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Brief Report

A new pod bioassay method to determine the toxicity of insecticides against Tea mosquito bug, *Helopeltis theivora*

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The insecticide susceptibility of *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) is being evaluated using shoot and glass-vial assay as described by IRAC. However, the reliability of the assay depends on feeding preference and contact toxicity. Hence, the cocoa pod was used as a substrate to test the susceptibility of *H. theivora* in comparison with existing methods. The experimental results revealed that the LC_{50} value of all of the insecticides was relatively lower in the pod bioassay than the other two methods and exhibited maximum mortality within 6 hr of post-exposure. Among insecticides, fipronil was the most effective molecule followed by lambda-cyhalothrin. *H. theivora*, which prefers to feed on a pod due to more tissue turgidity, thus facilitated adequate sap ingestion; whereas, these were limited in shoot and glass-vial bioassays. Therefore, it could be used as a methodology to determine the susceptibility of *H. theivora* against a wide range of insecticide molecules.

Keywords: Bioassay, cocoa, *Helopeltis theivora*, insecticides, susceptibility.

Introduction

Tea mosquito bug (TMB) (*Helopeltis* spp.) (Hemiptera: Miridae) is one of the serious pest on important agricultural, horticultural, and plantation crops like tea, cocoa, cashew, cotton, guava, eggplant and other crops in India.[1–3] It causes economic losses up to 25–40% in tea,[1] 35–60% in cocoa[4] and 30–50% in cashew[5]; however, the crop loss due to *Helopeltis* sp. may extend up to 100% under epidemic situations.[6,7] Under changing climatic scenarios, the pest complexities are increasing and responsible for huge economic losses in crop-based ecosystems.[8–10] However, expanding the host range for TMB from one crop ecosystem to another including weeds is a major concern; hence, they could multiply and survive even during off-season.[11]

Three species of *Helopeltis* are widely present in India, including *H. theivora* Waterhouse, *H. antonii* Signoret, and *H. bradyi* Waterhouse; however, normally they do not overlap each other and occur on specific crops at particular stages.[1] However, all three species of tea mosquito bug have been reported on cocoa and are responsible for causing potential damage both in qualitative and quantitative parameters; of these, *H. theivora* is the dominant species on cocoa followed by *H. bradyi* in India.[12] Both nymphs and adults suck the sap from tender shoots, cherelles and mature pods by inserting their proboscis which appears as discolored, necrotic lesions around the point of feeding. Damage on shoots results in a die-back appearance; whereas, on pods, it appears as dark circular lesions with cankers and gradually it become hardened with scars. A heavy infestation could result in pod malformation and premature drop; this pest occurs throughout the year, but the population reaches its peak during pre and post-monsoon season on cocoa.[11,12]

Management of hemipteran insects was quite difficult and involves various approaches including cultivation of resistant genotypes, use of bio-control agents and application of various insecticide molecules.[13,14] In fact, insecticide application is the

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primary resort to control TMB in many economically important crops due to its potential economic damage in a short period. However, intensive application of insecticides raises concerns about the development of resistant populations of TMB and limits the effectiveness of recommended insecticides for its control.[15,16] As a result, farmers switch to new molecules with unique modes of action over conventional insecticides.[17] With the extensive use of these novel molecules against TMB management, the relative susceptibility and bio-efficacy of these insecticides in the laboratory need to be evaluated before large-scale application at the field level. It includes development of appropriate testing methodologies to determine the toxicity of insecticide against TMB. Presently, leaf/shoot dip and glass vial bioassay methods are widely used to evaluate the toxicity of insecticides for mirid bugs.[18] However, there are some constraints in these methods; in the leaf dip method, the succulence and turgor pressure of leaf tissue is quite shorter; whereas, the reliability of glass vial assay depends on the movement of the insect on the treated surface (cuticular contact toxicity) without a feeding option. Furthermore, the choice of bioassay should be compatible and correspond to the feeding nature of an insect. In cocoa, the feeding affinity of TMB is highly skewed towards pods than leaf/shoots.[12] Additionally, cocoa pods can sustain turgidity for a longer time and facilitate adequate ingestion of sap for fragile feeding insects. With this background, we felt a need for comparative analysis to determine the consistency between various bioassay methods for representative insecticides used to control of TMB.

