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Toxicity and biochemical effects of some insecticides on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under laboratory conditions

Marwa F. K. Aly *and A. M. Ali

Plant Protection Department, Faculty of Agriculture, Minia University, El Minya, Egypt

ABSTRACT

The current study investigated the role of four insecticides; Novaluron, Chromafenozidae, Thiocyclam and Chlorantraniliprole, in *the* biochemical activity of 2nd and 5th larval instars of cotton leafworm (*Spodoptera littoralis*). The total carbohydrates, proteins and lipids were determined in both larval instars under insecticide application. Also, the impact of tested insecticides on the activity of digestive enzymes,α- esterase, β- esterase, carboxyesterase, phenoloxidase, chitinase and acetylcholinesterase in *S. littoralis* larvae was studied. The results revealed that all tested insecticides decreased total protein and lipid content except chlorantraniliprole, which increased the total protein content. The tested insecticides increased the total carbohydrates and invertase activity except Thiocyclam. Applying Thiocyclam increased the trehalase and amylase enzyme content in treated larvae. Applying Chromafenozidae and Chlorantraniliprole reduced the activity of trehalase and amylase in the two larval instars. Chlorantraniliprole and Thiocyclam activated the activity of β-esterase and Carboxyesterase. In contrast, Novaluron and Chromafenozidae inhibited the content of β- esterase and Carboxyesterase enzymes. The activity of α- esterase was induced by applying Chromafenozidae and Chlorantraniliprole and inhibited by applying Novaluron and Thiocyclam on the two larval instars. Chlorantraniliprole activated Phenoloxidase and Chitinase enzymes in larva*e,* but Novaluron and Chromafenozidae decreased the activity of Chitinase. Acelylcolinesterase has been activated by applying Chromafenozidae and deactivated by Thiocyclam and Chlorantraniliprole. The obtained results lead to a better understanding of the tested insecticides' mode of action by studying the insicticides' influence on the biochemical activities of the treated larvae of *S. littoralis.*

Key words: *Spodoptera littoralis***,** Toxicity**,** digestive enzymes, Acetylcholinesterase, Thiocyclam.

INTRODUCTION

The cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is attacking a wide range of economic crops such as cotton in Egypt. *Spodoptera littoralis* larvae mainly feed on leaves and stems and can postpone growth or decrease cotton production. Furthermore, during heavy infestations, CLW can also penetrate flowers and bolls, causing a

*Corresponding author email: marwa.ali@mu.edu.eg

substantial loss of up to 50% reduction in yield (Russell *et al*., 1993). The management tactic of cotton leafworm in Egypt mostly relies on rotating traditional insecticides such as organophosphates, carbamates, and pyrethroids yearly. Massive use of classical insecticides results in considerable problems like high resistance to various insecticides (Aydin and Gurkan, 2006; Miles and Lysandrou, 2002) and harmful hazards to the environment and beneficial organisms (Forgash, 1984; Hawkins *et al*., 2019).

Consequently, other approaches are required to develop alternative or further techniques, enabling insecticide rationalization and providing sufficient crop protection for sustainable production. The insect growth regulators (IGRs) are the most promising alternative control compounds. The IGRs received great attention for controlling insects in the future that showed diverse effects against *S. littoralis* and caused large selectivity to beneficial insects (Raslan, 2002; Grafton-Cardwell *et al*., 2005; El-Sheikh, 2015) and not targeting animals or humans (Abd El-Kareem *et al*., 2022).

Using IGRs pesticides reduces growth, inhibits molting, and causes abnormal anatomy as well as increases the mortality of a wide range of insect species belonging to Order Lepidoptera. The efficacy of the IGRs relies on the species of treated insect and the applied concentration (Khedr *et al*., 2005). IGRs disrupt the physiology and development of the targeted pests.

IGRs have many advantages compared to other traditional insecticides, which are selective compounds highly effective against the targeted insects and less toxic to the non-target organisms. (Gurr *et al*., 1999). Tunaz and Uygun (2004) stated that IGRs showed two different modes of action, they could inhibit the chitin synthesize (CSIs) and or interfere with pathways of the hormones in the treated insects (i.e., juvenile hormone analogues, and ecdysteroids).

