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DEVELOPMENT OF RESISTANCE TO FOUR INSECTICIDES AND ENHANCED DETOXIFYING ENZYME ACTIVITIES IN THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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ABSTRACT: The Egyptian cotton leafworm *Spodoptera littoralis* (Bosid.), one of the main pests in Egypt that causes damage to industrial, vegetable, and ornamental crops. The development of resistance and biochemical mechanism of *S. littoralis* to four insecticides (deltamethrin, spinetoram, pyridalyl and indoxacarb) were studied in the laboratory. Selection pressure in all experiments was carried out on 4th instar larvae for six generations by the leaf dipping technique. At the end of selection, the results indicated that the resistance ratios (RR) were 43.79-, 29.69-, 13.09- and 33.39-fold for deltamethrin, spinetoram, pyridalyl and indoxacarb, respectively, compared with the parent strain. At the end of selection pressure, detoxifying enzyme assays revealed that the α-esterases activity levels for such insecticides were 5542.64, 4024.44, 4223.64 and 3641.32, respectively compared with parent strain 447.89, whereas those of β-esterase activity were 4132.30, 2343.25, 2953.70 and 1294.04 for deltamethrin, spinetoram, pyridalyl and indoxacarb, respectively, higher than in the parent strain 400.61. In addition, there was a significant increase in levels of AChE, ACP, ALP and GSH activity at the end of selection with these insecticides in all selected generations. The results demonstrated that, treatment of *S. littoralis* with DMT, SPT, PYD and INC compared with the susceptible strain caused a significant decrease in the protein, carbohydrate and lipid contents.

Keywords: Insecticides, Resistance, Enzymatic activity, Cotton Leafworm, *Spodoptera littoralis.*

INTRODUCTION

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) commonly known as the Egyptian cotton leafworm (Hosny, 1980) is a serious polyphagous pest in both greenhouses and open fields on a broad range of ornamental, industrial, and vegetable crops all over the year (Hosny *et al.*, 1986). There are about 120 plant species known to be hosts, divided into 44 groups. It is found in the Near East, Mediterranean countries, Middle East, and northern Africa. In Egypt, 73 species were recorded that hosts this insect (ThÖming *et al.*, 2013), especially cotton plant (Hosny, 1980). Due to the widespread use of broad-spectrum pesticides in Egypt to control this pest, especially on cotton, resistance and cross-resistance have frequently evolved, making control of the pest even more challenging. (Miles and Lysandrou, 2002; Aydin and Gurkan, 2006).

One possible molecular basis of resistance is linked to decreased target-site sensitivity, whereas the other is based on elevated levels of detoxifying enzymes (Feyereisen, 1995; Ffrench-Constant, 1999; Hilliou *et al.*, 2021).

The focus has shifted to developing alternative management tactics, such as mixtures and rotating programs of insecticides with diverse modes of action, in order to postpone the development of pesticide resistance in lepidopterous pests. (Ahmad, 2004, 2009; Ascher *et al.*, 1986; Gunning *et al.*, 1999; Martin *et al.*, 2003; Ming *et al.*, 2021). Over the past 20 years, a few novel, effective compounds with various mechanisms of action have also been introduced into Egypt. Among those pesticides with exceptional insecticidal efficacy are deltamethrin, spinetoram, indoxacarb, and

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pyridalyl. Therefore, this study was accomplished to develop resistant strains of the cotton leafworm to four different insecticides (deltamethrin, spinetoram, indoxacarb and pyridalyl), individually under chemical pressure with clarifying the effect on detoxification enzymes including hydrolases (α- and β-esterases), transferases (glutathione *S*-transferase), and phosphatases (acid and alkaline phosphatase) as well as acetylcholine esterase (AChE) of all insecticides used.

MATERIALS AND METHODS

1. Insecticides used

Commercial formulations of the following insecticides were used in this study. Deltamethrin (Decis® 2.5 % EC, Cairo Chemicals company), spinetoram (RADIANT® 12 % SC, Corteva Agriscience company), indoxacarb (Avant® 15 % EC, DuPont Company) and pyridalyl (Pleo® 50 % EC, Shoura Chemicals Company). The insecticides used in bioassays are presented in Table 1.

2. Insect Strains and Rearing Technique

2.1. Susceptible laboratory strain (SUS-LAB): A colony of the cotton leafworm, *Spodoptera littorals*, which has been reared under laboratory conditions for more than two years was kindly supplied from the Central Laboratory of Pesticides, Ministry of Agriculture, Dokki, Giza.

2.2. Field strain (MNF-strain): Egg masses of *S. littoralis* were collected from three different locations of cotton fields in Menoufia governorate, during growing season of 2021-2022.

2.3. Resistant strains: Four *S. littoralis*-field populations were subjected individually to chemical pressure with each selected insecticide: viz. deltamethrin (DMT), spinetoram (SPT), pyridalyl (PYD), and indoxacarb (INC), for six successive generations (G_1- G_6) for developing insecticide resistance.

All strains were reared under constant laboratory conditions as described by El-Defrawi *et al.* (1964) at 25 ± 2°C, and 70 ± 5 % R.H.

