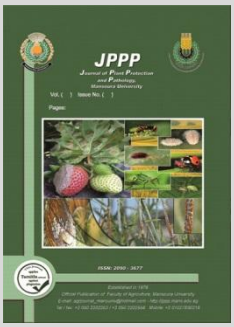
Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Comparison between *Spodoptera frugiperda* and *Spodoptera littoralis*: Insecticide Resistance, Detoxifying Enzymes and Protein Patterns

Abdullah, R. R. H.[1*] and Noha A. Sukar[2]

[1]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
[2]Agricultural Botany (Genetics) Dept., Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.



ABSTRACT

In 2019, researchers first discovered the highly invasive nocturnal pest *Spodoptera frugiperda* in Upper Egypt. This work investigated the sensitivity of *S. frugiperda* to six commercial pesticides in Egypt compared to *Spodoptera littoralis* and detected some biochemical changes (Detoxifying enzyme activities and protein patterns) in treated larvae of both insects. The results indicated that *S. frugiperda* was more sensitive to Spinosad (LC_{50} = 6.23 ppm) and Abamectin (LC_{50} = 27.75 ppm) than *S. littoralis* (LC_{50} = 22 and 30 ppm, respectively). However, *S. frugiperda* was more resistant to Tebufenozid (LC_{50} = 283.06 ppm) and Thiamethoxam (LC_{50} = 269.4 ppm) than *S. littoralis* (LC_{50} = 64.2 and 41.97 ppm, respectively). In addition, in comparison with *S. littoralis*, *S. frugiperda* was less sensitive to Alpha-Cypermethrin (LC_{50} = 53.05 ppm) and Indoxacarb (LC_{50} = 10.85 ppm), whereas *S. littoralis* was more sensitive to both pesticides (LC_{50} = 5.02 and 4.02 ppm, respectively). The data of detoxifying enzyme (AST, ALT, ACP, ALP, and GST) activities indicated that the evaluated pesticides led to different extents of inhibition or activation of estimated detoxifying enzymes in both insect species. The different degrees in the detoxifying enzyme activities may be the causes of the variations of the insect sensitivity or resistance to the tested pesticides. The SDS-PAGE analysis indicated that there were variations in the protein patterns among *S. littoralis* and *S. frugiperda* when treated by tested pesticides. Thus, this study recommended that Spinosad, Indoxacarb, and Abamectin could be used to control *S. frugiperda*.

Keywords: Fall armyworm, Cotton leafworm, Detoxifying enzymes, Insecticidal activity, SDS–PAGE

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* JE Smith (Lepidoptera: Noctuidae), is a destructive pest of many economic plants (Bueno *et al*., 2010; Nagoshi *et al*., 2007). It is indigenous to South and North America, but since 2016, it has begun to extend to Africa, where it has seriously harmed some cultivated crops, especially maize crops (Goergen *et al*., 2016; Kumela *et al*., 2018). After that, it spread throughout Asia. The *S. frugiperda* prefers several plant hosts and feeds on more than about three hundred and fifty different plant cultivars. The crop losses in sub-Saharan Africa due to damage from fall armyworm could reach up to 13 billion US dollars annually. In Egypt, as reported by the Agricultural Pesticide Committee of the Ministry of Agriculture and Land Reclamation, the first case of fall armyworm was discovered in a field of maize plants in Kom Ombo, Aswan governorate, Upper Egypt, within the 2019 season (Abdullah *et al*., 2024). Like northern Africa, the fall armyworm infestations is expected to occur on other economic crops, such as cotton, soybeans, and rice. Therefore, there should be additional control over the population of the fall armyworm *S. frugiperda*.

The cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is an economic insect pest that infests many crops and is found mostly in tropical and subtropical areas. Due to its polyphagous behavior, this species reduces the economic yield of multiple crops (Abdullah, 2023). Significant damage often occurs in many field crops, vegetables and some ornamental crops. There have been reports of *S. littoralis* on most continents of the world, especially Africa, Asia, and Europe. Apart from the direct harm resulting from a decrease in photosynthetic area, the presence of larvae, feeding damage, and excrement decreases the marketability of ornamentals and vegetables (El-Sheikh and Aamir, 2011).

Many enzymes, such as alkaline phosphatase (ALP), glutathione S-transferase (GST), aspartic transferase (AST), acid phosphatase (ACP), alanine transaminase (ALT), and acetylcholine esterase (AchE), are known detoxifying enzymes that are used to remove the toxic effects of pesticides in treated insect pests. GST stimulates the coupling of electrophile compounds with reduced glutathione (GSH), which makes the toxic compounds more soluble in water and less toxic. In addition to being contributed in the metabolism of organophosphorus and organochlorine, GSTs are crucial in the development of insecticide resistance (Zibaee *et al*., 2009). The ALP and ACP hydrolyze phosphomonoesters in alkaline and acidic conditions, respectively. For a number of metabolic processes, the ALP provides phosphate ions derived from mononucleotides and ribonucleoproteins. Additionally, the midgut has higher levels of ALP and ACP activity than other tissues, and ALP is involved in the trans-phosphorylation reaction. The production of nonessential amino acids, gluconeogenesis, nitrogen compound metabolism, and protein metabolism depend on transaminases, aspartic transferase and alanine transaminase (Hamadah, 2019).

There are many commercial pesticides against cotton leafworm in the Egyptian pesticide market, but there are only a few commercial pesticides against the fall armyworm. Thus,

* **Corresponding author.**
E-mail address: redakenany@yahoo.com
DOI: 10.21608/jppp.2025.349384.1296

this study aimed to detect the effective compounds for controlling the new invasive insect, *S. frugiperda*, in Egypt by determining its sensitivity to some commonly used pesticides in Egypt compared with that of *S. littoralis* and by estimating the activity of some detoxifying enzymes and protein patterns (SDS-PADE) in the treated larvae of both insects.

MATERIALS AND METHODS

Insect pests

Newly hatched larvae of *S. frugiperda* and a laboratory strain of *S. littoralis* were kindly obtained from the Plant Protection Research Institute, Agriculture Research Center, Egypt. Castor leaves were used to rear larvae of both insects at 27 ± 2 °C and 65 ± 5% R.H until reaching the suitable instar for experiments in the laboratory according to EL-Defrawi *et al*. (1964).

Pesticides

Six commercial formulations of pesticides from different chemical groups were acquired from the Plant Protection Research Institute, Agriculture Research Center, Egypt. Table 1 shows the common name, formulation, mode of action, chemical group and field recommended dose of tested pesticides.

