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Original Article

Esterase Activity in Homogenates of *Spodoptera Frugiperda* (J.E. Smith) Exposed to Low-Lethal Doses of Chlorantraniliprole and Emamectin Benzoate

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Abstract

Background: Chlorantraniliprole and emamectin benzoate have been widely used to control pests including FAW. For effective pest control, the sublethal effects of these insecticides should be considered.

Methods: The toxicity of chlorantraniliprole and emamectin benzoate to third-instar FAW larvae has been determined using topical application. Alpha esterase (EST-α) and beta esterase (EST-β) activity were also assessed after 1, 6, 12, 24, and 48 h of exposure of larvae to low doses of these insecticides.

Results: The susceptibility of FAW larvae to chlorantraniliprole and emamectin benzoate increased after 48 h, and the LD_{50} was 0.18 and 0.23 µg/larvae, respectively. EST-α and -β activity changed significantly in FAW larvae over time after exposure to insecticides. After only 1 h, EST-α, and -β activity after exposure of larvae to insecticides at LD_{10} showed no significant difference compared to control. However, under LD_{30} treatments, the activity of EST-α and -β decreased significantly. Significant reduction of EST-α and -β activity was observed in larvae after 48 h exposure to chlorantraniliprole at LD_{30} (0.5 and 0.15 nmol/min/mg protein, respectively) followed by emamectin benzoate (1.19 and 0.29 nmol/min/mg protein, respectively) compared with control activity.

Conclusion: Sublethal doses of chlorantraniliprole and emamectin benzoate affected activities of EST-α and -β of FAW.

Keywords: Alpha esterase, Beta esterase, Chlorantraniliprole, Emamectin benzoate, *Spodoptera frugiperda*, Sublethal effects.

Introduction

FAW is an invasive, highly destructive polyphagous pest that causes significant crop production losses. The use of insecticides remains the most common method of FAW control [1], although resistance is increasing. There are documented cases of FAW resistance to the main chemical groups of insecticides in different countries [2,3]. The development of insecticide resistance occurs mainly due to two main mechanisms, namely target site

insensitivity and detoxification by overproduction of various enzymes [4]. Hydrolysis of insecticides by esterase is an important biochemical mechanism that contributes to detoxification to protect insects from pesticides [4]. Increased esterase activity has been reported in insect resistance to insecticides [5,6]. Although FAW is a pest that has recently been observed in Egypt, this underscores the importance of information regarding the control of this type of pest.

Field insect populations are exposed to acute and chronic effects of sublethal concentrations of pesticides. It can lead to various consequences: death of insects, changes in their life cycle, fecundity, behavior, and physiological and biochemical parameters [7-9]. It is important to study the sublethal effects of exposure to pesticides, especially from new chemical groups with different mode of action, to avoid resistance and to formulate modern pest control methods [10].

In Egypt, chlorantraniliprole and emamectin benzoate are the main insecticides used to control fall armyworm. However, no information on the sublethal effects of these insecticides on FAW bioactivity. Thus, the present study was conducted to evaluate the sublethal effects of these insecticides on the esterases activity (EST-α and EST-β) of FAW.

Materials and Methods

Insects and Insecticides

FAW larvae were collected from maize fields on the 22 May 2022 in Sanores district (29°18′35.82″N, 30°50′30.48″E), Fayoum Governorate, Egypt. After being brought to the Central Agricultural Pesticides Laboratory, Egypt, they raised on artificial diet of Pinto *et al.* (2019) without exposure to insecticides. Insect cultures were maintained at 25 ± 1 °C, 60-65 % RH, and a photoperiod cycle of 14L:10D h. Newly moulted, 1-d-old third instar larvae of FAW were used in the experiments.

Chlorantraniliprole (95.3% purity) and emamectin benzoate (70% purity) were purchased from DuPont Crop Protection (Wilmington, DE, United States) and Guangxi Tianyuan Biochemical Co., Ltd. (Nanning, China), respectively.

The selection of these insecticides of different chemical groups and modes of action was purely based on recommendations from the Egyptian Ministry of Agriculture regarding their effectiveness in controlling FAW.

Bioassays

Topical application was performed to determine the toxicity level of chlorantraniliprole and emamectin benzoate on third instar larvae of FAW. Technical grade insecticides were individually dissolved in acetone to obtain a stock solution and six concentrations were prepared by serial dilution with solvent. One microliter of each insecticide was applied topically to the dorsum of the thorax of each larva using a micro-applicator. Acetone alone was used as the control for each insecticide. Each concentration had a population of 30 larvae and it was replicated thrice. Treated larvae were transferred to Petri dishes containing insecticide-free artificial diet. Mortality was assessed after 48 h, larvae that did not move after stimulation from a camel's hair brush were scored as dead. The LD_{50}, LD_{30}, and LD_{10} values of insecticides were estimated through probit analysis. All the experiments were carried out at 25±1 °C, 60±5% RH, and 14:10 hour of light:dark.

