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BEHAVIOURAL RESPONSE AND MASS TRAPPING OF MALES OF TEA MOSQUITO BUG *HELOPELTIS THEIVORA* WATERHOUSE

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ABSTRACT

Tea mosquito bug (TMB) *Helopeltis theivora* Waterhouse is one of the major pests in both south and north Indian tea plantations. This study was conducted to find out the behavioral responses of male TMB for developing a mass trapping technology. The study shows that TMB male's behavioral response was high towards the thorax extract of virgin females, virgin female body wash and live virgin females. Males' antennal responses were high towards to thorax extract of virgin females; the same sources performed well against male TMB under field conditions too. Caged virgin females provide prolonged attraction throughout their lifespan; in contrast, thorax and virgin female body extracts' attraction rate lasted only up to 24 hr but achieved a significant attraction rate over male TMB. Caged female and thorax extract could be the effective tools for mass trapping, monitoring and controlling of TMB through mating disruption.

Key words: *Helopeltis theivora*, male, female, body extracts, thorax, virgin female body extract, natural pheromones, electroantennogram, Y-tube, wind tunnel, attraction, antennal responses

The Miridae (plant bugs) being the largest family of the Heteroptera, has 1200 genera, including 10,000 described species. Mostly plant bugs are phytophagous. All phytophagous Miridae are considered as a serious pests of many crops because of their wide host range (Zhang and Aldrich, 2003). Among them, *Helopeltis theivora* Waterhouse commonly known as tea mosquito bug (TMB), severely damages the tea plant's young foliage and is thus a major pest in both north and south Indian tea plantations (Mukhopadhyay and Roy, 2009). Both nymphs and adults suck the sap of young buds, leaves and tender stems. While sucking, it releases toxic saliva at the feeding spots leading to the breakdown of tissues surrounding the puncture, which becomes dark brown shrunken spots each after 24 hr (Roy et al., 2009). Presently, effective management is been carried out through synthetic chemicals which are approved by the Tea Board of India through the Plant Protection Code (PPC). Since the continuous use of synthetic chemicals such as quinalphos, deltamethrin, thiomethoxam, thiocloprid and bifenthrin would lead to environmental pollution and also leads to secrete defensive enzymes in TMB (Mukhopadhyay and Roy, 2009). To overcome these, pheromone control is one of the tool for detection and monitoring of several agricultural and forest insect pests through mating disruption or mass trapping (Zhang and Aldrich, 2003). So, the present study was done to evaluate the different natural sources for the mass trapping of male TMB through the matting disruption.

MATERIALS AND METHODS

The nymphs and adults of *H. theivora* were collected from tea estates in Anaimalais, Coimbatore and laboratory mass rearing was done as per the method adopted by Sudhakaran (2000). Both male and females were segregated once the nymphs attained the fifth instar based on external appearance to carry out the pheromone studies. Volatile compounds were extracted following Golub and Weatherston (1984). Head, thorax and abdomen were dissected from virgin females (>5 and <10 days old) and extracts were derived from the respective body parts by using an acetone solvent. Acetone solvent was selected as per our previous studies (unpublished data). Similarly, the whole body extract was derived from virgin females. Freshly prepared extracts were used at every time.

The preliminary behavioral response of virgin male was studied with wind tunnel and Y-tube. Wind tunnel experiment was done as per the protocol of James et al. (2008). For Y-tube analysis, all the sources were evaluated individually against control. The total length of the Y-Tube was 100 cm and 5 cm in dia. The

wooden cube was filled with 100 µl of volatile extract then covered with aluminium foil and kept in one arm and another arm was contained an empty wooden cube which considered as a control. Virgin male was introduced into the base arm (insect releasing arm). Activated charcoal-filtered air was provided @ 0.5 ml/ sec to both arms. The treatment setup, replications and data collections were similar as in the wind tunnel experiment. During the study, the room temperature was 21±1°C. Male's distance travel was calculated with the formula (given below), and electroantennogram (EAG) studies done as per the protocol of James et al. (2008).

$$\text{Male's distance travelled \%} = \frac{\text{Male's travelled distance at end of the experiment}}{\text{Total length of the wind tunnel/ Y-tube}} \times 100$$