Inhibition of chitin synthesis impedes the biosynthesis of chitin in treated insects (Gijswijt *et al*., 1979). Malfunction in chitin biosynthesis prevents molting and or produces imperfect insect cuticle (Hammock and Quistad, 1981). In addition to chitin inhibition, IGRs induce hormones in treated insects, disrupting various physiological traits (Soltani *et al*., 1984). The impact of sublethal dose of insecticide on the insect biological parameters such as growth, fecundity, fertility and other developmental parameters has been mentioned in many studies (Pineda *et al*., 2007, Wang *et al*., 2008, Saber *et al*., 2013, Rehan and Freed, 2015). The sublethal dose of insecticides affect physiological parameters as well such as the metabolism of carbohydrates, lipids, and proteins (Vojoudi *et al*., 2017). On the other hand, Ishaaya *et al*., (2002) reported that IGRs showed no effect on the beneficial parasitoids and a minor non-harmful impact on other natural enemies. In addition, it has low toxicity

towards exposed mammals (Barazani, 2001). Despite its vital physiological effect on the treated insects, more information is needed to understand the IGRs mode of action in treated insects. Therefore, this work aims to study the influence of the LC_{50} of four insecticides, Novaluron, hromafenozide, Thiocyclam and Chlorantraniliprole, on the biochemical and physiological activity of the 2nd and 5th larval instars of *S. littoralis*.

MATERIALS AND METHODS

1. Insect rearing colony

Spodoptera littoralis were obtained as pupae from Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt, then kept in the growth chamber at 25 ± 2 °C and 65 ± 5% RH with a photoperiod of 16h:8h (light: dark) until adult emergence under Plant Protection Laboratory at Faculty of Agriculture, Minia University, El Minya, Egypt. The emerged adults were exposed to 10% (W/V) of honey solution for feeding. Small branches of oleander plants (*Nerium oleander*) were exposed to the emerging adults for oviposition. Oleander's leaves with freshly deposited eggs were collected daily and placed in a glass jar with fresh castor bean leaves.

Table1. Trade names, common names, chemical names, chemical group and mode of action for tested insecticides against *S. littoralis*

Trade name	Common name	Chemical name	Chemical group	Mode of action
Roxy 10%EC	Novaluron	(±)-1- [3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy) phenyl]-3-(2,6 difluorobenzoyl) urea.	Insect growth regulators pesticides	Chitin Synthesis Inhibitor.
Virtu® 5%	Chromafenozide	3,4-dihydro-5- methyl-2H-1-benzopyran-6-carboxylic acid 2-(3,5-dimethylbenzoyl)-2- (1,1-dimethylethyl) hydrazide.	Insect growth regulators pesticides	Is a novel dibenzoylhydrazine and is categorized to be an insect hormone ecdysone (moulting hormone agonists).
Evisect 50%SP	Thiocyclam hydrogen oxalate	*N*,*N*-dimethyltrithian-5-amine	Nereistoxin	Blocking cholinergic trans-mission resulting in paralysis and insect death.
Coragen20%SC	Chlorantraniliprole	3-Bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide	Anthranilic	Chlorantraniliprole binds to a specific receptor in muscles called the ryanodine receptor. When chlorantraniliprole binds to this receptor, it causes muscle cells to leak calcium. The muscles stop working normally. The insect is paralyzed and dies.

2.Tested insecticides.

Four insecticides were chosen to evaluate their toxicity and biochemical effects on the 2nd and 5th larval instars of *S. littoralis* (**Table 1**).

3.Bioassays and insecticides assessments

` The food dipping technique was used to determine the LC_{50} values of tested compounds against 2nd and 5th larval instars of *Spodoptera littoralis*. Six dilutions of each tested insecticide compound (100, 200, 400, 600, 800 and 1000 ppm) were prepared using sterilized water. Three replicates were used for each concentration. The assay repeated twice to confirm the obtained results. Ten larvae were used for each replicate. Castor leaves were dipped for 30 seconds in prepared concentrations. Castor leaves used for the control were dipped in water. The leaves left to dry under room temperature. After drying, the treated leaves were placed in glasses jars (1000 cm^3). The starving larvae in 2nd and 5th instars were transferred separately into the jars and allowed to feed on treated castor leaves. The assay continued for 96h the larvae mortalities were recorded daily. The median lethal concentration (LC_{50}) was calculated for the two larval stages. The LC_{50} of Novaluron, Chromafenozide, Thiocyclam and Chlorantraniliprole were 89.13, 117.49, 128.82 and 173.78ppm, respectively for the 2nd larval instar and were 107.15,141.25,186.21 and 457.09 for 5th larval instar. The LC_{50} values used for the upcoming experiments as described below.

