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RESEARCH

Laboratory rearing of tea mosquito bug, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) on cocoa (*Theobroma cacao* L.)

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Abstract Tea mosquito bug (TMB), *Helopeltis theivora* is a most serious pest on cocoa worldwide that inflicts huge economic losses under epidemic situations. Laboratory colonies are necessary to perform year-round research to develop suitable management strategies for this pest. This study aimed to establish a population of TMB on suitable cocoa-feeding substrates under laboratory conditions. We used green cocoa pods and tender shoots as a food substrate and compared the biological parameters of *H. theivora* between them. The adult females preferred to lay more eggs on pods (32.20 ± 1.86 eggs/female) either singly or in a small group, and low fecundity was observed on shoots (20.75 ± 1.16 eggs/female). Meanwhile, egg hatching was highest on pods (92.48 ± 2.91%) than on shoots (74.08 ± 3.73%). This pest consists of five nymphal instars and the total nymphal duration was 13.53 ± 1.26 and 14.73 ± 0.85 days on pods and shoots, respectively. In addition, the per cent survival of *H. theivora* was highest on pods (78.66 ± 3.46) and lowest on shoots (51.85 ± 2.33). Significant differences were recorded in adult longevity and the mean life span of males and females were 13.17 ± 1.58 and 16.63 ± 1.64 days on pods; 11.10 ± 1.41 and 14.36 ± 1.53 days on shoots, respectively. Further, females lived longer than males in both substrates. Although, TMB will survive both on pods and tender shoots; rearing on pods could be more suitable and reliable to establish a sizable population of *H. theivora* under laboratory conditions.

Keywords Cocoa · Fecundity · *Helopeltis theivora* · Pods · Shoots · Survival

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Introduction

Cocoa (*Theobroma cacao* L.: Malvaceae) is one of the important beverage crops and native to South America, specifically in the region of the American rainforest (Ploetz, 2007). It is widely cultivated in humid tropical countries around the world (WCF, 2014); however, the maximum production comes from West Africa, Latin America and South East Asia (Lahive et al., 2019). In India, it is cultivated approximately in 1.03 lakh hectares and produces around 27,000 metric tonnes annually (DCCD, 2023). The global demand for cocoa has increased over the past 15 years, leading to an expansion of cultivated area and intensification of production (WCF 2014: Tothmihaly et al., 2019). There are several production constraints limiting its productivity and among them, insect pests are the major factor affecting cocoa production (Madhu et al., 2023). More than 50 insect pests including sap feeders, defoliators, capsules and wood borers are known to infest globally; among them, the tea mosquito bug is a major concern for cocoa production (Entwistle, 1972; Shilpa et al., 2022; Thube et al., 2022).

Tea mosquito bug (TMB), *Helopetis theivora* (Heteroptera: Miridae) is a destructive pest on various agricultural, horticultural and plantation crops including tea, cocoa, cashew, guava, pomegranate and others (Roy et al., 2015). In India, three species of *Helopeltis* are present such as *H. theivora*, Waterhouse, *H. antonii* Waterhouse and *H. bradyi* Signoret (Thube et al., 2020*)*; all of them are commonly referred to as TMB and distributed both in southern and northern hemisphere that includes Africa, Australia and Asian continent (CABI, 1992; Stonedahl, 1991). All three species of *Helopeltis* have been reported on the cocoa ecosystem causing yield loss of up to 40% (CPCRI, 1993; Padi, 1997). Among these, *H. theivora* is the dominant species followed by *H. bradyi* (Thube et al., 2020). Both nymphs and adults of TMB suck the sap from young shoots, peduncles and pods by inserting its proboscis causing a necrotic spot. The affected shoots show a die back appearance; whereas on pods, it appears as a dark circular lesion and gradually becomes hardened with scars. Severe infestation results in pod malformation and premature drop (Alagar & Bhat, 2017).

A feasible laboratory-rearing protocol is key to conduct predefined experiments on target pests successfully. Presently, no artificial diets are available for rearing *H. theivora* and completely depend on natural hosts to sustain the populations under laboratory conditions. However, the information available on the establishment of *H. theivora* populations on respective hosts is scarce. This practically results in a critical gap between understanding pest behaviour and developing suitable management strategies. Therefore, the authors feel it is of utmost necessity to rear the insect on the natural host to understand pest interactions with climate change, natural enemies, insecticide molecules and others to develop appropriate management options against *H. theivora*.