Materials and methods

1. Insect collection and maintenance

The nymphs and adults of TMB were collected from the infested cocoa garden of the ICAR–Central Plantation Crops Research Institute, Research Station, Vittal (12°45 N, 75°4 E) with a fine brush (Cramlin No. 1), transferred into 20 mL glass vials (BOROSIL®) and brought to the laboratory. Species-level identity of *H. theivora* was confirmed using the keys as described by Stonedahl (1991). The population of *H. theivora* was released into the glass rearing cages (50×50×50 cm); maintained at 27± 2°C, 60±65% RH and a photoperiod of 16:8 (L:D) hr in the laboratory. Tender cocoa shoots dipped in 10% sugar solution in a glass vial and green cocoa pods (30–40 days old, 100–150 g) were provided as food substrates. The adults of *H. theivora* were maintained separately in a custom-made wooden cage with a glass top (40×40×40 cm) for mating and oviposition. The cage consists of a 20 cm diameter opening on the front side covered with sleeve cloth for insect handling. Cocoa shoots and pods were replaced regularly. Oviposited shoots/pods were transferred to new cages for emergence of nymphs and maintained the population from generation after generation using same feeding substrates. We used third to fourth instar nymphs from the established colony for insecticidal bioassay studies.

2. Insecticides used for the bioassay studies

Eight insecticide molecules belonging to different chemical groups with six different concentrations were used in this study. These includes: Thiamethoxam 25 WDG (Actara, Syngenta India Ltd.) at 25, 50, 100, 200, 400, 800; Emamectin benzoate 5 SG (Proclaim, Crystal Crop Protection Ltd.) at 10, 20, 40, 80, 160, 320; Pymetrozine 50 WG (Chess, Syngenta India Ltd.) at 50, 100, 200, 400, 800, 1,600; Lamda cyhalothrin 5 EC (Karate, Syngenta India Ltd.) at 10, 20, 40, 80, 160, 32; Clothianidin 50 WDG (Dantotsu, Sumitomo Chemical Ltd.) at 50, 100, 200, 400, 800, 1,600; Fipronil 5 SC (Regent, Bayer Crop Science Ltd.) at 5, 10, 20, 40, 80, 160; Spirotetramat+Imidacloprid 240 SC (Movento, Bayer Crop Science Ltd.) at 10, 20, 40, 80, 160, 320 and Azadirachtin 0.03% (Nimbicidine, T Stones & company Ltd.) at 250, 500, 1000, 2000, 4000, 8000 mg a.i L^{-1}, respectively.

3. Bioassays

Pod bioassay: Green cocoa pods (30–40 days old, 100–150 g) were collected from the cocoa garden at the Research Station, washed with running water for 10 sec and air dried under a ceiling fan. Insecticide stock solution was prepared with 0.1% Triton-X 100 in distilled water and six serial dilutions were made for each insecticide with six different concentrations. The pods were immersed in the corresponding solution for 15 sec and air-dried at room temperature by placing them on butter paper. Each pod pedicel was wrapped in cotton moistened with 10% sucrose solution to prevent dehydration and deterioration (Fig. 1). Treated pods were placed inside the insect breeding dishes (Himedia, 72×72×100 mm) and kept in a custom-made plexi-

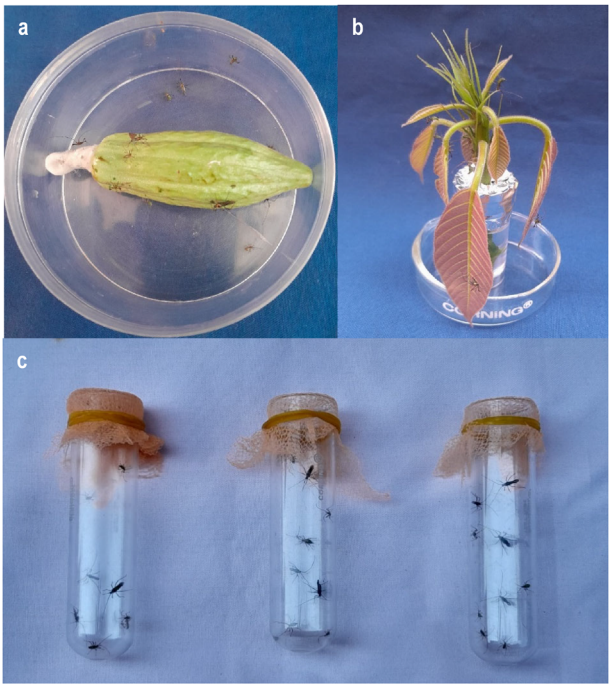

Fig 1. Insecticide toxicity bioassay for *H. theivora* (a) Pod, (b) Shoot and (c) Glass vial bioassay.

glass cage with sleeves (40×40×40 cm). About 10 nymphs (third to fourth instar) were released on each pod with four replicates for each treatment. The pods treated with distilled water alone were used as control.

