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RESEARCH ARTICLE

Detoxifying enzyme response in *Leucinodes orbonalis* under thermal and insecticide stress

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Abstract

The eggplant shoot and fruit borer (*Leucinodes orbonalis*) is a major pest of brinjal, causing significant yield losses due to increasing insecticide resistance. This study investigates the impact of sub-lethal doses (LD_{30}) of spinetoram, tetraniliprole and emamectin benzoate, combined with temperature variations (29 and 32 °C), on detoxifying enzyme activities in *L. orbonalis* larvae. Insecticide exposure significantly enhanced the activities of carboxylesterase (1.34- to 1.45-fold), glutathione S-transferase (1.73- to 1.86-fold) and cytochrome P450 (up to 2.6-fold) compared to the controls, with tetraniliprole inducing the highest responses. Thermal stress at 32 °C further amplified carboxylesterase and glutathione S-transferase activities compared to 29 °C, indicating enhanced detoxification under high temperature conditions. Larvae failed to survive at 22 and 37 °C, limiting enzymatic analysis to moderate temperatures. Among the plant extracts tested, *Nyctanthes arbor-tristis* (500 ppm) markedly increased cytochrome P450 activity, suggesting its potential as a synergist for resistance management. These findings highlight the role of detoxifying enzymes in mediating insecticide resistance and thermal resilience in *L. orbonalis*. Integrating biochemical markers into resistance monitoring and leveraging plant-derived compounds could improve sustainable pest management strategies to address challenges posed by resistance and climate variability.

Keywords: detoxifying enzymes; emamectin benzoate; insecticide resistance; *Leucinodes orbonalis*; spinetoram; tetraniliprole; thermal stress

Introduction

The persistent threat of several insect pests, particularly the shoot and fruit borer (*Leucinodes orbonalis*), looms large posing a significant risk to brinjal yield, the magnitude of yield losses has ranged from 20 % to 90 % in some instances (1). This voracious pest presents a major hurdle in brinjal production, with its larval stages exhibiting distinctive feeding behaviors on a monophagous diet, accompanied by homing and tunneling tendencies (2). While the primary target is brinjal, other plants within the Solanaceae family, including tomato, potato, nightshade and turkey berry, can also serve as hosts. Its activity persists year-round, particularly in regions with temperate climates where multiple generations overlap. The traditional approach to control this pest has relied heavily on scheduled pesticide applications, irrespective of actual pest activity, leading to higher production costs, environmental harm and the development of resistance, thus prompting an urgent need for alternative control strategies.

Resistance to insecticides is a formidable challenge in pest management, manifesting as an insect's ability to withstand standard doses due to physiological or behavioral adaptations (3). Mechanisms underlying resistance include heightened detoxifying enzyme activity, modified target sites and reduced cuticular penetration (4). Insects have evolved diverse detoxification mechanisms in response to both natural plant toxins and synthetic insecticides, with variations observed across different stages, populations and species (5). The rapid evolution of resistance underscores the urgency of devising proactive approaches. Enzymes such as cytochrome P_{450} monooxygenases, carboxyl esterases (CarE) and glutathione S-transferases (GSTs) play a vital role in xenobiotic detoxification. Their far-reaching influence extends to resistance development across multiple insecticide classes due to their broad substrate specificity (6). Understanding these enzymes' intricate roles sheds light on potential avenues for targeted pest management interventions, while alkaline and acid phosphatases also emerge as key players in insect metabolism.

As global temperatures surge due to climate change, the dynamics of pest management undergo a significant shift. Elevated temperatures not only alter the toxicity of insecticides but also accelerate pest metabolism. Temperature-induced fluctuations extend to major insecticide-detoxifying enzymes, significantly influencing the susceptibility of insect pests to control measures (7, 8). This intricate relationship between temperature and enzymatic activity adds another layer of complexity to pest management strategies. The interplay

necessitates a re-evaluation of resistance management approaches, as pests may develop resistance to insecticides at an accelerated pace (9). Against this background, the present investigation was carried out to examine the effect of one-time exposure of larvae to LD30 doses of select insecticides, alone and in combination with botanicals, on the detoxifying enzymes of *L. orbonalis*, as well as to determine the impact of single-generation exposure to varied temperature regimes on the properties of these enzymes.

Materials and Methods

The present study was carried out in the Toxicology Laboratory, Dept. of Entomology, OUAT, Bhubaneswar. The infested brinjal fruits were collected from the fields of the Bhubaneswar area for the 3rd/4th instar larvae of *L. orbonalis* (Guenee).