Material and methods

Insect collection

The nymphs (3rd to 5th instar) of TMB were collected from infested cocoa orchards of ICAR – Central Plantation Crops Research Institute, Regional Station, Vittal (12°45 N, 75°4 E) using a fine brush (Camlin SR 67 No.1) and transferred into a 20 ml glass vial (BOROSIL®). Insects were kept in a climate-controlled room and the rearing conditions were 27 ± 2 ^{0}C, $65 \pm 5\%$ RH and photophase of 16:8 h (L: D). The initial colony of TMB were maintained on green cocoa pods (30–40 days old, 100 – 150 g) till the adult stage in a customized wooden cage (40×40 x 40 cm). Species-level identity was confirmed using the keys as described by Stonedahl (1991). Among species, *H. theivora* was dominant on cocoa and we used the same for rearing under laboratory conditions.

Rearing of H. theivora on cocoa shoots and pods

Both cocoa shoots (6–8 cm) and green pods (30–40 days old, 100-50 g) were used as food substrates to study the demographic parameters of *H. theivora*. The shoot and pods were washed with deionized water and air dried under a ceiling fan at room temperature. Air dried shoots were trimmed with scissors, leaving only the top 2–3 leaves and placed in a 5 ml glass vial (BOROSIL®) containing 10% sucrose solution. Similarly, the pedicel of the pod was wrapped with 10% sucrose-moistened cotton to increase the shelf life. Both shoots and pods were kept in separate cages. About 200 adults were collected from the initial colony and sexed based on morphological characters (Roy et al., 2015). Ten adult couples (One day old) were chosen and released on each substrate with five replicates. The infested shoots/pods were checked daily and the ovipositional site was observed under a stereo microscope (Leica MZ 75). The oviposited shoots/pods were replaced with fresh shoots/pods on alternate days till the adult's life span. The oviposition site of *H. theivora* was located based on the presence of two unequal chorionic processes exposed outside for each egg (Fig. 1); these processes represent operculum and respiratory horns respectively (Gope & Handique, 1991). The oviposited shoots/pods were kept in separate plastic containers (45×45x45cm), covered with muslin cloth and observed for nymphal emergence. The emergence was calculated by the total number of nymphs that emerged divided by the total number of eggs laid on substrates $\times$ 100. The emerged nymphs from shoots/

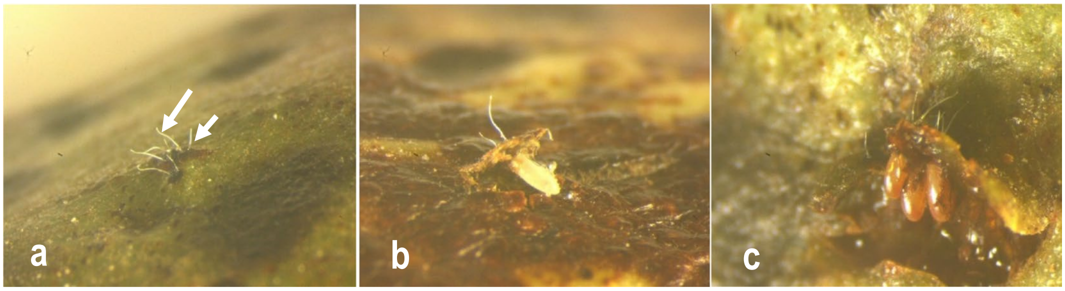

Fig. 1 Presence of unequal chorionic processes at the ovipositional site (**a**) and *H. theivora* lay egg either single (**b**) or in a small group (**c**)

pods were maintained on respective food substrate till the adult stage and recorded their developmental period at each instar. Upon adult emergence, males and females were paired and the same protocol was continued from generation after generation on respective food substrates.

Statistical analysis

Data on the developmental period of egg, nymph and adult longevity was analysed using One-way analysis of variance (ANOVA) with Tukey's honestly significant difference as *post-hoc* test at $\alpha = 0.05$ level. *t* test was performed to compare the percent nymphal emergence, survival rate and fecundity between shoots and pods; the percent data were arcsine root transformed to normalize the variance before being analysed in SPSS software (*ver 20*, IBM, NY, USA).