Table 1. Description of tested pesticides.

Common name	Formulation	Mode of action	Chemical group	Recommended rate against *S. littoralis*
Alpha-Cypermethrin	EC 10%	Neuro-active insecticides (Disruption of sodium channel function)	Pyrethroids	65 ml / 100 L
Tebufenozid	SC 20%	A molting hormone (It is an ecdysone receptor activator that causes larvae to molt too soon.)	IGR	75 ml / 100 L
Spinosad	SC 24%	Neuro-active insecticides (disruption of nicotinic acetylcholine receptors and GABA-gated)	Microbial metabolites (Spinosyn family)	20 ml / 100 L
Abamectin	EC 1.8%	Neuro-active insecticides (stimulate the gamma-aminobutyric acid (GABA) receptors)	Microbial metabolites (Avermectin family)	40 ml / 100 L
Thiamethoxam	WG 25%	Neuro-active insecticides (functioning as a nicotinic acetylcholine receptor agonist)	Neonicotinoids	30gm / 100 L
Indoxacarb	WG 30%	Neuro-active insecticides (by blocking neuronal sodium channels)	Oxadiazine	15gm / 100 L

Bioassay test

Four serial concentrations of each pesticide were prepared based on their field-recommended rate. Ten larvae of the third instar of *S. littoralis* and *S. frugiperda* were placed in separate jars (1 L) and left for two hours without feeding. The leaf-dip method was subjected to investigate the susceptibility of both insects to the tested pesticides. Clean castor leaves were dipped in each pesticide solution for 30 seconds and left to dry in room temperature then presented to the larvae. Control groups were fed on untreated leaves. The dead larvae count was recorded every day. Larval mortality percentages were corrected and calculated daily by Abbots' formula. This experiment was repeated four times.

Estimation of some detoxifying enzymes

The median lethal concentration was calculated and prepared for each tested pesticide. Twenty of the 3rd instar *S. littoralis* and *S. frugiperda* larvae were placed in jars (1 L) separately and starved for two hours before treatment. Clean castor leaves were dipped in the prepared pesticide solution (LC_{50}) for 30 seconds, allowed to dry, and presented to the larvae. Other untreated leaves were used for the control group larvae. After three days of treatment, the healthy larvae were collected, weighed, and frozen in centrifuge tubes (5 ml). Then, the frozen larvae were homogenated in phosphate buffer (pH 6.8) and centrifuged at 5000 rpm for 10 minutes under cooling conditions. The filtrate was poured in an Eppendorf tube and saved at -20 °C in a refrigerator as an enzyme source. This experiment was repeated four times. The activities of detoxifying enzyme were measured colorimetrically. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were measured at 520 nm (Reitman and Frankel 1957); acid phosphatase (ACP) and alkaline phosphatase (ALP) were measured at 510 nm (Powell and Smith 1954); and glutathione S-transferase (GS-T) were measured at 540 nm (Pan *et al.*, 2016).

Protein electrophoresis procedure (SDS-polyacrylamide gel electrophoresis)

Protein extraction was performed using 0.2 g of treated and untreated larvae, which were mixed separately with 600 µl of 1 M Tris-HCl buffer (pH 6.8) in an Eppendorf tube. The mixture was subsequently frozen, crushed, and centrifuged at 10000 rpm for 10 min while cooling (4 °C). The filtrate was placed in an Eppendorf tube and saved at -20 °C for protein electrophoresis analysis. Protein electrophoresis was carried out using 15% SDS–PAGE, as described by Laemmli (1970) and Davis (1964).

Statistical analysis

All the treatments were repeated four times. Abbot's formula is used to calculate and correct the percentages of larval mortality (Abbot, 1925). The median lethal concentration (LC_{50}) at a confidence limit of 95% and slope were subjected by the Finney method (Finney 1971) by using LCP-line software. The Sun equation was used to calculate toxicity index (Sun 1950). SAS software was used to do an analysis of variance (ANOVA) on the insect enzyme activity data (SAS 1997).

RESULTS AND DISCUSSION

Sensitivity of *S. littoralis* and *S. frugiperda* to the selected pesticides

In the present study, the toxicity of six pesticides (belonging to different chemical groups) was investigated against *S. littoralis* and *S. frugiperda*. The results in Table 2 indicate that Indoxacarb was the most toxic pesticide against *S. littoralis*, followed by Alpha-Cypermethrin, Spinosad, and Abamectin, whose LC_{50} values after three days of treatment

were 4.02 ppm, 5.02 ppm, 22.8 ppm, and 30.26 ppm, respectively. While Tebufenozid and Thiamethoxam were less toxic than the other tested pesticides were, their LC_{50} values after three days of treatment were 64.2 ppm and 41.97 ppm, respectively. The illustrated results in Table 3 show that Spinosad was the most toxic compound against *S. frugiperda* after three days of treatment, followed by Indoxacarb, Abamectin, and Alpha-Cypermethrin, whose LC_{50} values were 6.23 ppm, 10.85 ppm, 27.75 ppm, and 53.15 ppm, respectively. However, Tebufenozid and Thiamethoxam were less toxic than the other tested pesticides; their LC_{50} values after three days of treatment were 283.06 ppm and 269.4 ppm, respectively.

According to the LC_{50} values of the evaluated pesticides against both insects, *S. frugiperda* was more sensitive to Spinosad (LC_{50} = 6.23 ppm) and Abamectin (LC_{50} = 27.75 ppm) than was *S. littoralis* (LC_{50} = 22 and 30 ppm, respectively). However, *S. frugiperda* were more resistant to Tebufenozid (LC_{50} = 283.06 ppm) and Thiamethoxam (LC_{50} = 269.4 ppm) than was *S. littoralis* (LC_{50} = 64.2 and 41.97 ppm, respectively). In addition, the results indicated that, compared with *S. littoralis*, *S. frugiperda* was less sensitive to Alpha-Cypermethrin (LC_{50} = 53.05 ppm) and Indoxacarb (LC_{50} = 10.85 ppm) (LC_{50} = 5.02 and 4.02 ppm, respectively), as shown in Fig. 1.

Table 2. The lethal concentrations of tested pesticides against the 3rd instar *S. littoralis* larvae at laboratory conditions after three days.

