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Biochemical and molecular characterization of chlorantraniliprole resistance in *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

Resistance is one major problem that reduces the efficacy of insecticides against insect pests. Understanding the biochemical and molecular foundations of insect resistance to insecticides can help achieve the objectives of insect control and resistance management. In this study, resistance to chlorantraniliprole was developed in a field strain of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) over fifteen generations using the selection pressure. In addition, the biochemical basis of resistance and cross-resistance to other insecticides were investigated. Moreover, the expression of esterase and Cytochrome P450 (CYP450) genes was quantitatively estimated in *S. littoralis* on exposure to chlorantraniliprole by using real-time PCR.

The LC_{50} values of chlorantraniliprole to the developed resistant strain (CHL-R) and the susceptible strain (Sus) were 2.256 and 0.0056mg/L (ppm), respectively, representing a resistance ratio (RR) of 402.85. The CHL-R strain showed no cross-resistance to chlorpyrifos, methomyl, lambada-cyhalothrin, emamectin benzoate, and spinetoram (RR ranged from 0.97 to 1.89). However, a low cross-resistance level was developed to methoxyfenozide (RR = 4.04) and a moderate level was developed to indoxacarb (RR = 30.83). Synergism assays suggested that CYP450 may be involved in resistance development in the CHL-R strain. Nevertheless, a significant decrease in Carboxylesterases (CarE) and Glutathione S-transferase (GST) activities was observed. Consistently, over-expression of the CYP450 gene was recorded in the CHL-R strain, which provides a foundation for understanding the mechanism of *S. littoralis* resistance to chlorantraniliprole in Egypt. Generally, our results provide valuable information for monitoring and managing *S. littoralis* resistance in Egypt.

1. Introduction

The cotton leaf worm, *Spodoptera littoralis* (Boisd., 1833) is a widely spread pest. It is found in Southern Europe, Africa, the Middle East, and some islands of the Pacific Ocean (Amezian et al., 2021). *S. littoralis* causes losses in more than 80 important crops such as cotton, corn, peanut, and soybean (El-Sheikh et al., 2018). The frequent application of chemical insecticides against *S. littoralis* has developed resistance to most of them, particularly the conventional ones, including organophosphates, carbamates, and pyrethroids (Abo Elghar et al., 2005). Hence, there is a need for alternative groups of insecticides with a novel mode of action that can potentially delay or hinder resistance (Bolzan et al., 2019; Moustafa et al., 2021).

The diamide group is one of the promising groups as it has a good toxicological profile and displays high target mortality with fast action (Ebbinghaus-Kintscher et al., 2007; Lahm et al., 2009). Diamide insecticides are now registered in many countries and provide reliable levels of management against the lepidopteran species (Bolzan et al., 2019). These insecticides have a unique mode of action. It acts on insect ryanodine receptors located in the membrane of the sarcoplasmic reticulum of muscle tissues (Cordova et al., 2006; Nauen, 2006; Sparks and Nauen, 2015; Insecticide Resistance Action Committee). Chlorantraniliprole, a member of diamide group, is one of the effective insecticides against the lepidopteran insects, with minimal risk to mammals (Lahm et al., 2009; Hannig et al., 2009) and thus having the potential to be a successful agent in resistance management (Guo et al., 2013). Nevertheless, resistance to chlorantraniliprole has been reported in laboratory populations and several field populations (Lai and Su,

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2011; Cao et al., 2017; Richardson et al., 2020; Fouad et al., 2022).

Insects interact with chemical insecticides through several mechanisms including the biochemical mechanisms. For example, metabolic detoxification of insecticides involves Cytochrome P450 (CYP450) and Carboxylesterases (CarE) (Scott and Wen, 2001; Li et al., 2007), resulting in the oxidation or reduction of insecticide. Afterwards, glutathione *S*-transferases (GST) convert the detoxified molecule into a more water-soluble form, glutathione conjugation, which facilitates the rapid removal from the cell (Enayati et al., 2005). Alternatively, the modification of target site (mutation of amino acid residue) could result in insensitivity of insects to toxic chemicals (Park and Taylor, 1997; Franck et al., 2012). Indeed, the evolution of resistance can be influenced by both biochemical factors and genetic basis through the correlation between enzyme expression and the level of resistance (Campos et al., 2015; Li et al., 2017). The evolution of resistance is also influenced by cross-resistance resulting from previous selection by other insecticides (Bolzan et al., 2019).