All the field evaluations were done at the UPASI Experimental Farm (10°16'11.2''N 76°57'56.6"E), UPASI TRF Tea Research Institute, Valparai, Coimbatore. The field contains mixed tea clones with the planting spacing of 1.20 x 1.20 m; and 3rd and 4th year fields were selected with the study done during southwest (SW) and northeast (NE) monsoons of 2019. Initially, the TMB infestation was recorded as per the Standard Operating Protocol (SOP) of UPASI TRF TRI. Based on the results, highly TMB infested fields (> 30%) were chosen for further field studies. A newly emerged female was kept separately inside the nylon meshed cage (45x 30x 30 cm) and tied on above the tea shoots. The stalks of tea shoots were provided as food and the same was changed every day. The numbers of males attracted during the lifespan of the adult were recorded and attracted males were collected using long glass test tubes every day. Each treatment contains a single virgin female, replicated five times at five different fields. The gum trap method was adopted for field studies of volatile extracts. A 100 µl of each extract was separately loaded in a wooden cube then the cube was covered with aluminium foil and kept over the gum layered on the polytene cover in the yellow plastic board (30 × 30cm). Both gum and plastic board was obtained from the Fine trap Pvt. Ltd., India. A small tear was made over the aluminium foil while keeping it on the yellow plastic board. Due to the nocturnal behaviour of TMB, the traps were kept at evening time i.e. 5.30 pm. Attractive efficiency was noted every 24 hr until achieving the attractive rate of zero. Each treatment replicated five times at five different fields. All obtained data were subjected to one way ANOVA and independent T-test was performed on each sources between the wind tunnel and Y-tube using SPSS v16.0 software. Results with $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The male TMB's behavioral response was high towards to the thorax extract of virgin female and virgin female body extract in both wind tunnel and Y-tube. On the other hand, the males' responses were very low and sometimes nil to the head and abdomen extracts of virgin females. Males initially show some antennal behaviour like up and down movement for 5-10 min then few males approach the sources by slow and fast walking; some of them proceed with flight to reach the source and also shows wings opening and vigorous antennal movement in inside the wind tunnel. Similar observations were made on Y-tube. Once the source was identified, the males proceed both walking and flying towards the source. But due to space restriction for flying, it prefers walking rather than the flying. Among the tested sources the thorax extract and virgin female body extract were attracted 88 and 87% of males respectively in wind tunnel (Fig. 1); and males took 25 min to reach the source. Similarly, the same sources were found to achieve 94 and 93% of attractive rate, respectively in Y-tube studies and took 30 min to reach the source (Fig. 2). Both the extracts viz., thorax and virgin female body extract achieved significantly high attractive rate compared to other sources. The attractive rate of the virgin female body extract was significant in both wind tunnel and Y-tube studies ($F= 5.93$; $df=1$; $p<0.05$) while the thorax extract attractive rate was not

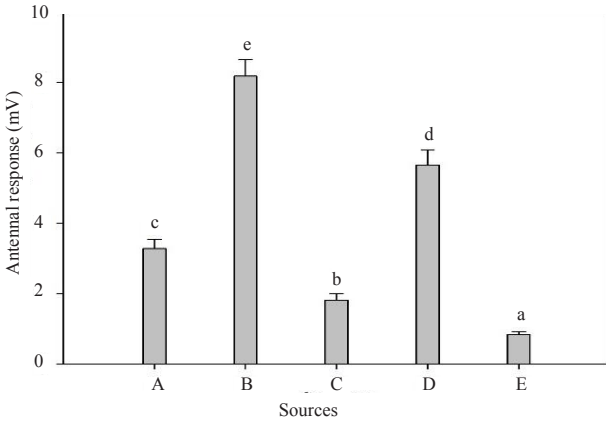

Fig. 1. Results of male responses to the various sources in wind tunnel. Bar diagram with error bar indicates mean ± SE of ten replications. Means followed by the same letter are not significantly different at 5% level by DMRT. (A=Live female TMB; B=Virgin female body extract; C=Head extract; D=Thorax extract; E=Adbomen extract; F=Control).

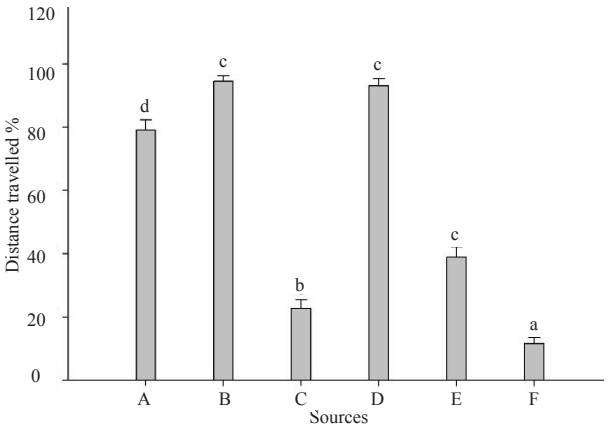

Fig. 2. Results of male responses to the various sources in Y-Tube studies. Bar diagram with error bar indicates mean ± SE of ten replications. Means followed by the same letter are not significantly different at 5% level by DMRT. (A=Live female TMB; B=Virgin female body extract; C=Head extract; D=Thorax extract; E=Adbomen extract; F=Control).