Pesticides	LC_{50} (ppm) C.L. 95%	LC_{90} (ppm) C.L. 95%	*Slope ± SE*	x^2	Toxicity index (%)
Alpha-Cypermethrin	5.02 (2.28 - 6.88)	11.6 (9.4 -13.87)	3.52 ± 0.82	0.65	80.0
Tebufenozid	64.2 (49.34 – 91.36)	893 (347 -14168)	1.12 ± 0.28	0.60	06.26
Spinosad	22.8 (13.38 – 54.81)	189 (71 -1029)	1.32 ± 0.31	2.61	17.63
Abamectin	30.26 (20.68 – 74.88)	197 (78 -2049)	1.57 ± 0.35	2.84	13.28
Thiamethoxam	41.97 (35.08 – 49.52)	216 (144 -464)	1.79 ± 0.29	0.56	09.57
Indoxacarb	4.02 (1.38 – 6.33)	16.66 (13.14 – 21.53)	2.07 ± 0.45	0.28	100.0

C.L. 95%: Confidence limit at 95%; Toxicity index= (LC_{50} of the most effective compound/LC_{50} of the tested compound) X 100

Table 3. The lethal concentrations of tested pesticides against the 3rd instar *S. frugiperda* larvae at laboratory conditions after three days.

Pesticides	LC_{50} (ppm) C.l. 95%	LC_{90} (ppm) C.l. 95%	*Slope ± SE*	x^2	Toxicity index (%)
Alpha-Cypermethrin	53.15 (39.91 - 148.39)	108 (63 -807)	4.15 ± 1.23	0.01	11.72
Tebufenozid	283.06 (254.73 - 327.28)	598 (475 – 881)	3.93 ± 0.54	1.87	02.20
Spinosad	6.23 (0.79 - 11.23)	24 (15 – 31)	2.17 ± 0.64	0.55	100.0
Abamectin	27.75 (20.27 - 61.19)	82 (43 – 446)	2.71 ± 0.63	0.05	22.45
Thiamethoxam	269.4 (201.27 – 574.32)	688 (386 – 3316)	3.14 ± 0.75	0.01	02.31
Indoxacarb	10.85 (7.58 – 13.28)	26.68 (23.1 – 32.76)	3.28 ± 0.55	3.43	57.42

C.L. 95%: Confidence limit at 95%; Toxicity index= (LC_{50} of the most effective compound/LC_{50} of the tested compound) X 100

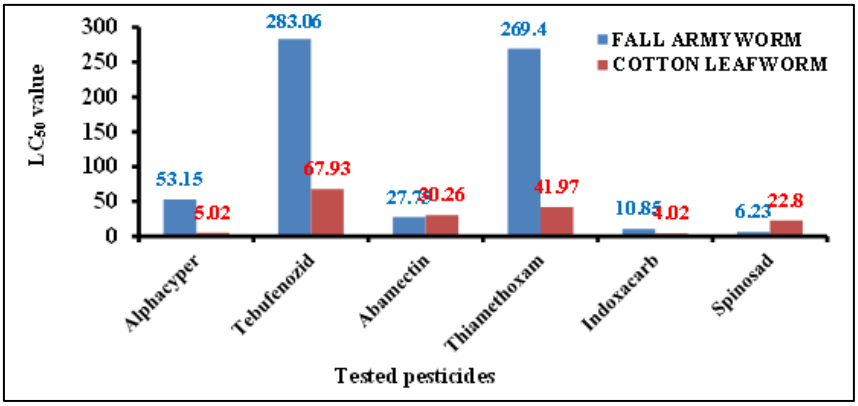


Fig. 1. Comparing the sensitivity of *S. frugiperda* and *S. littoralis* to the tested pesticides.

Activity of several detoxifying enzymes in larvae treated with the tested pesticides

Five detoxifying enzymes were estimated in the hemolymph of the treated larvae. In the case of *S. littoralis* (CLW), all the detoxifying enzymes were inhibited to different extents, except for the ALP enzyme, which was activated (43%) in the larvae treated with the Abamectin pesticide only, as shown in Table 4. The inhibition percentages of the estimated enzymes ranged from 15 to 40% for the AST enzyme, 5 to 76% for the ALT enzyme, 23 to 83% for the ACP enzyme, 31 to 89% for the ALP enzyme, and 21 to 47% for the GST enzyme (Table 4).

In the case of *S. frugiperda* (FAW), the ALT and ACP enzymes were inhibited by all the evaluated pesticides, as shown in Table 5. The inhibition percentages ranged from 27% to 75% for ALT and 14% to 80% for ACP. The AST enzyme was activated by Tebufenozid (6.5%) and Abamectin (8.4%) but inhibited by the other tested pesticides. In addition, the ALP enzyme was inhibited by all the evaluated pesticides except Thiamethoxam, which caused the activation of the ALP enzyme. However, the GST enzyme was activated by Alpha-Cypermethrin (24%), Tebufenozid (5%), and Indoxacarb (23%) and inhibited by Spinosad (42%), Abamectin (12%), and Thiamethoxam (5%) (Table 5). Notably, the level of enzyme activity in the cotton leafworm (CLW) was greater than that in the fall armyworm (FAW), whether the larvae were treated or not treated with pesticides, as shown in Tables 4 and 5.

Table 4. The changes in detoxifying enzyme activity in *S. littoralis* larvae, which were treated by tested pesticides.

Tested compounds	Enzymatic activity ± SE									
	AST U/ml	Ch %	ALT U/ml	Ch %	ACP U/L	Ch %	ALP U/L	Ch %	GST U/L	Ch %
Control	1317^{a}±2.96	00	1061^{a}±2.60	00	817^{a}±1.45	00	1207^{b}±2.02	00	4710^{a}±4.40	00
Alpha-Cypermethrin	945^{f}±2.88	-28	347^{f}±2.96	-67	150^{f}±1.45	-81	128^{g}±1.45	-89	3416^{c}±2.90	-27
Tebufenozid	785^{g}±1.45	-40	447^{e}±2.96	-57	325^{d}±1.52	-60	208^{f}±1.45	-82	3711^{b}±4.35	-21
Spinosad	959^{e}±3.17	-27	745^{d}±2.08	-29	137^{g}±1.45	-83	219^{e}±1.52	-81	3036^{e}±2.90	-35
Abamectin	1104^{c}±2.90	-16	1001^{b}±2.72	-5	626^{b}±0.88	-23	1731^{a}±1.52	+43	3163^{d}±4.91	-32
Thiamethoxam	1110^{b}±2.88	-15	922^{c}±2.33	-13	393^{c}±1.45	-51	826^{c}±2.90	-31	2755^{f}±1.66	-41
Indoxacarb	999^{d}±1.85	-24	254^{g}±1.85	-76	202^{e}±1.45	-75	319^{d}±1.52	-73	2488^{g}±1.45	-47
F test	***		***		***		***		***	
LSD (5%)	8.07		7.69		4.23		5.59		10.52	

Ch %: Change percentage against control; (-): inhibition; (+): activation. Means with different letters in the same column are significantly at P <.05.