Enzyme Preparation

In this experiment, leaves were dipped in concentrations of chlorantraniliprole and emamectin benzoate (LC_{10}, 0.06 and 0.11 µg/larvae) and (LC_{30}, 0.02 and 0.06 µg/larvae), respectively. 300 FAW larvae (the 3rd instar) were used for each treatment, and 60 larvae were evaluated each time. Assessments were performed at five times: 1, 6, 12, 24, and 48 h after exposure to insecticides. Twenty surviving larvae from each treatment (LC_{10}, and LC_{30} and control) were homogenized in 3 mL homogenization buffer (ice-cold 20 mM, pH 7.0 phosphate buffer). The crude homogenates were centrifuged at 10,000 rpm for 20 min at 4 °C. The resulting supernatants were used as enzyme solution.

Alpha Esterase (EST-α) and Beta Esterase (EST-β) Activity

Esterase activity was evaluated using the Van Asperen (1962) [12] method. An enzyme homogenate sample (30 µL) was mixed with 500 µL of substrate solution (0.3 mM α-naphthyl acetate buffer for EST-α) or (0.3 mM β-naphthyl acetate buffer for EST-β) and incubated at room temperature. After 30 min of incubation, 0.1 mL of chromogenic reagent (l percent fast blue B salt: 5 percent sodiumlauryl sulphate = 2: 5, v/v) was added. A red color was developed immediately, followed by changing to fairly stable blue color, measured at 590 nm. The results were expressed as nmole of α-naphthol or β-naphthyl released $minute^{-1}$mg of $protein^{-1}$.

Protein Contents Assay

Protein content of the enzyme homogenate was determined according to the method of Bradford (1976) [13] using bovine serum albumin as standard. The measurement was performed with the spectrophotometer at 595 nm.

Data Analysis

Bioassay data were analyzed using PoloPlus statistical software version 2.0 (LeOra Software Company, Beverly Hills, CA, USA) for probit analysis, and the LD_{10}, LD_{30} and LD_{50} values and their 95% confidence limits were calculated.

Results

LD Values for chlorantraniliprole and emamectin benzoate

The toxicity of chlorantraniliprole and emamectin benzoate to the 3rd instar larvae is indicated in Table 1. The LD_{10}, LD_{30}, and LD_{50} values for chlorantraniliprole were 0.06, 0.11, and 0.18 µg/larvae, respectively, whereas values for emamectin benzoate were 0.02, 0.06, and 0.23 µg/larvae, respectively.

Alpha-Esterase (EST-α) and Beta-esterase (EST-β) Activity

Esterase alpha (EST-α) and beta esterase (EST-β) activity showed a decrease after all exposure times to chlorantraniliprole and emamectin benzoate treatments at low doses compared to control (Figures 1 and 2). At 1 h, there was no significant difference between all treatments and the control (Figures 1A and 1B). Chlorantraniliprole and emamectin benzoate treatment at LD_{10} resulted, to some extent, in decreased EST-α activity after 6, 12, 24, and 48 h compared with the control groups (Figure 1A), while the LD_{30} treatment decreased significantly the EST-α activity (Figure 1B). At 48 h chlorantraniliprole and emamectin benzoate at LD_{30} treatments showed lower activity of EST-α (0.5 and 1.19 nmole/min/mg protein, respectively) compared with control (1.66 nmole/min/mg protein) (Figure 1B).

EST-β activity had not significantly changed in larvae 1 h after their exposure to chlorantraniliprole and emamectin benzoate at low doses (Figures 2A and 2B). Chlorantraniliprole and emamectin benzoate showed decreased EST-β activity (Figures 2A and 2B). Regarding the evaluation times of the activity, chlorantraniliprole and emamectin benzoate at LD_{10} and LD_{30} treatments presented decrease of the EST-β activity in larvae after 6, 12, 24, and 48 h compared to control (Figures 2A and 2B). Low activity of EST-β (0.15 and 0.29 nmole/min/mg protein, respectively) were observed in the larvae 48 h after an exposure to chlorantraniliprole and emamectin benzoate at a dose of LD_{30} when compared to the control (0.52 nmole/min/mg protein) (Figure 2B).