3. Bioassay Procedure

On a trial to buildup resistant strains of the cotton leafworm to the selected four insecticides with different mode of action: deltamethrin (Pyrethroids), spinetoram (Spinosyn), pyridalyl (Pyridine) and indoxacarb (Oxadiazine).

Table (1): List of insecticides used: commercial formulation, chemical group and mode of action.

Insecticide	Formulation (AI %)	Manufacturer	Chemical group	Mode of action
Deltamethrin	DECIS® (2.5 % EC)	Sumitomo Chemical Co., Ltd.	Pyrethroids	Delaying the closing of the activation gate of sodium ion channel.
Spinetoram	RADIANT® (12 % SC)	Dow AgroSciences, Indianapolis, IN, USA	Spinosyns	Interacts with both γ-aminobutyric acid (GABA) receptor and nicotinic acetylcholine (nACh) receptor.
Indoxacarb	AVANT ® (15 % EC)	Dupont, Wilmington, DE, USA	Pyridine	Blocking sodium channels in the insect nervous system.
Pyridalyl	PLEO® (50 % EC)	Sumitomo Chemical Co., Ltd.	Oxadiazine	Cytotoxicity and prevention of protein synthesis.

The subsequent technique had been adopted, briefly the susceptibility of the insecticide used in selection was attempted at the level of LC_{50} at each generation. The LC_{50} concentration of each insecticide was used as pressure dose for treating different successive generations. To protect the population from stronger pressure and sustain the strains for as long as feasible, the LC_{50} limit was selected. The selection was conducted by using leaf dipping technique at which, approximately 3000 larvae fourth-instar were allowed to feed on leaves treated for 24h, then the survivors were gathered, put in clean jars with brand-new caster bean leaves, and raised according to the previous procedures. This represented the first generation (G1). Similar technique of selection and rearing was followed for every successive generation until the 6th generation. The concentration-mortality curve of each insecticide was obtained by 5-7 concentrations with three replicates for each concentration using 10 larvae for each replicate. The data was corrected using Abbott's formula Abbott (1925), concentration mortality regression line was plotted out according to Finney (1971). Resistance ratio (level) was determined every generation by dividing LC50 of selected field strain by corresponding LC_{50} of SUS-LAB strain.

4. Biochemical Study

4.1. Preparation of Tissue Homogenate Samples for Biochemical Determination

Tissue homogenate samples were collected from the whole larvae by homogenizing in insect physiological saline (0.5 gm in 5 ml insect physiological saline) using Teflon homogenizer and collected in cold tubes (on ice). The samples were centrifugated at 4500 rpm for 5 minutes under cooling (4°C) to remove the tissues. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20°C until analysis.

4.2. Determination of Detoxifying Enzyme Activities

4.2.1. Non-specific Esterase Activities

Alpha-and Beta-esterases (α-E, β-E) activities were determined according to the method of Van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. The α-esterase activity was expressed as μg α-naphthol/min/ml); while β-esterase activity was expressed as μg β-naphthol/min/ml).

4.2.2. Acetylcholinesterase Activity (AChE)

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson *et al.* (1964). The AChE activity was expressed as μg acetyl-choline bromide/min/mL.

4.3. Phosphatases Activity

Acid phosphatase (ACP) and Alkaline phosphatase (ALP) activities were measured according to the method of Laufer and Schin (1971). Phosphatase activity was expressed as unit/ mL.

4.4. Determination of Reduced Glutathione (GSH) Level

The reduced glutathione level was determining spectrophotometrically at 340 nm using assay kit (Biodiagnostic and research agent, Egypt) according to the method of Beutler *et al.,* (1963). The reduced glutathione level was expressed as μM /min/mL.

4.5. Determination of total lipid content

The total lipid content of the homogenate was determined by the phosphovanillin method as described by Baronos and Blackstock (1973). Total lipid content was expressed as mg/ml.

4.6. Determination of total carbohydrate content

The total carbohydrate content of the insect homogenate was determined according to Singh and Sinha (1977) total carbohydrate content was expressed as mg/ml.

4.7. Determination of total protein content

The protein content of the haemgenate samples were determined using folin phenol reagent according to the method of Lowry *et al.* (1951) total protein content was expressed as mg/ml.

5. Statistical Analysis

All data were statically analyzed using Procs ANOVA and REG in SAS (Anonyms 1998) with P=0.05. For determining the increase of different studied variables among different generations Proc REG was used. The relation between different generations were determined to be second degree of polynomial (Y = a ± b1X ± b2 X2) (non-linear one). For significance between field and lab strains Proc ANOVA was used.

RESULTS AND DISCUSSION

1. Development of Resistance in *S. littoralis* to Selected Insecticides

The levels of resistance development in *S.littoralis* via successive to four selected insecticides are shown in the Table (2) when the successive generation were selected by the compound alone for the indicated generation.

Deltamethrin: The LC_{50} value of deltamethrin was 270.12 ppm in the first generation of the field-collected population of *S. littoralis*. This value dropped with subsequent generations, reaching a final value of 818.40 ppm in the 6^{th} generation Table (2).