Investigation of resistance to diamide insecticides has primarily focused on the insensitivity of the target-site of this insecticide, the ryanodine receptor (RyR), a ligand-gated calcium channel located in the sarcoplasmic reticulum and endoplasmic reticulum of the neuromuscular tissues (Sattelle et al., 2008). However, the underpinning mechanisms of metabolic resistance to this insecticide class are not well understood (Mallott et al., 2019). Therefore, the current research aimed to investigate the biochemical and molecular foundations of chlorantraniliprole resistance in *S. littoralis.* Cross resistance to other insecticides was also assessed.

2. Materials and methods

2.1. Strains

A field population of *S. littoralis* was collected from a grain production farm in El-Fayoum governorate (29.308374°N 30.844105°E), Egypt during the 2019 crop season (Fouad et al., 202). A laboratory strain of *S. littoralis* was used as a reference susceptible strain (Sus). This strain has been maintained at the Faculty of Agriculture, Cairo University, Egypt since 2017 without any insecticide selection pressure (Moustafa et al., 2021). Both strains were maintained in a rearing room at 25 ± 1 °C and 75 ± 5% relative humidity. The larvae were fed fresh castor bean leaves (*Ricinus communis*) whereas the moths were provided with 10% sugar solution for sustenance (Kandil et al., 2020).

2.2. Insecticides and chemicals

Formulated insecticides were used for bioassay. These insecticides included chlorantraniliprole (Coragen® 20%, SC, DuPont, France), chlorpyrifos (Dursban 48% EC, Dow AgroSciences, UK), methomyl (Neomyl, 90% WP; Rotam Agrochemical, China), lambada-cyhalothrin (Axon, 5% EC; Jiangsu Changqing Agrochemical, China), methoxyfenozide (Runner, 24% SC; Dow AgroSciences, UK), indoxacarb (Avant, 15% EC; Dupont, France), emamectin benzoate (Proclaim, 5% SG; Syngenta, Switzerland), and spinetoram (Radint, 12% SC; Dow AgroSciences, UK). Piperonyl butoxide (PBO), triphenylphoshate (TPP) and diethyl maleate (DEM) were purchased from Sigma-Aldrich, Germany.

2.3. Selection with chlorantraniliprole

A field-collected population of *S. littoralis* was selected for resistance to chlorantraniliprole (CHL-R) for 15 consecutive generations using the leaf dipping technique, as described by Fouad et al. (2022). The second instar larvae of the susceptible strain (Sus) and (CHL-R) strains were subjected to six different concentrations in each generation. Four replicates, each of twenty-five larvae, were used for each concentration. The larvae were allowed to feed on treated castor oil leaves in a glass jars (0.25 L) for 24 h. Mortality was recorded four days after treatment, and the surviving larvae were moved to clean jars with untreated leaves. Mortality data were subjected to Probit analysis (Finney, 1971) and the median lethal concentration (LC_{50}) of chlorantraniliprole was determined for each generation to be used as a selection pressure for the following generation. The developed resistance was expressed as a resistance ratio (RR), which was calculated by dividing the LC_{50} of chlorantraniliprole to the developed (CHL-R) strains by the LC_{50} to the Sus strain.

2.4. Cross-resistance patterns

The second instar larvae of the Sus and CHL-R strains of *S. littoralis* were exposed to other seven insecticides with different modes of action using the leaf dipping technique, and the cross-resistance was assessed. For both strains, four replicates (25 larvae for each) were used for each concentration. The median lethal concentration (LC_{50}) of each insecticide was determined for both the CHL-R and Sus strains. Cross-resistance was calculated by dividing (LC_{50}) for CHL-R by the (LC_{50}) of Sus strain.

2.5. Synergism test

For further understanding of chlorantraniliprole resistance in *S. littoralis,* toxicity synergism assays were performed on both the Sus and CHL-R strains. The potential synergistic effect of piperonyl butoxide (PBO) as CYP450 inhibitor, triphenyl phosphate (TPP) as an esterase(s) inhibitor, and diethyl maleate (DEM) as a GST inhibitor was tested using the leaf dipping technique. The toxicity of these synergists was first assessed to choose the suitable concentration that would have no larval mortality (Lai and Su, 2011). Concentrations up to 200 mg/liter of PBO, TPP or DEM were mixed with the concentrations of chlorantraniliprole. The synergism ratio (SR) was calculated by dividing the LC_{50} of chlorantraniliprole alone by the LC_{50} of the mixture of chlorantraniliprole and the synergist.