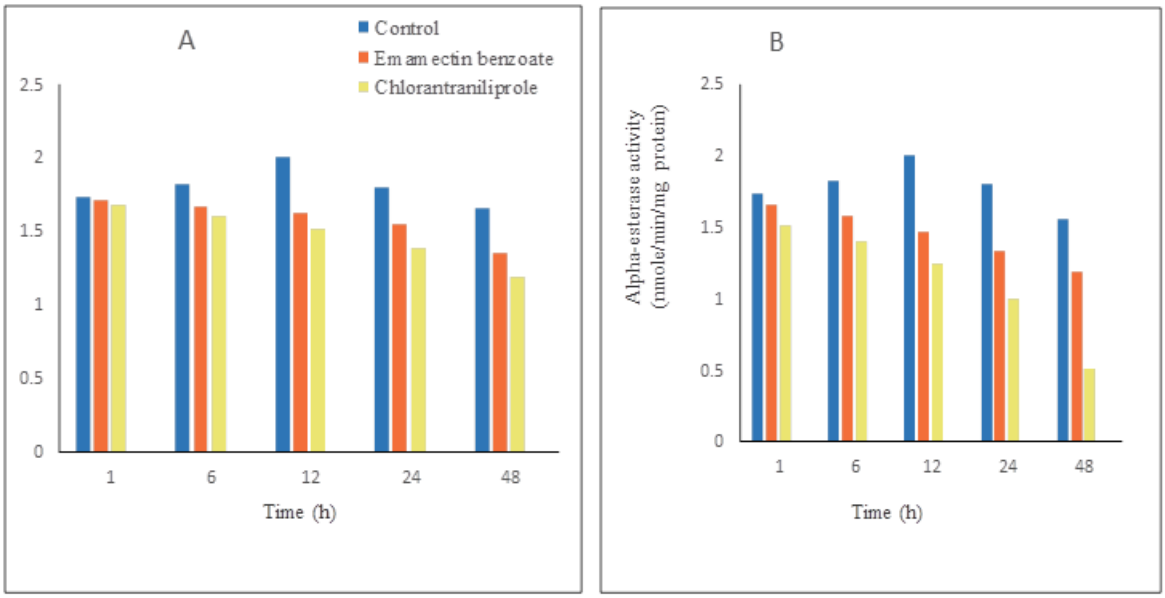

Figure 1 Alpha-esterase activity in third-instar larvae of *Spodoptera frugiperda* after exposure to low-lethal doses (A) LD_{10}, (B) LD_{30} of chlorantraniliprole and emamectin benzoate.

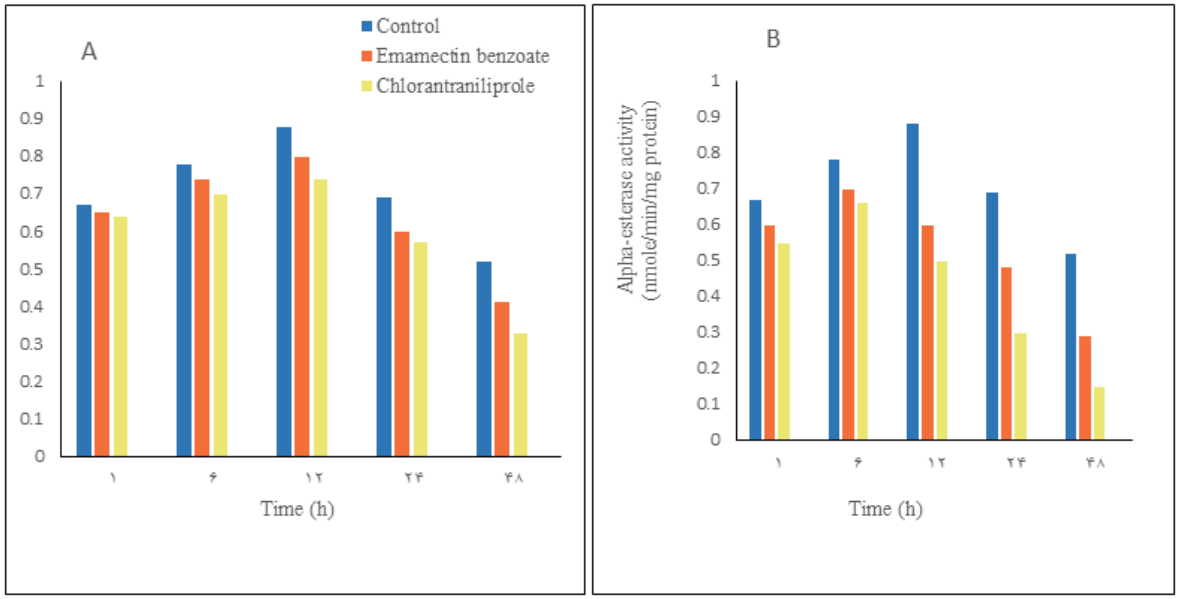

Figure 2 Beta-esterase activity in third-instar larvae of *Spodoptera frugiperda* after exposure to low-lethal doses (A) LD_{10} and (B) LD_{30} of chlorantraniliprole and emamectin benzoate