Shoot bioassay: Tender cocoa shoots were obtained from 6-month-old cocoa seedlings that were grown in the greenhouse and trimmed with fine scissor leaving only the top 3–4 leaves. The trimmed shoots (5–6 cm in length) were immersed in corresponding dilution for 15 sec, placed on butter paper and allowed to dry at room temperature. Treated shoots were placed inside the glass vial (Himedia, 5 mL) containing 10% sucrose solution and kept inside a cage. About 10 nymphs (third–fourth instar) of *H. theivora* were released on each treated shoot using a fine brush. Four replicates were maintained for each insecticide concentration (Fig. 1). The shoot bioassay is also referred as "Bouquet method" for insecticide evaluation against *H. theivora* recommended by the Insecticide Resistance Action Committee (IRAC) (Ranjthkumar *et al.* 2022).

Glass vial bioassay: Insecticides were serially diluted at the desired concentration from the stock solution with 0.1% Triton-X 100 in distilled water. 1 mL of the diluted solution was pipetted from each insecticide concentration and added to the glass vials (Himedia, 15 mL). The vials treated with distilled water was served as a control. To ensure uniform distribution, the vials were rolled on a modified hotdog roller with the heat unit disconnected (Bresco Group Ltd., India) in a fume hood until the water evaporated. About 10 nymphs (third–fourth instar) of *H. theivora* were released inside the treated vial and plugged with cotton. To make cuticular contact, the insects were allowed inside the vial for 15–20 min, and then released on untreated shoots/pods. Four replicates were maintained for each treatment. In all three bioassays, the mortality of *H. theivora* was recorded at every 6 hr interval when the bug did not show any coordinated movement after being released on the treated surface in each concentration. The time of mortality was recorded when at least one bug died in each concentration among released individuals.

4. Mortality of H. theivora at recommended field spray concentration

An attempt was made to determine their toxicity in pod, shoot and glass vial assay under laboratory conditions. Here, we used 1/10th recommended field spray concentration for all the molecules. These were thiamethoxam 1.25 mg a.i, clothianidin 20 mg a.i, fipronil 2.5 mg a.i, spirotetramat+imidacloprid 22 mg a.i, pymetrozine 12.5 mg a.i, lambda-cyhalothrin 5 mg a.i, emamectin benzoate 2.5 mg a.i and nimbicidine 20 mg a.i per liter respectively. The protocol was followed as described above for each bioassay. About 15 insects (third to fourth instar) were used for each insecticide concentration with four replicates. The pod, shoot and glass vials treated with 0.1% Triton-X 100 in distilled water were used as control. The mortality of *H. theivora* was recorded when they did not show any movement when probing with a brush. Observations were recorded up to 96 hr with 24 hr interval.

5. Statistical analysis

The mortality data of *H. theivora* in all three bioassays were corrected for the control mortality using Abbott's formula.[19] Corrected mortality data were subjected to Probit analysis with POLO PLUS software (LeOra Software Inc., Berkeley, CA) and estimated the LC_{50} (median lethal concentration) values with their fiducial limits and the slope of the regression line. The percent mortality of *H. theivora* for each assay with different insecticides was subjected to One-way analysis of variance (ANOVA) using a generalized linear model; and it was transformed using arcsine transformation to normalize the variance. The means were separated with Tukey's honestly significant difference test $\alpha=0.05$ level and analyzed in IBM SPSS software (*ver.* 20, Armonk, NY, USA).

Results

1. Susceptibility of H. theivora to different insecticides in pod bioassay

The toxicity of eight insecticide molecules against *H. theivora* in pod bioassay is shown in Table 1. The LC_{50} values of syn-