4. Preparation of insect samples

The 2nd and 5th larval instars of *Spodoptera littoralis* were treated with LC_{50} of tested insecticides for 24 hrs. Survived larvae were weighed and transferred into a 50 ml centrifuge tube, frozen directly for 24 h at -20 ºC, then homogenized. The homogenates obtained from the previous step were centrifuged for 15 min at 8000 rpm. under cooling condition of 4 °C. The supernatant was collected and the precipitates were discarded. The obtained supernatant was prepared for biochemical assay to measure the activity of targeted enzymes' activity.

5. Assessment of total proteins, total carbohydrates and total lipids

The impact of the LC_{50} of tested compounds on the total carbohydrates, total proteins and total lipids of the 2nd and 5th larval instars was assayed according to (Dubois *et al*., 1956), (Bradford, 1976) and (Knight *et al*., 1972), respectively.

6. Assessment of enzymes activity

The activities of different enzymes were measured including acetylcholinesterase which measured according to (Simpson *et al*.,1964). Also, chitinase activity was determined according to (Bade and Stinson, 1981). The activity of α- and β-esterases were measured according to (Van Asperen, 1962). Phenoloxidase was assessed according to (Ishaaya, 1971). The activity of Carboxylesterase enzyme was determined according to (Cao *et al*., 2008). Additionally digestive enzymes activity: Invertase, amylase and

trehalase activity was determined according to (Ishaaya and Swirski, 1976).

Statistical analysis

Based on control treatments, Abbott's formula was used to calculate the corrected mortality percentage (Abbott, 1925). The median lethal concentration (LC_{50}) and regression equation components of each insecticide toxicity line was calculated by using the statistically computed dose-response relationship curve (Finney, 1971). The Analysis of variance (ANOVA) using JMP Pro 16 (SAS 2013, Cary, NC) was used to determine the significance of the main differences in the biochemical parameters. The difference between 2nd and 5th larval instars was compared using an un-paired T-test. All data is presented as means±SE.

RESULTS

1.Toxicity of tested insecticides against *S. littoralis*

The effect of tested insecticides on 2nd and 5th larval instars of *S. littoralis* was estimated and shown in (**Table 2**). The obtained data revealed that novaluron had the highest acute toxicity against 2nd and 5th larval instars (89.13 and 107.15 ppm), respectively with high slope values of 1.824 ± 0.37 and 1.728 ± 0.38, respectively. Otherwise, Chlorantraniliprole recorded the lowest acute toxicity against 2nd and 5th larval instars (173.78 and 457.09 ppm, respectively) with the least slope values of 1.429 ± 0.30 and 1.044 ±0.56. Lethal concentrations values for Thiocyclam and Chromafenozidae were 128.82 and 173.78 ppm.

2. Total carbohydrates, proteins and lipids

The effect of evaluated insecticides in total carbohydrates, proteins and lipids for 2nd and 5th larval instars of *S. littoralis* was determined and presented in (**Table 3**). Results showed that Thiocyclam increased the total carbohydrates in 2nd larval instar (40.5 mg/ml) compared to control and other tested insecticides. While Novaluron and Thiocyclam increased the total carbohydrates in 5th larval instar (37.5 and 46.0 mg/ml) compared with controland other tested insecticides. It is obvious that Thiocyclam significantly increased total carbohydrates in 5th larval instar (46 mg/ml) than 2nd larval instar (40.5 mg/ml).

The Chromafenozidae, Novaluron and Thiocyclam, (20.1, 22.4 and 30.1 mg/ml, respectively) decreased total haemolymph protein content of 2nd larval instar on compared with control. Similar results were observed on the 5th larval instar, where the total protein was higher in the 5th larval instar treated with Chlorantraniliprole (38.5 mg/ml). In contrast, there was a reduction in total protein in larvae treated with Chromafenozidae, Novaluron and Thiocyclam ranged between 31.8-35.3 mg/ml than control (37.3 mg/ml). Otherwise, the reduction in total protein is significantly higher in the 5th larval instar compared to the 2nd larval instar for Chromafenozidae and Thiocyclam. All tested insecticides led to a decrease

in lipids, which was more obvious on Thiocyclam in 2nd and 5th larval instars

Table 2. Toxicity of Novaluron, Chromafenozide, Thiocyclam and *Chlorantraniliprole* on 2nd and 5th larval instars of *Spodoptera littoralis* 96 hr after treatment.