As shown in Table (2) it is obvious that LC_{50} and resistance levels exhibited slight and slow increase during the first three generations after selection with deltamethrin ranging between 14.46 and 17.09-fold. However, at the 4^{th} generations, the resistance levels increased, reaching 29.71, 33.58 and 43.79 times at G4, G5 and G6, respectively.

Spinetoram: The resistance levels of different generations of Spinetoram selected strain Table (2) followed the LC50 value showing that significantly increased in the tolerance ratios are compared from the LC50 values. The resistance levels were 2.97, 3.78, 4.79, 7.57, 13.09, 20.63 and 29.69-fold respectively, for the parent and selected six generations.

Pyridalyl: Pyridalyl LC_{50} value for the *S. littoralis* field population was 54.75 ppm for the first generation, and it dropped to 133.30 ppm for the 6_{th} generation indicat that there are a conservable and moderate resistance for Pyridalyl. The LC50 of values Pyridalyl reported for the next generations (G2 to G6) were 64.11, 83.82, 93.72, 122.27 and 133.30 ppm respectively, reporting resistance ratios by 4.24, 5.32, 6.29, 8.23, 9.20, 12.01 and 13.09-fold, for the parent and Pyridalyl–treated generations from 1st to 6th respectively.

Indoxacarb: The estimated LC50 values of indoxacarb as shown in Table (2) were 53.90, 65.35, 72.20, 130.70, 218.88, 417.74 and 533.28 ppm respectively, while the resistance ratios by: 3.38, 4.10, 4.53, 8.19, 13.70, 26.16 and 33.39-fold, for the parent and Indoxacarb–treated generations from 1^{st} to 6^{th} respectively.

The indiscriminate use of conventional and newer insecticides has caused the development of resistance to almost all kinds of insecticides in the Noctuidae species Fouad *et al.,* (2022), Zhang *et al.,* (2022), Garlet *et al.,* (2021), Awad *et al.,* (2022). Therefore, monitoring insecticides is considered a pre-requisite in IPM programs (Bull and Men 1990) and becomes a remarkable aspect of resistance management Abo-Elghar *et al.,* (2005).

The results of the present study clearly demonstrated that the development of resistance to deltamethrin was the most increase and exceed resistance levels exhibited slight and slow increase during the first three generations after selection with deltamethrin ranging between 14.46 and 17.09-fold. However, at the 4th generations, the resistance levels significant increased, 43.79 times at G6. Such increase was adopted indicate that there are a conservable and high resistance for deltamethrin. Likewise, Wang *et al.,* (2019), Silva *et al.,* (2011) recorded the same result that the cotton leaf worm, *A. argillacea*, populations to deltamethrin, chlorpyrifos and spinosad. Resistance ratios were estimated all populations evaluated showed varying levels of resistance to all insecticides tested, but only increased levels of resistance to deltamethrin. They demonstrated that

Spodoptera frugiperda reduced mobility on deltamethrin-treated surfaces, displaying immobilization and inhibiting food Vinha *et al.*, (2021).

Table (2): Levels of resistance development to deltamethrin, spinetoram, pyridalyl and indoxacarb in the 4th -instar *S. littoralis* larvae subjected for chemical pressure with each insecticide individually for six successive generations (G_1-G_6)

Insecticide	Selection[a]	Slope ± SE	LC_{50} (ppm)	Resistance Ratio[b]
Deltamethrin (DMT)	SUS-LAB	2.04±0.63	18.69	--
	MNF	1.61±0.53	135.81	7.26
	G_1	2.15±0.72	270.12	14.46
	G_2	3.12±1.01	319.45	17.09
	G_3	3.22±1.16	364.45	19.52
	G_4	2.19±1.82	555.16	29.71
	G_5	2.73±0.99	627.50	33.58
	G_6	9.25±2.77	818.40	43.79
Spinetoram (SPT)	SUS-LAB	2.31±0.64	21.59	--
	MNF	1.42±0.43	64.01	2.97
	G_1	1.85±0.63	81.49	3.78
	G_2	2.62±0.82	103.63	4.79
	G_3	4.34±1.35	163.52	7.57
	G_4	4.03±1.18	282.75	13.09
	G_5	4.34±1.51	445.47	20.63
	G_6	2.99±1.01	641.11	29.69
Pyridalyl (PYD)	SUS-LAB	1.07±0.37	10.18	--
	MNF	1.96±0.64	43.17	4.24
	G_1	2.65±0.08	54.25	5.32
	G_2	2.99±1.00	64.11	6.29
	G_3	3.87±1.35	83.82	8.23
	G_4	4.61±1.53	93.72	9.20
	G_5	4.32±1.48	122.27	12.01
	G_6	4.88±1.76	133.30	13.09
Indoxacarb (INC)	SUS-LAB	1.21±0.43	15.97	--
	MNF	2.81±0.83	53.90	3.38
	G_1	2.23±1.01	65.35	4.10
	G_2	2.15±0.64	72.20	4.53
	G_3	3.23±1.01	130.70	8.19
	G_4	1.67±0.63	218.88	13.70
	G_5	4.53±1.36	417.74	26.16
	G_6	5.37±1.87	533.28	33.39

