Research Article

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Comparative efficacy and residual toxicity of selective insecticides against *Helopeltis theivora* Waterhouse infesting Cocoa

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Abstract

Cocoa (*Theobroma cacao* L.) is an important beverage crop, commercially grown as a plantation crop. In the changed climate-scenario, tea mosquito bug species complex viz., *Helopeltis theivora, H. bradyii* and *H. antonii* are emerging as major threat to cocoa cultivation in India. Among these species, *H. theivora* is responsible for causing substantial crop-damage. The present investigation was carried out to evaluate the toxicity, efficacy, and residual effects of various test-insecticides (Bifenthrin, Clothianidin, Flonicamid, Imidacloprid, Quinolphos and Thiamethoxam) against *H.* theivora, a major pest of cocoa. The results revealed that bifenthrin exhibited the highest toxicity with an LC 50 value of 0.36 ppm, while flonicamid displayed the least toxicity with an LC50 value of 130.20 ppm. Quinalphos exhibited higher toxicity than flonicamid, with an LC50 value of 100.48 ppm. Imidacloprid and thiamethoxam recorded higher toxicity, recording lower LC 50 values of 47.23 and 64.98 ppm, respectively. Clothianidin showed moderate toxicity with an LC50 value of 75.31 ppm against *H. theivora*. Field experiments assessed the efficacy of the screened insecticides, revealing that bifenthrin and clothianidin were the most effective in managing *H. theivora* infestations, resulting in the lowest pod infestation percentages of 10.11% and 13.84%, respectively. Conversely, flonicamid exhibited the least effectiveness, with 37.82% pod infestation. Furthermore, this study investigated the residual toxicity of the insecticides in the field. Bifenthrin and clothianidin displayed persistent effects, causing over 25% mortality of *H. theivora* even up to 30 days after application. Flonicamid, however, showed rapid loss of residual toxicity compared to other insecticides, with less than 25% residual toxicity reported after 10 days. Imidacloprid and thiamethoxam demonstrated more than 25% residual mortality on day 20 after application. In conclusion, this research underscores the varying toxicities and efficacies of different insecticides against *H. theivora* in cocoa plantations, with implications for effective pest management strategies.

Keywords : *Helopeltis theivora, incidence, insecticides, efficacy, residues, toxicity*

Introduction

Cocoa (*Theobroma cacao* L.), originally native to South and Central America, holds significant economic value as a cash crop cultivated across various humid tropical countries worldwide. Its introduction to India dates back to 1798 (Ratnam, 1961), and it is predominantly grown as an understorey crop within arecanut, coconut, and oil palm plantations in southern states of India, such as Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh.

Presently, India's cocoa cultivation covers an area of 97,563 hectares, yielding around 25,783 tonnes annually (DCCD, 2020). However, the majority of global cocoa production is led by West Africa, accounting for over 70% of the total output, followed by South-East Asia and Latin America (Lahive et al., 2019). As a fundamental component of the chocolate industry and confectionaries, cocoa holds immense importance. The country's demand for dry cocoa beans reaches approximately 30,000

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tonnes annually, but local production falls short, resulting in significant imports from other cocoa-producing nations (DCCD, 2020).

Despite its economic significance, cocoa cultivation faces formidable challenges stemming from various biotic and abiotic factors. Notably, insect pests and diseases stand as primary impediments to cocoa production and productivity. Among these biotic stresses, the tea mosquito bug, *Helopeltis* spp. (a species complex; Heteroptera: Miridae), has emerged as a major pest in South India (Thube et al., 2019). Within this species complex, *H. theivora* prevails as the dominant species, contributing to more than 40% potential yield reduction in Indian cocoa plantations. This mirid pest primarily infests the economically crucial cocoa pods. Even minor feeding damage caused by these insects can result in substantial quantitative and qualitative losses (Muhamad and Way, 1995).