Table 5. Effects of the tested pesticides on the activity of several detoxifying enzymes in treated *S. frugiperda*

Tested compounds	Enzymatic activity ± SE									
	AST U/ml	Ch %	ALT U/ml	Ch %	ACP U/L	Ch %	ALP U/L	Ch %	GST U/L	Ch %
Control	568^{c}±3.05	00	923^{a}±1.45	00	145^{a}±2.33	00	133^{b}±2.96	00	2497^{d}±2.96	00
Alpha-Cypermethrin	558^{d}±3.05	-1.7	584^{c}±2.90	-36	124^{b}±2.02	-14	126^{c}±2.90	-5	3112^{a}±5.81	+24
Tebufenozid	605^{b}±2.88	+6.5	541^{d}±0.88	-41	28^{e}±1.45	-80	33^{f}±2.33	-75	2623^{c}±2.96	+5
Spinosad	435^{f}±2.33	-23	227^{g}±2.08	-75	47^{d}±0.88	-67	50.00^{e}±2.08	-62	1425^{g}±2.60	-42
Abamectin	616^{a}±2.90	+8.4	527^{e}±2.96	-43	64^{c}±0.88	-55	73^{d}±1.45	-45	2184^{f}±2.90	-12
Thiamethoxam	524^{e}±2.90	-7.7	670^{b}±2.33	-27	50^{d}±0.88	-65	172^{a}±1.45	+29	2370^{e}±2.08	-5
Indoxacarb	522^{e}±3.05	-8.0	441^{f}±1.45	-52	48^{d}±0.88	-66	19.00^{g}±0.88	-85	3087^{b}±1.45	+23
F test	***		***		***		***		***	
LSD (5%)	8.78		15.55		4.40		6.48		9.79	

Ch %: Change percentage against control; (-): inhibition; (+): activation. Means with different letters in the same column are significantly at P <.05.

As shown in Fig. 2, all the pesticides inhibited the AST enzyme in the larvae of both treated insect species except for Tebufenozide and Abamectin, which activated the AST enzyme in the fall army worm (FAW) larvae. Notably, the percentage of inhibition of the AST enzyme was greater in cotton leafworm (CLW) than in FAW. However, the activity of the ALT enzyme was inhibited in the larvae of both treated insects (CLW and FAW) by all the tested pesticides (Fig. 2).

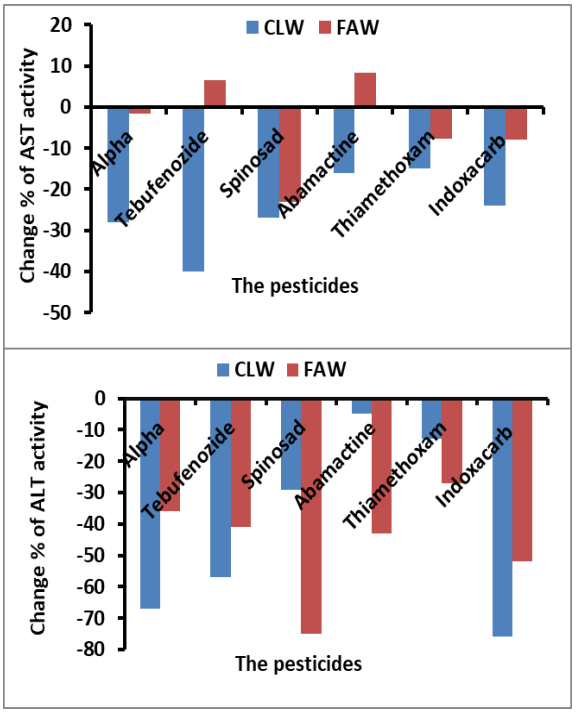


Fig. 2. Changes in the percentages of AST and ALT enzyme activities in the treated *S. littoralis* (CLW) and *S. frugiperda* (FAW) larvae.

The activity of the ACP enzyme was inhibited in CLW and FAW treated with all the pesticides. However, the activity of the ALP enzyme was inhibited in CLW treated with Abamectin alone or in FAW treated with only Thiamethoxam (Fig. 3). In addition, Alpha-Cypermethrin, Tebufenozide, Spinosad and Indoxacarb led to the inhibition of the ALP enzyme in treated CLW and FAW (Fig. 3).

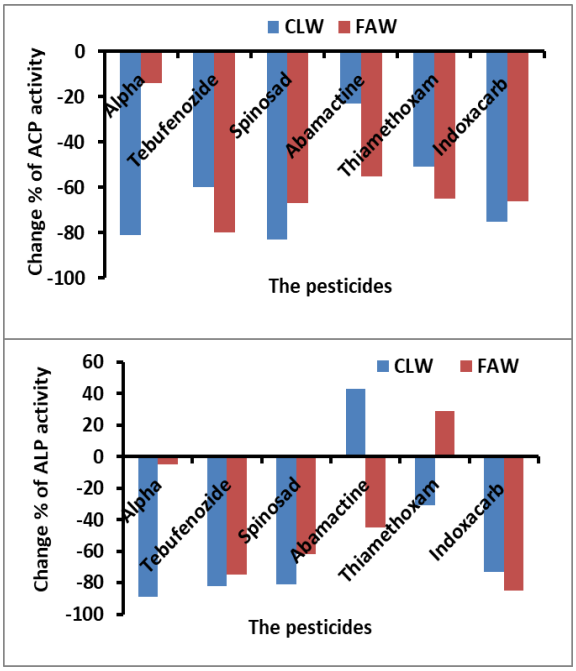


Fig. 3. Changes in the percentages of ACP and ALP enzyme activities in the treated *S. littoralis* (CLW) and *S. frugiperda* (FAW) larvae.

Figure 4 shows that Alpha-Cypermethrin, Tebufenozid, and Indoxacarb led to activation of the GST enzyme in the FAW larvae but inhibited the GST enzyme in the CLW larvae. However, Spinosad, Abamectin and Thiamethoxam led to the inhibition of the GST enzyme in both insects, with different percentages of change.

