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Assessment of insecticide resistance and enzymatic mechanisms in *Helopeltis theivora* from tea plantations of Assam

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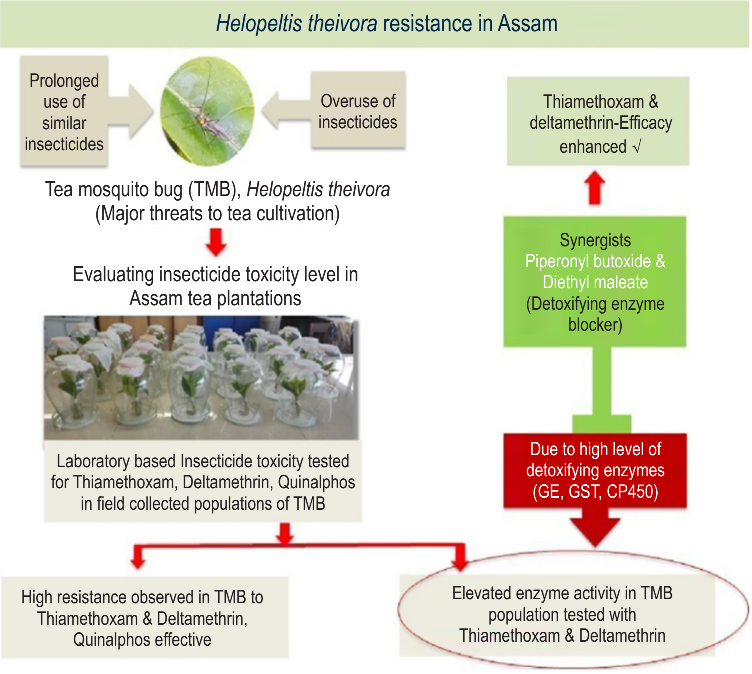
Abstract

Aim: To investigate the extent of insecticide resistance in *H. theivora* within Assam's tea-growing areas and to understand the defence mechanism that contribute to its development.

Methodology: Adult population of *H. theivora* from an organic tea plantation were collected for susceptible laboratory culture and other seven adult populations were collected from major tea-growing parts of Assam following conventional practices and reared under controlled laboratory conditions. The leaf dip method was used to conduct concentration mortality bioassays with thiamethoxam, deltamethrin and quinalphos. Biochemical assays were carried out to assess the activity of major detoxifying enzymes in the insects, including general esterases involved in hydrolysis, oxidative enzymes of cytochrome P450 family, and transferases that conjugate glutathione to xenobiotics. Furthermore, synergistic assays with piperonyl butoxide and diethyl maleate were carried out to evaluate their influence on the efficacy of insecticides.

Results: Resistance to thiamethoxam and deltamethrin was quite significant, with resistance coefficients ranging 2.04-22.14 and 1.56-23.90 times, respectively. All tested populations remained susceptible to quinalphos (RC<1). Increased enzyme activities exhibited a strong positive correlation with LC_{50} values, while the use of synergists significantly improved the insecticide efficacy.

Interpretation: The findings highlight the field-evolved resistance in *H. theivora* and its association with elevated detoxifying enzymes. This study recommends the strategic use of insecticides, rotation, and synergists for better resistance management, contributing to more sustainable pest control in tea plantations.



Key words: Esterase, *H. theivora,* Insecticides, Tea mosquito bug

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Introduction

Tea plantations are threatened by several pests, among them Helopeltis theivora, commonly known as the tea mosquito bug, is regarded as one of the most serious and damaging pests posing a major threat to tea cultivation. It is a sap-sucking, polyphagous pest, known to damage economically important crops such as cocoa, cashew, pepper, camphor, and especially tea (Roy *et al.,* 2015; Goswami *et al.,* 2023). This Hemipteran pest belongs to family Miridae that causes damage to tea crops by piercing tender plant tissues (leaves, buds and stems) with its proboscis to extract sap, simultaneously injecting toxic saliva (Roy *et al.,* 2015; Sankarganesh *et al.,* 2020). This results in localized tissue death, initially visible as ring-shaped discoloration that progresses to necrotic spots. As lesions merge, leaves curl, deform, and eventually fall, with severe infestations potentially causing total crop loss (Muraleedharan, 1992; Vishnupriya *et al.,* 2021). All the life stages right from nymphs to adults are equally capable of damaging the tea crop severely.