Insecticides	Toxicity line equation	LC_{50} as ppm	95% CL		Slope ± SE
			Lower	Upper	
2nd larval instar					
Novaluron	y = 1.824x + 1.451	89.13	71.38	110.56	1.824 ± 0.37
Chromafenozide	y = 1.762x + 1.350	117.49	99.15	130.22	1.762 ± 0.20
Thiocyclam	y = 1.554x + 1.715	128.82	105.72	144.11	1.554 ± 0.22
Chlorantraniliprole	y = 1.429x + 1.806	173.78	144.22	196.43	1.429 ± 0.30
5th larval instar					
Novaluron	y = 1.728x + 1.499	107.15	89.98	25.82	1.728 ± 0.38
Chromafenozide	y = 1.551x + 1.659	141.25	118.36	168.49	1.551 ± 0.31
Thiocyclam	y = 1.547x + 1.493	186.21	145.58	298.06	1.547 ± 0.22
Chlorantraniliprole	y = 1.044x + 2.224	457.09	299.77	632.96	1.044 ± 0.56

(2.5 and 2.6 mg/gm tissue, respectively) than control (3.6 and 3.9 mg/gm tissue, respectively). While total lipids were 2.8, 3.0 and 3.2 mg/gm tissue for Novaluron, Chromafenozidae and Chlorantraniliprole, respectively in the 2nd larval instar and were 2.9, 3.2 and 3.5 *mg/gm tissue*, respectively in the 5th larval instar. No significant differences were found between both larval instars in total lipids for tested insecticides.

3. **Carbohydrates hydrolyzing enzymes**

Three digestive enzymes; amylase, trehalase and invertase were evaluated in 2nd and 5th larval instars which treated with LC_{50} of tested insecticides

Table 3. Effect of Novaluron, Chromafenozide, Thiocyclam and Chlorantraniliprole at LC_{50} c total carbohydrates, proteins and lipids content in the 2nd and 5th larval instars of *S. littoralis.*

Treatments	Physiological traits		
	2nd larval instar	5th larval instar	*P-value*
	Total carbohydrates (mg/ml)		
Control	31.1±2.1	32.1±2.1	0.754 NS
Novaluron	33.7±2.2	37.5±2.4	0.068 NS
Chromafenozide	32.3±2.3	32.4±2.3	0.481 NS
Thiocyclam	40.5±1.4	46.0±1.4	0.046*
Chlorantraniliprole	33.7±1.8	35.7±1.9	0.564NS
F value	11.7 *	9.3 *	-
	Total proteins (mg/gm tissue)		
Control	37.7±2.2	37.3±1.2	0.732 NS
Novaluron	22.4±2.3	32.7±2.1	0.063 NS
Chromafenozide	20.1±2.1	31.8±1.3	0.045*
Thiocyclam	30.1±1.8	35.3±2.8	0.050*
Chlorantraniliprole	38.3±1.4	38.5±2.0	0.0867NS
F value	7.8 *	5.4 *	-
	Total lipids (mg/gm tissue)		
Control	3.6±1.1	3.9±2.1	0.835 NS
Novaluron	2.8±1.9	2.9±2.0	0.785 NS
Chromafenozide	3.0±1.5	3.2±2.5	0.654 NS
Thiocyclam	2.5±1.1	2.6±1.0	0.854 NS
Chlorantraniliprole	3.2±2.1	3.5±1.2	0.645NS
F value	2.8*	1.5*	-

*Significant at 0.05 Probability level. NS is not significantly different.

and presented in (**Table 4**). The obtained data indicated that the invertase activity was increased in2nd larval instar treated with Novaluron, Chromafenozidae and greatly higher. with Chlorantraniliprole (230.8, 250.5 and 280.8 µg glucose /g.b.wt, respectively**)** compared to control (221.5 µg glucose /g.b.wt).

However, Thiocyclam decreased invertase activity (207.3 µg glucose /g.b.wt) than control and other tested insecticides. Similar results were observed in 5th larval instar, where Chlorantraniliprole had the highest invertase activity *(310.5* µg glucose /g.b.wt) than control and other tested insecticides. In contrast, Thiocyclam decreased the invertase activity and reached to 209.9. On the other hand, Chromafenozidae sharply decreased the trehalase activity in 2nd and 5th larval instars (73.5 and 85.4)

Table 4. Effect of LC_{50} of the tested insecticides on the carbohydrates hydrolyzing enzymes assessed in 2nd and 5th larval instars of *S. littoralis*