Table 1. Toxicity of insecticides on *H. theivora* in Pod bioassay

Insecticide	$N^{a)}$	Time of mortality (hours)$^{b)}$	LC_{50}/mg a.i L^{-1} (95% Fiducial limits)$^{c)}$	Slope±S.E.$^{d)}$	$X^{2e)}$	$df^{f)}$	Toxicity Index$^{g)}$
Thiamethoxam	240	6	12.62 (2.23–25.45)	1.206±0.29	1.24	4	43.34
Clothianidin	240	6	51.52 (27.06–73.70)	1.923±0.35	1.59	4	10.61
Spirotetramat+Imidacloprid	240	6	30.94 (16.39–48.07)	1.025±0.19	0.21	4	17.67
Pymetrozine	240	6	40.62 (12.07–72.89)	1.189±0.25	0.39	4	13.46
Lambda-cyhalothrin	240	6	14.54 (7.06–22.40)	1.357±0.24	0.28	4	37.62
Emamectin benzoate	240	6	30.74 (16.90–47.21)	1.092±0.192	2.18	4	17.80
Azadirachtin	240	72	1,833.75 (1,074.95–3,765.33)	0.835±0.21	0.18	4	0.30
Fipronil	240	6	5.47 (1.63–10.06)	0.965±0.22	0.31	4	100

$^{a)}N$—Total number of insects used in the bioassay. $^{b)}$Mortality reading at 6, 12, 18, 24 and 72 hr after treatment. $^{c)}LC_{50}$—Expressed in mg a.i/liter (a.i=active ingredient); 95% Fiducials limits are given in parenthesis. $^{d)}$SE—Standard error. $^{e)}$Chi-square test of dose mortality response. $^{f)}df$—Degrees of freedom. $^{g)}$Toxicity index=LC_{50} of the most effective compound/LC_{50} of each compound×100.

Table 2. Toxicity of insecticides on *H. theivora* in Shoot bioassay

Insecticide	$N^{a)}$	Time of mortality (hr)$^{b)}$	LC_{50}/mg a.i L^{-1} (95% Fiducial limits)$^{c)}$	Slope±S.E.$^{d)}$	$X^{2e)}$	$df^{f)}$	Toxicity Index$^{g)}$
Thiamethoxam	240	12	96.84 (52.08–137.97)	1.354±0.28	0.14	4	7.10
Clothianidin	240	12	63.85 (24.68–99.38)	1.274±0.29	0.83	4	10.77
Spirotetramat+Imidacloprid	240	12	34.09 (18.24–53.02)	1.000±0.21	0.94	4	20.18
Pymetrozine	240	24	123.77 (60.64–192.95)	1.023±0.21	0.42	4	5.56
Lambda cyhalothrin	240	12	16.47 (6.70–27.20)	1.070±0.21	0.43	4	41.78
Emamectin benzoate	240	12	57.58 (32.59–95.57)	0.905±0.177	0.16	4	11.94
Azadirachtin	240	96	1,862.96 (1,218.35–3,170.98)	1.051±0.21	0.33	4	0.37
Fipronil	240	6	6.88 (1.24–14.21)	0.706±0.18	0.23	4	100

$^{a)}N$—Total number of insects used in the bioassay. $^{b)}$Mortality reading at 6, 12, 18, 24 and 72 hr after treatment. $^{c)}LC_{50}$—Expressed in mg a.i/L (a.i=active ingredient); 95% Fiducials limits are given in parenthesis. $^{d)}$SE—Standard error. $^{e)}$Chi-square test of dose mortality response. $^{f)}df$—Degrees of freedom. $^{g)}$Toxicity index=LC_{50} of the most effective compound/LC_{50} of each compound×100.

thetic insecticides ranged from 5.47 to 51.52 mg a.i L^{-1} and 1,833.75 mg a.i L^{-1} in botanical insecticide. Among tested insecticides, the susceptibility of *H. theivora* for fipronil, thiamethoxam and lambda-cyhalothrin were relatively higher and their LC_{50} values were 5.47, 12.62 and 14.54 mg a.i L^{-1} respectively. Based on the toxicity index, fipronil was the most effective insecticide (100%), followed by thiamethoxam (43.34%). However, the toxicity of emamnectin benzoate, spirotetramat+imidacloprid, pymetrozine and clothianidin were relatively lower. Further, all synthetic insecticides at tested concentrations exhibited mortality of *H. theivora* within 6 hr of post-exposure; while, nimbicidine was least toxic and showed mortality 72 hr of post-treatment.

2. Susceptibility of H. theivora to different insecticides in shoot bioassay

In shoot bioassay, the LC_{50} values ranged from 6.88 to 123.77 mg a.i L^{-1} for synthetic insecticide tested against *H. theivora;* whereas, 1862.96 mg a.i L^{-1} in botanical insecticide (Table 2). The highest toxicity index was observed in fipronil (100%), followed by lambda-cyhalothrin (41.78%). Similar to the pod assay, the toxicity of emamectin benzoate, clothianidin, pymetrozine and spirotetramat+imidacloprid were comparatively lower; and nimbicidine was least toxic (0.37%). Further, the mortality period of *H. theivora* in shoot assay was slightly higher as compared with pod bioassay for most of the insecticides except fipronil.