While it was initially discovered in Java in 1847, this pest has now spread widely across South-east Asia, Africa and Northern Australia, particularly in tropical climates. On the global scale, *H. theivora* is regarded as a serious pest in tea-growing regions across Africa and Asia (Muraleedharan, 1992; Hazarika *et al.,* 2009; Ahmed and Mamun, 2014; Vishnupriya *et al.,* 2021). In India, it infests about 80% of tea-growing regions, causing yield reduction up to 50% (Roy and Gurusubramanian, 2013; Kalita *et al.,* 2016; Goswami *et al.,* 2023). In India, the major tea cultivation is done in the North-eastern states including Assam, Tripura and West Bengal, with Assam contributing roughly 51% of the national output, producing 630-700 million kilograms annually. Historically, a wide range of insecticides from various groups were crucial in reducing the economic impact of *H. theivora* (Saha *et al.,* 2012; Roy *et al.,* 2015; Roy and Prasad, 2018). However, with the introduction of the Plant Protection Code (PPC) by the Tea Board of India in 2014, the use of insecticides in tea plantations has been restricted to a few comparatively safer options, selected on the basis of maximum residue limits (MRL) and pre-harvest intervals.

Consequently, a few insecticides from the neonicotinoid, pyrethroid, and organophosphate groups are permitted for managing *H. theivora* in tea plantations (Roy and Prasad, 2018). Moreover, bioassay results have shown that *H. theivora* exhibits varying degrees of susceptibility to different insecticides suggesting low to moderate resistance to thiamethoxam, thiacloprid, cypermethrin, lambda-cyhalothrin, quinalphos and imidacloprid (Roy *et al.,* 2015; Das *et al.,* 2024; Naskar *et al.,* 2025). Lately, tea growers across several regions of Assam have observed a reduced efficacy of insecticides with their recommended doses against *H. theivora*, allowing it to remain a persistent pest in tea plantations. Key factors contributing to the failure in controlling *H. theivora* include high reproductive potential, multiple annual generations, and the repeated use of same insecticides (Roy *et al.,* 2011; Yao *et al.,* 2025). Repeated applications of these insecticides have led to claims of field control failures in some areas of Assam. Moreover, intensive insecticide use exposes natural enemies to excessive levels of these chemicals (Das *et al.,* 2005, Goswami *et al.,* 2023). Therefore, it is crucial to evaluate *H. theivora* resistance to various insecticides and understand the underlying mechanisms to implement effective resistance management strategies.

Resistance development in *H. theivora* to commonly used insecticides has been documented in the Dooars (Roy *et al.,* 2010; 2011) and Darjeeling regions (Bora *et al.,* 2007) of West Bengal, as well as in the Jorhat region of Assam (Bora and Gurusubramanian, 2007). Field population of H. theivora exhibits high resistance to organochlorines, moderate resistance to organophosphates, and low resistance to neonicotinoids (Roy *et al.,* 2011). Furthermore, resistance to the pyrethroid insecticide deltamethrin has also been reported (Roy and Gurusubramanian, 2013; Das *et al.,* 2024). In *H. theivora,* enhanced detoxification enzyme activity has been found one of the key mechanisms underlying such increased resistance (Roy *et al.,* 2015; Das *et al.,* 2024; Naskar *et al.,* 205). Specifically, general esterase (GE), cytochrome P450-mediated mono-oxygenases (CYP) and glutathione S transferase (GST) exhibit enhanced activity in resistant field populations compared to susceptible populations (Saha *et al.,* 2012, 2013; Das *et al.,* 2024; Naskar *et al.,* 2025).

Evaluating the susceptibility of *H. theivora* to commonly used insecticides, and uncovering its resistance, is key to develop a practical and location-specific strategies to manage this pest effectively. However, information on its susceptibility to widely used insecticides in Assam is scarce. In light of recent reports of insecticide failures in major tea-producing areas, evaluating the present resistance status of field populations has become imperative. Continuous and prolonged use of a limited range of chemical actives approved by PPC has likely accelerated resistance development. With only a few insecticides recommended for *H. theivora* control, tracking resistance trends and identifying alternative or rotation-compatible actives are essential to avert further efficacy loss and ensure sustainable, long-term pest management.

Materials and Methods

Collection of insects: Adult *H. theivora* insects were collected from an organic tea estate in the Karbi Anglong district of Assam (26.11° N, 93.44° E), where insecticide applications have been banned for the past 27 years (certified organic since 1997). We considered this organic population as susceptible population. Field-collected adult populations were obtained from seven major tea-producing districts of Assam: Tinsukia (27.48° N, 95.35° E), Dibrugarh (27.47° N, 94.91° E), Sibsagar (26.98° N, 94.64° E), Jorhat (26.75° N, 94.20° E), Golaghat (26.52° N, 93.96° E), Udalguri (26.74° N, 92.09° E), and Cachar (24.78° N, 92.85° E) that practice conventional chemical management. Both susceptible and conventional populations were maintained on the shoots of tea cultivar TV1 separately, as per the rearing protocol of Roy *et al.* (2010), in wooden cages measuring 70 × 62 × 75 cm.

Insects were maintained under standardized laboratory conditions at 25 ± 2 °C, with relative humidity of 75 ± 5% and a 16:8 hr light-to-dark cycle.