Throughout the history of modern agriculture, the use of chemical pesticides to control insect pests remains a cornerstone of plant protection strategies. To endorse the application of specific insecticides against target insect pests, thorough investigations into their efficacy within both laboratory and field settings are imperative.

Despite being a recently emerged cocoa insect pest, there has been a lack of scientific studies focusing on the screening of novel compounds against the tea mosquito bug (TMB) in India. This pest has the potential to inflict economic losses of up to 75% in cocoa crops (Alagar and Bhat, 2017). Given the backdrop of a changing climate, the situation becomes even more complex if TMB populations exceed economic threshold levels, especially in the absence of labeled chemical solutions. Consequently, there is a pressing need to study and screen new, effective chemical insecticides for managing this target pest efficiently.

Pesticides are extensively employed for target insect pest management through various application methods. Once novel insecticides have been screened under laboratory conditions, their performance in the field requires immediate follow-up assessment. Furthermore, subsequent to the application of insecticides in real field conditions, the evaluation of their efficacy over time becomes a vital aspect of entomological research. In the context of effectively managing TMB in cocoa, the evaluation of residual toxicity of the screened insecticides in field conditions holds significant importance.

Hence, investigations were carried out to evaluate the toxicity, efficacy, and residual effects of various test-insecticides against *H. theivora* both under *in-vitro* and field conditions.

Materials and methods

In-vitro screening of insecticides

Test insecticides

Insecticides for conducting bioassays against *Helopeltis* spp. were chosen based on recommendations provided by the Central Insecticide Board (CIB), State Agricultural Universities (SAUs), and research institutes affiliated with the Indian Council of Agricultural Research (ICAR) for managing TMB in tea and cashew ecosystems. Further details regarding the insecticides employed in the bioassay are provided in Table 1.

Rearing of *Helopeltis theivora*

The aluminium cages, originally developed by Sundararaju & John (1992), were utilized with slight modifications (Thube et al., 2021) for laboratory rearing of TMB. Gravid TMB females were sourced from the experimental farm at ICAR-CPCRI-Regional Station, Vittal, and their identity was authenticated following the Stonedahl (1991) method. These gravid females were allowed to lay eggs on fresh cocoa pods placed within the rearing cages. Egg deposition was confirmed by the presence of two distinct respiratory filaments on the pod's surface. Upon hatching, the first instar nymphs were promptly transferred to separate rearing cages containing fresh pods, with humidity maintained through the use of damp muslin cloth.

Subsequent TMB instars were sustained on fresh cocoa pods, with a pod replacement cycle every three days to ensure continuous nutrition. Following the moult of fifth instar nymphs, adult males and females were distinguished based on abdominal shape and size (pointed in females and blunt in males), and each pair was relocated to individual cages for copulation. All mated females were provided with fresh pods for nourishment and oviposition. The TMB culture required for bioassay was maintained at the prevailing room temperature.

Bioassay

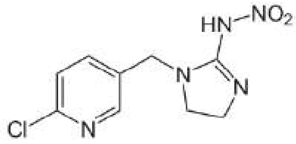
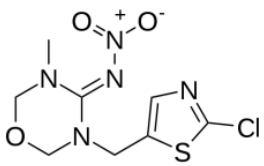
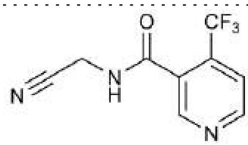
The target insecticides were diluted using deionized water to create the intended concentrations. Initially, a bioassay was established using a broad range of concentrations to verify the mortality range of 20-80%. Once an appropriate mortality range was determined, five concentrations of each insecticide were selected for the bioassay, aiming to compute the median lethal dose (LD50). Fresh cocoa pods were collected from the experimental farm at ICAR-CPCRI, Regional Station, Vittal. The desired concentrations of each insecticide (five in total) were individually applied to the cocoa pods through spraying. Subsequently, the treated pods were air-dried under natural conditions and then placed in separate cages corresponding to their respective concentrations. Ten recently emerged adult individuals were introduced into each replication, and three replications were maintained throughout the experiment. Mortality data for the adult insects were recorded at 24-hour intervals. Adults displaying immobility (moribund) were considered as deceased. To mitigate experimental errors, the entire procedure was repeated twice.