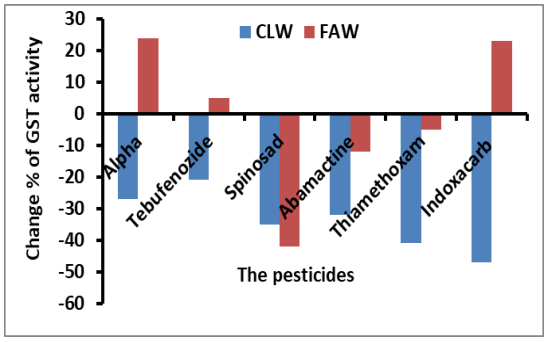


Fig. 4. Changes in the percentage of GST enzyme activity in the treated *S. littoralis* (CLW) and *S. frugiperda* (FAW) larvae.

Electrophoretic fraction protein patterns (SDS–PAGE) of *S. littoralis* and *S. frugiperda*- treated larvae compared to those of the control group.

Changes in the protein profile were detected in the 4th instar larvae of treated *S. littoralis* and *S. frugiperda* by the LC_{50} of some commercial pesticides. The electrophoretic protein patterns (SDS–PAGE) are presented in Tables 6 and 7. The results revealed that the protein profiles of *S. frugiperda* larvae had changes after treatment with Alpha-cypermethrin (9 bands), Abamectin (11 bands), and Thiamethoxam (10 bands) compared with those of the control (12 bands), as shown in Table 6.

Table 6. Molecular weight and number of SDS–PAGE protein bands detected in electropherograms of the 4th larval instar of *S. frugiperda* hemolymph

Band No.	M.W kDa.	The protein bands in the hemolymph of *S. frugiperda* larvae							Polymorphism
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	
1	224	1	1	1	1	1	1	1	Monomorphic
2	192	1	0	1	1	0	1	1	Polymorphic
3	128	1	1	1	1	1	1	1	Monomorphic
4	87	1	1	1	1	1	0	1	Polymorphic
5	69	1	1	1	1	1	1	1	Monomorphic
6	57	1	1	1	1	1	1	1	Monomorphic
7	53	1	1	1	1	1	1	1	Monomorphic
8	46	1	0	1	1	1	1	1	Polymorphic
9	38	1	1	1	1	1	1	1	Monomorphic
10	23	1	1	1	1	1	1	1	Monomorphic
11	21	1	1	1	1	1	1	1	Monomorphic
12	18	1	0	1	1	1	0	1	Polymorphic
Total		12	9	12	12	11	10	12	

M.W. = Molecular Weight, Lane 1 = Control, Lane 2 = Alpha-Cypermethrin, Lane 3 = Tebufenozid, Lane 4 =Spinosad, Lane 5 = Abamectin, Lane 6 = Thiamethoxam, Lane 7 = Indoxacarb

Table 7. Molecular weight and number of SDS–PAGE protein bands detected in electropherograms of the 4th larval instar of *S. littoralis* hemolymph

Band No.	M.W kDa.	The protein bands in the hemolymph of *S. littoralis* larvae							Polymorphism
		Lane 8	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14	
1	224	1	0	0	0	0	0	0	Polymorphic
2	192	1	1	1	1	0	0	0	Polymorphic
3	128	1	1	0	0	1	0	0	Polymorphic
4	87	1	1	1	1	1	0	0	Polymorphic
5	69	1	1	1	1	1	1	1	Monomorphic
6	57	1	1	1	1	1	1	1	Monomorphic
7	53	1	1	0	1	1	1	1	Polymorphic
8	46	1	1	1	1	1	1	0	Polymorphic
9	38	1	1	1	1	1	1	1	Monomorphic
10	23	1	1	1	1	1	1	1	Monomorphic
11	21	1	1	1	1	1	1	1	Monomorphic
12	18	1	1	1	1	1	1	1	Monomorphic
Total		12	11	9	10	10	8	7	

M.W. = Molecular Weight Lane 8 = Control, Lane 9 = Alpha-Cypermethrin, Lane 10 = Tebufenozid, Lane11 =Spinosad, Lane 12 = Abamectin, Lane 13 = Thiamethoxam, Lane 14 = Indoxacarb

In addition, the band molecular weights ranged from 18 to 224 kDa. Additionally, the polymorphism percentage among the treatments reached 33.3%, as shown in Fig. 5.

However, in the case of *S. littoralis*, the protein profiles changed in larvae treated with Alpha-cypermethrin (11 bands), Tebufenozid (9 bands), Spinosad (10 bands), Abamectin (10 bands), Thiamethoxam (8 bands), and Indoxacarb (7 bands) compared with those in the control (12 bands), as shown in Table 7. Additionally, the band molecular weights ranged from 18 to 224 kDa. However, the polymorphism percentage among the treatments reached 50%, as shown in Fig. 6. The protein profiles in both insects confirmed that there was variation in the sensitivity of *S. littoralis* and *S. frugiperda* to the evaluated pesticides.

The highly invasive nocturnal pest *S. frugiperda* was discovered first time in Upper Egypt in 2019. In field populations of *S. frugiperda*, insecticide toxicity was tested to determine the resistance in *S. frugiperda* in Egypt (Salem *et al.*, 2023). Various classes of chemical or biochemical insecticides are used to control cotton leafworm *S. littoralis* in Egypt. These include pyrethroids, organophosphorus, IGRs, diamides, oxadiazine, spinosyns, emamectin benzoate, and *B. thuringiensis*. It is important to identify pesticides that are suitable for controlling the fall armyworm *S. frugiperda* among the pesticides used to control cotton leafworm *S.*

littoralis in Egypt. In addition, the extent of the sensitivity and the range of naturally occurring tolerance found in field populations should be investigated, as these factors should be considered when evaluating the toxicity of pesticides. (Sawicki, 1987).