Discussion

Chlorantraniliprole and emamectin benzoate are novel insecticides that are effectively used to control many lepidopterous pests in different crops [6,14-16]. Low concentrations of different insecticides can have significant effects on the reproductive capacity and biochemical parameters of insects [9]. Chlorantraniliprole and emamectin benzoate showed highly toxic effects against FAW larvae in the present study (Table 1).

Table 1. Lethal toxicity of chlorantraniliprole and emamectin benzoate for 3rd instar larvae of *Spodoptera frugiperda*

Treatment	n	Slope (±SE)	LD_{10} (95% CL) (µg/larvae)	LD_{30} (95% Cl) (µg/larvae)	LD_{50} (95% Cl) (µg/larvae)	χ^2 (df)	p-value
Chlorantraniliprole	300	2.34 (0.41)	0.02 (0.01–0.03)	0.06 (0.04–0.09)	0.18 (0.15–0.23)	7.66 (3)	0.82
Emamectin benzoate	300	1.21 (0.14)	0.06 (0.03–0.08)	0.11 (0.09–0.14)	0.23 (0.17–0.31)	4.50 (3)	0.96

n, LD, CL, χ^2, df, and p-value indicate total number of larvae used, lethal dose, confidence limit, chi-square, degrees of freedom, and p-value as calculated by probit analysis with SPSS 23.0, respectively.

The toxicity of chlorantraniliprole was 0.06, 0.11, and 0.18 µg/larvae at LD_{10}, LD_{30}, and LD_{50}, respectively, while for emamectin benzoate, it was 0.02, 0.06, and 0.23 µg/larvae, respectively. The high toxicity of chlorantraniliprole and emamectin benzoate to FAW shows the susceptibility of this pest to these insecticides due it being the latest in use with high insecticidal activity. High toxicity of chlorantraniliprole and emamectin benzoate also was reported by Ismail (2020) [6]; XingChuan *et al.* (2019) [14]; Zhang *et al.* (2022) [15]; and Zhang *et al.* (2023) [16] who studied the toxicity of these insecticides in FAW and were recommended for FAW control. A study by Zhang *et al.* (2023) [16] showed that the susceptibility of second instar larvae of FAW to chlorantraniliprole and emamectin benzoate.

In this study, it was found that low-doses exposure to chlorantraniliprole and emamectin benzoate treatments resulted in significant reduction in EST-α and EST-β activity compared to the control (Figures 1 and 2). A significant difference was observed between the treatments and the control, and this difference increased with increasing concentration. These results were consistent with those of Yu *et al.* (2003) [17]; Carvalho *et al.* (2018) [18]; Samanta *et al.* (2023) [19]. However, this result was different from the results of the study conducted by Carneiroi *et al.* (2019) [5]; Ismail (2020) [6], in which the higher alpha- and beta-esterase activity observed in other insects such as; *Helicoverpa armigera* (Hübner) and *S. littoralis* (Boisd.) population indicates a higher metabolic rate, which may mean some level of resistance.

In general, a negative correlation was observed between EST-α and -β activity in FAW larvae and their sensitivity to insecticides. This study suggests that chlorantraniliprole and emamectin benzoate exhibit strong efficacy as pesticides against FAW populations as they have been observed to significantly reduce both the EST-α and -β of FAW when administered in sublethal doses.

Conclusion

Based on the results of this study, the new insecticides chlorantraniliprole and

emamectin benzoate have high toxicity against FAW larvae. Low concentrations also reduce the activity of EST-α and -β in FAW. Hence, the inclusion of low concentrations of both insecticides in integrated pest management (IPM) programs constitutes a highly desirable alternative to rational application in the field to control this pest. Therefore, field studies are required to evaluate and validate the laboratory results of the present study.

List of abbreviations

FAW: fall armyworm
EST-α: alpha esterase
EST-β: beta esterase

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests in this article.

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Authors' contributions

S.M.I. subject selection, study design, carried out the experiments, writing manuscript, collecting, interpretation of the data, and performing statistical analysis. The author read and approved the final manuscript.

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