2.6. Enzyme assays

2.6.1. Enzyme preparation

The second instar larvae (50 mg) of the Sus and CHL-R strains of *S. littoralis* were isolated and starved to remove any remnants of digested food particles. Afterwards, the whole larvae were homogenized in 0.1 M phosphate buffer with the suitable pH values in a ratio of 1:10 (W: V) (Moustafa et al., 2021). Five replicates were used for each strain.

2.6.2. The mixed function oxidases (MFO) assay

The assay of MFO was conducted according to Hansen and Hodgson (1971). The larvae were homogenized in a phosphate buffer (pH 7.8) and then centrifuged at 15,000g for 15 min at 4 ^{O}C. A mixture of 2 mM p-nitroanisole (100 μL) with enzyme stock solution (90 μL) was added to each well of a microplate. After incubation for 2 min at 27 ^{O}C, 9.6 mM of NADPH (10 μL) were added to initiate the reaction. The activity of MFO was measured at 405 nm for 15 min using the molecular devices of the Vmax kinetic microplate reader.

2.6.3. The carboxylesterase (CarE) assay

The activity of alpha (α)- and beta (ß)- esterase was examined according to Van Asperen (1962). The larvae were homogenized in a phosphate buffer (pH 7.0) and then centrifuged at 12,000 *g* at 4 °C for 15 min. A mixture of the supernatant (50 μL) with 30 mM (α) or (ß)—naphthyl acetate (NA) (50 μL) was incubated at 30 °C for 15 min. The reaction was stopped by adding 50 μL of the stop solution, which consisted of Fast Blue B (1%) and sodium dodecyl sulphate (5%) (2:1). The hydrolysis of α-NA and ß- NA was measured at 600 nm and 550 nm, respectively, using a UV/Vis Spectrophotometer (Jenway 7205UV/Vis). The mean levels of the CarE activity were derived from the standard curves of the protein content and α and ß-naphthol.

2.6.4. The glutathione S- transferase (GST) assay

The GST activity was measured as indicated by Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The larvae were homogenized in a phosphate buffer (pH 6.5) and then centrifuged at 12,000g for 15 min at 4 °C. The reaction solution consisted of 10 μL supernatant, 25 μL of 30 mM CDNB and 25 μL of 50 mM glutathione (GSH). The GST activity was determined with a UV/Vis Spectrophotometer (Jenway 7205UV/Vis) at 340 nm using a kinetic mode for 5 min.

2.6.5. Extraction of total RNA and quantitative real-time PCR (RT-qPCR) analysis

The surviving larvae of *S. littoralis* (0.5g) were collected from two groups and were pooled as three biological replicates. The RNA was extracted as described in the instruction manual of Thermo- Scientific Gene JET RNA purification kit #K0731, #0732. Genomic DNA was removed from the RNA preparations using DNase1, free nuclease #EN0521. Quantitative real-time PCR (RT-qPCR) analysis was performed on ABI 7500 Quantitative Real-Time PCR system (Applied Biosystems, USA) using SYBR® Premix Ex Taq™ (Takara, Dalian, China). The RT-qPCR primers for all genes were synthesized by Macrogen. The oligonucleotide primers are listed in Table 1 considering the sequences of Cytochrome P450 and esterase genes (GenBank Acc. No. JQ979050.1, DQ680828.1) respectively. One gene actin (ACT) was used as an internal reference gene to normalize the expression levels of the target gene. (Shu et al., 2018).

The 20 μL RT-qPCR reaction mixtures contained 10 μL SYBR®Premix Ex Taq, 1 μL of cDNA, 0.4 μL of each primer, 0.4 μL of ROX Reference Dye II, and 7.8 μL of RNase-free water. The RT-qPCR analysis included three technical and three independent biological replicates for each treatment. The amplification process started with an initial denaturation step at 95 °C for 30 s, followed by 40 cycles at 95 °C (for 5 s) and 60 °C (for 34 s), then a dissociation step was performed. For amplification efficiencies and cycle threshold (Ct), a standard curve was established for each primer pair with serial dilutions of cDNA (1, 1/10, 1/100, 1/1000, 1/10000, and 1/100000). Quantification of the target gene expression level was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6.6. Statistical analysis