[a]SUS-LAB, Susceptible laboratory strain; MNF, Menoufia field strain,

[b] $Resistance\ Ratio\ (R.R) = \frac{\text{LC50 of field strain}}{\text{LC50 of susceptible strain}}$

Resistance to Spinetoram had significantly increased, the rate of development of resistance increased remarkably and reached 20.63 and 29.69-fold at the 5th and 6th selected generations. In general, the resistance ratios indicated clearly that the spinetoram results are congruent with previous studies by Ahmed *et al.* (2016) and Tamilselvan *et al.* (2021) who found that spinosad and other bioinsecticides, such as emamectin benzoate and spinetoram, were more toxic to *S. littoralis* and *Plutella xylostella* than conventional insecticides. These findings are consistent with those of Tamilselvan *et al.* (2021), who reported that indoxacarb and cypermethrin were the least hazardous to a susceptible population of *P. xylostella*, while spinetoram, spinosad, and emamectin benzoate were more toxic.

The results of the present study showed moderate resistance to pyridaly where the tolerance ratios at the 5th and 6th generations were 12.01 and 13.09-fold respectively. Based on the LC50 values, pyridalyl exhibited a moderate toxic effect. Ismail, (2018) indicated that pyridalyl was a successful insecticide at sub-lethal dose, which may be used to prevent or delay appearance of resistance to conventional pesticides and save the environment.

Our current findings revealed that selection of *S. littoralis* of resistance development to Indoxacarb for six consecutive generations significantly increased, resulted in the resistance development at the 5th and 6th generations were 26.16 and 33.39 -fold respectively. The same result was found by Moataz *et al.,* (2021) the authors found that indoxacarb insecticide exhibit good efficiency for control lepidopteran pests. These results indicated that indoxacarb could be effective for *S. littoralis* control. According to Song *et al.* (2011), indoxacarb was found to be more effective when taken orally as opposed to when applied topically. This was linked to the insecticide's ability to inhibit sodium channels.

2. Enzymatic Activity

2.1. Deltamethrin-Resistant(DMT) Strain

Data in Table 3 presented the activity of Esterases enzymes determined by α- and β-naphthyl acetate. Esterases activities of both substrates show significant alteration in the resistant strain to deltamethrin (DMT), the highest level of α-Estrase activity was found in the sixth generation (5542.64) compared to the susceptible strain (12.61) and field strain (447.89). The data obtained from β-esterase were similar to that obtained from α-Esterases recorded the higher level of β-esterase in the sixth generation (4132.30) compared to the susceptible strain (16.88) and field strain (400.61).

The resulted showed in (Table 4) recorded that the highest level of the acetylcholinesterase (AChE) was found in the sixth generation (7151.35) compared to the susceptible strain (35.85) and field strain (516.93).

The resulted of acid phosphatase and alkaline phosphatases in each of S. *littoralis* selected to deltamethrin presented in Table (4) showed the changes in ACP and ALP activity of resistance strain to deltamethrin. The highest level of the enzyme activity was found in the sixth generation (65.97 and 21.92) of resistant strain compared to (3.78 and 1.86) of field strain for ACP and ALP, respectively.

Glutathione reduced activity (GSH) data given in (Table 4) showed that the resistant strain of deltamethrin recorded (79.25) for the 6th generation compared to the susceptible strain was (14.56) and field strain was (19.38).

2.2. Spinetoram-Resistant (SPT) Strain

It is clearly seen that there were a significant increase of α-Esterases and β-esterase activity in 4th instar larvae of *S. littoralis* treated with spinetoram (Table 4). The highest increase in α-Esterases and β-esterase (4024.44 and 2343.25) were recorded in G6 compared to the susceptible strain was (12.61 and 16.88), field strain was (447.89 400.61) for both α-Esterases and β-esterase, respectively.

As for the data showed increase in results AChE in spinetoram selected strain. A higher level of AChE in G_6 of resistant strain (3068.81), compared to the susceptible strain was (35.85), field strain was (516.92).

Table (3): Enzymatic activity levels of esterases, phosphatases (α-Esterase, β-Esterase, AChE, ACP, ALP), and reduced glutathione (GSH) of the 6th-*S.littoralis* instar larvae from SUS-LAB, MNF and DMT-developed resistant strains.