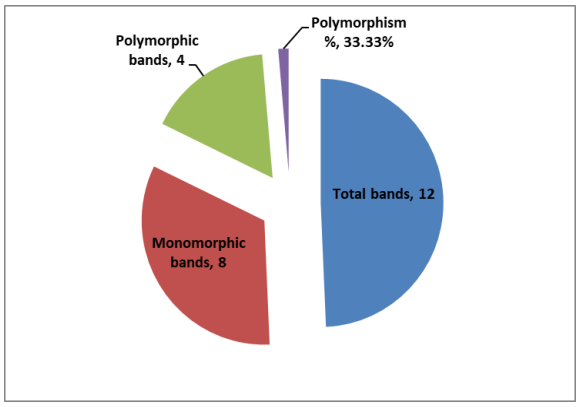


Fig. 5. The quantity, kinds, and percentage of polymorphisms in *S. frugiperda* larval protein bands

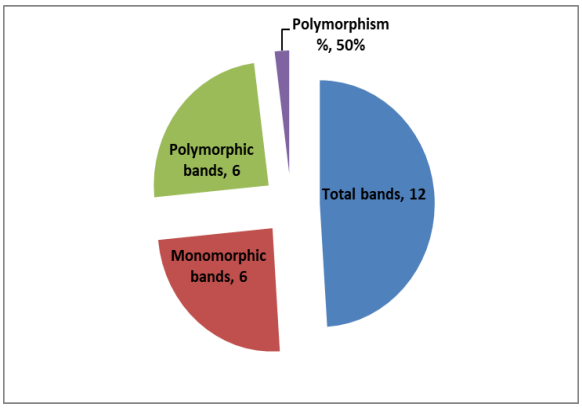


Fig. 6. The quantity, kinds, and percentage of polymorphisms of *S. littoralis* larval protein bands

The present research was carried out to investigate the sensitivity of the fall armyworm *S. frugiperda* to several commonly used pesticides in Egypt compared with that of the laboratory strain of *S. littoralis* and to determine the biochemical reactions of these pests. The results indicated that *S. frugiperda* was more tolerant or less sensitive to Alpha-cypermethrin (10.59-fold), Tebufenozid (4.41-fold), Thiamethoxam (6.42-fold) and Indoxacarb (2.69-fold) than was *S. littoralis*. On the other hand, *S. frugiperda* was found to be more sensitive to Spinosad (0.27-fold) than was *S. littoralis*, but for the Abamectin pesticide (0.92-fold), both insects showed approximately equal degrees of sensitivity, as shown in Table 8.

Table 8. Resistance ratio of *S. frugiperda* to several commonly used pesticides compared with that of the laboratory strain *S. littoralis*

Treatments	LC_{50} (ppm)		Resistance ratio of *S. frugiperda* (Fold)
	S. frugiperda	*S. littoralis*	
Alpha-Cypermethrin	53.15	5.02	10.59
Tebufenozid	283.06	64.2	4.41
Spinosad	6.23	22.8	0.27
Abamectin	27.75	30.26	0.92
Thiamethoxam	269.4	41.97	6.42
Indoxacarb	10.85	4.02	2.69

Resistance ratio = LC_{50} of *S. frugiperda* / LC_{50} of *S. littoralis*

In a similar study by Bird *et al*. (2022), *S. frugiperda* exhibited a significant reduction in sensitivity to Methoxyfenozide and Indoxacarb compared with the laboratory strain of *Helicoverpa armigera*. In addition, *S. frugiperda* was less sensitive to synthetic pyrethroids than was *H. armigera*, where *S. frugiperda* was 44–132 times less toxic when exposed to Alpha-cypermethrin than was *H. armigera*.

In our study, *S. frugiperda* was found to be highly sensitive to Spinosad more than was the lab. strain of *S. littoralis*. The results in our study are compatible with those of Zhao *et al*. (2020), who reported that *S. frugiperda* is highly susceptible to Spinosyn, revealed that both Spinosyn insecticides (Spinosad and Spinotram) are to be effective control agents. Nonetheless, Spinosad resistance has been observed in *S. frugiperda* from Central and South America, suggesting that selection for resistance may take place if the usage of these insecticides increases (Okuma *et al*., 2017; Lira *et al*., 2020). In addition, our research reported that *S. frugiperda* and *S. littoralis* exhibited approximately equal sensitivities to the Abamectin pesticide. However, in a similar study, larvae of *S. frugiperda* were less sensitive (2- to 3-fold) to emamectin benzoate than larvae of *H. armigera* (Bird, 2015).

According to Bird *et al*. (2022), these studies may help with *S. frugiperda* control decisions, particularly when compared to the known practical importance of pesticide resistance in *H. armigera*. Monitoring resistance will be essential for managing *S. frugiperda* resistance because metabolic detoxification is similarly connected to insect resistance to diamides in the closely related species *S. litura* (Muthusamy *et al*., 2014). The three main mechanisms of insecticide resistance are increased detoxification (Enayati *et al*., 2005), decreased penetration (Ahmad and McCaffery, 1999), and target-site insensitivity (Soderlund and Knipple, 2003).

In our study, most of the evaluated pesticides led to the inhibition of detoxifying enzymes (AST, ALT, ACP, ALP, and GST) in *S. frugiperda* and *S. littoralis*. Additionally, the inhibition of enzymes activity was higher in treated *S. frugiperda* than that of *S. littoralis* with the most evaluated pesticides. This may explain the lower sensitivity of the fall armyworm *S. frugiperda* to some pesticides than the cotton leafworm *S. littoralis*. The expression of insecticide-induced hormesis and the upregulation or overexpression of detoxifying enzymes are two possible causes of the intraspecific heterogeneity in pesticide sensitivity. (Cutler, 2013; Guedes and Cutler, 2014). Both situations may help reduce pests' sensitivity to pesticides and enhance arthropods' ability to detoxify insecticidal substances. (Cutler and Guedes, 2017; Guedes *et al*., 2019).

In the present investigate; the larvae of *S. frugiperda* treated with Alpha-cypermethrin were more tolerant than were those of *S. littoralis*. The resistance of *S. frugiperda* to Alpha-cypermethrin may be caused by detoxification by microsomal oxidases, GS-T, and carboxylesterases (Carvalho *et al*., 2013; Yu *et al*., 2003). On the other hand, 3 amino acid substitutions at VGSCs (L1014F, L932F, and T929I) were present in pyrethroid-resistant strains of *S. frugiperda* from Brazil. These substitutions are known to confirm the target-site resistance to pyrethroids in a number of insect species (Carvalho *et al*., 2013).