Chemicals used: Commercially used insecticide from different chemical groups *viz.,* thiamethoxam (Actara® 25% WG, Syngenta India Ltd.), deltamethrin (Decis® 2.8% EC, Bayer Crop Science Ltd.) and quinalphos (Ekalux® 25% EC, Syngenta India Ltd.) were selected for the bioassay experiments. All three are approved under the Plant Protection Code (PPC) (Tea Board, 2024) and are authorized for application against *H. theivora*. The synergists used were piperonyl butoxide (PBO, 99%, CAS 51-03-6) and diethyl maleate (DEM, 97%, CAS 141-05-9) which were procured from Sigma-Aldrich (3050 Spruce St., Saint Louis, MO, United States, 63103).

Insecticide assays: Bioassays were performed on adult *H. theivora* using a previously described leaf dip method (Roy and Prasad, 2018) to evaluate the concentration-responses to different insecticides. In brief, twenty adult *H. theivora* both organic and conventional tea plantations were treated to different concentrations of insecticides. Based on preliminary results that showed mortality rates between 20 to 100%, subsequent assays were conducted using six to nine graded concentrations. Tea leaves immersed in demineralized water served as untreated controls. For each insecticide and control treatment, three replicates were maintained. Mortality was recorded after 48 hr, and lethal effects were expressed as percentage mortality at each concentration, calculated by Abbott's formula (Abbott, 1925).

Estimation of enzyme activities: Twenty adult *H. theivora* both from organic and conventional plantations were starved for 3 hr to clear the gut. These insects were first grounded in 0.1 M sodium phosphate buffer, (pH 7.0) to obtain a uniform homogenate, thereafter the homogenate was centrifuged at 12000 × g for 20 min at 4°C. (Saha *et al.,* 2012). The resulting supernatant was divided into 100 µl aliquots, transferred into 0.5 ml microcentrifuge tubes, and stored at −20°C until further use. Each aliquot was subsequently used to assess the activity of major detoxification enzymes, *viz.,* general esterases (GE), cytochrome P450 monooxygenases (CYP), and glutathione S-transferases (GST). The total protein content in the enzyme extracts was estimated by the method of Lowry *et al.* (1951).

The GST level in *H. theivora* homogenate was measured using the substrate 1-chloro-2,4-dinitrobenzene (CDNB) (Habig *et al.,* 1974). The reaction mixture was prepared by combining 0.1 M phosphate buffer (2.78 ml of pH 6.5) containing 1 mM EDTA with 50 µl of 1-Chloro-2,4-dinitrobenzene (50 mM prepared in ethanol), along with 150 µl of reduced glutathione (50 mM prepared in 0.1 M PB, pH 6.5). Following this, to initiate the reaction, 20 µl of enzyme supernatant was introduced into the mixture and mixed well. A 300 µl portion from each reaction was transferred to a microplate, and using the kinetic (time-scan) function of a microplate reader, the absorbance was read at 340 nm over a period of 10 min. GE activity in *H. theivora* from field populations was determined with α-naphthyl acetate at a final concentration of 30 mM (Van Asperen, 1962). For the assay, 200 µl of substrate solution was dispensed into each well of a 96-well plate, after which 20 µl of the enzyme sample was added. wells. Following a 15 min pre-incubation at 25°C, 50 µl of the staining solution was pipetted into the wells. Control wells without enzyme served as blanks. Absorbance readings were obtained at 570 nm with a 5-minute gap between each measurement using a Multiskan GO microplate spectrophotometer (Thermo Scientific). GE activity was quantified as µmol of α-naphthol formed each minute for every milligram of protein.

CYP activity was determined using a haem peroxidase substrate, as haem protein forms the primary component (Penilla *et al.,* 2007). For the assay, 20 µl of enzyme homogenate was combined with 200 µl of 3,3',5,5'-Tetramethylbenzidine solution (TMBZ) (prepared by mixing 0.01 g of TMBZ in 5 ml methanol and adding 15 ml of 0.25 M sodium acetate buffer, pH 5.0), 25 µl of 3% H_2O_2. and 80 µl of 0.0625 M PB (pH 7.2). Following a 30-minute incubation at 25°C, absorbance at 630 nm was recorded using the microplate reader. A standard curve was prepared using cytochrome C derived from horse heart (type IV), and the activity of CYP was determined as nanomoles of CYP equivalent units formed per minute per mg protein.

Synergism assays: The detoxification ability of *H. theivora* towards insecticides was studied using two synergists *i.e.* PBO and DEM in the assays conducted through the standard leaf dip method. Synergist solutions were prepared alone and in combination with the recommended doses of deltamethrin and thiamethoxam respectively across seven to nine selected concentrations at a concentration of 100 ppm. Tea leaves treated with a synergist solution (100 ppm) served as the control. Ten adult bugs were introduced to 5-6 sets of different concentrations of each synergist with insecticide combination. Each treatment was replicated three times.