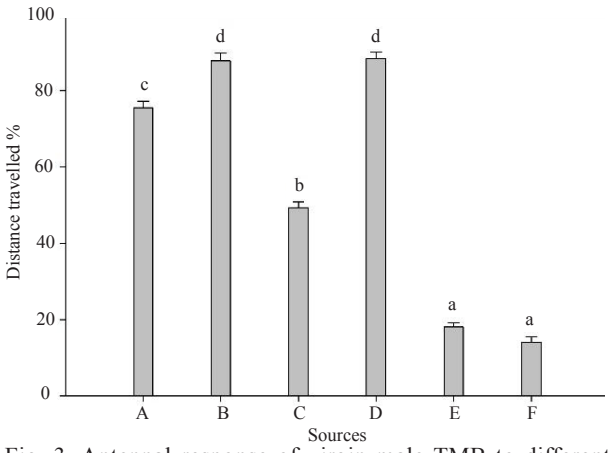

Fig. 3. Antennal response of virgin male TMB to different body extracts. Bar diagram with error bar indicates mean± SE of five replications. Means followed by the same letter is not significantly different at 5% level by DMRT. (A=Head extract; B=Thorax extract; C=Abdomen extract; D=Virgin female body extract; E=Control).

significant indicating that males' behavioral responses were always same towards to the thorax extract. The comparison of attraction rate between the Y-tube and wind tunnel analysis shows that the male's attraction was not significant towards to the sources such as live virgin female, virgin female body extract and thorax. However, a significant attraction was noted in head extract ($F=4.33$; $df=18$; $p<0.001$) and abdomen extract ($F=10.01$; $df=18$; $p<0.001$) in Y-tube and wind tunnel analysis.

In EAG studies also revealed that males' antennal response were high towards the thorax and virgin female body extracts (8.17 and 5.65 mV, respectively). Head (3.28± 0.25 mV) and abdomen (1.81± 0.18 mV) extracts of virgin females were achieved with low male antennal response (Fig. 3). These preliminary results indicate that *H. theivora* females produce sex pheromones and releases to attract its males. These results are on par with the previous study done by Sudhakaran et al. (2000); also other mirid species when identified as female produced sex pheromones such as those of cocoa capsid *Distantiella theobromae* (King, 1973), the green apple bug *Lygocoris communis* (Boivin and Stewart, 1982), the mullein bug *Campylomma verbasi* (Smith et al., 1991), the apple brown bug *Attactotomus mali* (Smith and Gaul, 1994), *Phytocoris relativus* (Millar et al., 1997), *Phytocoris californicus* (Millar and Rice, 1998) and *Lygus hesperus* (Ho and Millar, 2002).

In an infield caged study, a live virgin female started to attract males on an average of 3-4 days after the emergence of an adult; an average of 206 males

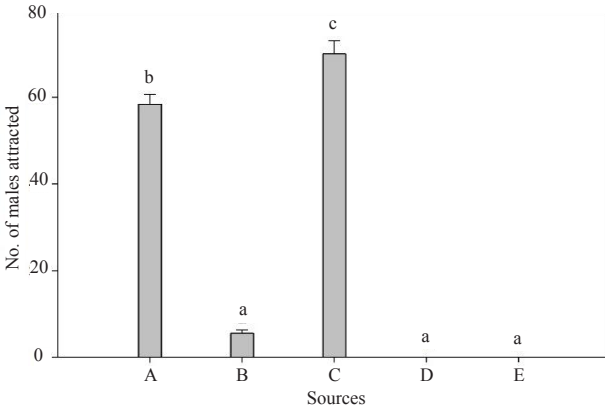

Fig. 4. Attractive efficiency of different sources against the TMB males under field conditions. (A=Virgin female body extract; B=Head extract; C=Thorax extract; D=Abdomen extract; E=Control).

was trapped and a single virgin female can attract a maximum of 220 males during their maximum lifespan of 30 days. Insect life span also varied during the study period; maximum and minimum lifespans were observed at 33 and 28 days, respectively. The caged virgin females attracted 7.6 males/ day during their attractive period (Fig. 4). Boivin and Stewart (1982) observed that unmated female of *Lygocoris communis* attracted males up to 15 days, which later decreased sharply. In the present field studies, an exponential attractive rate was noted between 8- 24 days. The attractive rate declined after the 25th day and reached nil before the death of the female. Yasuyo et al., (2007) stated that the young unmated females captured more males than the old females and they assumed that old

females were reduced or stop the pheromone release. In the present study, the attraction of males decreased as per the age of the female. There might be a positive correlation between age, pheromone release and attractions. Moreover, the insects release an extremely low quantity of pheromones from a few nanograms to micrograms/ unit time (Sunil et al., 2014; but the caged virgin female was able to release constant amount of pheromones throughout its lifespan. This may be the reason for attracting more males than other sources. Furthermore, nymphs of *H. theivora* and other insects' attraction was not noted during the caged study. This indicates that female *H. theivora* attracts only the same species of males.