Bioassay

Three insecticides, such as emamectin benzoate, spinetoram and tetraniliprole were used to study the toxicity towards 3rd/4th instar larvae of *L. orbonalis*. The insecticide stock solutions were prepared to deliver 1 mg/larva using the formula of Kranthi (10). The stock solutions were further diluted to total of six dilutions such as 1×10^{-3}, 5×10^{-4}, 1×10^{-4}, 5×10^{-5}, 1×10^{-5}, 1×10^{-6} mg/larva and acetone as control. The insecticides were applied topically at the dorsal thoracic segments of 3rd/4th instars larvae using a hand-held Hamilton micro dispenser (1 mL/larvae) to record mortality after 24 hr. Percent mortality was computed by Abbot's correction formula (11).

Extracts preparation

The fresh tender leaves of *Alstonia scholaris*, *Spathodea campanulata*, *Eupatorium odoratum*, *Vitex negundo*, *Nyctanthes arbor-tristis* and bark of *Terminalia arjuna* were collected and made into powder using a mortar and pestle. The leftovers after evaporation were collected in glass vials until further use.

Determination of LD_{50} and LD_{30} values and their 95 % fiducial limits (FL) were obtained by maximum likelihood logit regression analysis using SPSS software. Bioassay data were analysed by probit analysis using SPSS v. 27.0. Dosage-mortality regression lines, obtained by probit analysis (12) provided LD_{50} values for each insecticide synergist solution.

Effect of sub-lethal doses of insecticides on detoxifying enzyme activity of *L. orbonalis*

The collected larval population was treated with sub-lethal (LD_{30}) dosage of test insecticides using a Hamilton hand micro applicator (1 mL/larva) and were reared on potato. The detoxifying enzyme activities were determined in the F_1 generation.

Effect of thermal stress on detoxifying enzyme activity of *L. orbonalis*

The collected larvae were reared on the potato for one generation and the second instar larvae of F_1 generation were exposed to varied temperature regimes *viz*. 22, 29, 32 and 37 in Rivotek incubator up to 3rd/4th instars and enzyme assay was conducted. However, larvae did not thrive at the temperatures of 22 and 37°C.

Carboxylesterase

Carboxylesterase activity was estimated using α-naphthyl acetate as the substrate and α-naphthol as the standard, following the method of Devonshire and Moores (13) with modifications by Eziah et al. (14) and further optimized according to our laboratory protocols.

Calculation of enzyme units

Calibration of enzyme activity was done from α-naphthol standard curve and expressed as mmol of α-naphthol released/ min/mg of protein.

Estimation of glutathione-S-transferase

Determination of glutathione-S-transferase enzyme was done following the method Habig et al. (15), modified Zhang et al. (16) and partially modified according to our lab requirements.

Calculation of enzyme activity

CDNB-glutathione conjugate formed in m moles min^{-1} mg^{-1} protein

$$= \frac{ABS(increase\ in\ 5\ \text{min}) x\ 3\ x\ 1000}{9.6\ x\ 5\ x\ protein\ in\ mg}$$

In the above formula, 3 represents the volume of the reaction mixture used. The activity of an enzyme is measured in units, which is the amount of enzyme that can convert 1 mmol of GSH and CDNB to S-2,4-dinitrophenylglutathione per min at 30 °C. The specific activity of an enzyme is the number of units of enzyme activity per mg of protein. The value 9.6 corresponds to the difference in the millimolar extinction coefficient between the CDNB-GSH conjugate and CDNB.

Mixed function oxidase enzyme assay

Mixed function oxidase was assayed to identify their roles in imparting resistance. Enzyme determination was performed following the method in the earlier studies (10), with partial modifications to suit the study requirements.

Determination of cytochrome b_5

Cytochrome b_5 in the reduced condition shows a maximum Soret band at 424 nm. Three milliliters of enzyme solution was pipetted out into 4 mL quartz cuvette. Ten milligrams of sodium dithionite was added to the enzyme in the cuvette, mixed thoroughly and incubated for 2 min at room temperature to reduce the enzyme. The difference in the absorbance at 424 and 409 nm was taken and used to calculate the amount of cytochrome b_5, using an extinction coefficient difference of 184 cm^{-1} mM^{-1}.

$$\text{Cytochrome } b_5\ (\text{mM}) = \frac{(\text{ABS at } 424 - \text{ABS at } 409) \text{ x } 1000}{184}$$