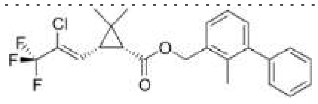
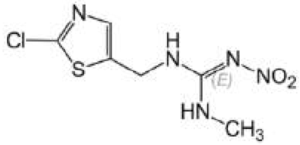
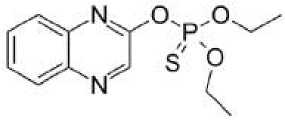
Residual toxicological assay

Pods growing under natural field conditions were treated once with the test insecticides at the field-recommended dose. As there are no label claim insecticides available for cocoa, the term 'field-recommended dose' here refers to the recommendations provided by the CIB, Research Institutes, and SAUs for managing *Helopeltis* (spp.) in alternate hosts like tea and cashew. The insecticidal doses employed in the study are outlined in the Materials and Methods section. Groups of 10 pods were collected at intervals of 1, 10, 20 and 30 days after spraying (DAS) and were subjected to a residual toxicological assay with adult TMBs under laboratory conditions.

Ten insects were released per treatment, and each treatment was replicated four times, totalling 40 target insects per treatment. Mortality of the insects was recorded 24 hours after their exposure to treated cocoa pods. The percentage mortality data underwent arcsine transformation and were subjected to analysis of variance. Mean values were distinguished using Duncan's Multiple Range Test (SPSS, 2007) at a significance level of $P = 0.05$.

Table 1. Details of insecticides used in bioassay

Insecticide	Trade name	Molecular structure	Source
Imidacloprid 17.8% SL($C_9H_{10}ClN_5O_2$)	Confidor®		Indofil Industries Ltd.,Mumbai
Thiamethoxam 25% WG(C8H10ClN5O3S)	ExtraSUPER™		Crystal Crop Protection Pvt. Ltd.Jammu
Flonicamid 50% WG($C_9H_6F_3N_3O$)	ULALA®		UPL, India Ltd.,Mumbai

Bifenthrin 10% EC ($C_{23}H_{22}ClF_3O_2$)	Barristar®		Tamron Astra Bio-Chemicals Pvt. Ltd.Bangalore
Clothianidin 50 WDG ($C_6H_8ClN_5O_2S$)	DANTOTSU®		Sumitomo Chemical India Pvt. Ltd. Mumbai
Quinalphos 25% EC ($C_{12}H_{15}N_2O_3PS$)	KEMLOX®		Sumitomo Chemical India Pvt. Ltd. Mumbai

Results and Discussion

Insecticide bioassay

Since TMB has recently emerged as a significant pest in cocoa cultivation, there have been practically no scientific studies to screen insecticides against TMB within the cocoa ecosystem. Consequently, as of the present date, no insecticides with label claims from the Central Insecticide Board (CIB) are available. To address this gap and screen a range of insecticides against TMB (*H. theivora*), the current investigation conducted bioassays.

Toxicity assessments were carried out on three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam), one synthetic pyrethroid (bifenthrin), one organophosphate (quinalphos), and one chordotonal organ modulator (flonicamid) against the adult TMB population (*H. theivora*) in laboratory conditions.

The probit estimates for all the target insecticides are presented in Table 2. Bifenthrin exhibited the highest toxicity to the laboratory-reared *H. theivora* population, with the lowest LC50 value recorded at 0.36 ppm. Flonicamid displayed the least toxicity against *H. theivora,* yielding the highest LC50 value of 130.20 ppm. In comparison to flonicamid, quinalphos demonstrated higher toxicity, with a lower LC50 value of 100.48 ppm. Imidacloprid and thiamethoxam exhibited increased toxicity, with each displaying lower LC50 values of 47.23 and 64.98 ppm, respectively. The *H. theivora* population showed moderate toxicity to clothianidin, with an LC50 value of 75.31 ppm.