3. Susceptibility of H. theivora to different insecticides in Glass vial bioassay

The LC_{50} of synthetic insecticides in glass vial assay ranged from 7.29 to 60.61 and 2353.44 mg a.i L^{-1} in botanical insecticide (Table 3). The toxicity of tested insecticides was slightly on par with pod bioassay, and relatively higher than shoot bioassay. Among insecticides, fipronil exhibited highest toxic index (100%) followed and lambda cyhalothrin (31.26%) and their LC_{50} values were 7.29 and 23.32 mg L^{-1} respectively. However, except for fipronil, the mortality period of *H. theivora* for all the insecticides was quite longer than pod bioassay.

4. Mortality of H. theivora at field spray concentration

The toxicity of insecticides on *H. theivora* showed a significant difference in pod (F=5.919, df=7, p<0.001), shoot (F=3.55, df=7, p<0.001) and glass vial assay (F=4.317, df=7, p<0.001) at recommended concentration. The percent mortality of *H.*

Table 3. Toxicity of insecticides on *H. theivora* in Glass vial bioassay

Insecticide	$N^{a)}$	Time of mortality (hr)$^{b)}$	LC_{50}/mg a.i L^{-1} (95% Fiducial limits)$^{c)}$	Slope±S.E.$^{d)}$	$X^{2e)}$	$df^{f)}$	Toxicity Index$^{g)}$
Thiamethoxam	240	12	33.01 (11.72–55.88)	1.042±0.22	0.17	4	22.08
Clothianidin	240	12	59.77 (21.98–94.26)	1.277±0.29	1.86	4	12.19
Spirotetramat+Imidacloprid	240	12	40.22 (23.33–61.01)	1.064±0.22	2.17	4	18.12
Pymetrozine	240	24	60.61 (8.66–121.80)	0.749±0.21	0.21	4	12.02
Lambda Cyhalothrin	240	12	23.32 (8.32–40.19)	0.855±0.20	0.45	4	31.26
Emamectin benzoate	240	24	51.47 (28.56–83.93)	0.919±0.178	0.28	4	14.16
Azadirachtin	240	96	2,353.44 (1,393.68–5,585.46)	0.833±0.21	0.30	4	0.31
Fipronil	240	6	7.29 (3.86–10.90)	1.428±0.24	2.41	4	100

$^{a)}N$—Total number of insects used in the bioassay. $^{b)}$Mortality reading at 6, 12, 18, 24 and 72 hr after treatment. $^{c)}LC_{50}$—Expressed in mg a.i/L (a.i=active ingredient); 95% Fiducials limits are given in parenthesis. $^{d)}$SE—Standard error. $^{e)}$Chi-square test of dose mortality response. $^{f)}df$—Degrees of freedom. $^{g)}$Toxicity index=LC_{50} of the most effective compound/LC_{50} of each compound×100.

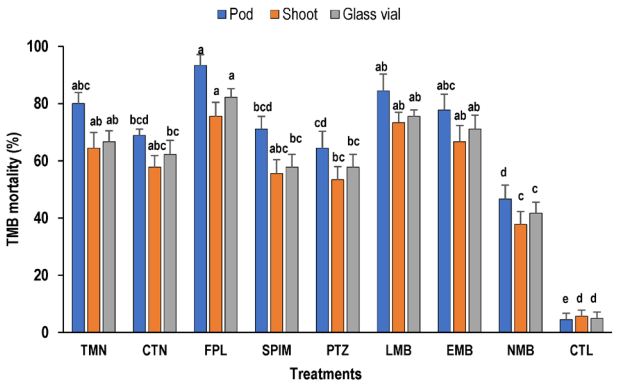

Fig. 2. Mean percent mortality (±S.E.) of *H. theivora* in Pod, Shoot and Glass vial bioassay. TMN—Thiomethaxam, CTN—Clothianidin, FPL—Fipronil, SPIM—Spirotetramat+Imidacloprid, PTZ—Pymetrozine, LMB—Lambda Cyhalothrin, EMB—Emamectin benzoate. NMB—Nimbicidine, CTL—Control. Different letters indicate statistical differences ($P>0.05$, Tukey's honestly significant difference test).

theivora in all the treatments was highest in pod bioassay. Of these insecticides, the toxicity of fipronil was higher and caused percent mortality of 93.33±3.84, 82.20±2.96 and 75.56±4.87 in the pod, glass vial and shoot assay, respectively; followed by lambda-cyhalothrin, emamectin benzoate and thiamethoxam (Fig. 2). However, clothianidin, spirotetramat+imidacloprid, and pymetrozine showed mortality of *H. theivora* from 53.34 to 71.11%; while, the lowest mortality was recorded with nimbicidine.