Determination of cytochrome P_{450}/P_{420}

Three milliliters of reduced enzyme was pipetted out into 4 mL quartz cuvette and 10 mg of sodium dithionate was added, then it was transferred for carbon monoxide to get bubbled for 1-2 min into the glass test tube. It was ensured that the tube containing the enzyme was covered with aluminium foil to avoid light. Then the enzyme content was transferred into a quartz cuvette and placed inside a spectrophotometer. The difference in absorbance between 450 and 490 nm was used to calculate the cytochrome P_{450} using an extinction coefficient

difference of 91 cm^{-1} mM^{-1}. The difference in absorbance between 420 and 490 nm was used to calculate the cytochrome P_{420} content using an extinction coefficient difference of 110 cm^{-1} M^{-1}.

$$\text{Cytochrome } P_{450} \text{ (mM)} = \frac{(\text{ABS at } 450 - \text{ABS at } 490) \times 1000}{110}$$

$$\text{Cytochrome } P_{420} \text{ (mM)} = \frac{(\text{ABS at } 420 - \text{ABS at } 490) \times 1000}{91}$$

$$\text{Activity ratio} = \frac{\text{Enzyme activity in treated stage}}{\text{Enzyme activity in control}}$$

Acid and alkaline phosphatases assay

Acid and alkaline phosphatase activities were determined according to the method of Bessey et al. (17), as modified by Bilal et al. (18).

Protein analysis

Protein assay was done by using folin-ciocaltou reagent and BSA as a standard as originally described in the previous study (19). 50 mg of Bovine Serum Albumin (BSA) was dissolved in distilled water and the volume was made up to 50 mL from which 5 mL of the stock standard solution was diluted to 50 mL with distilled water. One milliliter of this solution contains 100 mg protein.

Assay

Protein concentrations ranging from 20 to 100 mg was prep aired by pipetting 0.2, 0.4, 0.6, 0.8 and 1.0 mL working standard solution into a series of test tubes and volume is made up to 1 mL in each test tube with distilled water. A test tube with 1 mL distilled water is taken as blank. Then 5 mL of reagent C and 0.5 mL of Folin's reagent (1N) were added to each test tube mixed well and kept aside for colour development for 15 min, not for more than 30 min colour development was measured at 660 nm against blank.

Reaction mixture

Reaction mixture was prepared by mixing 1 mL of tissue homogenate into each test tube along with 5 mL of reagent C and 0.5 mL of Folin's reagent and the colour development was measured at 660 nm at least after 15 min. The quantity of protein was calculated from the standard graph.

Statistical analysis

All enzyme assays were conducted with 10 replicates per treatment group for insecticide (spinetoram, tetraniliprole, emamectin benzoate and control), temperature (29 °C, 32 °C and control at 25 °C) and plant extract (*Vitex negundo*, *Eupatorium odoratum*, *Spathodea campanulata*, *Nyctanthes arbor-tristis*, *Terminalia arjuna*, *Alstonia scholaris* at 100-500 ppm and control) treatments. Each replicate consisted of a pooled sample of $3^{rd}/4^{th}$ instar *L. orbonalis* larvae, with a total weight of approximately 200 mg for plant extract assays and equivalent biomass for insecticide and temperature assays. Enzyme activities (carboxylesterase, glutathione S-transferase, cytochrome P450, cytochrome b5, acid phosphatase and alkaline phosphatase) were quantified as described above and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA with SPSS v. 27.0 software, followed by Duncan's multiple-range test (20) ($p = 0.05$) to compare means across treatments. Critical difference (CD) values and standard error of the mean (SE(m)) were calculated to assess treatment effects.

Effect of plant extracts on microsomal oxidases and carboxylesterases of *L. orbonalis* larvae reared at different temperature regimes

The plant extracts prepared were made into different concentrations ranging from 100 to 500 ppm of 5 mL volume with analytical acetone as solvent. 0.1 g of calcium silicate was added to reduce the colour. After addition, concentrations were left untouched for 24 hr and then filtered to remove calcium silicate. Three larvae totalling 200 mg body weight priorly exposed to different temperature regimes was taken for study. The plant extract concentrations of 1 mL each were added to the reaction mixture of enzyme assay. A control treatment i.e., larvae reared at room temperature was also used. We failed to rear the larvae at 22 and 37 °C because of the mortality of released second instars larvae on potato tubers and keeping them in an incubator after temperature regimes were fixed. Hence larvae reared in the rest two temperature regimes i.e., 29 and 32 °C were used for study.

Results and Discussion

Effect of insecticide stress on detoxifying enzyme activity in *L. orbonalis*

The study revealed significant induction of detoxifying enzymes in *L. orbonalis* larvae exposed to sub-lethal doses (LD_{30}) of insecticides (21-23). Carboxylesterase (CaE) activity was highest in larvae treated with spinetoram (49.13 ± 1.43 µmol/min/g protein), followed by tetraniliprole (46.12 ± 1.15 µmol/min/g protein) and emamectin benzoate (45.43 ± 1.32 µmol/min/g protein). The lowest activity was recorded in the control group (33.79 ± 2.00 µmol/min/g protein). Compared to control, spinetoram, tetraniliprole and emamectin benzoate increased carboxylesterase activity by 1.45-fold, 1.36-fold and 1.34-fold, respectively ($p = 0.05$).

