International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 22-11-2022, Accepted: 07-12-2022, Published: 24-12-2022
Volume 7, Issue 12, 2022, Page No. 138-142

Toxicological and biochemical effects of certain insecticides against *Spodoptera littoralis* (Boisd.) in cauliflower fields

Ali R El-Gabaly, Mohamed F Abdel Aziz, Hassan F M Abdel-Hamid
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Abstract
This study was conducted to evaluate four insecticides; namely methoxyfenozide & chlorfluazuron (as insect growth regulators) and deltamethrin & beta-cyfluthrin (as pyrethroids) against the 2nd and 4th larval instars of *Spodoptera littoralis* after treatment cauliflower fields in Beheira and Fayoum Governorates under semi-field condition and corrected percentage larval mortality were recorded at intervals. The results in the governorates of Beheira and Fayoum are similar, where in Fayoum Governorate, the mortality percentage of insect growth regulators (methoxyfenozide & chlorfluazuron) declined compared to pyrethroid compounds (deltamethrin & beta-cyfluthrin) post treatment against 2nd & 4th instars, where the mortality percentage were 96 & 92% for deltamethrin and 94 & 91% for beta-cyfluthrin against second & fourth instar larvae, respectively at first day of post treatments (initial kill), and the mortality percentage (initial kill) reached to 69.5 & 67% post treatment with methoxyfenozide and 70.5 & 68% post treatment with chlorfluazuron for second & fourth instar larvae, respectively. In case of residual effect, the mortality percentages for 2nd & 4th instar larvae recorded 65.5 & 65%, 64.5 & 61.5%, 87.5 & 85% and 85.5 & 84% post treatment with deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron, respectively. Biochemical studies, indicated that the four tested insecticides caused significant increasing in chitinase activity and the most effective compound was noticed by chlorfluazuron and methoxyfenozide. all tested insecticides caused highly significant reduction at range -34.36 and -52.68% compared to untreated in total proteins. On the other hand, all insecticides achieved high acetylcholinesterase activity compared with untreated and the highest significant increase of acetylcholinesterase activity obtained of deltamethrin followed by beta-cyfluthrin. In case of carbohydrate digestive enzymes (invertase, trehalase and amylase), the amylase enzyme achieved no significantly differences between all treatments and untreated, while the activity of trehalase enzyme was significant decreased as affected by all treatments compare to untreated, Likewise invertase enzyme, it was remarkable decreases in the activity of enzyme for treated with deltamethrin, beta-cyfluthrin and methoxyfenozide, but risen in case of chlorfluazuron.

Keywords: *Spodoptera littoralis*, methoxyfenozide, chlorfluazuron

Introduction
The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd). (Lepidoptera: Noctuidae) is one of the most notorious and destructive phytophagous insect pests in Egypt, not only to cotton but also to other crops and vegetables (Kandil *et al*., 2003). It control program is based mainly on use of insecticides which created some problems such as insecticides resistance environmental pollution and hazard to natural enemies and beneficial insects (Nada, 1990) [24]. The larvae feed mainly on leaves causing seriously to the crop growth and reducing the production of the cotton crop. Moreover, they can infest the squares, flowers and green bolls (Ghonein *et al* 2017 and Mokbel *et al.*, 2017) [23]. IGRs are compounds used in insect control known as insect developmental inhibition and these compounds used against a wide range of insect species (Pineda et al., 2007; Wang &Tian 2009 and Bakr *et al.* 2010) [32, 8]. Also, Pyrethroids compounds are currently among the major insecticides applied to control several noctuid pests against the deleterious larval stages (Usmani and Knowles 2001) [31]. The changes in the biochemical especially the carbohydrate, hydrolyzing enzymes such as Trehalase, invertase, amylase and protein content have an important role in biological and physiological activities of insects (Khedr, 2002) [22]. This work was designed to achieve the following purposes:

- Study toxicity of different compounds deltamethrin, beta cyfluthrin, methoxyfenozide and chlorfuazuron against *S. littoralis* under the semi field conditions after treatment cauliflower fields in Beheira and Fayoum Governorates.
- Assessment of carbohydrate digestive enzymes (amylase, trehalose, and invertase), total protein, chitinase and acetylcholine esterase (AchE) to *S. littoralis*.