The corrected mortality percentages according to (Abbott, 1925) were statistically analyzed according to the method of Finney (1971) using Probit-MSChart computer program (Chi, 2023). Levels of resistance were classified according to **Ahmad and Iqbal Arif (2009) as follows**: susceptible (RR ≤ 1-fold), very low resistance (RR = 2-10-fold), low (RR = 11–20) moderate resistance (RR = 21–50), high resistance (RR = 51–100) and very high (RR > 100). Detoxification enzyme activities were statistically analyzed and graphically represented using the Paired *t*-test within GraphPad Prism 9, with statistical significance set at $p < 0.05$. The relative changes in gene expression were determined using the comparative $2^{-\Delta\Delta Ct}$ method, where ΔCt for the basal level (untreated control) = (Ct_R- Cts) and ΔCt for the gene induction (treated versus untreated control) = ($Ct_{treated}$- $Ct_{control}$).

Table 1
The oligonucleotide primers used in quantitative real time PCR.

No.	Oligo Name	5′-Oligo Seq.- 3′
1	*Spli*-EST1- F	5′-CGGCGGCAACCCTAACGAC-3′
	Spli-EST1- R	5′-CCCACGGGACCAATGAAGAGC-3′
2	*Spli*-CytoP450- F	5′-CCTATCATCGGGCAGTTCG-3′
	Spli-CytoP450- R	5′-GGTACATCCGCTCTATTTCATCC-3′
3	*Spli*-Actin- F	5′-GCGTCGCCCCTGAGGAACAC-3′
	Spli-Actin- R	5′-CGACGTACATGGCGGGGGAG-3′

3. Results

3.1. Selection for resistance

As shown in Table 2, selection for resistance to chlorantraniliprole for 15 consecutive generations in El-Fayoum field population resulted in reasonable level of resistance. The resistance ratio (RR) increased over generations until it reached 402.85 in G15, compared to 2.32 in the parent generation.

3.2. Cross-resistance

As shown in Table 3, CHL-R strain exhibited a moderate level of cross-resistance to indoxacarb (RR = 30.83) and a Low level to methoxyfenozide (RR = 4.04). In contrast, no cross resistance (RR < 2-fold) was recorded to chlorpyrifos, methomyl, lambada-cyhalothrin and emamectin benzoate.

3.3. Synergistic effects

As shown in Table 4, the LC_{50} value of chlorantraniliprole to CHL-R strain decreased after exposure to PBO while it increased after the exposure to TPP. PBO and DEM caused slight synergism levels (SR = 1.32- and 1.06- fold, respectively). In contrast, TPP, as an esterase(s) inhibitor, had no synergistic effect on CHL-R strain (SR = 0.67).

3.4. Detoxification enzyme assays

As shown in Fig. 1, the CHL-R strain exhibited a higher MFO activity

Table 2
Selection of chlorantraniliprole resistance in *S. littoralis*.

Generations	Slope ± SE	LC_{50} (μg/ml) (95% CL)	Fit of probit line			[a]RR	[b]RR
			χ^2	*df*	*P*		
Sus	0.50 ± 0.12	0.005 (0.00–0.03)	0.30	2	0.86	–	
Parent	0.91 ± 0.16	0.01 (0.00–0.03)	1.48	3	0.68	2.32	–
G1	2.34 ± 0.20	0.05 (0.04–0.06)	4.78	4	0.31	9.28	5
G2	2.00 ± 0.23	0.11 (0.10–0.12)	0.11	3	0.99	19.82	11
G3	1.97 ± 0.33	0.20 (0.17–0.23)	0.79	4	0.94	35.89	20
G4	1.38 ± 0.25	1.18 (0.98–1.42)	0.28	3	0.96	210.36	118
G5	2.17 ± 0.31	1.18 (0.75–1.94)	3.22	3	0.36	210.89	118
G6	2.75 ± 0.47	1.53 (1.44–1.63)	0.13	3	0.98	273.03	153
G7	2.11 ± 0.36	1.52 (1.30–1.79)	0.49	3	0.92	271.60	152
G8	1.26 ± 0.23	1.97 (1.66–2.32)	0.43	4	0.98	350.89	197
G9	1.34 ± 0.27	2.11 (1.36–4.46)	1.53	3	0.68	376.78	211
G10	2.52 ± 0.38	1.88 (1.68–2.04)	0.24	3	0.97	334.82	188
G11	1.74 ± 0.23	2.26 (1.95–2.63)	0.72	4	0.95	403.92	226
G12	2.29 ± 0.36	1.77 (1.25–2.43)	2.70	3	0.44	315.17	177
G13	1.98 ± 0.36	1.87 (1.37–2.47)	1.18	3	0.76	333.92	187
G14	1.97 ± 0.34	2.43 (2.13–2.75)	0.25	3	0.97	433.75	243
G15	2.70 ± 0.48	2.26 (1.70–2.84)	0.41	3	0.81	402.85	226