Treatments	Mean enzyme activity (µg glucose /g.b.wt) ± SE		
	2nd larval instar	5th larval instar	*P-value*
Invertase			
Control	221.5±6.3	222.7±5.5	0.756 NS
Novaluron	230.8±6.2	238.8±7.7	0.073 NS
Chromafenozide	250.5±5.8	270.4±6.5	0.064 NS
Thiocyclam	207.3±6.8	209.9±5.5	0.754 NS
Chlorantraniliprole	280.8±8.1	310.5±7.1	0.008*
F value	13.5 *	11.7 *	-
Trehalase			
Control	119.7±5.5	120.5±5.1	0.654 NS
Novaluron	86.4±5.4	97.2±3.6	0.063 NS
Chromafenozide	73.5±5.4	85.4±3.5	0.057 NS
Thiocyclam	122.2±3.7	130.7±4.3	0.074 NS
Chlorantraniliprole	100.4±4.7	109.8±3.6	0.074 NS
F value	10.6 *	8.2 *	-
Amylase			
Control	54.7±4.5	55.4±2.8	0.453 NS
Novaluron	55.4±5.3	60.5±3.1	0.050*
Chromafenozide	23.5±3.4	38.6±2.5	0.073 NS
Thiocyclam	65.5±4.2	75.5±5.3	0.075 NS
Chlorantraniliprole	39.7±2.2	48.3±2.0	0.088*
F value	8.5*	5.7*	-

*Significant at 0.05 Probability level. NS is not significantly different.

compared to control (119.7 and 120.5). In contrast, Thiocyclam increased trehalase activity in both 2nd and 5th larval instars (122.2 and 130.7) compared to Novaluron (86.4 and 97.2) and Chlorantraniliprole (100.4 and 109.8). Likewise, Chromafenozidae and Chlorantraniliprole affected and decreased the amylase activity *in both* 2nd and 5th larval instars (23.5 and 38.6). In comparison, Thiocyclam significantly increased amylase activity in 2nd and 5th larval instars (65.5 and 75.5) followed by Novaluron (55.4 and 60.5). Compared to control (54.7 and 55.4).

4. α- esterase, β- esterase and Carboxyesterase

Alph and beta esterase and Carboxyesterase enzymes were evaluated in 2nd and 5th instars and illustrated in (**Table 5**). The obtained data clearly show that Novaluron and Thiocyclam greatly decreased alpha esterase activity (611.7 and 830.2 ug α -naphthol/min/g.b.wt), respectively for 2nd larval instar and (806.3 and 1089 ug α -naphthol/min/g.b.wt) for 5th larval

Table 5. Effect of LC_{50} of the tested insecticides on the α-, β- esterase and carboxyesterase assessed in 2nd and 5th instar larvae of *S. littoralis*

Treatments	Mean enzyme activity (ug α-or β-naphthol/min/g.b.wt) ± SE		
	2nd larval instar	5th larval instar	*P-value*
	α- esterase		
Control	1162.9±12.1	1164.8±15.2	0.645 NS
Novaluron	611.7±11.8	806.3±13.3	0.0095*
Chromafenozide	1299.9±13.5	1325.8±14.3	0.0065*
Thiocyclam	830.2±13.1	1089.5±13.2	0.0089*
Chlorantraniliprole	1276.9±13.0	1536.3±16.5	0.0043**
F value	108.9 **	103.7 **	-
	β- esterase		
Control	257.9±8.6	258.2±10.7	0.489 NS
Novaluron	84.0±8.3	107.9±9.3	0.008*
Chromafenozide	115.3±7.7	210.5±9.6	0.006*
Thiocyclam	355.7±7.4	435.3±9.1	0.006*
Chlorantraniliprole	300.2±9.4	375.1±8.1	0.008*
F value	18.4 **	15.8 **	-
	Carboxyesterase		
Control	123.2±6.7	143.8±5.8	0.736 NS
Novaluron	45.1±6.8	90.4±5.5	0.006*
Chromafenozide	105.1±5.5	120.7±4.6	0.006*
Thiocyclam	163.8±6.4	180.3±5.5	0.008**
Chlorantraniliprole	130.9±5.7	162.9±6.8	0.007*
F value	13.2**	11.6**	-

*Significant at 0.05 Probability level. NS is not significantly different.

instar, respectively compared to control. While Chromafenozide and Chlorantraniliprole significantly increased alpha esterase activity to in 2nd (1299.9 and 1276.9) and (1325.8 and 1536.) in 5th larval instars compared to control (1162.9 and 1164.8). Chlorantraniliprole and Thiocyclam enzyme, where Chlorantraniliprole and Thiocyclam increased carboxyesterase enzyme to (130.9 and 163.8) in 2nd instar larvae and 5th larval instars (162.9 and 180.3). Carboxyesterase were decreased to (45.1 and 105.1) on 2nd instar larvae and (90.4 and 120.7) in 5th larval instars when treated with activated β- esterase in both 2nd (300.2 and 355.7) and 5th larval instars (375.1 and 435.3) compared to control. While Novaluron and Chromafenozide inhibited β- esterase enzymes in 2nd (84.0 and 115.3) and (107.9 and 210.5)5th larval instars. Similar results were observed for carboxyesterase Novaluron and Chromafenozide.