Table 2. Relative susceptibility of tea mosquito bug, *Helopeltis theivora* to different insecticides

Insecticides	*df*	Slope ± SE	χ^2	LC_{50} value (CI 95%)	Fiducial limit
Imidacloprid	4	1.06 ± 1.032	7.82	47.23	42.14 to 52.15
Thiamethoxam	4	1.109 ± 1.05	4.69	64.98	57.52 to 71.44
Flonicamid	4	0.116 ± 0.340	5.18	130.20	91.47 to 170.05
Clothianidin	4	0.69 ± 0.83	6.6	75.31	65.126 to 84.443
Bifenthrin	4	0.45 ± 0.67	5.05	0.36	0.32 to 0.41
Quinalphos	4	0.334 ± 0.578	4.6	100.48	83.67 to 116.39

Considering the unavailability of label-claimed insecticides or CIB recommendations specifically for *Helopeltis* spp. in cocoa, we undertook an in-vitro investigation into insecticidal screening. In the current study, TMB adults exhibited the highest susceptibility to bifenthrin (a Synthetic Pyrethroid-Neurotoxin) and the lowest susceptibility to flonicamid (a Pyridin Organic Compound-Chordotonal organ modulator), as evidenced by the respective LD50 values (Figure 5.4). Although flonicamid is recognized as a novel chemical and a feeding channel blocker, its specific binding site in the chordotonal organ remains unknown. Notably, flonicamid has demonstrated high effectiveness against aphids and whiteflies (Morita et al., 2007; Roditakis et al., 2014); however, these pests are typically high-density, whereas TMB is a low-density pest.

Quinalphos was found to be the least toxic after flonicamid. Quinalphos, a conventional organophosphate insecticide, has been utilized for blunt in males), and each pair was relocated to individual cages for copulation. All mated females were provided with fresh pods for nourishment and oviposition. The TMB culture required for bioassay was maintained at the prevailing room temperature.

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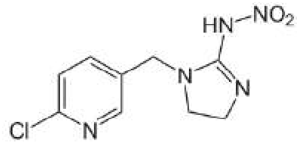
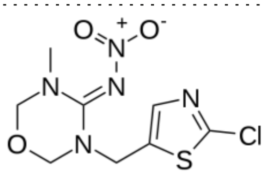
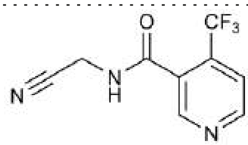
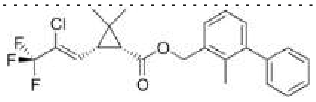
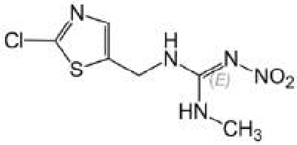
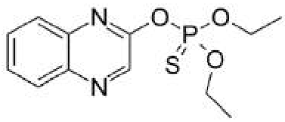
Imidacloprid 17.8% SL($C_9H_{10}ClN_5O_2$)	Confidor®		Indofil Industries Ltd.,Mumbai
Thiamethoxam 25% WG(C8H10CIN5O3S)	ExtraSUPER™		Crystal Crop Protection Pvt. Ltd.Jammu
Flonicamid 50% WG($C_9H_6F_3N_3O$)	ULALA®		UPL, India Ltd.,Mumbai

Bifenthrin 10% EC ($C_{23}H_{22}ClF_3O_2$)	Barristar®		Tamron Astra Bio-Chemicals Pvt. Ltd.Bangalore
Clothianidin 50 WDG ($C_6H_8ClN_5O_2S$)	DANTOTSU®		Sumitomo Chemical India Pvt. Ltd. Mumbai
Quinalphos 25% EC ($C_{12}H_{15}N_2O_3PS$)	KEMLOX®		Sumitomo Chemical India Pvt. Ltd. Mumbai

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Quinalphos was found to be the least toxic after flonicamid. Quinalphos, a conventional organophosphate insecticide, has been utilized for years to manage TMB in cashew and tea ecosystems (Sundararaju, 1984; Devasahayam and Nair, 1986). In the context of cashew management, quinalphos has been recommended for tackling TMB with a 375 ppm dose (Ahmed and Mamun, 2014). Gurusubramanian and Bora (2008) reported approximately 62-fold resistance development of *H. theivora* to quinalphos, indicating the possibility of the development of tolerant or resistant strains within this population.