Material and Methods
Mass rearing of cotton leaf worm, *Spodoptera littoralis*
The strain of cotton leaf worm *Spodoptera littoralis* used in this paper is a laboratory strain that has been reared for several generations without any contamination with insecticides for approximately one year in the Cotton Leafworm Research Department, Plant Protection Research Institute, Dokki, Giza, Egypt under constant laboratory condition at 26± 2ºC and 65± 5% R.H. and fed daily on fresh caster bean leaves according to El-Defrawi *et al.* (1964).

Chemicals
Four tested insecticides use in this study
1. Deltamethrin (Sodium channel modulators) belongs to group Pyrethroids formulated as Rooftox 5% EC rate of 100 ml/feddan.

2. beta-Cyfluthrin (Sodium channel modulators) belongs to group Pyrethroids formulated as Belando extra 10% EW rate of 60ml/ feddan.
3. Methoxyfenozide (Ecdysteroid agonist) belongs to group Diacylhydrazines formulated as Banzer 24% SC rate of 37.5 ml/ feddan.
4. Chlorfluazuron: (chitin synthesis inhibitor) belongs to group Benzoylureas formulated as Caprice 5% EC rate of 400 ml/ feddan.

Semi-field Technique

Field –laboratory experiments were carried out to evaluate the initial as well as residual effects of the tested insecticides namely, deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron against laboratory strain of the 2nd and 4th instar larvae of *S. littoralis.*

The insecticide applications were implemented by using a knapsack sprayer on February20th in cauliflower fields at two Governorates, the first in Beheira Governorate at Etay El-Baroud district and the second in Fayoum Governorate, Tamiya district during season 2022 using semi-field technique. Complete randomized blocks design was used in the area of band 8 carats which divided into five parts each part contains three replicates, first part included control (sprayed with water). The other 4 parts were sprayed with deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron using recommended rate.

Samples of cauliflower, *Brassica oleracea var. botrytis* leaves were collected randomly directly after spraying at zero time and continue to 10 days post sprayed. Then the treated and untreated cauliflower leaves were transferred to the laboratory and offered to the 2nd and 4th instar larva which categorized into five groups. Each group has one hundred larvae divided into 5 replicates (each replicate = 20 larvae) for 2nd or 4th instar larvae. Corrected percentage larval mortality according to Abbotts formula (Abbott, 1925) [1] was recorded after 24 hrs, 3 days, 5 days, 7 days and 10 days for each group from treated.

Enzymes Assay

For biochemical studies, LC_{50} of the tested insecticides were prepared according to probit- analysis method of Finney (1971) [17] by using dipping technique where fed the 4th instar larvae for 48 hrs. and the total body samples of the 6th instar were collected from the surviving treated as well as untreated larvae.

Preparation of insect's homogenates for analysis: Batched of the treated larval were homogenized in distilled water. Homogenates were centrifuged at 8000r.p.m for 15 minutes at 4°C in refrigerated centrifuge. The deposits were discarded and the supernatants were kept in deep freezer at –20 °C until biochemical analysis. The supernatants were used to assess the activity of carbohydrate digestive enzymes (amylase, trehalose, and invertase), total protein, chitinase and acetylcholine esterase (AchE) using methods described by Ishaaya and Swirski (1976) [20] (Gornall *et.al.*1949) [19], Bade and Stinson (1981) [6] and Simpson *et al.*, (1964) [29], respectively. Biochemical tests were determined in Physiology Department, Plant Protection Research Institute, Dokki, Egypt.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test was used to determine significant differences between means (P= 0.05) using CoStat system for Windows, Version 6.4.0

Results and Discussion

Semi-filed Technique

Concerning the efficiency of the tested insecticides against the 2nd & 4th instar larvae after different feeding period of 1, 3, 5, 7 and 10 days, implemented in Beheira Government during 2022 as presented in Table (1). The obtained data showed that deltamethrin was the most toxic compound against the 2nd instar larvae at different periods of feeding post treatment of cauliflower with the tested insecticides, recorded 98 and 68 % mortality for initial and residual effect, respectively, while beta-cyfluthrin recorded the highest % mortality against 4th instar larvae and reached to 94 and 74% for initial and residual effect, respectively.