Selection*	α-Esterase	β-Esterase	Acetylcholin-esterase (AChE)	Acid Phosphatase (ACP)	Alkaline Phosphatase (ALP)	Glutathione Reduced (GSH)
SUS-LAB	12.61±0.73^{d}	16.88±1.02^{d}	35.85±2.07^{e}	2.10 ± 0.05^{d}	0.86± 0.03^{c}	14.56±0.40^{e}
MNF (G_0)	447.89±1.78^{c}	400.61±2.12^{c}	516.93±2.44^{d}	3.78 ± 0.28^{c}	1.86 ± 0.03^{c}	19.38±0.71^{d}
G_1	505.16±1.21^{c}	418.53±3.06^{c}	692.54±1.02^{c}	4.92 ± 0.12^{c}	1.55 ± 0.05^{c}	23.30±1.02^{c}
G_3	2210.06±0.49^{b}	1779.92±1.68^{b}	1565.7±2.52^{b}	16.13 ± 1.11^{b}	5.47± 0.17^{b}	28.03±1.23^{b}
G_6	5542.64±1.38^{a}	4132.30±2.40^{a}	7151.3±3.03^{a}	65.97 ± 3.25^{a}	21.92±0.67^{a}	79.25±3.47^{a}
LSD	1.597	1.357	1.597	1.117	1.695	1.597

Data are expressed as mean ± SE (n=5). Data are analyzed by one-way ANOVA. Means with the same letter are not significantly different (P < 0.05).

* SUS-LAB, Susceptible Laboratory strain; MNF, Menoufia-field strain; G1-G6, Generations subjected to six selected pressure with Deltamethrin (DMT).

Table (4): Enzymatic activity levels of esterases, phosphatases (α-Esterase, β-Esterase, AChE, ACP, ALP), and reduced glutathione (GSH) of the 6th-*S.littoralis* instar larvae from SUS-LAB, MNF and SPT-developed resistant strains

Selection*	α-Esterase	β-Esterase	Acetylcholin-esterase (AChE)	Acid Phosphatase (ACP)	Alkaline Phosphatase (ALP)	Glutathione Reduced (GSH)
SUS-LAB	12.61±0.73^{d}	16.88±1.02^{d}	35.85±2.07^{e}	2.10 ± 0.05^{d}	0.86± 0.03^{c}	14.56±0.40^{e}
MNF (G_0)	447.89±1.78^{c}	400.61±2.12^{c}	516.93±2.44^{d}	3.78 ± 0.28^{c}	1.86 ± 0.03^{c}	19.38±0.71^{d}
G_1	526.67±1.15^{c}	386.66±2.08^{d}	605.57±1.70^{c}	4.43±0.30^{c}	1.21±0.04^{cd}	19.45±1.04^{c}
G_3	720.89±1.91^{b}	830.13±1.02^{b}	1103.93±1.75^{b}	8.86±0.61^{b}	2.48±0.08^{b}	23.53±0.78^{b}
G_6	4024.44±3.35^{a}	2343.25±2.30^{a}	3068.81±4.35^{a}	28.95±1.35^{a}	7.73±0.24^{a}	65.13±1.26^{a}
LSD	1.304	1.494	1.433	1.562	0.585	0.286

Data are expressed as mean ± SE (n=5). Data are analyzed by one-way ANOVA. Means with the same letter are not significantly different (P < 0.05).

* SUS-LAB, Susceptible Laboratory strain; MNF, Menoufia-field strain; G1-G6, Generations subjected to six selected pressure with Spinetoram (SPT).

As well as for testing the resistant strain to spinetoram was increased of the activity of (ACP and ALP) in the resistant strain (G_6) was (28.95 and 7.73) compared to the susceptible strain was (2.10 and 0.86) for both ACP and ALP, respectively.

Similarly, a significant increase detected in 4th instar larvae of *S. littoralis* treated with spinetoram, the highest increase in GSH content (4.47-fold) were recorded in G_6 (Table 4).

2.3. Pyridalyl-Resistant (PYD) Strain

As well as for testing the resistance strain to pyridalyl in (Table 5) was results increased of the activity of α-esterases and β-Estrase in the resistant strain. The highest increase in α-esterases was recorded in G_6 (4223.64) compared to the susceptible strain was (12.61), field strain was (447.89). Also, the results given β-esterases were similar to that obtained from α-esterases.

Data in Table (5) showed increase in the activity of AChE in pyridalyl selected strain. A higher level of AChE in G_6 of resistant strain (1580.81), compared to the susceptible strain was (35.85), field strain was (516.92).

The resulted in Table (4) showed changes in the activity of ACP and ALP of pyridalyl resistance strain. The highest level of the enzyme activity was found in G_6 of resistant strain (17.61 and 17.08-fold) compared to the susceptible strain.

GSH data given in (Table 5) showed that the resistant strain of pyridalyl recorded (4.99-fold) for the 6th generation compared to the susceptible strain.

2.4. Indoxacarb-Resistant(INC) Strain

Data represented in Table (6) showed that there were a significant increase of α-Esterases and β-esterase activity in 4th instar larvae of *S. littoralis* treated with indoxacarb. The highest increase in α-Esterases and β-esterase (3641.32 and 1294.04) were recorded in G_6 compared to the susceptible strain was (12.61 and 16.88), field strain was (447.89 400.61) for both α-Esterases and β-esterase, respectively.

Likewise, a significant increase in detected in 4th instar larvae of *S. littoralis* in Indoxacarb selected strain. The highest increase in AChE (1415.20) were recorded in G6 compared to the susceptible and field strain (Table 6).