Phenoloxidase, Chitinase and Acetylcolinesterase

The impact of evaluated insecticides on Phenoloxidase, Chitinase and Acelylcolinesterase were assessed in the 2nd and 5th larval instars (**Table 6**).

Table 6. Effect of LC_{50} of the tested insecticides on the activity of phenoloxidase, chitinase and acetylcholinesterase assessed in 2nd and 5th instar larvae of *S. littoralis*

Treatments	Mean enzyme activity ± SE		
	2nd larval instar	**5th larval instar**	***P-value***
	Phenoloxidase (O.D. units/min/g.b.wt)		
Control	13.1±1.3	14.0±2.3	0.835NS
Novaluron	14.3±2.6	18.2±3.5	0.0500*
Chromafenozide	13.2±1.6	14.7±2.8	0.056NS
Thiocyclam	13.9±2.2	20.4±3.3	0.079 NS
Chlorantraniliprole	16.8±3.8	22.2±1.9	0.049*
F value	5.5 *	4.2 *	-
	Chitinase (µg NAGA/min/g.b.wt)		
Control	1445.3±9.1	1448.3±10.6	0.612NS
Novaluron	1239.7±11.8	1310.5±10.8	0.065 NS
Chromafenozide	1348.2±10.9	1356.5±9.9	0.065NS
Thiocyclam	1535.4±12.9	1798.7±13.7	0.084 NS
Chlorantraniliprole	1623.8±13.7	1835.5±14.7	0.072 NS
F value	119.3 **	115.5 *	-
	AchE (µg AchBr/min/g.b.wt)		
Control	323.5±4.3	323.5±4.3	0.458NS
Novaluron	342.0±6.0	465.4±6.0	0.024*
Chromafenozide	465.2±5.8	587.7±5.8	0.063 NS
Thiocyclam	205.8±4.4	306.3±4.4	0.063 NS
Chlorantraniliprole	212.0±6.3	317.0±6.3	0.054*
F value	10.5*	9.4*	-

*Significant at 0.05 Probability level.
** Significant at 0.01 Probability level. NS is not significant different.

Results showed that Phenoloxidase enzyme values ranged between 13.1 - 14.3 O.D. units/min/g.b.wt for control, Novaluron, Chromafenozide and Thiocyclamin the 2nd larval instar . But larvae treated with Chlorantraniliprole had a higher level of Phenoloxidase enzyme, reaching 16.8. The fifth larval instar treated with Chromafenozide did not differ from the control (14.7 and 14.0). However, larvae treated by Chlorantraniliprole followed by Thiocyclam and Novaluron showed higher values of Phenoloxidase enzyme, ranging between 18.2 - 22.2, than control.

Chitinase enzyme was high after treated 2nd instar with Chlorantraniliprole followed by Thiocyclam (1623.8 and 1535.4 µg NAGA/min/g.b.wt, respectively) and (1835.5 and 1798.7) after treated 5th instar. On the other hand, Novaluron and Chromafenozide decreased Chitinase enzyme to (1239.7 and 1348.2) 2nd instar and (1310.5 and 1356.5) in 5th instar than control (1445.3 and 1448.3).

Acelylcolinesterase (AchE) was significantly activated with Chromafenozide in the 2nd and 5th instars (465.2 and 587.7 µg AchBr/min/g.b.wt, respectively). The control showed similar AchE value (323.5) for 2nd and 5th instars. The AchE was inhibited by applying Thiocyclam and Chlorantraniliprole (205.8 and 212.0) in 2nd instar and (306.3 and 317.0) and in 5th instar, respectively.

Discussion

The present study evaluated the impact of chitin synthesis inhibitors and new insecticides on the activation and inhabitation of 2nd and 5th larval instars of *S. littoralis* enzymes under laboratory conditions. Results showed that the 2nd larval instar was more susceptible to insecticide application than the 5th larval instar, where total carbohydrates in the 2nd larval instar were impacted and lowered by Thioclcyam more than the 5th larval instar. The same pattern was observed for total protein, where Chromafenozide and Thiocyclam had more effective and decreased total protein in the 2nd larval instar than the 5th larval instar. Novaluron and Chlorantraniliprole were negatively affected amaylase phenoloxidase and acetylcholinesterase enzaymes on the 2nd larval instar compared to the 5th larval instar. Additionally, all insecticicdes affected and decreased α-esterase, β- esterase and Carboxyesterase enzymes in 2nd larval instar much more than in the 5th larval instar. The susceptibility of the 2nd instar compared to the 4th instar was previous reported by Abdu-Allah (2011), Bengochea *et al*., (2014) and Qayyum *et al*., (2020), the susceptibility of the second instar is dure to the smaller size of the larvae compared to older instars as well as the developed defense mechanisms in the older instars.