Glutathione S-transferase (GST) activity was significantly enhanced upon insecticide exposure, with the highest activity observed in tetraniliprole-treated larvae (535.95 ± 3.09 µmol/min/mg protein), followed by emamectin benzoate (506.34 ± 3.57 µmol/min/mg protein) and spinetoram (496.93 ± 4.01 µmol/min/mg protein). Compared to control (286.98 ± 5.48 µmol/min/mg protein), these treatments resulted in a 1.86-fold, 1.76-fold and 1.73-fold increase, respectively ($p = 0.05$).

Similarly, mixed-function oxidases (MFO) showed a significant increase in cytochrome b_5 activity, with tetraniliprole-treated larvae recording the highest activity (2.76 ± 0.11 nmol/mg protein), followed by emamectin benzoate (2.66 ± 0.19 nmol/mg protein) and spinetoram (2.65 ± 0.10 nmol/mg protein). The lowest activity was in the control group (1.46 ± 0.19 nmol/mg protein). The increase in cytochrome P_{450} activity followed a similar trend, with tetraniliprole showing the highest induction (5.88 ± 0.36 nmol/mg protein) compared to control (2.29 ± 0.21 nmol/mg protein).

Acid phosphatase (ACP) activity was significantly increased in tetraniliprole-treated larvae (340.13 ± 3.30 µmol/min/mg protein), followed by emamectin benzoate (337.47 ±

5.89 µmol/min/mg protein) and spinetoram (282.36 ± 7.41 µmol/min/mg protein), with the lowest activity in the control group (237.54 ± 4.36 µmol/min/mg protein). Tetraniliprole, emamectin benzoate and spinetoram increased acid phosphatase activity by 1.43-fold, 1.42-fold and 1.18-fold, respectively. The differences in acid phosphatase activity between the control and the insecticide -treated groups were statistically significant ($p = 0.05$), whereas between the tetraniliprole and spinetoram it was non-significant, LC_{30} of each tested insecticide was applied topically on 3rd/4th instar larvae and detoxifying enzyme activities were quantified in the same stage of the succeeding generation. The enzyme activities recorded under different insecticide treatments are presented in Table 1.

Effect of thermal stress on detoxifying enzyme activity in *L. orbonalis*

Larval survival was significantly impacted by extreme temperatures, with complete mortality observed at 22 and 37 °C, precluding enzymatic analysis at these temperatures. At 22 °C, larvae exhibited lethargy, reduced feeding activity and cessation of movement within 48 hr, leading to 100 % mortality. At 37 °C, larvae showed signs of rapid desiccation, paralysis and darkened cuticles, resulting in complete mortality within 24 hr. These observations suggest critical thermal thresholds for *L. orbonalis* larvae, warranting further investigation into physiological mechanisms underlying temperature-induced mortality (24-26). The impact of thermal stress on detoxifying enzyme activity is summarized in Table 2 (27-29).

At 32 °C, carboxylesterase activity was higher (39.11 ± 3.60 µmol/min/g protein) than at 29 °C (37.23 ± 2.42 µmol/min/g protein) and control (33.79 ± 3.35 µmol/min/g protein). GST activity exhibited a temperature-dependent increase, with maximum levels at 32 °C (334.29 ± 6.87 µmol/min/mg protein), followed by 29 °C (286.98 ± 5.48 µmol/min/mg protein) and control (256.39 ± 5.67 µmol/min/mg protein). Cytochrome P_{450} and cytochrome b_5 activities also showed an increasing trend with temperature.