Data in Table (6) showed that significantly increased in the activity of (ACP and ALP) to indoxacarb especially in 6th generation (71.07 and 27.05, respectively) compared to the susceptible strain and field strain.

Similarly, a significant increase detected in 4th instar larvae of *S. littoralis* treated with indoxacarb. The highest increase in GSH content (6.61-fold) were recorded in G_6 (Table 6).

The detoxification enzymes, which include the esterase enzymes, remove all foreign substances from an insect's body. Esterase is one of the enzymes that strongly respond to environmental stimulation (Hemingway and Karunaratne, 1998). In addition, treatment with all selected compounds boosted the non-specific esterases activity compared to the control. Their excessive activity might signify resistance development and the insect's reaction to bodily poisoning (Serebrov *et al*., 2006; Chen *et al.,* 2017; Ahmed and Freed, 2021).

Table (5): Enzymatic activity levels of esterases, phosphatases (α-Esterase, β-Esterase, AChE, ACP, ALP), and reduced glutathione (GSH) of the 6th-*S.littoralis* instar larvae from SUS-LAB, MNF and PYD-developed resistant strains

Selection*	α-Esterase	β-Esterase	Acetylcholin-esterase (AChE)	Acid Phosphatase (ACP)	Alkaline Phosphatase (ALP)	Glutathione Reduced (GSH)
SUS-LAB	12.61±0.73^{d}	16.88±1.02^{d}	35.85±2.07^{e}	2.10 ± 0.05^{d}	0.86± 0.03^{c}	14.56±0.40^{e}
MNF (G_0)	447.89±1.78^{c}	400.61±2.12^{c}	516.93±2.44^{d}	3.78 ± 0.28^{c}	1.86 ± 0.03^{c}	19.38±0.71^{d}
G_1	336.76±2.62^{d}	298.25±3.60^{d}	496.83±1.99^{d}	4.46±0.31^{c}	1.77±0.02^{c}	24.72±1.08^{c}
G_3	912.09±3.53^{b}	803.36±2.71^{b}	809.23±2.01^{b}	10.36±0.40^{b}	4.64±0.14^{b}	29.01±1.27^{b}
G_6	4223.64±3.06^{a}	2953.70±3.64^{a}	1580.81±2.42^{a}	36.98±1.31^{a}	14.69±0.45^{a}	72.74±3.19^{a}
LSD	1.599	1.956	2.396	1.562	0.292	1.456

Data are expressed as mean ± SE (n=5). Data are analyzed by one-way ANOVA. Means with the same letter are not significantly different (P < 0.05).

* SUS-LAB, Susceptible Laboratory strain; MNF, Menoufia-field strain; G1-G6, Generations subjected to six selected pressure with Pyridalyl (PYD).

Table (6): Enzymatic activity levels of esterases, phosphatases (α-Esterase, β-Esterase, AChE, ACP, ALP), and reduced glutathione (GSH) of the 6th-*S.littoralis* instar larvae from SUS-LAB, MNF and INC-developed resistant strains

Selection*	α-Esterase	β-Esterase	Acetylcholin-esterase (AChE)	Acid Phosphatase (ACP)	Alkaline Phosphatase (ALP)	Glutathione Reduced (GSH)
SUS-LAB	12.61±0.73^{d}	16.88±1.02^{d}	35.85±2.07^{e}	2.10 ± 0.05^{d}	0.86± 0.03^{c}	14.56±0.40^{e}
MNF (G_0)	447.89±1.78^{c}	400.61±2.12^{c}	516.93±2.44^{d}	3.78 ± 0.28^{c}	1.86 ± 0.03^{c}	19.38±0.71^{d}
G_1	392.89±3.39^{d}	322.30±3.90^{d}	533.29±2.90^{c}	8.13±0.26^{c}	1.55±0.05^{c}	28.49±1.36^{c}
G_3	1276.93±2.55^{b}	683.10±2.26^{b}	733.96±1.80^{b}	24.84±1.38^{b}	8.50±0.26^{b}	34.04±1.49^{b}
G_6	3641.32±5.06^{a}	1294.04±3.64^{a}	1415.20±2.08^{a}	71.07±2.99^{a}	27.05±0.83^{a}	96.27±4.22^{a}
LSD	1.321	1.419	2.728	1.562	0.363	1.503

Data are expressed as mean ± SE (n=5). Data are analyzed by one-way ANOVA. Means with the same letter are not significantly different (P < 0.05).

* SUS-LAB, Susceptible Laboratory strain; MNF, Menoufia-field strain; G1-G6, Generations subjected to six selected pressure with Indoxacarb (INC).

Acetylcholinesterase is key physiological role in the turnover of the neurotransmitter acetylcholine. This enzyme is found in, or attached to, cellular or basement membrane of presynaptic cholinergic neurons. Abd EI-Mageed and Shalaby (2011) have found similar results in the same insect using IGR's. Spinetoram generated a moderate increase in acetylcholinesterase activity by (El-Barky et al., 2008, Fahmy and Dahi 2009 and Rashwan, 2013).