Table 1: Initial kill and residual effects of deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron for the 2nd and 4th instar larvae of *S. littoralis* under semi field conditions in Beheira Governorate during 2022**.**

Treatments	Instar	% corrected mortality						
		Initial				Residual Effect		
		1 day	3day	5 day	Mean	7 days	10 days	Mean
deltamethrin	2nd	98	-	-	98	72	64	68
	4th	93	-	-	93	71	62	66.5
beta-cyfluthrin	2nd	96	-	-	96	68	66	67
	4th	94	-	-	94	77	71	74
methoxyfenozide	2nd	-	69	75	72	84	86	85
	4th	-	64	73	68.5	81	83	82
chlorfluazuron	2nd	-	68	75	71.5	83	88	85.5
	4th	-	67	71	69	80	86	83

In the case of insect growth regulators (methoxyfenozide & chlorfluazuron) the mortality percentage declined compared to pyrethroid compounds (deltamethrin & beta-cyfluthrin) and methoxyfenozide and chlorfluazuron are similar in effect post treatment against 2nd & 4th instars. In initial effect, the mortality percentages of methoxyfenozide were 72 & 68.5% for 2nd & 4th instars larvae, respectively whereas in case of chlorfluazuron were 71.5 & 69% for 2nd & 4th instars larvae, respectively and gradually increased to reach maximum in residual effect where methoxyfenozide were 85 & 82% for 2nd & 4th instars larvae, respectively and post treatment of chlorfluazuron the mortality percentages were 85.5 & 83% for 2nd & 4th instars larvae, respectively.

Results represented in Table (2) were related to Fayoum Governorate and cleared that, data are like to Beheira Governorate, where the initial kill of pyrethroid compounds (deltamethrin & beta-cyfluthrin) recorded increasing of percentage mortality compared to insect growth regulators (methoxyfenozide & chlorfluazuron), and the opposite was in the case of the residual effect. Whereas the mortality percentage were 96 & 92% for deltamethrin and 94 & 91% for beta-cyfluthrin against second & fourth instar larvae, respectively at first day of post treatments (initial kill), and the mortality percentage (initial kill) reached to 69.5 & 67% post treatment with methoxyfenozide and 70.5 & 68% post treatment with chlorfluazuron for second & fourth instar larvae, respectively. In case of residual effect, the mortality percentages for 2nd & 4th instar larvae recorded 65.5 & 65%, 64.5 & 61.5%, 87.5 & 85% and 85.5 & 84% post treatment with deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron respectively.

In agreement by (Awad *et al.* 2014) reported that treatments of the cotton bollworms (*Pectinophora gossypiella* and spiny bollworm *Earias insulana*) by conventional insecticides of cotton plant, higher reduction was recorded

83.60% with Sumi-alfa KZ® (ES-fenvalerate) followed by 83.2% with Lamda-Z® (lambda-cyhalothrin), while, Buldock® (beta cyfluthrin) gave a more lower reduction of 80.8%. while, for the other insecticides percentages of reduction values were 82.6% for Icton® (lambda-cyhalothrin), 82% for Pulsar® (lambda-cyhalothrin), 82.1% for Dora® (chlorpyrifos) and 81.1% for Fenerate-S® (ES-fenvalerate). El-Hamaky *et al.* (1990) [14] applied cyfluthrin to cotton fields in Egypt and found that treatment caused 95% initial mortality to 2nd and 4th instar larvae of S. littoralis and positive correlations were found between residual mortality and concentration of cyfluthrin. (Bakr *et al.* 2013, Sabry and Khedr 2014) [28] they found that, Insect Growth Regulators (IGRs) belonging to group, chitin synthesis inhibitors was more toxic than IGRs belonging to group, moulting hormone agonist against larval instars of *S. littoralis*. (El-Zahi 2015) [15] reported that, when the 4th instar larvae of *S. littoralis* were fed on treated castor bean plants, pyridalyl and hexaflumuron resulted in the superior percentages of larval mortality. Flufenoxuron causing significantly increased the larval and pupal durations, while reduce the percentages of pupation, adult emergency, fecundity and fertility of the eggs, also, flufenoxuron significantly induced larval mortalities (Reda *et al.* 2010) [27]. On the other hand, (Eldesouky *et al.* 2018) [13] reported that, treatments with chlorfluazuron and lambda-cyhalothrin significantly reduced some biological parameters such as larval weight, pupal weight, percentage of pupation, and percentage of adult emergence compared to untreated.