Discussion

Sensitive and reliable bioassay protocols are necessary to assess the toxicity of insecticides before novel chemicals are brought into field application.[20,21] The insecticides used in this study were selected because of their prevalent use in various crops against *H. theivora* and their ability to induce lethal effects on other sucking pests as well.[22,23] The early concerns about *Helopeltis* toxicological bioassays were centered around contact toxicity using glass vial *versus* ingestion toxicity through leaf dip/shoot bioassay.[16,24] Hence, we tested the toxicity of insecticides for *H. theivora* using cocoa pods in comparison with existing bioassay methods as recommended by IRAC.

The results from this study revealed the susceptibility of *H. theivora* in three different bioassay protocols. With respect to pod bioassay, the susceptibility level of *H. theivora* to all the tested insecticides were higher as compared to other two methods. In addition, the mortality time was considerably lower and exhibited mortality occurred within 6 hr of exposure to synthetic insecticides. This difference is due to the feeding nature of *H. theivora* and considerably it spent more time on cocoa pods and ingested more sap than shoots. To support this, a choice test was conducted and reported that *H. theivora* have a preference towards cocoa pods for feeding and egg laying than shoots.[12] In the glass vial assay, the mortality period of *H. theivora* to all the tested insecticide molecules was substantially longer than pod bioassay, which is likely due to longer insecticide exposure from the point of contact to the target site. In addition, the glass vial assay is not representative of the major group of systemic insecticides and the insecticidal action is dependent on tarsal contact.[25] Furthermore, the sensitivity and successful bioassay method for insecticide toxicity can be assessed based on feasibility which exhibit insect mortality in relatively less time.[26] The pod bioassay met all these parameters and longevity of the tissue was retained for up to 8 days; whereas it was much shorter in shoot assay (<24 hr). Therefore, the use of pod bioassay method enables better discrimination of the susceptible or resistant populations of *H. theivora* to various insecticides in cocoa.

The increased incidence of *H. theivora* on economically important crops has led to the application of various synthetic chemicals at an alarming rate; yet, the control was not satisfactory. In our study, the susceptibility of *H. theivora* to fipronil was extremely high in all methods (5.47, 6.88 and 7.29 mg a.i L^{-1}) with the highest toxicity index followed by lambda-cyhalothrin and thiamethoxam. A report on tamarind showed that the application of fipronil at 0.2% was most effective in reduction of *H. antonii*.[27] Recently, a study from Ghana reported that fipronil was not permissible to use in the cocoa ecosystem, although it exhibits 90–100% mortality on target pests such as mirid, stink and coreid bugs due to its detrimental effect on non-target organisms.[28] Since the median lethal concentration of fipronil and lambda-cyhalothrin was on par in this study; lambda-cyhalothrin could be used to mitigate the populations of *H. theivora* in cocoa. Similarly, the use of lambda-cyhalothrin induced high mortality against *Helopeltis spp* with minimal effect on natural enemies in cocoa.[11,29] The effect of emamectin benzoate and spirotetramat+imidacloprid was moderate showed high toxicity index than clothianidin and pymetrozine. The tea shoots treated with emamectin benzoate and spirotetramat+imidacloprid at the field recommended dose showed 90 and 81.87% mortality of *H. theivora*[23]; whereas, azadirachtin remained least effective on *H. theivora* and *H. antonii*.[30,31]