This investigation demonstrates that the susceptibility of *H. theivora* to thiamethoxam and imidacloprid lies at a moderate level. Srikumar et al. (2017) conducted an electro-antennographic response study of *H. theivora* against quinalphos, bifenthrin, thiamethoxam, and deltamethrin, concluding that all stages of the insect are capable of perceiving quinalphos from a distance, suggesting a strong electro-antennographic response. This response might lead to the development of repellent behavior against quinalphos. This study's findings align with the development of repellency behavior concerning quinalphos. Srikumar et al. (2017) also reported a low level of electro-antennographic response to thiamethoxam. According to the ad-hoc recommendation, imidacloprid has been in use against TMB in cocoa or cashew for the past five to six years (ICAR-CPCRI). Over time, due to annual applications on cashew and cocoa, the population may have developed some level of tolerance or resistance.

Efficacy of screened insecticides under field conditions

Based on the LC50 values of the in-vitro screened insecticides, a single dose (approximately 10 times higher than the LC50 dose) of each insecticide (as shown in Table 3) was selected for field evaluation during the pre-monsoon season (January-April, 2018). The experiment was designed following a randomized block design (RBD), with treatment assignments determined using a random number table. Insecticides were uniformly sprayed across the full canopy of cocoa trees. Application of treatments took place during the last week of January, and data regarding the percentage of TMB infestation was collected within the first fortnight of April. The percentage of infestation in both control and insecticidal treatments was compared, and the data was analyzed using the SPSS package (version 15.0).

Efficacy of screened insecticides under field conditions

The investigation revealed notable variations in the efficacy of all the tested insecticides. A significantly low percentage of pod infestation was observed in the bifenthrin treatment (10.11%), followed by clothianidin (13.84%). Flonicamid exhibited the least effectiveness against *H. theivora*, resulting in a pod infestation rate of 37.82%. The percentages of infestation in treatments involving imidacloprid, thiamethoxam, and quinalphos were statistically similar (Fig. 1.)

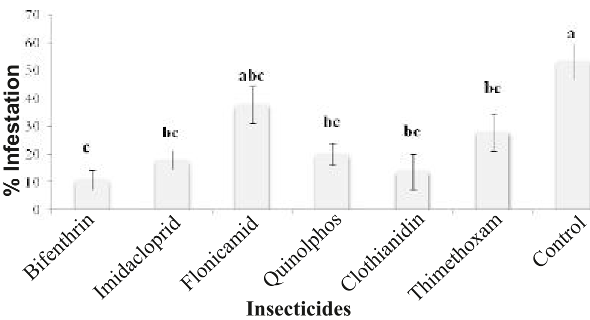

Fig. 1. Field efficacy of test insecticides against *Helopeltis theivora*

Field efficacy of the in-vitro screened test insecticides were assessed against TMB.. During this study, bifenthrin and clothianidin were identified as the most effective insecticides for managing TMB in cocoa. Therefore, these two chemicals may warrant further evaluation for developing an effective IPM package to manage TMB in cocoa. Comprehensive reports on the field efficacy of insecticides against TMB in cocoa for comparison with our study are currently unavailable.

Residual toxicity

In the study of residual toxicity at 1 DAS, bifenthrin-treated pods displayed significantly higher mortality at 95%, followed by thiamethoxam at 85%, compared to other insecticides. Imidacloprid, quinalphos, and clothianidin-treated pods exhibited considerable mortality at 77.50%, 75%, and 77.50%, respectively, which were statistically comparable. The lowest residual toxicity was observed in pods treated with flonicamid.