[a] Resistance ratio (RR) = LC_{50} of resistant strain/LC_{50} of susceptible strain.
[b] RR = LC_{50} of resistant strain/LC_{50} of parent generation.

Table 3
Susceptibility of resistant (Chlorant-FR) and susceptible (Sus) strains of *Spodoptera littoralis* to seven insecticides of different modes of action.

Population	Insecticide	Slope ± SE	LC_{50} (CI 95%) (μgmL^{-1})	Fit of probit line			[a]RR
				χ2	df	*P*	
Susceptible							
	Chlorpyrifos	2.36 ± 0.34	1.61 (0.91–2.66)	4.96	3	0.17	–
	Methomyl	2.01 ± 0.25	71.91 (4.96–100.77)	4.78	4	0.31	–
	Lambada-cyhalothrin	1.69 ± 0.27	11.12 (8.42–14.73)	1.04	3	0.79	–
	Indoxacarb	0.63 ± 0.11	0.001 (0.00–0.004)	0.20	3	0.98	–
	Methoxyfenozide	3.21 ± 0.53	4.99 (4.99–5.002)	0.001	3	0.99	–
	Emamectin benzoate	0.62 ± 0.11	0.004 (0.001–0.012)	0,90	3	0.63	–
	Spinotram	0.72 ± 0.12	0.09 (0.08–0.10)	0.03	3	0.99	–
R-Strain							
	Chlorpyrifos	3.13 ± 0.68	1.83 (1.09–2.84)	1.33	2	0.51	1.14
	Methomyl	1.79 ± 0.36	85.26 (80.31–90.51)	0.05	3	0.99	1.18
	Lambada-cyhalothrin	1.73 ± 0.33	10.85 (8.70–13.48)	0.53	3	0.91	0.97
	Indoxacarb	0.82 ± 0.12	0.04 (0.01–0.1)	3.06	3	0.38	30.83
	Methoxyfenozide	1.81 ± 0.30	20.20 (15.61–26.14)	0.97	3	0.81	4.04
	Emamectin benzoate	0.66 ± 0.10	0.007 (0.003–0.02)	1.92	3	0.59	1.75
	Spinotram	2.18 ± 0.37	0.17 (0.15–0.19)	0.24	3	0.97	1.89

[a] Resistance ratio (RR) = LC_{50} of resistant strain/LC_{50} of susceptible strain.

Table 4
Synergistic effect of piperonyl butoxide (PBO), Triphenyl phosphate (TPP), and diethyl maleate (DEM) on the toxicity of chlorantraniliprole to Chlorant-FR and Sus strains of *S. littoralis*.

Treatment	Slope ± SE	LC_{50} (CI 95%) (μgmL^{-1})	Fit of probit line			SR
			χ2	df	*P*	
Sus-strain						
Chlorantraniliprole	0.50 ± 0.12	0.005 (0.001–0.022)	0.30	2	0.86	–
Chlorantraniliprole + PBO	2.27 ± 0.41	0.09 (0.07–0.11)	0.85	3	0.84	0.06
Chlorantraniliprole + TPP	1.89 ± 0.32	0.13(0.11–0.16)	0.64	3	0.88	0.04
Chlorantraniliprole + DEM	2.22 ± 0.35	0.24 (0.22–0.27)	0.15	3	0.98	0.02
R-strain						
Chlorantraniliprole	2.63 ± 0.37	2.22 (1.94–2.53)	0.49	3	0.92	–
Chlorantraniliprole + PBO	2.13 ± 0.39	1.70 (1.42–2.05)	0.74	3	0.86	1.30
Chlorantraniliprole + TPP	2.33 ± 0.43	3.32(3.14–3.51)	0.08	3	0.99	0.67
Chlorantraniliprole + DEM	3.20 ± 0.56	2.10 (1.80–2.44)	0.94	3	0.82	1.06

*Synergistic ratio (SR) = LC_{50} of chlorantraniliprole alone/LC_{50} of chlorantraniliprole with the synergist.

and lower esterase and GST activities, compared with the Sus-strain.