Acid phosphatase is known as lysosomal maker enzyme and active in guts (Ferreira and Terra 1980, Csikos and Sass 1997). Alkaline phosphatase is especially active in tissue with active membrane transport, such as intestinal epithelial cells and Malpighian tubules (Ferriera and Terra 1980). So could be used as parameter for determine antifeeding activity (Abd-El aziz, 2000). These results are in agreement with those obtained on *S. littoralis* by (Hamadah *et al.,* 2016) using novel chitin synthesis inhibitors with significant increase activities of acid (ACP) and alkaline (ALP) phosphatases in two larval tissues of *S. littoralis*. Also, increase in the activity of acid phosphatase in the same insect by (Sokar, 1995).

The most abundant intracellular antioxidant, glutathione reduced (GSH), is involved in the protection of cells against oxidative stress (Shi *et al.,* 2015). GST also plays a vital role in stress physiology which catalyze the conjugation GSH with numerous compounds containing an electrophilic center and have been implicated in intracellular transport and various biosynthetic pathways (Wilce and Parker, 1994). The present study showed that deltamethrin, spinetoram, pyridalyl and indoxacarb induced an increase in GSH activity. Pesticide tolerance or resistance is caused by detoxifying enzymes such as Glutathione-S-transferases activated by insecticide exposure (Vojoudi *et al.,* 2017).

3. Total Nutrient Contents of Insects

Effects on total protein, total carbohydrate, and total lipid contents

Data in Table (7) showed the effects of deltamethrin, Spinetoram, Pyridalyl and indoxacarb insecticides on main insect metabolites such as total protein, total carbohydrate and total lipid after treatment of the 6th instar larvae of *S. littoralis* for six generations. Results indicated that the four tested insecticides induced highly significant reduction in the total protein contents especially in 6th generation (236.10, 277.27, 286.67 and 255.30 for DMT, SPT, PYD and INC, respectively) compared with SUS-LAB strain (320.53).

Table (7): Total content of proteins, carbohydrates and lipids of the 6th-*S.littoralis* instar larvae from SUS-LAB, MNF and developed resistant strains

Selection*	Total protein	Total carbohydrates	Total lipids
DMT Strain			
SUS-LAB	320.53±2.64^{a}	423.00±2.51^{a}	369.00±2.44^{a}
MNF	282.53±1.95^{e}	349.77±2.28^{g}	257.20±3.60^{i}
G_1	284.43±2.98^{de}	374.13±2.95^{c}	310.73±1.96^{d}
G_3	264.47±1.49^{h}	364.80±2.38^{e}	284.23±2.51^{f}
G_6	236.10±2.52^{j}	344.30±1.37^{h}	267.30±2.37^{g}
SPT Strain			
G_1	281.57±3.77^{e}	370.63±2.39^{d}	336.67±2.63^{b}
G_3	263.50±2.08^{h}	329.80±1.16^{i}	322.00±2.91^{c}
G_6	277.27±2.46^{f}	309.50±2.15^{j}	243.07±2.22^{j}
PYD Strain			
G_1	288.60±0.46^{c}	356.97±2.95^{f}	263.57±2.04^{h}
G_3	273.07±2.58^{g}	309.80±2.81^{j}	241.07±2.87^{j}
G_6	286.67±1.14^{cd}	264.60±2.91^{k}	226.77±1.92^{k}
INC Strain			
G_1	293.40±1.50^{b}	406.17±3.29^{b}	335.17±2.69^{b}
G_3	282.27±1.21^{e}	367.47±2.27^{de}	311.70±2.32^{d}
G_6	255.30±2.59^{i}	329.87±1.50^{i}	292.30±2.76^{e}
LSD	2.932	3.618	3.818

*Data are expressed as mean ± SE (n=14). Data are analyzed by one-way ANOVA Procedure. Means with the same letter are not significantly different ($P < 0.05$).

* SUS-LAB, Susceptible Laboratory strain; MNF, Menoufia-field strain; G1-G6, Generations subjected to six selected pressure with Deltamethrin (DMT), Spinetoram (SPT), Pyridalyl (PYD) and Indoxacarb (INC).

Similarly, treatment of the 6th-instar larvae of *S. littoralis* with the four tested insecticides for six generations, significantly decreased in total carbohydrate contents. Mean values of this reduction were 264.60 mg/ml for Pyridalyl, followed by 309.50 mg/ml for spinetoram, 329.87 for indoxacarb and 344.30 mg/ml for deltamethrin as compared with susceptible strain (423 mg/ml).

The four tested compounds caused highly significant reduction in lipid contents as compared with control. Obviously, treatment with DMT recorded 267.30 mg/ml while SPT, PYD and INC were 243.07, 226.77 and 292.30 mg/ml, respectively compared with susceptible strain (369 mg/ml).

In general, the main components required for an organism to develop, grow, and carry out its essential functions are lipids, total proteins, and carbohydrates. Changes in the insect's energy stores, such as proteins, lipids, and carbohydrates, show how susceptible it is to insecticides and how their effects may impact its functionality.