Results

Developmental period of H. theivora on shoot vs pod

Adults of *H. theivora* laid eggs inside the tissue projecting two unequal chorionic processes outside the ovipositional site and the incubation period was varied from 1.26 ± 0.07 to 1.33 ± 0.08 days on pods and shoots; upon hatching it underwent five nymphal instars before reaching adult stage (Fig. 2) and the total nymphal period was varied

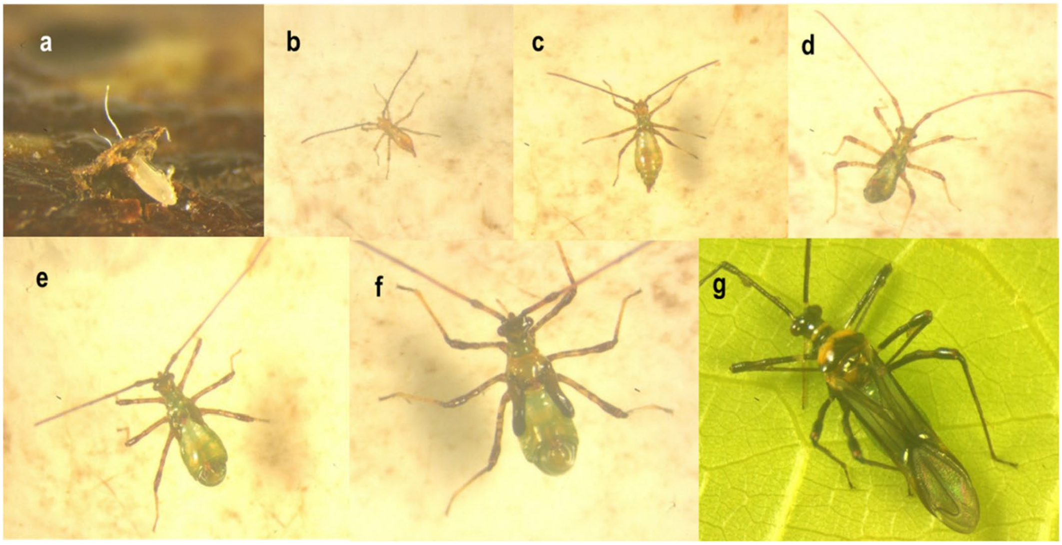

Fig. 2 Developmental stages of *H. theivora*. (**a**) egg, (**b**) I instar (**c**) II instar, (**d**) III instar, (**e**) IV instar, (**f**) V instar, (**g**) Adult

Table 1 Developmental period of *H. theivora* on cocoa shoots and pods

Treatments	*N*	Developmental period (days)						Total nymphal duration (days)	Adult longevity (days)	
		Egg	I instar	II instar	III instar	IV instar	V instar		Male	Female
Shoots	200	1.33 ± 0.08	2.30 ± 0.14	2.64 ± 0.18	2.96 ± 0.12	3.33 ± 0.28	3.50 ± 0.13	14.73 ± 0.85	11.10 ± 1.41	14.36 ± 1.53
Pods	200	1.26 ± 0.07	2.08 ± 0.12	2.56 ± 0.19	2.67 ± 0.24	2.94 ± 0.31	3.28 ± 0.40	13.53 ± 1.26	13.17 ± 1.58	16.63 ± 1.64
P value		0.581	0.528	0.691	0.400	0.008	0.412	0.434	0.023	0.132

N – Number of individuals used for the study. Developmental duration was analysed using One-way ANOVA with Tukey's honestly significant difference as post hoc test at $\alpha = 0.05$ level

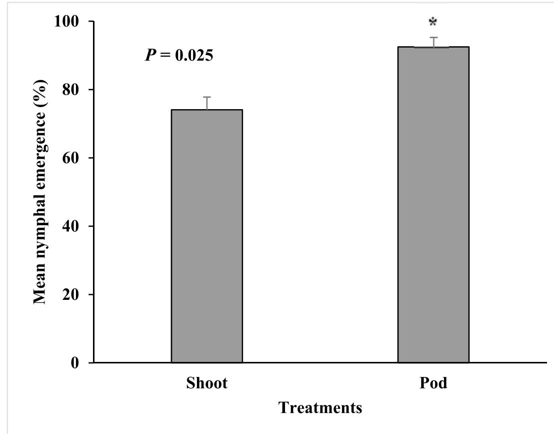

Fig. 3 Mean nymphal emergence of *H. theivora* on shoots and pods. Bars labelled with the asterisk symbol are significantly different according to ANOVA, $p < 0.05$ with Tukey honestly significant difference test