Effect of plant extracts on microsomal oxidases and carboxylesterases

The application of plant extracts significantly influenced detoxifying enzyme activities in *L. orbonalis* larvae (30) (Table 3 and 4). Among the tested botanicals, *N. arbor-tristis* at 500 ppm exhibited the highest induction of cytochrome P_{450} activity (54.05 ± 0.14 nmol/min/mg protein at 32 °C) and cytochrome b_5 (37.28 ± 0.18 nmol/min/mg protein at 32 °C), significantly higher than the control (12.42 ± 0.10 nmol/min/mg protein for cytochrome P_{450}; 7.25 ± 0.08 nmol/min/mg protein for cytochrome b5; $p < 0.05$, Duncan's multiple-range test). *S. campanulata* and *E. odoratum* also significantly increased cytochrome P_{450} and b_5 activities compared to the control ($p < 0.05$), with activity ratios ranging from 3.05-5.14 at 32 °C to 3.00-6.02 at 29 °C (Table 3). Carboxylesterase activity was significantly enhanced by *N. arbor-tristis* (84.24 ± 3.19 µmol/min/mg protein at 32 °C) and *S. campanulata* (91.03 ± 2.37 µmol/min/mg protein at 32 °C) compared to the control (28.55 ± 0.95 µmol/min/mg protein; $p < 0.05$), with activity ratios of 2.95 and 3.19, respectively (Table 4). *V. negundo* and *E. odoratum* showed moderate but significant increases in carboxylesterase activity at higher concentrations ($p < 0.05$). Statistical analysis using one -way ANOVA confirmed significant differences between plant extract treatments and the control ($p < 0.05$), with Duncan's multiple-range test indicating distinct treatment groups (Table 3 and 4). Similar trends were observed at 29 °C, though enzyme induction was generally lower than at 32 °C.

The effects of plant extracts on carboxylesterase activity across concentrations (100-500 ppm) at 29 °C and 32 °C are illustrated in Fig. 1, showing a dose-dependent increase with *S. campanulata* and *N. arbor-tristis* exhibiting the highest activities. Fig. 2 depicts the activity ratios of microsomal oxidases (cytochrome P_{450}, P_{420} and b_5), highlighting stronger induction at 32 °C compared to 29 °C, particularly for *N. arbor-tristis* at 500 ppm.

Table 1. Effect of insecticide stress on detoxifying enzyme activity of 3rd/4th instar larvae *L. orbonalis* (Guenee)

Insecticide	LD_{30} (ng/larva)	CaE (µmol/min/g protein)	GST (µmol/min/mg protein)	Cyt-b5 (nmol/mg protein)	Cyt-P_{450} (nmol/mg protein)	ACP (µmol/min/mg protein)
Spinetoram	0.010	49.13 ± 1.43	496.93 ± 4.01	2.65 ± 0.10	3.25 ± 0.37	282.36 ± 7.41
Tetraniliprole	0.048	46.12 ± 1.15	535.95 ± 3.09	2.76 ± 0.11	5.88 ± 0.36	340.13 ± 3.30
Emamectin benzoate	0.095	45.43 ± 1.32	506.34 ± 3.57	2.66 ± 0.19	4.23 ± 0.22	337.47 ± 5.89
Control (Untreated)	-	33.79 ± 2.00	286.98 ± 5.48	1.46 ± 0.19	2.29 ± 0.21	237.54 ± 4.36

Data represent mean ± SD of 10 replicates.

CaE: carboxylesterase; GST: glutathione S-transferase; Cyt-b5: cytochrome b5; Cyt-P_{450}: cytochrome P_{450}; ACP: acid phosphatase.

Table 2. Effect of thermal stress on detoxifying enzyme activity in L. orbonalis (3rd/4th instar larvae)

Temperature (°C)	CaE (µmol/min/g protein)	GST (µmol/min/mg protein)	Cyt-b5 (nmol/mg protein)	Cyt-P450 (nmol/mg protein)	ACP (µmol/min/mg protein)
22	-	-	-	-	-
29	37.23 ± 2.42	286.98 ± 5.48	4.99 ± 0.19	2.34 ± 0.26	242.92 ± 4.82
32	39.11 ± 3.60	334.29 ± 6.87	6.04 ± 0.09	3.51 ± 0.25	291.54 ± 4.94
37	-	-	-	-	-
Control (25)	33.79 ± 3.35	256.39 ± 5.67	3.55 ± 0.52	1.64 ± 0.155	237.54 ± 4.36

Data represent mean ± SD of 10 replicates.

No survival at 22 °C and 37 °C.

Table 3. Effect of various leaf extracts on microsomal oxidases and activity ratio of *L. orbonalis*