Calculations and data analysis: For each insecticide, the expected effective concentration (EEC) was derived from its LC_{95} value, and the corresponding expected effective dose (EED) was obtained following Bora and Gurusubramanian (2007). LC_{50} values (ppm) were calculated through Finney's probit analysis with SPSS version 19.0 (SPSS Inc., USA) (Finney, 1973). Following Zhang *et al.* (2017), the resistance ratio (RR) was determined, and the resistance coefficient (RC) was estimated based on the procedure of Wegorek *et al.* (2009). RC classification followed Roy *et al.* (2021): RC=1 denoted susceptibility, RC = 2 to 10 indicated low resistance, RC = 11 to 30 meant moderate level of resistance, RC = 31 to 100 indicated high resistance, and very high resistance with RC > 100. Differences among treatments were tested via one-way ANOVA, and mean comparisons were carried out using Tukey's post hoc test at $p \leq 0.05$. The association between LC_{50} values and enzyme activity levels was evaluated using Spearman's rank-order correlation in SPSS. Synergism ratios (SR) were computed following the procedure outlined by Hsu *et al.* (2004).

Results and Discussion

Prolonged and indiscriminate use of insecticides develops resistance in many pest species, rendering conventional control methods less effective (Venkatesan *et al.,* 2022). The successful management of insecticide resistance necessitates a multifaceted approach that includes both monitoring resistance levels and comprehending the underlying resistance mechanisms (Malathi *et al.,* 2017). In this context, the resistance profile of *H. theivora* was evaluated against three widely used insecticides, namely thiamethoxam, deltamethrin, and quinalphos which varied evidently among the eight field populations of *H. theivora* assessed in this study (Table 1). These regional differences are illustrated in a resistance map, emphasising the influence of localised insecticide usage patterns on resistance dynamics (Fig. 1). Compared to the susceptible Karbi Anglong strain, five populations exhibited moderate resistance to thiamethoxam (RC=13.28-22.14), while the Udalguri (RC=9.26) and Cachar (RC=2.04) populations displayed either tolerance or low resistance, respectively which is probably a consequence of its widespread and recurrent use in tea plantations since the early 2000s for managing sucking pests (Roy *et al.,* 2015; Chen *et al.,* 2021). This pattern is further facilitated by favourable maximum residue limits and pre-harvest intervals that encourage its continuous application. Deltamethrin resistance ranged from low to high across populations, reflecting its extensive use as a low-cost pyrethroid, especially following restrictions on organophosphate insecticides. For deltamethrin, moderate resistance was observed in four populations (RC = 14.69-23.90) whereas Jorhat, Udalguri and Cachar showed low resistance, with RC values of 8.45, 4.35 and 1.56, respectively.

Previously, *H. theivora* populations from Kalchini, North Bengal showed varied resistance levels, including extremely high resistance to quinalphos and endosulfan, and moderate resistance to thiamethoxam and cypermethrin (Roy *et al.,* 2011; 2015). Similarly, Saha *et al.* (2012) documented strong resistance to quinalphos in Terai and Dooars populations. High resistance to organophosphates such as profenofos and chlorpyrifos has also been reported from Kerala (Shanmugapriyan *et al.,* 2013), and diminished susceptibility to deltamethrin and imidacloprid was observed in Assam's Jorhat region (Bora *et al.,* 2008). In contrast,