In the gum trap method, the thorax extract alone attracted a maximum of 81 males within 24 hr; thorax and virgin female body extracts attracted 70 and 58 males, respectively. Its attractive rate lasted for up to 24 hr and a very minimum or nil attractive rate was observed later (Fig. 4). The head extract results were on par with nil attractive treatments such as abdomen and control. It indicates that pheromone secretion and release is present in the thorax region of the *H. theivora*. However, pheromone secreting gland's location varied with insect orders, most of these such as Lepidoptera, Diptera, Blattodea and Coleoptera secrete their pheromone at the base of the abdomen; and in contrast, some insects such as cockroaches, phasmid, hemipteran bugs, scorpion flies, carabid beetles, tenebrionid beetles, and ants produce their pheromone at the thorax region (Elofsson and Lofqvist, 1974); and female orthopterans such as *Poecilocerus pictus* and *Taeniopoda eques* (Gupta, 1978), the bagworm moths, *Kotochalia junodi* Heyl. (Bosman and Brand, 1971), *Thyridopteryx ephemeraeformis* (Leonhardt et al., 1983), the male ant lion, *Euroleon nostras* (Elofsson and Lofqvist, 1974), *P. relativus* (Millar et al., 1997) and *Pityogenes chalcographus* (Birgersson et al., 1990) produced their pheromones at the thorax region. The present study reveals that *H. theivora* also produce their sex pheromones at the thorax region and it might be located at the metathorax region.

From the present observations, eventhough the caged female gave a prolonged efficiency and attracted more males than other sources, it required an average of 27 days. Similarly, the thorax extract alone could attract an equal number of males within 2.9 days when compared with caged female. Based on the study, both caged female and thorax extract has the potential to attract males in both laboratory and field conditions (Fig. 5, 6). Both the methods can be used for monitoring of the TMB as well as its control through the mating disruption. So far there is no report on the parthenogenesis in TMB. Hence, females require males

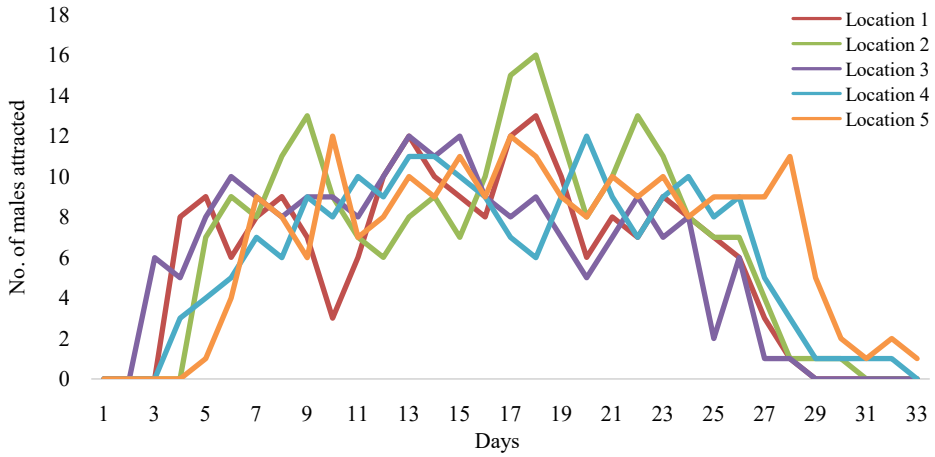

Fig. 5. Attractive efficiency of caged virgin females against males under field conditions

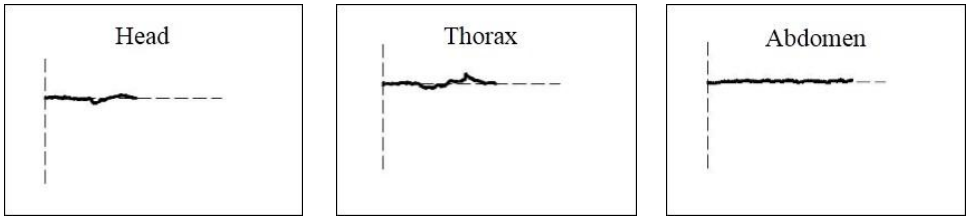

Fig. 6. Male TMB's antennal responses to virgin female body parts' extracts (head, thorax, abdomen)

for sexual reproduction and the same will be disrupted by catching of TMB males. The results show that the pheromone gland might be located in the thorax region of the female. Further studies will be required to find and locate the pheromone gland for a clear understanding of its physiological role in attraction.

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AUTHORS CONTRIBUTION STATEMENTS

PM and NSB has designed the experiments. NSB and AA conducted the experiments. NSB and TPR contributed in statistical analysis. NSB and PM wrote the manuscript. All authors read and approved the manuscript.

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

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