from 14.73 ± 0.85 to 13.53 ± 1.26 days on cocoa shoots and pods, respectively (Table 1). No significant differences were recorded in early (I and II) instars, whereas, later instars (III to V) exhibited significant differences. The developmental duration of immature stages of *H. theivora* on pods was relatively shorter than on shoots. However, there were significant differences in the longevity of male (df = 1, F = 5.44, p = 0.023) and female adults (df = 1, F = 2.33, p = 0.132), and they lived longer on pods than on shoots.

Mean emergence of nymphs and Survival rate

A significant difference was recorded both in the nymphal emergence ($P = 0.020$) and survival ($P = 0.025$) of *H. theivora*, when reared on cocoa pods and shoots. The mean emergence of nymphs and the rate of survival was relatively higher on pods as compared to that of shoots and these were 92.48 ± 2.91 and 74.08 ± 3.73 (Fig. 3); 78.66 ± 3.46 and 51.85 ± 2.33% (Fig. 4), respectively.

Fecundity and adult longevity of H. theivora

Significant differences were recorded in the fecundity of *H. theivora* between the two substrates ($P = 0.026$). The gravid female laid the maximum

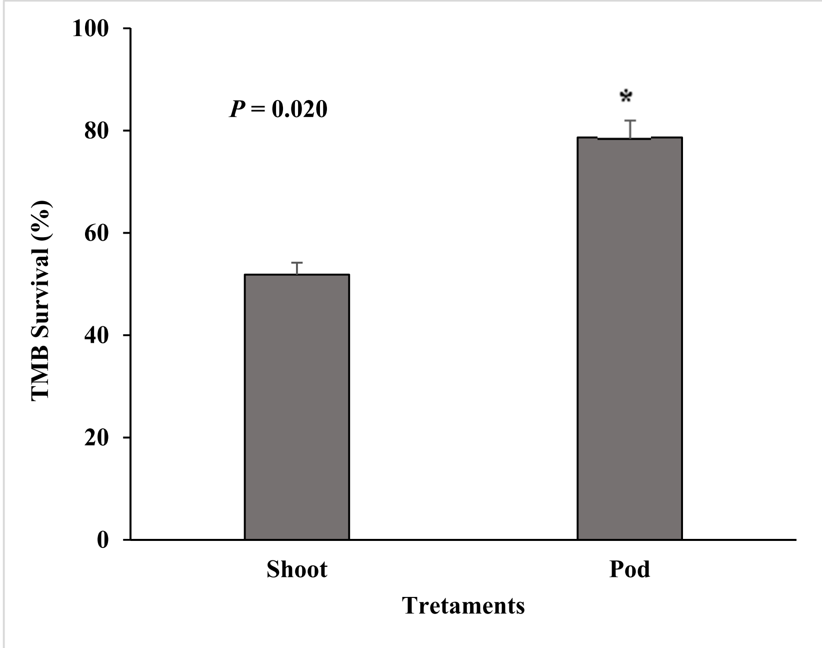

Fig. 4 The percent survival of *H. theivora* on shoots and pods. Bars labelled with the asterisk symbol are significantly different according to ANOVA, $p < 0.05$ with Tukey honestly significant difference test

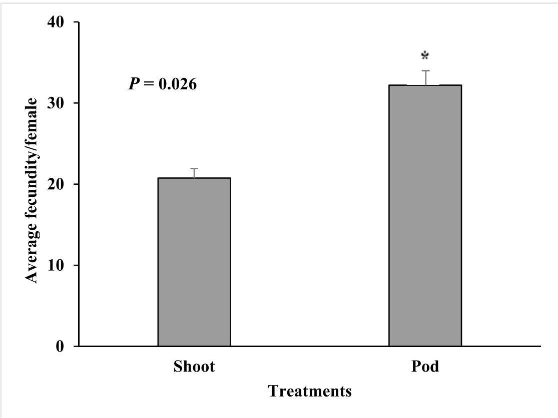

Fig. 5 Average fecundity (eggs/female) of *H. theivora* on shoots and pods. The total number of eggs laid on pods and shoots are significantly different according to ANOVA, $p < 0.05$ with Tukey honestly significant difference test

number of eggs on pods as compared to shoots and the mean fecundity was 32.20 ± 1.86 and 20.75 ± 1.16 eggs/female, respectively (Fig. 5). However, no significant differences were observed in adult longevity and the longevity of males and females were 13.17 ± 1.58 and 16.63 ± 1.64 days on pods; 11.10 ± 1.41 and 14.36 ± 1.53 days on shoots, respectively. Furthermore, females lived longer than males in both food substrates (Table 1).