3.5. Quantitative detoxification gene expression

As shown in Fig. 2, the CHL-R strain showed a down expression of esterase gene estimated by 0.92- fold compared with over expression estimated by 20.76- fold for CYP450 gene, in comparison with the Sus-strain.

4. Discussion

Understanding the mechanisms conferring insecticide resistance is crucial to the development of more efficient resistance management. The control of lepidopteran pests such as *Plutella xylostella* (L.) and *Tuta absoluta* (Meyrick) failed due to the resistance to the diamide group including chlorantraniliprole insecticide (Ribeiro et al., 2014; Silva et al., 2016). The present study aimed to examine the effectiveness of chlorantraniliprole against *S. littoralis* by investigating the metabolic mechanism of resistance. As reported in this study, the parent field strain developed a chlorantraniliprole resistance level of 173.64-fold after selection for only 15 generations. This is consistent with the results of Bolzan et al. (2019) who found that chlorantraniliprole resistance also developed rapidly (237-fold) in *Spodoptera frugiperda* (Smith).

Identifying the cross-resistance to insecticides is important for developing strategies for deferring resistance evolution in the field (Wang et al., 2013). In the current study, the CHL-R strain exhibited a moderate level of cross-resistance to indoxacarb and a low level to methoxyfenozide. In contrast, no cross-resistance was recorded to chlorpyrifos, methomyl, lambada-cyhalothrin, spinetoram, and emamectin benzoate. In fact, the positive correlation between chlorantraniliprole and indoxacarb resistance was shown in Diamondback moth, *Plutella xylostella,* field strains (Tamilselvan et al., 2021). However, the spinetoram-resistant population of *Thrips hawaiiensis* exhibited a lack of cross-resistance to chlorantraniliprole (Fu et al., 2018). In addition, the chlorantraniliprole resistant field population of beet armyworm, *Spodoptera exigua,* had developed moderate level of resistance to indoxacarb and emamectin benzoate. Nevertheless, methoxyfenozide and chlorfenapyr were highly effective against the chlorantraniliprole-resistant populations (Huang et al., 2021).

Enhancement of the activities of cytochrome P450 monooxygenases, esterases and glutathione-*S*-transferases through gene amplification or up-regulation has been implicated in insecticide resistance (Whalon et al. 200; Bass and Field, 2011). The induction of some P450 expression genes by insecticides is a common phenomenon in insecticide resistance (Bai-Zhong et al., 2020). In the current study, the cytochrome P450 exhibited a higher activity in the CHL-R strain, and 1.30-fold of synergism was recorded when PBO was added to chlorantraniliprole. Moreover, as detected with qRT-PCR, cytochrome P450 gene was overexpressed (20.76- fold). Consistently, PBO had a synergistic effect on chlorantraniliprole in the fourth-instar larvae of *Leptinotarsa decemlineata* populations in China (Jiang et al., 2012). In addition, chlorantraniliprole in combination with PBO showed a good amount of synergism (2.2-fold) on the resistant strain of *S. litura* (Muthusamy et al., 2014). The higher dose of PBO (80 μg/individual) had a higher synergistic effect on chlorantraniliprole in *S. frugiperda* populations (Liu et al., 2022). Consequently, the up-regulated P450s provided the primary detoxification mechanism for chlorantraniliprole in *C. suppressalis* (Meng et al., 2019). On the other hand, a moderate correlation between cytochrome- P450-dependent monooxygenases and susceptibility of *T. absoluta* populations to chlorantraniliprole was detected (Campos et al., 2015).