Bird, (2015) mentioned that *S. frugiperda* were less sensitive (2- to 3-fold) to emamectin benzoate (the same chemical group of Abamectin) than larvae of *H. armigera*. This result may reveal that genes that confirm practical

resistance to this insecticide are not found in *S. frugiperda*. This also confer the idea that small variance in intraspecific sensitivity to these compounds is likely to reverse naturally occurring variability in this species (Bird, 2015). Moreover, detoxification process is also contribute for the insect resistance to diamides in the closely related species *S. litura* (Muthusamy *et al*., 2014), and monitoring resistance is a key agent of resistance management for *S. frugiperda*. In addition, Global Indoxacarb use might not be the cause of an increase in selection for resistance to this insecticide; alternatively, selection for more generalist resistance mechanisms, such as metabolic detoxification systems, might result in decreased sensitivity to Indoxacarb. Compared to other effective pesticides evaluated on *S. frugiperda* populations from China and India, Indoxacarb was found to be relatively less toxic (Zhao *et al*., 2020; Deshmukh *et al*., 2020).

To achieve effective pest management plans, it is crucial to conduct an evidence-based evaluation of the sensitivity status of recently established insect pest species. The toxicity tests conducted in this research will be beneficial tools for increasing the capacity for early monitoring the pesticide resistance in *S. frugiperda* in Egypt. In the present study, *S. frugiperda* was found to be more sensitive to Spinosad, Indoxacarb, and Abamectin, but it was more resistant to Tebufenozid, Thiamethoxam, and Alpha-cypermethrin than *S. littoralis*.

REFERENCES

Abbot, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267. https://doi.org/10.1093/jee/18.2.265a.

Abdullah, R.R.H. (2023). Comparing the efficiency of fungal spores and their metabolites in some entomopathogenic fungi against cotton leafworm, *Spodoptera littoralis.* Journal of Entomology and Zoology Studies, 11(5): 40-44. https://doi.org/10.22271/j.ento.2023.v11.i5a.9230

Abdullah, R.R.H., Abd El-Wahab, A.H. and Abd El-Salam, S.A. (2024). Insecticidal activity and possible modes of action of secondary metabolites of some fungal strains and wild plants as natural pesticides against *Spodoptera frugiperda*. *Beni-Suef Univ J Basic Appl Sci* 13, 9 (2024). https://doi.org/10.1186/s43088-024-00467-z

Ahmad, M. and McCaffery, A.R. (1999). Penetration and metabolism of trans-cypermethrin in a susceptible and a pyrethroid-resistant strain of *Helicoverpa armigera.* Pestic. Biochem. Physiol., 65 (1999), pp. 6-14. https:/ /doi. org/10.1006/pest.1999.2420

Bird, L., Miles, M., Quade, A. and Spafford, H. (2022). Insecticide resistance in Australian *Spodoptera frugiperda* (J.E. Smith) and development of testing procedures for resistance surveillance. PLoS ONE 17(2): e0263677. https://doi.org/10.1371/journal.pone.0263677

Bird, L. (2015). Baseline susceptibility of *Helicoverpa armigera* (Hu¨bner) (Lepidoptera: Noctuidae) to indoxacarb, emamectin benzoate and chlorantraniliprole in Australia. J Econ Entomol., 108: 294–300. https:/ /doi. org/ 10.1093/jee/tou042

Bueno, R.C.O.F., Carneiro, T.R., Bueno, A.F., Pratissoli, D., Fernandes, O.A. and Vieira, S.S. (2010). Parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) eggs. Brazilian Archives of Biology and Technology 53(1): 133-139. https:/ /doi.org/ 10.1590/S1516-89132010000100017.

Carvalho, R.A., Omoto, C., Field, L.M., Williamson, M.S. and Bass, C. (2013). Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLOS ONE, 8(4): e62268. https://doi.org/10.1371/journal.pone.0062268.

Cutler, G.C. and Guedes, R.N.C. (2017). Occurrence and significance of insecticide-induced hormesis in insects. In: Duke SO, Kudsk P, Solomon K, editors. Pesticide dose: effects on the environment and target and nontarget organisms. Washington: American Chemical Society; pp. 101–119

Cutler, G.C. (2013). Insects, insecticides and hormesis: evidence and considerations for study. Dose–Response, 11: 154–177. https://doi.org/10.2203/dose-response.12-008.

Davis, B.J. (1964). Disc electrophoresis II. Methods and application to human serum proteins. Annals of the New York Academy of Sciences. 1964; 121:404-427.

Deshmukh, S., Pavithra, H.B., Kalleshwaraswamy, C.M., Shivanna, B.K., Maruthi, M.S. and Mota-Sanchez, D. (2020). Field efficacy of insecticides for management of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on maize in India. Fla Entomol. 103: 221–227.

EL-Defrawi, M., Toppozada, A., Mansour, N. and Zeid, M. (1964). Toxicological studies on the Egyptian cotton leafworm, *Prodenia litura*. 1-Suceptibility of different larval instars to insecticides. J. Econ. Entomol., 57 (4): 591–593.

El-Sheikh, E.A. and Aamir, M.M. (2011). Comparative effectiveness and field persistence of insect growth regulators on a field strain of the cotton leafworm, *Spodoptera littoralis*, Boisd (Lepidoptera: Noctuidae). Crop Protection 30 (2011) 645-650. https://doi:10.1016/j.cropro.2011.02.009.

Enayati, A.A., Ranson, H. and Hemingway, J. (2005). Insect glutathione transferases and insecticide resistance. Insect Mol Biol. 14(1):3-8. https://doi.org/10.1111/j.1365-2583.2004.00529.x.

Finney, D.J. (1971). Probit analysis. 3rd edition. Cambridge university press. 318 pp.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. and Tamo, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), a new alien invasive pest in west and Central Africa. PloS ONE, https://doi.org/10.137/Journal.pone.0165632.

Guedes, R.N.C. and Cutler, G.C. (2014). Insecticide-induced hormesis and arthropod pest management. Pest Manag Sci. 70:690–697. https://doi.org/10.1002/ps.3669.

Guedes, R.N.C., Roditakis, E., Campos, M.R., Haddi, K., Bielza, P. and Siqueira, H.A.A. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. J Pest Sci. 92: 1329–1342.

Hamadah, K.S. (2019). Disturbance of phosphatase and transaminase activities in grubs of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) by certain insecticidal compounds. Journal of Basic and Applied Zoology (2019) 80:52. https://doi.org/10.1186/s41936-019-0123-1.

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Gohole, L. and Tefera, T. (2018). Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. International Journal of Pest Management 65(1): 1-9. https://doi.org/10.1080/09670874.2017.1423129.

Laemmli, U.K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature. 1970; 227(5259):680-685.