The results demonstrated that treating the larvae with the LC_{50} of tested insecticides decreased the total carbohydrates in the larvae except Thioclcyam. Additionally, all tested insecticides lowered total protein and total lipids except chlorantraniliprole. It has been reported by Piri *et al*., (2014) that changes in energy resources i.e., lipids, carbohydrates, glycogen and proteins is due to function alteration and indicator of insect susceptibility to particular insecticide. Chapman (1998) explained the role of carbohydrates in insect bodies as they develop metabolism, metamorphosis, flight muscles, insect reproduction, and embryonic development. Carbohydrates probably turn into lipids and can contribute to amino acid production. In general, sugars in the larvae stimulate their feeding (Genç, 2006). Abd El-Kareem *et al*., (2022) stated that under toxicant stress, the carbohydrate content shortage could result in an increase in the metabolism. Also, low carbohydrates in stress conditions could activate the glycogenolysis and glycolytic pathway to provide excess energy (Franeta *et al*., 2018; Vojoudi *et al*., 2017). Proteins are important for vital organism activities such as development, growth, and performance. Thus, the impact of

protein results in cell disorder. The decrease in total proteins in our assessment may be due to the high toxicity of tested insecticides. Moreover, the total protein reduction might be to the breakdown of protein into amino acids; consequently, with amino acids entering the tricarboxylic acid cycle (TCA) as a keto acid, they will provide insects with energy. Under insecticidal stress, protein depletion in hemolymph tissues could retain free amino acid content,, which intermediates the TCA cycle, leading to physiological and compensatory stimulation (Nath *et al*., 1997). The reduction of soluble protein contents in various insects after IGRs application was reported by Assar *et al*., (2016) and Saleh and Abdel-Gawad (2018) on cotton leafworm and Talleh *et al*., (2020) on *Tuta absoluta*.

The stress resulted by the application of the IGRs changes the rate of protein synthesis and biodegradation; that imbalance alters the total protein reserves in the tissue of the larvae, that influences the enzymes and/or the exchange of chemical information which subsequently changes the behavioral patterns of treated larvae (Muthusamy and Ramkumar, 2011). Also, our results showed that the total protein was increased in larvae treated with LC_{50} of Thiocyclam. The natural increase of protective hydrolytic and detoxifying enzymes usually occurs directly after insecticide application increases the treated larvae's total protein (Assar *et al*., 2016).

Insect cell walls contain phospholipids, fatty acids, and sterols, which enhance the cell wall's structure and contribute to various biochemical and physiological functions (Piri *et al*., 2014). The reduction in total lipids in our research occurred when *S. littoralis* larvae were treated with tested insecticides except chlorantraniliprole. Similar results were reported by Assar *et al*., (2016) and Awadalla *et al*., (2017) when *S. littoralis* larvae were treated with emamectin and IGRs. The accumulation of the lipids is more likely directly related to a lack of juvenile hormones (Hill and Izatt 1974). Xu *et al*., (2016) explained that the detoxification process in larvae after insecticide treatment requires a transformation of large quantity of consumed food into energy which leads to reduction in total lipid contents. Furthermore, our results showed that Chromafenozide and Chlorantraniliprole decreased trehalase and amylase enzyme activity in *S. littoralis* larvae. Meanwhile, a reduction in invertase enzyme concentration was observed with thiocyclam.

In *S. littoralis* a significant reduction in amylase, invertase, total carbohydrates and trehalase activities in larvae after feeding on diet treated with abamectin, coragen, clothianidin and metaflumizone which caused larvae anti feed and inability to digest diet and consequently affected on growth and reproduction (Abo El Ghar *et al*., 1995; Abd El –Aziz *et al*., 2017; Hatem *et al*., 2017). Changes in pH usually followed the higher activity of digestive enzymes that influences. The rates of reactions

and changes in the charge state of the active site of the substrate and or the enzymes. In addition, it disrupts the hydrogen bonds leading to enzymes deactivation and protein denaturation (Zheng and Cohen, 2000). Our results supported with several studies that showed similar finding when tested the impact of Spinetoram on trehalase and amylase in *S. littoralis* (El-Barky *et al*. 2008; Rashwan 2013), also teflubenzuron was reported to be fluctuated changes of trehalase in treated larvae (Sheikh *et al*., 2013). Florkin and Jeanuiaux, (1964) stated that a tissue with low trehalase is a term that explains the high metabolic activity of the epidermis which unable to utilize trehalose and subsequently leads to a rapid decrease of glucose concentration at the end of the last larval instar.

