovipositional site preference study. Female TMBs mostly prefer the site that stalks between the 1st and 2nd leaf, harboring 86 eggs (37.1%) followed by the lower side of the 2nd leaf mid rib and stalks between the 2nd and 3rd leaf with 41 (17.7%) and 40 (17.2%) eggs, respectively (Table 3). Similarly, the sites that stalk between the 1st and bud, and below the 3rd leaf are least preferred by female TMBs for egg laying. In a study similar to the present one, Sudhakaran, (2000) reported that stalks (broken end of plucked shoots) are the most preferred ovipositional site followed by green shoots, leaf petioles, the lower side of the mid rib and axillary buds, with egg distribution percentages of 52%, 26%, 17%, 4% and 1%, respectively. Additionally, Bhuyan and Bhattacharyya (2006) support the fact that tea mosquitoes predominantly prefer tea stems over leaves for oviposition. The higher oviposition rate in stem and stalks may be due to the softness of the tissues, making it easier to insert the ovipositor compared to other parts of the shoots.

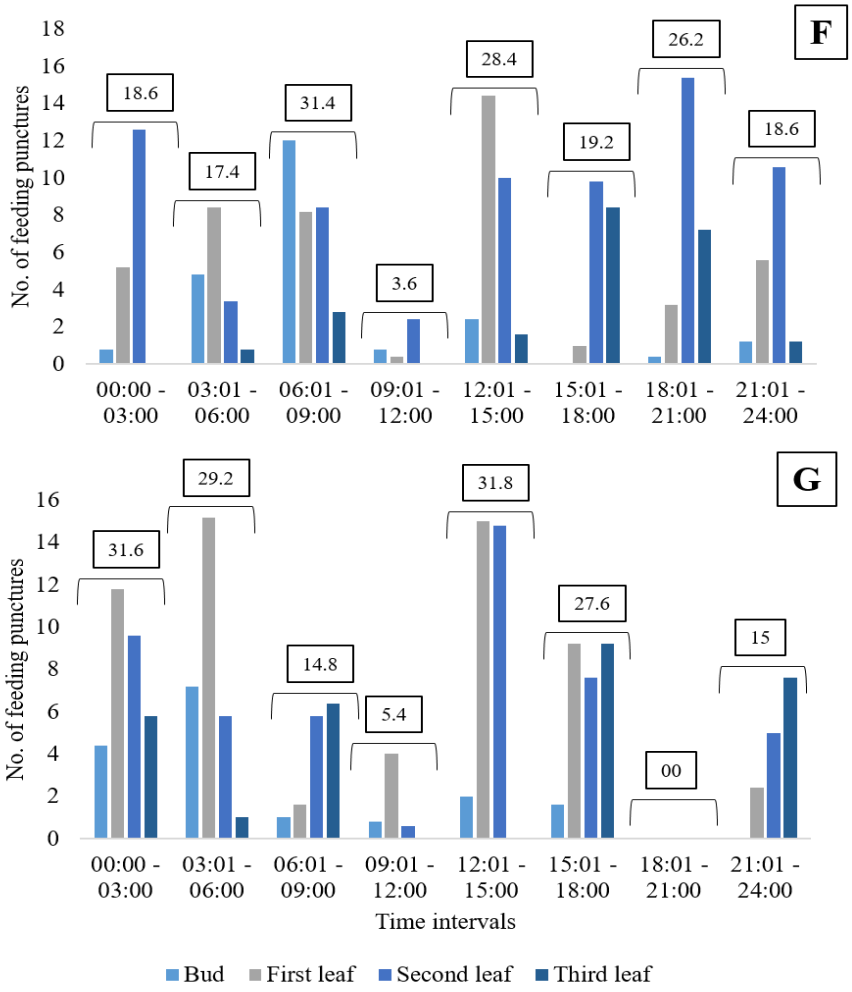

Fig. 5. Number of feeding punctures produced by male (M) and female (F) TMBs during different time intervals (The values in the box represent the total number of feeding punctures)

4. CONCLUSION

The study results revealed that the total number of feeding punctures per day and the total leaf area damaged by feeding gradually increased from the early nymphal stage to the adult stage of the tea mosquito bug. Similarly, all stages of the tea mosquito bug exhibited significant feeding behavior in all time intervals, with the highest feeding activity observed between 12:01 and 15:00. The study on oviposition site preference showed that female tea mosquitoes mostly prefer sites between the 1st and 2nd leaf for egg laying compared to other sites. Planters can identify pests in their crops by measuring the size of the punctures they make while feeding. This helps them take steps to manage infestations, such as closely monitoring plucking rounds, picking out plants with bugs, and using synthetic chemical insecticides. These study results could potentially lead to the development of new cultural practices for better management of the tea mosquito bug in tea ecosystems.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

The authors are thankful to the Director, UPASI TRF Tea Research Institute, Valparai for his support during the study period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4367