Discussion

The establishment of TMB populations using appropriate feeding substrates is necessary to conduct basic and applied research aspects to develop sustainable management strategies. In our study, rearing *H. theivora* on green cocoa pods appears to be a promising method, as the pods remain in good condition for an extended period, effectively supporting the growth and development of all life stages of *H. theivora*. Gravid females laid eggs inside the tissue by protruding two unequal chorionic processes for each egg; however, cocoa pod favours egg maturation relatively earlier than shoot and contributes to maximum emergence of nymphs. In addition, the development period of nymphs of *H. theivora* from the first to fifth instar was quite shorter (13.40 ± 1.33 days) when reared on cocoa pods; whereas, it spends relatively more time on tender cocoa shoots to complete its nymphal period (14.73 ± 0.75 days). The possible reason could be the difference in nutritional value between the pod and shoot to sustain the early stages of *H. theivora* (Hougni et al., 2021). To support our study, Awang et al. (1998) from Malaysia reported that the nutritional value of cocoa shoots was inferior as compared to pods; hence, the development of *H. theivora* on pods was faster than on shoots. Few studies reported that the mean egg hatchability of *H. theivora* between cashew and cocoa was 66 and 79%, respectively, and this may be due to differences in nutritional composition (Srikumar & Bhat, 2012; Thube et al., 2020). In contrast to our study, Alagar and Bhat (2017) reported that the egg hatchability of *H. bradyi* was higher on tender shoots (77.1%) than on pods (56.5%). This could have been attributed to the species preference of TMB on different substrates. Furthermore, the shelf life of pods can be enhanced to some extent by wrapping of pod pedicel with cotton moistened with 10% sucrose solution to avoid loss of moisture and also prevent pod decay from secondary infection of microorganisms.

In general, fecundity is a key indicator to measure the reproductive fitness of any insect (Pincheira-Donoso & Hunt, 2017; Leather, 2018; Madhu & Mohan, 2021, Madhu & Muralimohan, 2022; Madhu et al., 2021). In our study, significant differences were recorded in the egg laying potential of *H. theivora* between cocoa pods and shoots. After mating, gravid females laid the maximum number of eggs on cocoa pods (32.20 ± 1.86/female), whereas it was substantially lower on shoots (20.75 ± 1.15 eggs/female). Similar to our study, Thube et al. (2020) showed that the preference of *H. theivora* was comparatively higher on pods and laid the maximum number of eggs, whereas *H. bradyi* preferred cocoa shoots and laid more eggs than pods (Alagar and Bhat, 2017). The variations in preference from species to species were quite natural when they occurred simultaneously on the same host to avoid competition by partitioning the resources (Liu et al., 2023). Although, both male and female adults of *H. theivora* lived slightly longer on cocoa pods than on shoots; yet, no significant differences were observed between them. Nevertheless, female adults outlived males in both pods and shoots. This was similar to the observations of Thube et al. (2020), who reported that adult longevity of males and females of *H. theivora* on cocoa was 15.67 ± 0.68 and 20.87 ± 0.72 days, respectively, and females of *Helopeltis spp.* lived longer than males (Roy et al., 2015).

In conclusion, our results demonstrated that green cocoa pods would be the most suitable feeding substrate for laboratory rearing of *H. theivora* compared to shoots. The growth and development of *H. theivora* reared on cocoa pods is quite stable and the biological parameters thus obtained are reasonably comparable with those obtained by rearing it on shoots. However, cocoa pods are seasonal and available twice a year; hence, cocoa shoots can be used to maintain the population of *H. theivora* during off season.

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Author contributions MTN, SEK—Conducted the experiment and wrote the manuscript. RTTP, SM—Prepared the Figs. 2–5. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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