J. Plant Prot. and Path., Mansoura Univ., Vol.5 (5): 613-632, 2014

DEVELOPMENT OF RESISTANCE TO SOME INSECTICIDES AND ITS RELATION TO SOME BIOCHEMICAL CHANGES IN *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The development of resistance and biochemical mechanism of the cotton leafworm, *Spodoptera littoralis* (Boisd.), to five insecticides (chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron) were studied in the laboratory. The parent strain of *S. littoralis* was collected from El-Fayom Governorate at the cotton season 2005 and reared under laboratory conditions for seven generations, then subdivided into five sub-strains, three of them were selected by chlorpyrifos, profenofos and cypermethrin for fourteen generations and two were selected by spinosad and flufenoxuron for twelfth generations at LC_{30} level. Selection pressure in all experiments was carried out on 4th instar larvae by the leaf dipping technique. At the end of selection, the results indicated that the resistance ratios (RR) were 16.30-, 40.23-, 1070.42-, 145.14- and 8.24-fold for chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron, respectively, compared with the parent strain. At the end of selection pressure, detoxifying enzyme assays revealed that the α-esterase activity levels for such insecticides were 4.64, 4.67, 3.10, 3.40 and 1.24 times, respectively, higher than in the parent strain whereas those of β-esterase activity were 4.16, 4.07, 3.11 and 3.64 times for chlorpyrifos, profenofos, cypermethrin and spinosad, respectively, higher than in the parent strain, on the contrast the β-esterase activity for flufenoxuron was 0.96 time lower than in the parent strain. In addition, the levels of glutathione S-transferase (GST) activity at the end of selection with these insecticides were 6.62, 6.43, 2.11, 4.69 and 1.32 times, respectively, higher than in the parent strain. The results showed a correlation between the activity of both non-specific esterases (α- and β-esterases) and GST and resistance level to the tested insecticides. The results, also, indicated that the broad spectrum of insecticide observed in the field populations was due to multiple resistance mechanisms, including their increased detoxification. Finally, the rapid assessment of esterases and GST activities may be useful for monitoring resistance to these insecticides in *S. littoralis*. Therefore, differential levels of such enzymes could likely be used to detect the development of resistance during the early stages of insecticide resistance in the field.

Keywords: *Spodoptera littoralis*, Insecticide resistance, Non-specific esterases, Glutathione S-transferase.

INTRODUCTION

The cotton leafworm, *S. littoralis* (Boisd.), is a major polyphagous pest and is considered one of the most dangerous pest attacking cotton plants and more than 29 hosts from other crops and vegetables of economic importance in Egypt (Magd El-din and El-Gengaihi 2000). Their infestation rates can reach up to 50,000 egg-masses/acre, causing severe damage to leaves, buds, flowers and bolls (Temerak 2002). Therefore, various insecticides from organochlorine, organophosphate, carbamate, pyrethroid,

antichitin synthesis chemicals and new chemistry classes were introduced to control this pest of in the field.

The widespread and intensive use of insecticides and their spray cocktails the life cycle of this insect which has not hibernation period and its destructive feeding habits encourage this pest to develop resistant to most of the conventional insecticides (Ezz El-Din *et al.* 2009) and insect growth regulators (IGRs) (El-Guindy *et al*. 1983 and 1989; Abo-Elghar and Hussein 1992) registered for its control. Resistance to insecticides was diagnosed in Egypt for several years in colonies of the cotton leafworm, *S. littoralis* by several investigators (El-Guindy *et al*. 1982; Keddis *et al*. 1988; Ghoneim *et al*. 1994, 2002 and 2012; Betana *et al.* 2000; Gamal *et al.* 2009).

Insecticide resistant involves mainly three mechanisms: decrease penetration (Ahmad and McCaffery 1999; Yu and Nguyen 1996), enhanced detoxification (Enayati *et al*. 2005; Ishaaya 1993) and target-site insensitivity (Soderlund and Knipple 2003; Li and Han 2004).

The target of the present work was to study the development of resistance of cotton leafworm, *S. littoralis* (Boisd), to some selected insecticides (chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron) of different mode of action throughout several successive generations of selection pressure. The organophosphate, chlorpyrifos and profenofos act as acetylcholinesterase inhibitors, the pyrethroid cypermethrin it act on the nervous system of the insect, disturb the function of neurons by interaction with sodium channel, spinosad appears to be unique, with a primary site of attack being the nicotinic acetylcholine receptor and a secondary site of attack being Gama Amino Bytyric Acid (GABA) receptors and the insect growth regulator flufenoxuron that interfere with insect growth and development by inhibiting chitin synthesis in insect. Moreover, the correlation between some biochemical changes in *S. littoralis* strains selected with the tested insecticides as α- and β-esterases as well as GST activities with the resistance levels was also investigated.