In conclusion, our study indicated that the cocoa pod bioassay method is a very promising and reliable for studying the susceptibility of *H. theivora* to shoot and glass vial assay in the cocoa ecosystem. In addition, this method could easily be scaled up to screen a large number of insecticide molecules. Among tested insecticides, the efficacy of fipronil and lambda-cyhalothrin was significantly higher and exhibited maximum mortality. Further, the detrimental effect of fipronil on non-target organisms needs to be tested. Therefore, field application of lambda-cyhalothrin and thiamethoxam on a rotation basis is suggested to control *H. theivora* infestation to avoid development of resistance. Overall, this data provides essential information to assess the toxicity of insecticides using pod bioassay along with sustainable management of *H. theivora* in cocoa.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1) S. Roy, N. Muraleedharan, A. Mukhapadhyay and G. Handique: The tea mosquito bug, *Helopeltis theivora* Waterhouse (Heteroptera: Miridae): Its status, biology, ecology and management in tea plantations. *Int. J. Pest Manage.* **61**, 179–197 (2015).
2) K. K. Srikumar and P. S. Bhat: Biology of the tea mosquito bug (*Helopeltis theivora* Waterhouse) on *Chromolaena odorata* (L.) R.M. King & H. Rob. *Chil. J. Agric. Res.* **73**, 309–314 (2013).
3) P. D. Jayanthi, T. Nagaraja, T. Raghava and V. Kempraj: Pomegranate, a newly documented host plant of tea mosquito bug, *Helopeltis antonii* Signoret. *Pest Manage. Hortic. Ecosyst.* **22**, 88–90 (2016).
4) B. Padi: Prospects for the control of cocoa capsids: Alternatives to chemical control. In "Proceedings First International Cocoa Pests and Diseases Seminar," Accra, Ghana, pp. 28–36, 1997.
5) E. Eradasappa, K. Vanitha, M. Babli and C. Anilkumar: Deciphering genotype by year interaction for tea mosquito bug incidence (*Helopeltis* spp.) in Indian cashew (*Anacardium occidentale* L.) germplasm. *Genet. Resour. Crop Evol.* **71**, 3735–3744 (2024). https://doi.org/10.1007/s10722-024-01892-w
6) N. Muraleedharan: Bio-ecology and management of tea pests in southern India. *J. Plant. Crops* **20**, 121 (1992).
7) L. K. Hazarika, M. Bhyuan and B. N. Hazarika: Insect Pest of tea and their management. *Annu. Rev. Entomol.* **54**, 267–284 (2009).
8) T. N. Madhu, R. T. P. Pandian, S. E. Apshara, A. Bhavishya, A. Josephrajkumar, B. N. Kumar and P. S. Kumar: New occurrence of the *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) infestation on cocoa in India. *J. Lepid. Soc.* **77**, 110–115 (2023).
9) R. T. P. Pandian, S. H. Thube, Bhavishya, M. Babu, H. Rajashekara, T. N. Madhu, Y. Diwakar, P. Santhoshkumar, B. J. Nirmalkumar, B. Patil and V. Hegde: *Colletotrichum kahawae subsp. cigarro* causing leaf spot disease on arecanut, *Areca catechu* L. in India: A first report. *Crop Prot.* **179**, 106607 (2024).
10) M. T. Nagaraju, K. M. Mohan, M. C. Keerthi, T. Prabhulinga, S. Thube, V. Shah, H. O. Elansary, I. M. Mousa and M. A. El-sheikh: Effect of temperature on the biological parameters of pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae). *Sci. Rep.* **14**, 15047 (2024). https://doi.org/10.1038/s41598-024-65241-8
11) S. H. Thube, R. T. P. Pandian, M. Babu, A. Josephrajkumar, P. H. Mhatre, P. S. Kumar, B. N. Kumar, V. Hegde and S. N. Chavan: Evaluation of a native isolate of *Metarhizium anisopliae* (Metschn.) Sorokin TM BMA1 against tea mosquito bug, *Helopeltis theivora* infesting cocoa (*Theobroma cacao L.*). *Biol. Control* **170**, 104909 (2022).
12) T. N. Madhu, E. K. Saneera, R. T. P. Pandian, Bhavishya, M. Chaithra, M. Suchithra, N. R. Nagaraja, B. J. N. Kumar and S. E. Apshara: Laboratory rearing of tea mosquito bug, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) on cocoa (*Theobroma cacao* L.). *Phytoparasitica* **52**, 64 (2024). https://doi.org/10.1007/s12600-024-01182-2
13) M. S. A. Mamun and A. V. K. Iyengar: Integrated approaches to tea pest management in south India. *Int. J. Sustain. Agril. Tech.* **6**, 27–33 (2010).