The current study found weak and insignificant correlation between esterase activity and susceptibility to chlorantraniliprole. This may suggest that esterase activity does not play a significant role in chlorantraniliprole resistance in *S. littoralis*. Compared to the susceptible

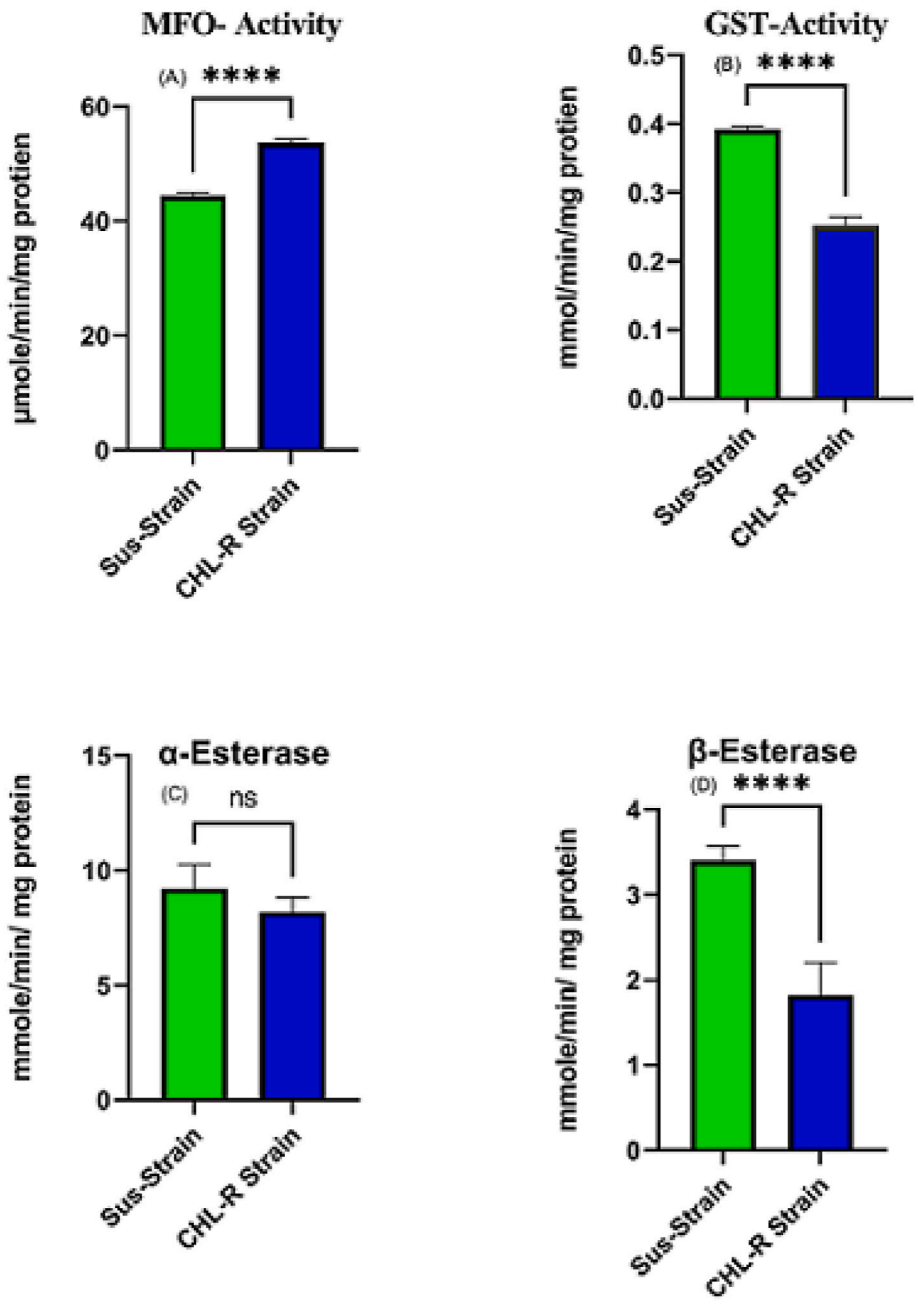

Fig. 1. Mean (±SE) of detoxification enzymes activity in *Spodoptera littoralis* strains.

stain, CHL-R strain exhibited significant decrease in esterase activity associated with less effect of TPP and low expression level of esterase' genes (0.67- fold). Although, the role of esterase activity in CHL-R strain is unclear, its reduced activity may interpret the obvious cross resistance to indoxacarb in the chlorantraniliprole-resistant strain. Indoxacarb is a pro-insecticide that is converted by esterase and/Or amidase to more toxic form in several lepidopteran larvae (Wing et al., 1998, 2010). Therefore, the reduction in esterase activity in CHL-R strain was associated with reduced sensitivity to indoxacarb, and consequently, may increase cross-resistance to indoxacarb.In accordance with these results, the weak and insignificant correlations between the activity of esterases and susceptibilities of *T. absoluta* populations to chlorantraniliprole were reported (Campos et al., 2015). Oppositely, other reports confirmed the role of esterase in chlorantraniliprole resistance in *Musca domestica* L. (Diptera: Muscidae) (Shah and Shad, 2021), *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae) (Muthusamy et al., 2014), *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) (Sial et al., 2011) However, the increase of TPP dose caused no or negative change in the synergistic ratio (SR) of chlorantraniliprole on the resistant population of *S. frugiperda* (Liu et al., 2022).