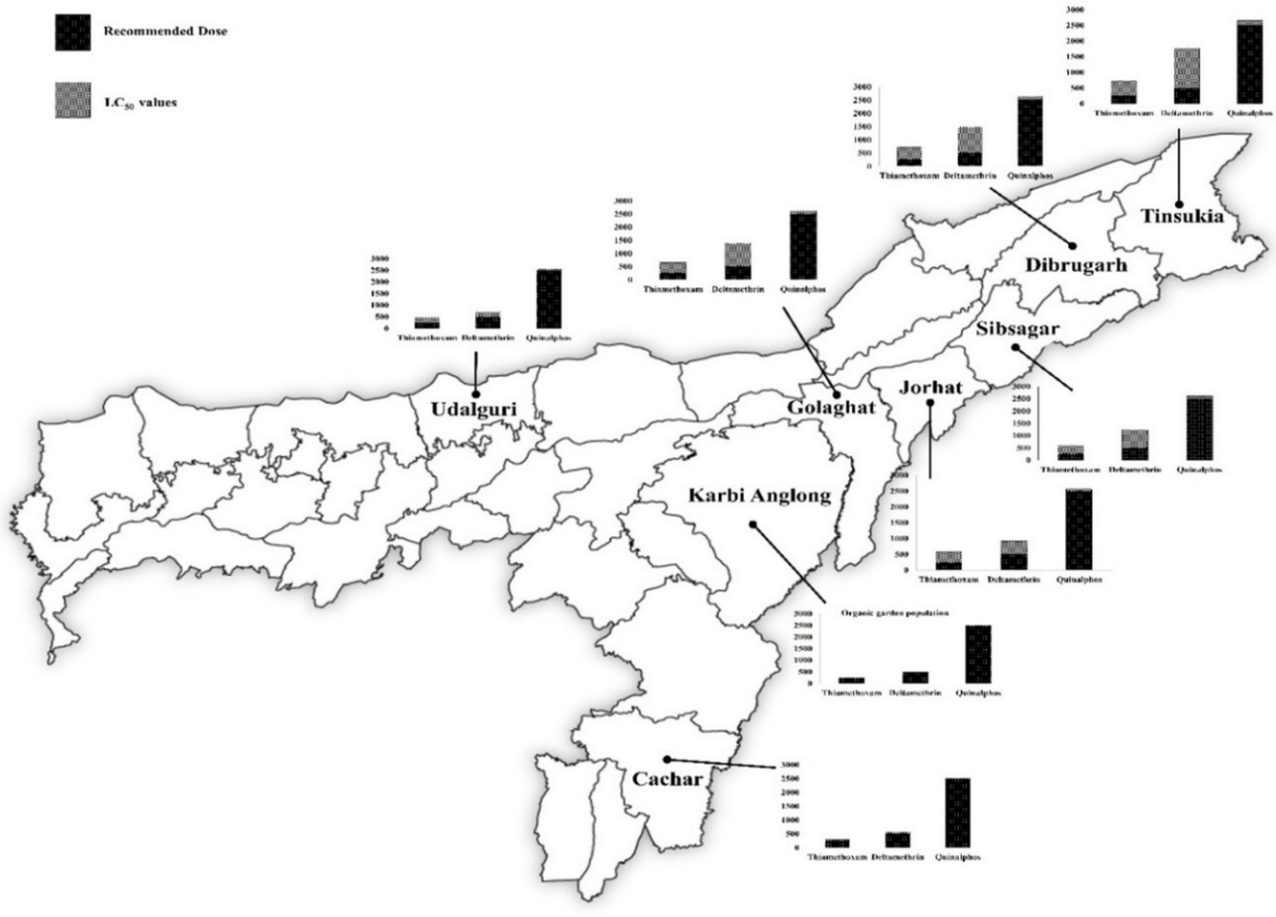

Fig. 1: Resistance map depicting varying degree of resistance across different regions of Assam by adult *Helopeltis theivora.*

Table 1: Susceptibility to three insecticides in eight field populations of adult *Helopeltis theivora*

Insecticides	Regions	n^a	LC$_{50}$ (95% FL^b) (ppm)	Slope ± SE	χ^2 (df)	RR^c	LC$_{95}$ (ppm)	RD^d	RC^e
Thiamethoxam	Tinsukia	210	489.29 (371.03-645.25)	1.57 ± 0.001	3.76 (6)	163096.67	5535.12	250	22.14
	Dibrugarh	180	461.45 (336.91-632.01)	1.59 ± 0.002	6.05 (5)	153816.67	5000.44	250	20.00
	Sibsagar	180	349.89 (261.42-468.29)	1.65 ± 0.001	6.13 (5)	116630.00	3504.71	250	14.02
	Jorhat	180	323.11 (234.34-445.52)	1.64 ± 0.002	3.88 (4)	107703.33	3321.05	250	13.28
	Golaghat	180	403.55 (303.81-536.04)	1.65 ± 0.001	4.31 (5)	134516.67	4089.23	250	16.36
	Udalguri	180	217.80 (162.79-291.39)	1.61 ± 0.001	2.67 (5)	72600.00	2315.17	250	9.26
	Cachar	210	43.72 (32.73-58.41)	1.63 ± 0.002	3.55 (6)	14573.33	456.09	250	1.83
	Karbi Anglong	180	0.0030 (0.0022-0.0040)	1.59 ± 0.002	0.21 (4)	1.00	0.030	250	0.00
Deltamethrin	Tinsukia	180	1265.91(946.45-1693.19)	1.69 ± 0.001	6.96 (5)	234427.78	11948.39	500	23.90
	Dibrugarh	210	976.80 (743.91-1282.60)	1.64 ± 0.001	2.65 (6)	180888.89	9929.10	500	19.86
	Sibsagar	180	740.11 (539.52-1015.27)	1.66 ± 0.002	3.36 (5)	137057.41	7342.89	500	14.69
	Jorhat	180	416.83 (312.15-556.50)	1.65 ± 0.001	5.58 (5)	77190.74	4227.28	500	8.45
	Golaghat	180	898.56 (673.99-1197.96)	1.65 ± 0.001	5.32 (5)	166400.00	8997.38	500	17.99
	Udalguri	180	216.77 (160.97-291.75)	1.65 ± 0.001	6.30 (5)	40142.59	2176.93	500	4.35
	Cachar	180	80.01 (58.56-109.31)	1.69 ± 0.002	4.98 (4)	14547.27	766.21	500	0.63
	Karbi Anglong	180	0.0054 (0.0039-0.0075)	1.66 ± 0.002	0.83 (4)	1.00	0.05	500	0.00
Quinalphos	Tinsukia	180	156.06 (117.12-207.95)	1.69 ± 0.001	6.09 (5)	1793.79	1482.73	2500	0.59
	Dibrugarh	180	139.59 (82.79-235.34)	1.65 ± 0.005	1.46 (5)	1604.48	1404.75	2500	0.56
	Sibsagar	180	111.86 (83.13-150.53)	1.64 ± 0.001	4.35 (5)	1285.75	1147.94	2500	0.46
	Jorhat	210	85.88 (63.44-116.27)	1.56 ± 0.002	4.41 (6)	987.13	995.69	2500	0.40
	Golaghat	180	124.18 (93.07-165.70)	1.63 ± 0.001	1.65 (5)	1427.36	1289.74	2500	0.52
	Udalguri	180	60.72 (42.09-87.61)	1.68 ± 0.002	3.00 (5)	697.93	583.56	2500	0.23
	Cachar	150	22.43 (16.24-30.98)	1.63 ± 0.002	4.22 (4)	257.81	279.66	2500	0.11
	Karbi Anglong	180	0.0087 (0.0060-0.0120)	1.65 ± 0.002	2.84 (4)	1.00	0.087	2500	0.00