Lira, E.C., Bolzan, A., Nascimento, A.R.B., Amaral, F.S.A., Kanno, R.H. and Kaiser, I.S. (2020). Resistance of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to spinetoram: inheritance and cross-resistance to spinosad. Pest Manag Sci., 76: 2674–2680. https://doi.org/10.1002/ps.5812

Muthusamy, R., Vishnupriya, M. and Shivakumar, M.S. (2014). Biochemical mechanism of chlorantraniliprole resistance in *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). J Asia Pac Entomol., 17: 865–869.

Nagoshi, R.N., Adamczyk, J.J., Meagher, J., Gore, R.L. and Jackson, R. (2007). Using stable isotope analysis to examine fall armyworm (Lepidoptera: Noctuidae) host strains in a cotton habitat. Journal of Economic Entomology 100(5): 1569-1576. https://doi.org/10.1093/jee/100. 5.1569.

Okuma, D.M., Bernardi, D., Horikoshi, R.J., Bernardi, O., Silva, A.P. and Omoto, C. (2017). Inheritance and fitness costs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to spinosad in Brazil. Pest Manag Sci. 4: 1441–1448.

Pan, L., Renm, L., Chen, F., Feng, Y. and Luo, Y. (2016). Antifeedant activity of *Ginkgo biloba* secondary metabolites against *Hyphantria cunea* larvae: Mechanisms and applications. PloS ONE 11 (5): e0155682. https://doi.org/10.1371/journal.pone.0155682.

Powell, M.E.A. and Smith, M.J.H. (1954). The determination of serum acid and alkaline phosphatase activity with 4-amino antipyrine (A. A. P). Journal Clinical pathology 7(3): 245-248. https://doi.org/10.1136/jcp.7.3.245.

Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. American Journal of Clinical Pathology 28(1): 56-63. https:// doi. org/ 10.1093/ajcp/28.1.56.

Salem, S.A.R., Dahi, H.F., Abdel-Galil, F.A. and Mahmoud, M.A.B. (2023). Efficacy of Common Synthetic Insecticides for Management of Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt. Egypt. Acad. J. Biology. Sci., 15(1):157-170(2023). https:// doi .org/ 10.2 1608 /EAJBSF.2023.303457.

SAS Institute. (1997). SAS/STAT User's Guide for Personal Computers, SAS Institute, Cary, NC, 18 pp.

Sawicki, R.M. (1987). Definition, detection and documentation of insecticide resistance. In: Ford MG, Holloman DW, Khambay BPS, Sawicki RM, editors. Combating resistance to xenobiotics; biological and chemical approaches. Chichester, United Kingdom: Ellis Harwood; 1987. pp. 105–117.

Soderlund, D.M. and Knipple, D.C. (2003). The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochemistry and Molecular Biology, 33:(6), 563-577. https://doi.org/10.1016/S0965-1748(03)00023-7.

Sun, Y.P. (1950). Toxicity index on improved method of comparing the relative toxicity of insecticides. *Journal of Economic Entomology* 43(1): 45-53. https:// doi.org /10.1093/ jee/43.1.45.

Yu, S.J., Nguyen, S.N. and Abo-Elghar, G.E. (2003). Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Pestic. Biochem. Physiol., 77: 1–11.

Zhao, Y.X., Huang, J.M., Ni, H., Guo, D., Yang, F.X. and Wang, X. (2020). Susceptibility of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), to eight insecticides in China, with special reference to lambda-cyhalothrin. Pestic Biochem Physiol. 2020; 168: 1046.

Zibaee, A., Sendi, J., Alinia, F., Ghadamyari, M. and Etebari, K. (2009). Diazinon resistance in different selected strains of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), rice striped stem borer, in the north of Iran. *Journal of Economic Entomology* 102, 1189- 1196.

مقارنة بين *Spodoptera frugiperda* و*Spodoptera littoralis*: مقاومة المبيدات الحشرية، والإنزيمات المزيلة للسموم وأنماط البروتين

رضا راضي حسن عبدالله[1] ونها الدسوقي عبد السميع سكر[2]

[1]معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة – مصر
[2]قسم النبات الزراعي (الوراثة) - كلية الزراعة بالقاهرة – جامعة الأزهر

الملخص

في عام 2019، اكتشف الباحثون لأول مرة دودة الحشد الخريفية في صعيد مصر. أجريت هذه الدراسة لتحديد حساسية دودة الحشد الخريفية لستة مبيدات حشرية شائعة الاستخدام في مصرومقارنتها بدودة ورق القطن ودراسة التغير في نشاط الانزيمات المزيلة للسموم وكذلك أنماط البروتين المختلفة لكلا الأفتين. أشارت النتائج أن دودة الحشد الخريفية كانت أكثر حساسية لسبينوساد (LC_{50} = 6.23 ppm) وأباماكتين (LC_{50} = 27.75 ppm) من دودة ورق القطن (LC_{50} = 22 , 30 ppm ، على التوالي). ومع ذلك، كانت دودة الحشد الخريفية أكثر مقاومة لتيبيوفينوزيد (LC_{50} = 283.06 ppm) وثيوميثوكسام (LC_{50} = 269.4 ppm) من دودة ورق القطن (LC_{50} = 64.2 , 41.97 ppm ، على التوالي). وبالمقارنة مع دودة ورق القطن كانت دودة الحشد الخريفية أقل حساسية للمبيد ألفا سيبرمثرين (LC_{50} = 53.05 ppm) و اندوكساكارب (LC_{50} = 10.85 ppm)، في حين كانت دودة ورق القطن أكثر حساسية لكلا المبيدين (LC_{50} = 5.02 , 4.02 ppm ، على التوالي). أيضا أشارت النتائج أن المبيدات الحشرية المختبرة أدت إلى درجات مختلفة في نشاط إنزيمات إزالة السموم (AST و ALT و ACP و ALP و GST) المقدرة في كلا الافتين. وربما كانت الدرجات المختلفة لنشاط إنزيمات إزالة السموم في الافتين هي من أسباب التباين في حساسيتهما للمبيدات المختبرة. وكذلك أشارت نتائج تحليل SDS–PAGE إلى وجود تباين في أنماط البروتين بين يرقات دودة ورق القطن ودودة الحشد الخريفية المعاملة مقارنة بتلك الموجودة في المجموعة الضابطة. أوصت هذه الدراسة بإمكانية استخدام الاسبينوساد و الأباماكتين والاندوكساكارب في مكافحة دودة الحشد الخريفية بشكل فعال.