At 10 DAS, residual toxicity analysis indicated the highest mortality in pods treated with bifenthrin and clothianidin, both exhibiting 75% mortality in their respective insecticidal treatments. Flonicamid-treated cocoa pods displayed the least residual toxicity, with 25% mortality. Moderate residual toxicity was observed in both imidacloprid and thiamethoxam-treated pods, with 65% mortality.

At 20 DAS, a significant decline in residual effects was noted for quinalphos and flonicamid, with insecticide-treated pods recording 15% and 2.50% mortality, respectively. Similar to the 10 DAS results, the highest residual effect was observed in pods treated with bifenthrin and clothianidin, both exhibiting 60% mortality. In comparison to other insecticides, imidacloprid and thiamethoxam demonstrated moderate residual toxicity, with 35% and 25% mortality, respectively.

The 30 DAS residual toxicity analysis demonstrated a notable reduction in residual effects for all insecticides, except bifenthrin and clothianidin. Pods treated with bifenthrin and clothianidin retained a toxic residual effect even up to 30 DAS, displaying more than 25% mortality in these treatments (Fig. 2).

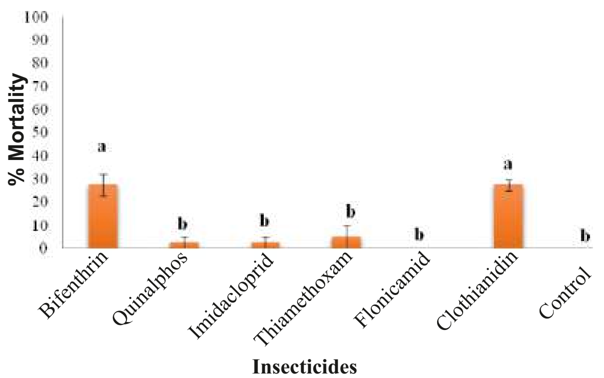

Fig. 3. Residual toxicity of test insecticides on *Helopeltis theivora* after 30 days spraying

The residual toxicity of field-recommended doses of all insecticides against *H. theivora* was investigated. Following the application of any recommended insecticide under field conditions, it is crucial to assess its persistence to judge its efficacy and benefit-cost ratio. This study found that both bifenthrin and clothianidin maintained their persistence in field conditions for up to 30 days after spraying (DAS), causing over 25% mortality of *H. theivora* (Fig. 3). In contrast, the loss of residual toxicity for flonicamid was significantly rapid compared to other test insecticides, resulting in less than 25% residual toxicity on 10 DAS. Imidacloprid and thiamethoxam reported over 25% residual mortality on 20 DAS. Consequently, the current investigation suggests that the duration between two applications of test insecticides can be determined based on these findings. Bifenthrin and clothianidin offer protection to cocoa crops for up to 30 DAS, thereby recommending a minimum interval of 30 days between successive applications of these insecticides. For neonicotinoid insecticides such as imidacloprid and thiamethoxam, the interval between applications should not be less than 20 days. Unfortunately, a comprehensive report on the residual toxicity of insecticides against TMB *Helopeltis* spp. is not available for direct comparison with our findings.

Conclusion

In conclusion, this study provides valuable insights into the varying toxicities and efficacies of different insecticides against *Helopeltis theivora*, a major pest of cocoa. Bifenthrin emerged as the most toxic insecticide, while flonicamid displayed the least toxicity. Bifenthrin and clothianidin were identified as the most effective in managing *H. theivora* infestations, showcasing low pod infestation rates. Additionally, the investigation highlighted the persistence of bifenthrin and clothianidin in the field, causing sustained mortality even up to 30 days post-application. These findings contribute to a comprehensive understanding of insecticide performance, aiding in the formulation of targeted pest management strategies for sustainable cocoa production.

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