^{a}Number of adults tested; *df* = Degrees of freedom; ^{b}Fiducial limit; ^{c}Resistance ratio (RR) = LC$_{50}$ of field population/LC$_{50}$ of the susceptible population; ^{d}RD = Recommended Dose (g a.i./ ha); ^{e}Resistance coefficient (RC) = Expected effective dose /Recommended field dose

Table 2: Synergistic effect of piperonyl butoxide and diethyl maleate on thiamethoxam and deltamethrin to resistant field populations of adult *Helopeltis theivora*

Treatments	n^a	*df*	Slope ± SE	LC$_{50}$ (ppm)	95% FL^b of LL (ppm)	LC$_{50}$ UL (ppm)	χ^2	SR
Thiamethoxam 25WG	210	6	1.57 ± 0.0014	489.29	371.03	645.25	3.76	1.00
Thiamethoxam 25WG + PBO	180	5	1.63 ± 0.0022	169.22	121.15	236.36	5.69	2.89
Thiamethoxam 25WG + DEM	180	5	1.64 ± 0.0022	240.57	172.91	334.70	4.36	2.03
Deltamethrin 2.8EC	180	5	1.69 ± 0.0017	1265.91	946.45	1693.19	6.96	1.00
Deltamethrin 2.8EC + PBO	210	6	1.66 ± 0.0014	304.05	230.64	400.83	4.45	4.16
Deltamethrin 2.8EC + DEM	210	6	1.62 ± 0.0014	556.04	421.15	734.14	6.51	2.28

*PBO = Piperonyl butoxide, DEM = Diethyl maleate; ^{a}Number of insect tested (Adults); df = Degrees of freedom; $_b$Fiducial limit; LC$_{50}$: lethal concentration for 50%; SR (Synergistic ratio) = LC$_{50}$ of insecticide along/LC$_{50}$ of the combination treatment

the findings of this study reveal that all populations remained susceptible to quinalphos (RC < 1), which could be attributed to the implementation of the Plant Protection Code (PPC) for tea in 2014, which led to a gradual decline in the use of quinalphos owing to its long pre-harvest interval and an Indian MRL of 0.7 ppm. Consequently, the reduced application probably diminished the selection pressure on *H. theivora,* facilitating a reversion towards susceptibility and reflecting the positive impact of regulatory measures in restricting its usage (Roy *et al.,* 2015). Such rapid reversion of organophosphate resistance upon discontinuation or limited use is well documented in other pest species (Low *et al.,* 2013; Gong *et al.,* 2020). Additionally, the pest's high reproductive rate and multiple generations per year (Gurusubramanian and Bora, 2008) as well as differing insecticide application intensities across regions (Zhao *et al.,* 2018; Naskar *et al.,* 2025) facilitates dilution of resistance alleles in the absence of continuous insecticide exposure (Carriere and Tabashnik, 2001; Alyokhin *et al.,* 2008).