Plant extract	Concentration (ppm)	Cytochrome b5 (nmol/min/mg protein)		Cytochrome P450 (nmol/min/mg protein)		Cytochrome P420 (nmol/min/mg protein)	
		32 °C	Activity ratio	29 °C	Activity ratio	32 °C	Activity ratio
Vitex negundo	100	12.64 ± 0.09^{b}	1.74	12.46 ± 0.08^{b}	2.02	36.21 ± 0.11^{c}	2.92
	200	15.76 ± 0.11^{b}	2.17	12.93 ± 0.12^{b}	2.09	38.18 ± 0.13^{c}	3.07
	300	17.57 ± 0.12^{b}	2.42	13.81 ± 0.14^{b}	2.24	40.61 ± 0.14^{c}	3.27
	400	20.65 ± 0.14^{b}	2.85	15.72 ± 0.15^{b}	2.55	43.33 ± 0.16^{b}	3.49
	500	22.28 ± 0.16^{b}	3.07	16.30 ± 0.17^{b}	2.64	48.79 ± 0.18^{b}	3.93
Eupatorium odoratum	100	16.18 ± 0.10^{b}	2.23	15.86 ± 0.09^{b}	2.57	35.76 ± 0.12^{c}	2.88
	200	17.57 ± 0.12^{b}	2.42	16.01 ± 0.13^{b}	2.59	37.88 ± 0.14^{c}	3.05
	300	18.30 ± 0.13^{b}	2.52	16.45 ± 0.14^{b}	2.67	37.27 ± 0.15^{c}	3
	400	18.84 ± 0.15^{b}	2.6	17.04 ± 0.16^{b}	2.76	39.70 ± 0.16^{c}	3.2
	500	22.10 ± 0.17^{b}	3.05	18.51 ± 0.17^{b}	3	41.52 ± 0.18^{b}	3.34
Spathodea campanulata	100	23.73 ± 0.11^{b}	3.27	18.51 ± 0.10^{b}	3	38.18 ± 0.13^{c}	3.07
	200	25.36 ± 0.13^{b}	3.5	19.39 ± 0.14^{b}	3.14	40.91 ± 0.15^{c}	3.29
	300	25.18 ± 0.15^{b}	3.47	23.50 ± 0.14^{a}	3.81	41.24 ± 0.16^{b}	3.32
	400	26.09 ± 0.16^{b}	3.6	22.18 ± 0.16^{b}	3.59	42.12 ± 0.17^{b}	3.39
	500	27.36 ± 0.17^{b}	3.77	26.29 ± 0.18^{a}	4.26	45.76 ± 0.19^{b}	3.68
Nyctanthes arbor-tristis	100	23.55 ± 0.12^{b}	3.25	21.15 ± 0.11^{b}	3.43	23.55 ± 0.14^{d}	3.27
	200	28.26 ± 0.14^{b}	3.9	27.76 ± 0.15^{a}	4.5	28.26 ± 0.16^{d}	3.59
	300	31.52 ± 0.15^{a}	4.35	33.05 ± 0.16^{a}	5.36	31.52 ± 0.15^{d}	3.83
	400	33.33 ± 0.16^{a}	4.6	34.52 ± 0.17^{a}	5.59	33.33 ± 0.17^{c}	4.07
	500	37.28 ± 0.18^{a}	5.14	37.16 ± 0.19^{a}	6.02	37.28 ± 0.12^{c}	4.54
Control	-	7.25 ± 0.08^{c}	-	6.17 ± 0.07^{c}	-	12.42 ± 0.10^{e}	-

Data represent mean ± SD of three replicates. Means within a column followed by different letters (a, b, c, d, e) are significantly different ($p < 0.05$, Duncan's multiple-range test).

Plant extracts significantly influenced detoxifying enzyme activities in *L. orbonalis* larvae, with *N. arbor-tristis* at 500 ppm inducing the highest cytochrome P_{450} (54.05 ± 0.14 nmol/min/mg protein at 32 °C) and cytochrome b5 (37.28 ± 0.18 nmol/min/mg protein at 32 °C) activities compared to controls (12.42 ± 0.10 and 7.25 ± 0.08 nmol/min/mg protein, respectively) (30). These effects are likely driven by phytochemicals such as flavonoids, alkaloids and terpenoids, which modulate enzyme activity through induction or inhibition. Flavonoids in *N. arbor-tristis* may upregulate cytochrome P_{450} by activating transcriptional factors like the aryl hydrocarbon receptor, enhancing the larvae's metabolic capacity to detoxify xenobiotics (31). Similarly, *S. campanulata* and *E. odoratum* increased cytochrome P_{450} and carboxylesterase activities, likely due to their saponins, phenols and rutin content, which can stimulate enzyme synthesis or alter metabolic pathways (32). In contrast, some phytochemicals, such as chromolaenide in *E. odoratum*, exhibit inhibitory effects by competitively binding to enzyme active sites, reducing detoxification efficiency and enhancing insecticide efficacy, as seen in *Spodoptera frugiperda* (33). For instance, terpenoids like limonene and 1,8-cineole from plant extracts have been shown to inhibit glutathione S-transferase and esterases, disrupting resistance mechanisms and acting as synergists (34). These dual roles—induction for metabolic adaptation and inhibition for synergism—highlight the potential of plant-derived compounds in integrated pest management (35). By incorporating enzyme inhibitors, resistance can be mitigated, while inducers provide insights into

Table 4. Effect of various leaf extracts on carboxylesterases and activity ratio of *L. orbonalis*