Table 2: Initial kill and residual effects of deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron for the 2nd and 4th instar larvae of *S. littoralis* under semi field conditions in Fayoum Governorate during 2022.

Treatments	Instar	% corrected mortality						
		Initial				Residual Effect		
		1 day	3day	5 day	Mean	7 days	10 days	Mean
deltamethrin	2nd	96	-	-	96	69	62	65.5
	4th	92	-	-	92	67	63	65
beta-cyfluthrin	2nd	94	-	-	94	65	64	64.5
	4th	91	-	-	91	62	61	61.5
methoxyfenozide	2nd	-	66	73	69.5	86	89	87.5
	4th	-	62	72	67	84	86	85
chlorfluazuron	2nd	-	65	76	70.5	85	87	85.5
	4th	-	63	73	68	83	85	84

Biochemical studies

Chitinase, total proteins and acetyl-cholinesterase activities

It can be seen in Table (3), The four tested insecticides caused increasing in chitinase activity where the most effective compound was noticed by chlorfluazuron and methoxyfenozide with significant rise of 32.74 and 18.04%, respectively while beta-cyfluthrin and deltamethrin recorded non-significant rise the enzymatic activity by 14.78and 8.61%, respectively compared with untreated control.

The effect of four tested insecticides on total proteins in *S. littoralis* larvae as represented in Table (3) showed that no significant between all the tested compounds, also all compounds caused highly significant reduction at range -34.36 and -52.68% than untreated, where means recorded 261.67, 253.33, 207.67, 188.67 and 398.67 µg /ml for deltamethrin, beta-cyfluthrin, methoxyfenozide, chlorfluazuron and untreated, respectively.

On the other hand, all insecticides achieved high acetylcholinesterase activity compared with untreated was 159.67 µg Ach Br /min/g.b.wt and the highest significant increase of acetylcholinesterase activity obtained of deltamethrin followed by beta-cyfluthrin with values were 465.33 (191.44% change) and 320.33 (100.63% change) µg Ach Br /min/g.b.wt, respectively while in the case of insect growth regulators (methoxyfenozide and chlorfluazuron), there is no significant difference between them with values 245 and 247 µg Ach Br /min/g.b.wt, respectively.

It is generally suggested that chitin synthesis inhibitors interfere with the molting process by inhibiting the Bio synthesis of chitin Molting fluid secretion and ecdysal membrane formation molting fluid contains protease and Chitinase enzymes that digest the main constituents of the old endocuticle protease and Chitinase enzymes that digest the main constitutes of the old endocuticle (Bakr *et al.* 2010; Abdel-Aziz and El-Gohary 2013) [8, 3]. Novaluron (IGRS) interfere with the Chitin synthesis and suppressed it or prevented deposition of chitin on the exoskeleton or in the normal deposition of new cuticle during a polysis which leading to abnormalities, Ghoneim *et al.* (2017) [18].

In agreement by (Farag, 2001 [16]; Abdel-Aal (2006) [2] reported that treatment of *Spodoptera littoralis* larvae with diflubenzuran or chlorfluazuron caused significant increase in chitinase activity compared to control. Tolba (2006) [30] reported an increase in chitinase activity of *Agrotis ipsilon* when treated with flufenoxuron.

Table 3: Changes in chitinase, total proteins and acetyl- cholinesterase activities of *Spodoptera littoralis* after treated with LC_{50} values of the tested insecticides.

Treatments	Chitinase (µg NAGA/min/g.b.wt)		Total proteins (µg /ml)		Acetyl- cholinesterase (µg Ach Br /min/g.b.wt.)	
	Mean ± S.E	Change %	Mean ±S.E	Change %	Mean ±S.E	Change %
deltamethrin	1935 ± 45.37 bc	8.61	261.67 ± 26.19 b	-34.36	465.33 ± 13.23 a	191.44
beta-cyfluthrin	2045 ± 61.71 bc	14.78	253.33 ± 24.55 b	-36.45	320.33 ± 3.51 b	100.63
methoxyfenozide	2103 ± 56.77 ab	18.04	207.67 ± 58.92 b	-47.91	245.00 ± 14.50 c	53.44
chlorfluazuron	2365 ± 137.69 a	32.74	188.67 ± 44.37 b	-52.68	247.00 ± 27.85 c	54.70
untreated	1781.67 ± 85.65 c	-	398.67 ± 47.35 a	-	159.67 ± 5.78 d	-

Means with the same letter are not significantly different at $p < 0.05$.