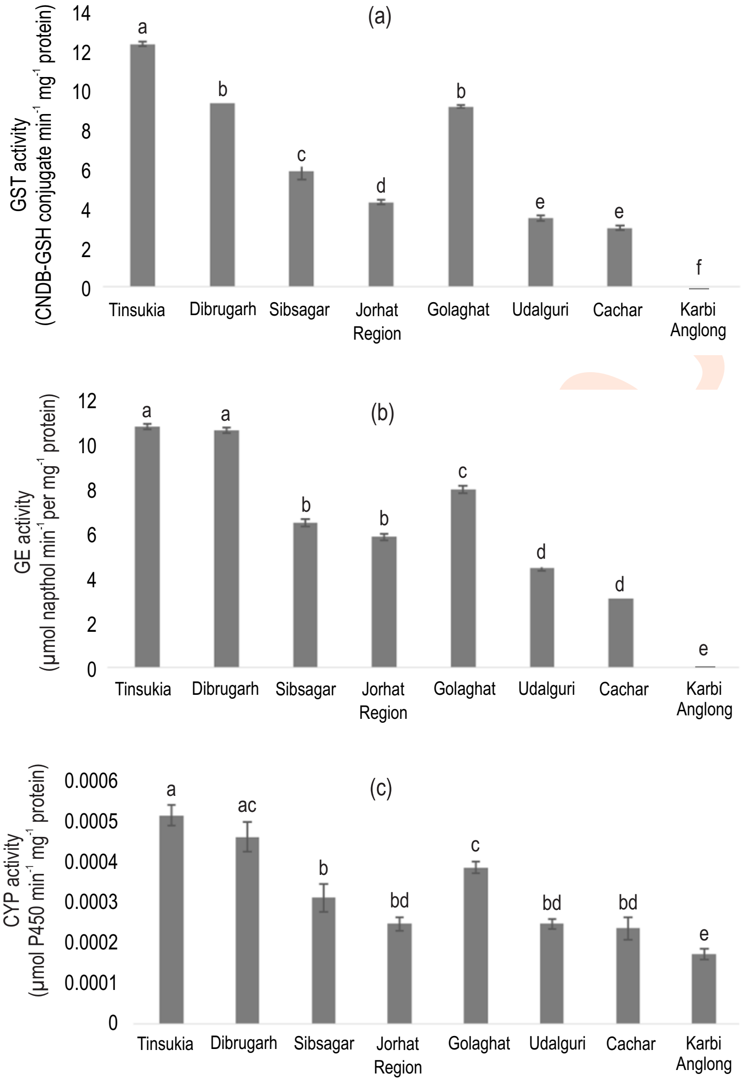

Fig. 2: Detoxifying enzyme activities of glutathione S-transferase (A), general esterase (B), and cytochrome P450 (C) in eight field populations of adult *Helopeltis theivora*. (n=10), *Mean values ± SE. #Means with different letters indicate significant difference @p ≤ 0.05: analysed by One-way ANOVA based on LSD.

Biochemical assays revealed significant variation in detoxification enzyme activities across the populations. Glutathione S-transferase (GST) activity among resistant strains ranged from 3.10 to 12.81 CNDB-GSH conjugate min^{-1} mg^{-1} protein, with the Tinsukia population recording the highest mean activity-177.92-fold greater than the susceptible strain followed by Dibrugarh (138.75-fold) and Golaghat (132.08-fold) (Fig. 2A). This indicates that GSTs are likely important contributors to the resistance observed in field populations of *H. theivora*. Similarly, general esterase (GE) and cytochrome P450 (CYP) activities were elevated in resistant populations, consistent with enhanced metabolic detoxification contributing to resistance. GE activity was substantially high, with Tinsukia and Dibrugarh registering values of 11.51 and 10.98 μmol α-naphthol min^{-1} mg^{-1} protein, respectively (Fig. 2B). CYP activity followed a comparable trend with Tinsukia exhibiting a 3.00-fold increase, Dibrugarh 2.69-fold and Golaghat 2.25-fold relative to the susceptible reference (Fig. 2C). Statistical analysis indicated no significant difference in enzyme activities among some populations, such as Udalguri and Cachar, consistent with their lower resistance levels.

Results also indicated that there were strong, statistically significant positive correlations between LC_{50} values for all three insecticides and the levels of GST, general esterase, and cytochrome P450 activities. GST showed the strongest correlations, with r^2 values of 0.9190 for thiamethoxam, 0.9001 for deltamethrin, and 0.9603 for quinalphos (Fig. 3A). General esterase activity also correlated strongly (r^2 = 0.8416, 0.9514, and 0.9196 (Fig. 3B), as did cytochrome P450 activity (r^2 = 0.8546, 0.9806, and 0.9180 (Fig. 3C).