Plant extract	Concentration (ppm)	32 °C (µmol/min/mg protein)	Activity ratio	29 °C (µmol/min/mg protein)	Activity ratio
Vitex negundo	100	53.98 ± 3.98^{b}	1.89	43.64 ± 3.64^{b}	1.91
	200	55.59 ± 2.27^{b}	1.95	43.83 ± 2.16^{b}	1.92
	300	64.67 ± 2.55^{b}	2.27	49.42 ± 2.41^{b}	2.16
	400	72.92 ± 2.98^{a}	2.55	55.10 ± 2.98^{b}	2.41
	500	85.02 ± 3.19^{a}	2.98	68.16 ± 3.17^{a}	2.98
Eupatorium odoratum	100	34.02 ± 1.66^{c}	1.19	24.90 ± 1.62^{c}	1.09
	200	38.98 ± 1.99^{c}	1.37	29.81 ± 1.78^{c}	1.3
	300	47.38 ± 2.57^{c}	1.66	37.13 ± 2.20^{c}	1.62
	400	56.85 ± 2.27^{b}	1.99	40.66 ± 2.02^{b}	1.78
	500	73.24 ± 2.55^{a}	2.57	46.11 ± 2.42^{b}	2.02
Spathodea campanulata	100	64.71 ± 2.84^{b}	2.27	50.28 ± 2.71^{b}	2.2
	200	72.92 ± 3.14^{a}	2.55	55.26 ± 3.05^{b}	2.42
	300	81.17 ± 3.19^{a}	2.84	61.90 ± 3.17^{a}	2.71
	400	89.54 ± 2.18^{a}	3.14	69.60 ± 2.19^{a}	3.05
	500	91.03 ± 2.37^{a}	3.19	72.33 ± 2.32^{a}	3.17
Nyctanthes arbor-tristis	100	62.35 ± 2.66^{b}	2.18	49.96 ± 2.41^{b}	2.19
	200	67.58 ± 2.83^{b}	2.37	52.95 ± 2.55^{b}	2.32
	300	75.87 ± 2.95^{a}	2.66	55.03 ± 2.55^{b}	2.41
	400	80.86 ± 3.19^{a}	2.83	58.37 ± 3.17^{a}	2.55
	500	84.24 ± 3.19^{a}	2.95	63.41 ± 3.17^{a}	2.55
Control	-	28.55 ± 0.95^{d}	-	22.85 ± 0.89^{d}	-

Data represent mean ± SD of three replicates. Means within a column followed by different letters (a, b, c, d) are significantly different ($p < 0.05$, Duncan's multiple-range test).

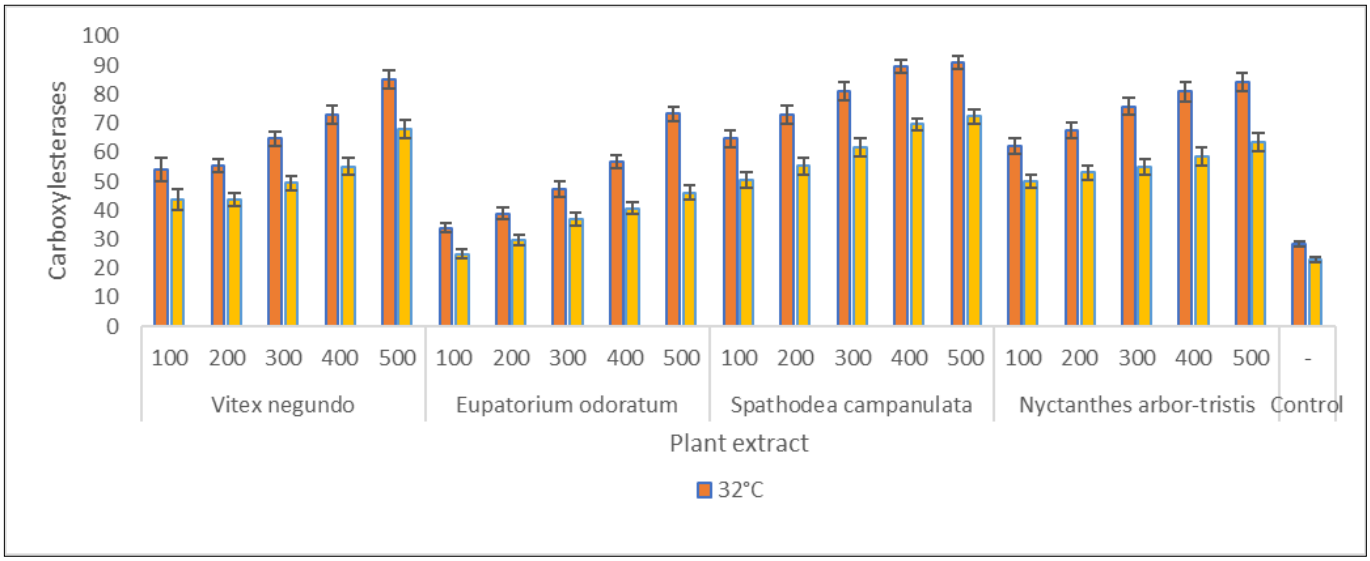

Fig. 1. *In vitro* effect of various leaf extracts on carboxylesterases of *L. orbonalis* at different temperatures.