Change % (%Increase or decrease than control) = ((Treated-Control) / Control) * 100

Activity of digestive enzymes

Data in Table (4) showed changes in carbohydrate digestive enzymes (invertase, trehalase and amylase) when it determined in the larvae of *S. littoralis.*

a. Amylase

Deltamethrin and beta-cyfluthrin caused decreasing the amylase activities were found to be 1936.33 and 1943.00 µg glucose/min/g.b.wt, respectively while activity of amylase

was increased to 1975.67 and 1968.33 µg glucose/min/g.b.wt achieved by methoxyfenozide and chlorfluazuron treatments respectively. Although, the results obtained of the activity of amylase achieved no significantly differences between all treatments and untreated.

b. Trehalase

In insects, carbohydrates reserves are present as trehalose which can be readily converted into glucose for the support of all life processes. Table (4) indicate that, the activity of trehalase enzyme was significant decreased as affected by all treatments than untreated and the mean recorded for deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron were1010.67, 1110.67, 1230.33 and 1350.00 µg glucose/min/g.b.wt respectively compared with 1996.67 µg glucose/min/g.b.wt for untreated. It was obvious that all insecticides reduced trehalase activity remarkably than untreated. Reduction gradually, reached -49.38, -44.37, -38.38 and -32.39% compared to untreated for deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron respectively.

c. Invertase

Concerning invertase enzyme, there was remarkable decreases in the activity of enzyme for deltamethrin, beta-cyfluthrin and methoxyfenozide, but risen in case of chlorfluazuron. The activity recorded mean of 166.33, 158.33, 213.33 and 219.67 µg glucose/min/g.b.wt for deltamethrin, beta-cyfluthrin, methoxyfenozide and chlorfluazuron, respectively compared with 215.67 µg glucose/min/g.b.wt for untreated. considering the change in activity it was obvious that deltamethrin and beta-cyfluthrin exhibited remarkably significant reduction of -22.87 and -26.58% relative to untreated, respectively whereas chlorfluazuron achieved increasing in invertase enzyme activity reached + 1.85% relative to untreated. (El- Sheikh 2012) [11] evaluated pyrethroid compound (cypermethrin) against 4th instar larvae of S. littoralis and recorded different levels of significant changes in the total protein, carbohydrate contents and carbohydrate hydrolyzing enzymes.

Also, the results obtained agree with (Khaled and Farag 2015) [21] where reported that treatment of larvae of *S. littoralis* with indoxacarb and methoxyfenozide, data were recorded a significant reduction in the activities of carbohydrate digestive enzymes (invertase, trehalase and amylase) where impairments in the activity of carbohydrate digestive enzymes cause The disturbance in carbohydrate in treated larvae (Radwan *et al.* 1984 and Eid, 2002) [25, 10].

Table 4: Activities of carbohydrate digestive enzymes (invertase, trehalase and amylase) of S. littoralis after treated with LC_{50} values of the tested insecticides.

Treatments	Enzyme activity (µg glucose/min/g.b.wt)					
	Amylase		Trehalase		Invertase	
	Mean ± S.E	Change %	Mean ±S.E	Change %	Mean ±S.E	Change %
deltamethrin	1936.33 ± 32.92 a	-1.12	1010.67 ± 39.47 c	-49.38	166.33 ± 7.54 b	-22.87
beta-cyfluthrin	1943.00 ± 54.06 a	-0.78	1110.67 ± 81.49 c	-44.37	158.33 ± 9.49 b	-26.58
methoxyfenozide	1975.67 ± 94.42 a	0.89	1230.33 ± 87.14 bc	-38.38	213.33 ± 12.02 a	-1.08
chlorfluazuron	1968.33 ± 40.45 a	0.51	1350.00 ± 75.06 b	-32.39	219.67 ± 9.84 a	1.85
untreated	1958.33 ± 34.44 a	-	1996.67 ± 26.03 a	-	215.67 ± 2.60 a	-

Means with the same letter are not significantly different at $p < 0.05$.

Change % (%Increase or decrease than control) = ((Treated-Control) / Control) * 100

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