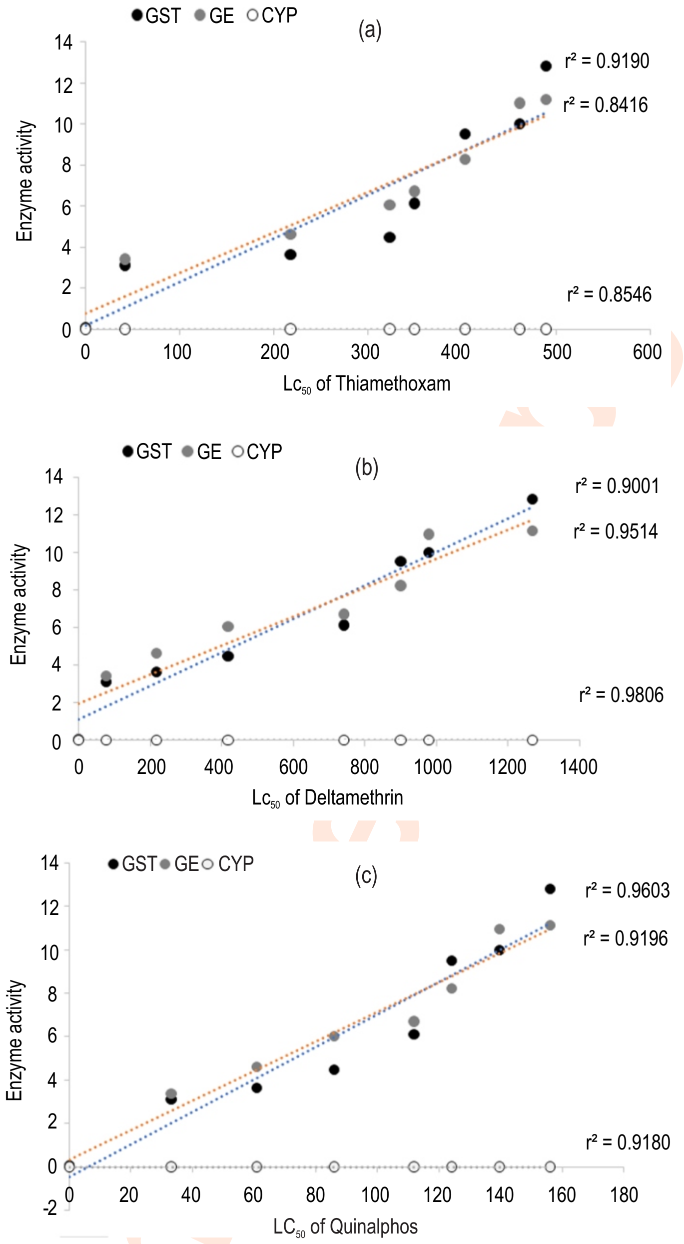

Fig. 3: Correlation coefficients of LC_{50} values of thiamethoxam (A), deltamethrin (B) and quinalphos (C) correlated with the detoxifying enzyme activities of glutathione S-transferase, general esterase, and cytochrome P450 in eight field populations and a susceptible population of adult *Helopeltis theivora*.

The data collectively suggest that resistance in *H. theivora* is primarily supported by enhanced activity of detoxification pathways. GSTs detoxify insecticides by conjugation (Li *et al.,* 2007; Pavlidi *et al.,* 2018; Yang *et al.,* 2020), with previous studies linking increased GST activity to resistance against thiamethoxam (Yang *et al.,* 2016; Jameel *et al.,* 2020; Das *et al.,* 2024) and deltamethrin (Fragoso *et al.,* 2003; Tao *et al.,* 2022; Das *et al.,* 2024). The elevated levels of both GST and GE in resistant *H. theivora* populations suggest that these enzymes are significantly involved in breaking down and metabolizing the insecticides (Saha *et al.,* 2012; Qian *et al.,* 2024). CYP-associated resistance arises mainly from elevated oxidative metabolism, often due to altered expression or function of P450 enzymes (Oppenoorth, 1984; Naskar *et al.,* 2025). These changes are crucial in promoting the development of resistance across various insect species (Zhu *et al.,* 2008; Liu *et al.,* 2011; Yang *et al.,* 2020). Synergistic bioassays using piperonyl butoxide (PBO) and diethyl maleate (DEM) further confirmed the involvement of these enzymes. Application of the synergists PBO and DEM in bioassays with the resistant Tinsukia population provided further confirmation of metabolic involvement. PBO significantly increased the toxicity of both thiamethoxam (synergistic ratio, SR = 2.89) and deltamethrin (SR = 4.16), whilst DEM yielded SRs of 2.03 and 2.28 for these insecticides, respectively (Table 2). Neither synergist alone produced any observable mortality, implying that the observed increase in toxicity is due to their inhibition of key detoxification enzymes, rather than intrinsic toxicity. The combination of PBO, an inhibitor of GE and CYP activity (Young *et al.,* 2006; Panini *et al.,* 2017) and DEM, a GST inhibitor (Memarizadeh *et al.,* 2013; Yang *et al.,* 2020; Das *et al.,* 2024), significantly increased the toxicity of thiamethoxam and deltamethrin in resistant populations. This demonstrates the combined role of GST, GE, and CYP enzymes in reducing insecticide efficacy. Similarly, there have been reports of PBO enhancing the efficacy of pyrethroids affecting their metabolic processing, with GST and CYP enzymes serving key roles in modulating insecticide efficacy (Roy *et al.,* 2009; El-Sayed *et al.,* 2023; Das *et al.,* 2024).

In conclusion, this study demonstrated that *H. theivora* populations exhibit differing susceptibility levels to three key insecticides. Gaining insights into the metabolic processes involved in insecticide detoxification can help improve control strategies by using synergists that block these pathways, thereby enhancing insecticide effectiveness and supporting the development of robust resistance management programs. Future research should focus on exploring the genetic factors and specific resistance mechanisms involved, which will be critical for designing effective resistance management strategies for *H. theivora* populations in Assam.

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