Glutathione-S-transferases (GST) plays an essential role in cell protection and repair processes via detoxification of peroxides and oxidized DNA bases (Forman et al., 2009). Resistant strains of Lepidoptera has often exhibited a surge in GST activity (Ortelli et al., 2003). However, our study showed that GST activity is not correlated to chlorantraniliprole resistance in *S. littoralis*. In agreement with this, Lai and Su (2011) reported that diethyl-maleate (DEM) did not enhance the toxicity of chlorantraniliprole to three *S.exigua* strains. Nonetheless, it is likely that GST is the main detoxification enzyme responsible for chlorantraniliprole resistance in *P. xylostella* (Hu et al., 2014).

Finally, understanding the biochemical and molecular foundations of insect resistance to chlorantraniliprole, can contribute in the

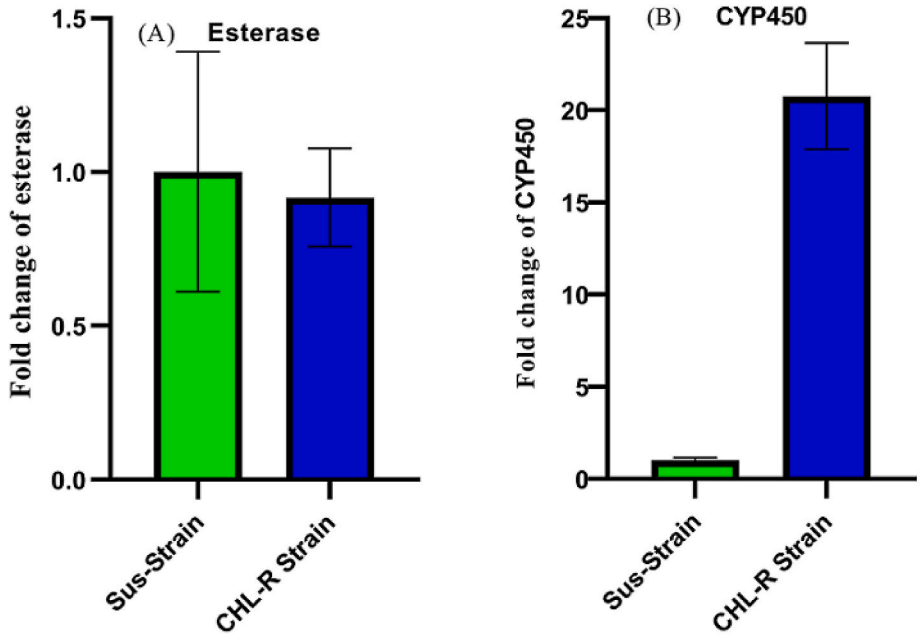

Fig. 2. Relative quantity (mean ± SE) of esterase and CYP450 gene for Sus and CHL-R strains.

development of effective strategies for resistance management. Furthermore, monitoring the expression of specific genes, such as esterase and Cytochrome P450 (CYP450), can provide insights into the development of resistance and help in the early detection of resistant populations. In addition, the results of cross-resistance studies can be used to suggest an appropriate rotation of pesticide application and to identify alternative groups of insecticides that can potentially delay or hinder resistance resurgence. Finally, continued research is needed to further understand the mechanisms of resistance and develop effective strategies for managing resistance in *S. littoralis*.

Author contributions

MAMM participated in designing the study, investigation, methodology, and writing the original draft. EAO designed the primers and worked on the Real-time- PCR experiments. EMSM participated in data writing and analyzing. EAF participated in designing the study, investigation, methodology, writing the original draft, and analyzing the data. All authors read and approved the final manuscript.

Ethical approval

This article does not contain any studies conducted on human participants or animals. The authors consent to the publication of the manuscript in its current form.

Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

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