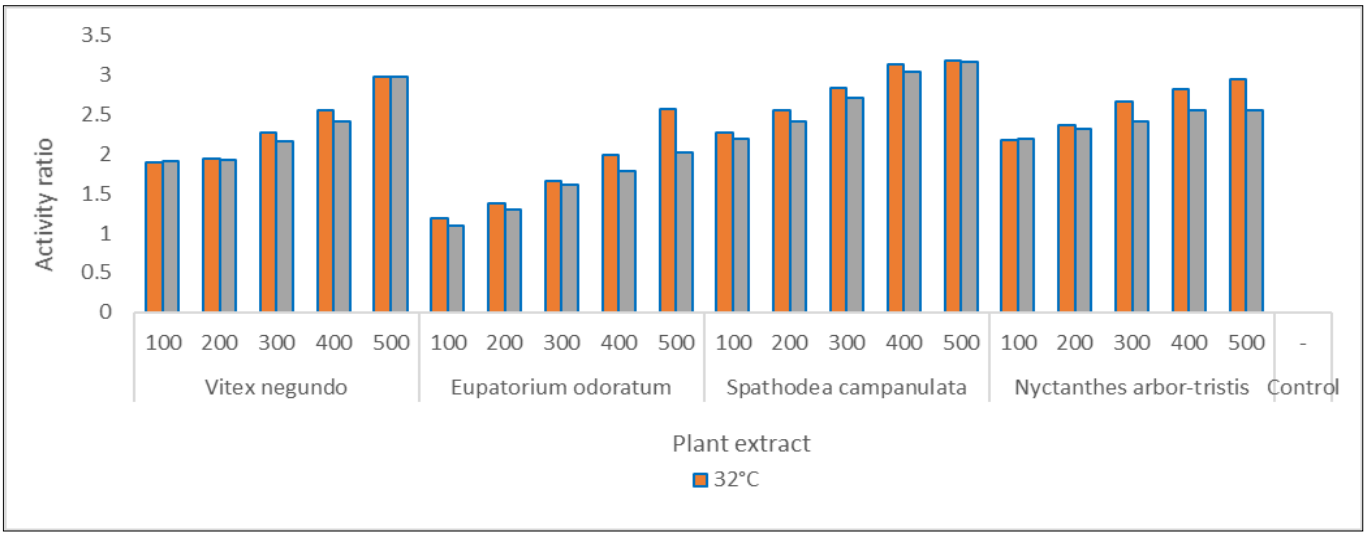

Fig. 2. *In vitro* effect of various leaf extracts on activity ratio of *L. orbonalis* at different temperatures.

resistance evolution. Future research should explore specific phytochemical-enzyme interactions using molecular assays to optimize their application in sustainable pest control.

Conclusion

This study elucidates the biochemical adaptations of *L. orbonalis* to insecticide exposure, thermal stress and plant-based interventions, revealing significant upregulation of detoxifying enzymes like carboxylesterase, GST and cytochrome P_{450} under sub-lethal insecticide doses and elevated temperatures. The inability of larvae to survive at 22°C and 37°C, coupled with enhanced enzyme activity at 32°C, highlights the influence of climatic variations on pest resilience and resistance evolution. Plant extracts, notably *N. arbor-tristis*, demonstrated potential as synergists by inducing enzyme activity, offering a sustainable pest management tool. Integrating biochemical assays into resistance monitoring can provide early detection of resistance, while future research should explore molecular mechanisms and field validation of these findings to combat insecticide resistance effectively.

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Authors' contributions

GVSS conducted the experiments, collected the data and contributed to the initial draft of the manuscript. MKT designed the study, supervised the research and provided critical input for data analysis and manuscript revision. SSD performed statistical analyses, interpreted the results and assisted in writing and editing the manuscript. MRK contributed to the conceptualization of the study, provided expertise on enzyme assays and reviewed the manuscript for technical accuracy. SKD supported fieldwork, assisted in sample collection and contributed to the discussion and finalization of the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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