Lai and Su, (2011) explained the mode of action of Chlorantraniliprole as it causes Ca^{2+} depletion by stimulating the unregulated release of internal calcium stores, which prevents feeding, enhances lethargy, paralysis the muscle, and eventually leads to death. The low toxicity of chlorantraniliprole against non-targeted organisms, particularly animals, makes it eco-friendly and makes it a promising alternative to conventional insecticides (Han *et al*., 2012).

Chromafenozide belongs to a novel class of IGRs, the molting accelerating compounds or non-steroidal ecdysteroid agonists. It mimics the mode of action of the natural insect molting hormones by true binding on the ecdysteroid receptors of the epidermal cells and inducing precocious molting (Smagghe *et al*., 2004). They act more slowly than neurotoxin insecticides because they disrupt insects' hormonal system or physiological development rather than kill through direct toxic action (Biddinger *et al*., 2006). Furthermore, our results showed that Novaluron and Chromafenozide decreased β- esterase, carboxyesterase, chitinase and phenoloxidase enzymes. Bakr *et al*., (2013) reported that treating *S.littoralis* with IGR's could cause significant changes in alpha and beta esterases. However, Rashwan (2013) found that alpha esterases slightly increased in larvae treated with Spinetoram compared to control. and beta esterases was highly increased with application of emamectin. Feng *et al*., (2018) stated that carboxylesterase provides the key mechanism of insecticide resistance, particularly organophosphate, which is responsible for cross-resistance to different insecticides.

Novaluron is considered a chitin synthesis inhibitor that influences insect’s ability to produce new exoskeletons when molting. Additionally, it blocked and or inhibited the synthesis of chitin which directly impacts the larvae growth and development. Also, results in increasing egg mortality. Chitin synthesis inhibitors include conventional benzoylureas, triazine/pyrimidine derivatives, and buprofezin (Perveen, 2012). Otherwise, Chlorantraniliprole activated chitinase enzyme in larvae of *S. littoralis*. These

results are in agreement with (Rashwan, 2013), who used spinetoram on *S.littoralis* and stated that there is an increase in chitinase activity; while there was a recorded of fluctuated changes was reported by El-Sheikh *et al*., (2013) in chitinase when teflubenzuron and spinetoram was applied to *S.littoralis*. Mostafa (1993) reported a reduction in phenoloxidase activity after application of teflubenzuron to *S. littoralis*. Revenis (2011) also mentioned that insects expressed several defense reactions such as the induction of proteolytic cascades which leads to localization of melanization and coagulation in response to microbial infection. The activation of prophenoloxidase (pro po) to its active form phenoloxidase (po) required for Melanization. Phenoloxidase is an important enzyme that enhances the formation of melanin around intruding microorganisms at the wound sites and in the haemolymph.

Also, our results showed that Thiocyclam decreased activity of acetylcholinesterase; this inhibition is due to blocking the action potential of the nervous system resulted treating the larvae with Thiocyclam which blocks the Nicotinergic acetylcholine, leading to blocking of ganglionic action on the central nervous system which causes paralysis for treated larvae. These findings in agreement with (Ismail, 2020), who reported a significant decrease in the activity of AChE in *S. littoralis* larvae after application of profenofos because organophosphorus insecticides acting as inhibitors of neuronal cholinesterase activity and are considered neurotoxic. Abd El- Mageed and Shalaby (2011) and Mostafa (1993) also recorded a reduction in acetylcholinesterase that appeared in *S. littoralis* larvae after using teflubenzuron. Additionally, different studies by El-Barky *et al*., (2008), Fahmy and Dahi (2009) and Rashwan (2013) found that Spinetoram induced a moderate increase in acetylcholin-esterase activity.

Conclusion

Because of the intensive use of traditional insecticides, insects started to have a resistance mechanism; consequently, new control agents are required and need more investigation. Therefore, our study recommended using Novaluron, Chromafenozidae, Thiocyclam and Chlorantraniliprole in controlling *S. littoralis* because of their mode of action on activation or inhibition of body enzymes of larvae. These results are promising for using these insecticides in Integrated Pest Management programs.

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