14) T. N. Madhu, R. Kumar, V. C. Naik, T. Prabhulinga, S. Santosh, N. Chandrashekar and P. Verma: Attraction of leaf hopper, *Amrasca biguttulla biguttulla*, and whitefly, *Bemisia tabaci*, toward natural essential oils in cotton. *Anim. Biol. Leiden Neth.* **73**, 27–41 (2023).
15) S. Bora, M. Sarmah, A. Rahaman and G. Gurusubramanian: Relative toxicity of pyrethroid and non-pyrethroid insecticides against male and female tea mosquito bug, *Helopeltis theivora* Waterhouse (Darjeeling strain). *J. Entomol. Res.* **31**, 37–41 (2007).
16) S. Roy, A. Mukhopadhyay and G. Gurusubramanian: Resistance to insecticides in field-collected populations of tea mosquito bug (*Helopeltis theivora* Waterhouse) from the Dooars (North Bengal, India) tea cultivations. *J. Entomol. Res. Soc.* **13**, 37–44 (2011).
17) D. Saha, S. Roy and A. Mukhopadhyay: Insecticide susceptibility and activity of major detoxifying enzymes in female *Helopeltis theivora* (Heteroptera: Miridae) from sub-Himalayan tea plantations of North Bengal, India. *Int. J. Trop. Insect Sci.* **32**, 85–93 (2012).
18) G. L. Snodgrass and W. P. Scott: Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi River Delta. *Southwest. Entomol.* **27**, 191–199 (2002).
19) W. S. Abbott: A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265–267 (1925).
20) K. J. Brent: Detection and monitoring of resistant forms: An overview. In "Pesticide Resistance: Strategies and Tactics for Management," National Research Council. National Academy Press, Washington, DC, pp. 298–312, 1986.
21) N. Prabhaker, S. Castle, F. Byrne, T. J. Henneberry and N. C. Toscano: Establishment of baseline susceptibility data to various insecticides for *Homalodisca coagulata* (Homoptera: Cicadellidae) by comparative bioassay techniques. *J. Econ. Entomol.* **99**, 141–154 (2006).
22) S. Roy, A. Mukhopadhyaya and G. Gurusubramanian: Sensitivity of the tea mosquito bug (*Helopeltis theivora* Waterhouse), to commonly used insecticides in 2007 in Dooars tea plantations, India and implication for control. *Am.-Eurasian J. Agric. Environ. Sci.* **6**, 244–251 (2009).
23) R. S. Chowdhury, M. Ahmed, M. S. A. Mamun and S. K. Paul: Relative efficacy of some insecticides for the control of tea mosquito bug, *Helopeltis theivora* (Waterhouse) in Bangladesh. *J. Plant Prot. Sci.* **5**, 50–54 (2013).
24) R. Ranjithkumar, M. Kalaynasundaram, M. Kannan, J. S. Kennedy, C. R. Chinnamuthu and P. Paramaguru: Bio-efficacy of newer molecules insecticides against tea mosquito bug, *Helopeltis theivora* waterhouse under laboratory condition. *Int. J. Plant Soil Sci.* **34**, 73–80 (2022).
25) C. M. Tibola, L. Silva, F. Sgubin and C. Omoto: Monitoring resistance of *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) to insecticides by using encapsulated artificial diet bioassay. *Insects* **12**, 599 (2021). https://doi.org/10.3390/insects12070599
26) T. C. Sparks, D. G. Riley, A. M. Simmons and L. Guo: Comparison of toxicological bioassays for whiteflies. *Insects* **11**, 789 (2020).
27) H. N. Sanjay, R. Manjunatha, G. Narasareddy, V. Mulimani and K. M. Mohan: Evaluation of different insecticides against tea mosquito bug, *Helopeltis antonii* sign (Miridae: Hemiptera) on tamarind. *Pharma Innov.* **12**, 1–3 (2023).
28) S. W. Avicor, G. K. Awudzi, R. Adu-Acheampong, P. Boamah-Dankyi and S. Adu-Acheampong: Contact toxicity and proximate effect of fipronil on insect pest and predatory ant community structure in cocoa agro-ecosystem. *J. Agric. Food Res.* **14**, 100909 (2023).
29) M. Algar: Cumulative effects of insecticides on generalist predators and parasitoids population in cocoa ecosystem. *J. Plant. Crops* **43**, 245–249 (2015).

30) S. Roy, G. Gurusubramanian and A. Mukhopadhyay: Neem-based integrated approaches for the management of tea mosquito bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) in tea. *J. Pest Sci.* **83**, 143–148 (2010).
31) V. C. B. Naik, M. K. Narode, S. Kumbhare, D. S. Ghongade, T. N. Madhu, J. S. V. Murthy, N. G. Narkhedkar and Y. G. Prasad: Efficacy of novel insecticides and their combinations against pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in cotton. *Int. J. Trop. Insect Sci.* **43**, 397–407 (2023). https://doi.org/10.1007/s42690-022-00939-8