The level of protein synthesis, protein breakdown, and even water movement between tissues all affect the amount of protein in a

larvae's body. The results presented here demonstrated that, treatment the 6th instar larvae of *S. littoralis* with selected to deltamethrin, Spinetoram, Pyridalyl and indoxacarb compared with the susceptible and Field strain caused a significant decrease in the protein, carbohydrate and lipid contents. Changes in energy stores, including carbohydrates, lipids, proteins, and glycogen, reflect the insect's sensitivity to insecticides and variations in its function (Piri *et al.,* 2014).

In agreement results were obtained by El-Barky et al. (2008), who estimated reduction in carbohydrate content of 4th instar larvae of *S. littoralis* after treatment with spinosad compared to untreated control. Spinosad treatments significantly decreased the total protein and carbohydrate contents in the 6th-instar larvae of *S. littoralis* (El-Sheikh, 2012). In addition, the total protein content significantly decreased in imidacloprid-treated larvae of *S. littoralis* when compared with control (El-Saleh *et al.,* 2016).

In addition, the lower protein level may result from the degradation of protein into amino acids, which, entering the tricarboxylic acid (TCA) cycle as keto acids, will assist in providing energy for the insect. Therefore, protein depletion in tissues may be a physiological process and play a role in compensatory mechanisms during insecticidal stress by preserving the free amino acid content in hemolymph to supply intermediates for the TCA cycle (Nath *et al.,* 1997).

A mechanical formation of lipoprotein that will be uses to repair damaged cells, tissues, and organs could be the cause of the decrease in protein content (Saravana Bhavan & Geraldine, 2001; Ribeiro *et al.,* 2001; Mosleh *et al.,* 2003). Also, the reduction of protein level might be due to the destructive effect on some of the cerebral neurosecretory cells of the brain responsible for secretion of the protein of the treated larval instars of *S. littoralis* (Hamouda and Dahi, 2008) who proved that spintoram has a neurotoxic effect manifested as defined in histopathological changes in nerve and neurosecretory cells of *S. littoralis*.

Lipids are important structural component of cell membrane and cuticle. They supplied a rich source of metabolic energy. The obtained results declare that the four tested insecticides caused a highly significant decrease in lipid contents as compared with susceptible strain. The breakdown of lipids into simpler moieties that could be utilized as a carbon source for growth may be the cause of the significant decrease in total lipids. According to Bennett and Shotwell (1972), infected larvae may generate an enzyme that uses lipids as a source of energy.

CONCLUSION

Based on the findings of the current study, it can be concluded that there was a significant increase in resistance levels when the insect was subjected to selection pressure by different insecticides deltamethrin, spinetoram, pyridayl and Indoxacarb pressure generation after six generations. The results demonstrated that the development of resistance to deltamethrin was the most increased and exceeded resistance, the resistance levels significantly increased, 43.79 times at G_6, Followed by Indoxacarb 33.39- fold at G_6. In addition, there was a significant increase in levels of α&β-EST, AChE, ACP, ALP and GSH activity at the end of selection with these insecticides in all selected generations. The assessment of the activity of these enzymes may be useful for monitoring resistance to these insecticides in *S. littoralis*. Our results also showed that, treatment of *S. littoralis* with DMT, SPT, PYD and INC compared with the susceptible strain caused a significant decrease in the protein, carbohydrate and lipid contents.

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تطور المقاومة لأربع مبيدات، وزيادة نشاط إنزيمات إزالة السمية في حشرة دودة ورق القطن (*Spodoptera littoralis*)

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[٢] معهد بحوث وقاية النبات، مركز البحوث الزراعية، الدقي، مصر.

الملخص العربي

تعتبر دودة ورق القطن واحدة من أهم الآفات المدمرة للعديد من محاصيل الزينة والخضروات في مصر. تم دراسة تطور صفة مقاومة دودة ورق القطن لأربعة مبيدات حشرية (دلتامثرين، سبينوترام، بيرادليل- واندوكساكارب) ف المعمل. تم تعريض العمر اليرقي الرابع لدودة ورق القطن لضغط انتخابي من المبيدات المختبرة لمدة ستة أجيال متتالية باستخدام طريقة غمر الأوراق، حيث أوضحت النتائج حدوث زيادة كبيرة في مستوى المقاومة وصلت لـ ٤٣,٧٩، ٢٩,٦٩، ١٣,٠٩ و٣٣,٣٩ ضعف لكلا من مبيد دلتامثريت، سبينوترام، بيرادليل واندوكساكارب، على التوالي بالمقارنة بالسلالة الحساسة. أوضحت نتائج نشاط انزيمات إزالة السموم في نهاية تجربة الضغط الانتخابي حدوث زيادة معنوية في نشاط انزيمات الفا، بيتا-استريز، انزيم الاستيل كولين وانزيمات الفوسفات الحامضي والقاعدي، بالمقارنة بالسلالة الحساسة والسلالة الحقلية. أوضحت النتائج أيضاً حدوث نقص معنوي في معدل البروتين، الكربوهيدرات والدهون الكلية بالمقارنة بالسلالة الحساسة.