MATERIALS AND METHODS

Strain of cotton leafworm

The strain of *S. littoralis* (Boisd.) used in this study was obtained from Plant Protection Institute, Agriculture Research Center, Dokki, Giza, Egypt. This strain originally collected from cotton fields of Fayoum Governorate in 2006. It was reared ever since free from any insecticide contamination.

Before selections started, the strain was reared in our laboratory under constant conditions of 25 ± 2 ^{O}C, 65 ± 5 % relative humidity and photoperiod (12:12 light: dark), for seven successive generations absence of insecticides contamination at the Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt as described by Eldefrawi *et al*. (1964).

The pesticides used

Organophosphates

Chlorpyrifos (Dursban® 48% EC) Dow AgroSciences Co.

Profenofos (Curacron® 72% EC) Syngenta Agro Co.

Pyrethroids

Cypermethrin (Cyperco® 20% EC) United Phosphorus Co.

Spinosyn

Spinosad (SpinTor® 24% SC) Dow AgroSciences Co.

Chitin synthesis inhibitors

Flufenoxuron **(**Cascade® 10% DC) BASF Agro Co.

Bioassay of insecticides.

Sensitivity of the strain to insecticides

The sensitivity of the parent *S. littoralis* strain was measured by determination of LC_{50} values of the tested insecticides using leaf dipping technique. The insecticide concentrations were prepared by water dilution. The fresh castor bean leaves were dipped in the prepared insecticide concentrations for 20 seconds, then air-dried at room temperature. After drying, the 4th instar larvae of 40±5 mg average body weight were placed into glass jar (0.5 liter) and provided with treated castor bean leaves, covered with muslin cloth. Check control larvae were fed on untreated leaves. Six different concentrations for each tested insecticide were used. Four replicates of ten larvae each were used for each concentration. The larvae were allowed to feed on treated leaves for 24 hours under laboratory conditions. Mortality counts were recorded after 24 hours of exposure to treated leaves for chlorpyrifos, profenofos and cypermethrin, after 48 hours for spinosad and after 72 hours for flufenoxuron.

Mortality percentages were corrected according to Abbott's formula (1925). LC_{50} and slope values were determined by probit analysis program according to (Finney 1971).

Selection procedure

After rearing in the laboratory for seven generations under free insecticidal contaminations, the 4th instar larvae of *S. littoralis* were selected for development of resistance.

Selection in all experiments was carried out using calculated LC_{30} values of the tested insecticides. About 2000 larvae were subjected to selection pressure in each generation. The survived larvae were reared to complete their development, and the emerged 4th instar larvae were subjected to different concentrations of each insecticide. The LC_{50} values for each generation were estimated as mentioned in sensitivity test. Higher selection concentrations were used in subsequent generations with increasing the resistance levels. The resistance ratio for each generation was calculated by using the following equation:

$$\text{Resistance Ratio (RR)} = \frac{LC_{50} \text{ of the selected strain}}{LC_{50} \text{ of the parent strain}}$$

Biochemical analysis

Enzyme preparation

Ten larvae of *S. littoralis* were homogenized using glass homogenizer at 4 ^{O}C in 3 ml homogenization buffer pH 7.8 containing 50 mM Tris, 15% glycerol, 10 mM ethylene diaminetetra-acetic acid (EDTA), and 0.005% phenylthiourea, KOH or KH_2PO_4 solution was used for the

adjustment of pH to 7.8. The homogenate was centrifuged at 5000 r.p.m. at 4 ^{O}C for 15 min. The supernatant fraction was used for determining activities of α- and β-esterases and glutathione S-transferase (GST).

Protein contents assay

Protein contents of the enzyme homogenate were determined in the 4th instar larvae of *S. littoralis* by using diagnostic kit produced by Diamond Company according to the method described by Young (2001). The measurement was performed with the wavelength of 550 *nm* by Jenway 6105 spectrophotometer.

Non-specific esterases assay

The activities of total esterases were measured in 4th instar larvae using the procedure described by Van Asperen (1962), using α- and β-naphthyl acetate as substrates which are hydrolyzed by esterase enzymes to form α- and β-naphthol. The produced α- and β-naphthol is converted, by adding diazoblue B sodium lauryl sulphate (Diazblue-SDS) solution, to strong blue and red colors which may be spectrophotometrically measured at 600 and 550 *nm* wavelength, respectively. The esterase activity was calculated using the extinction coefficient according to Grant *et al*. (1989). The specific esterases activities were expressed as nMole/min/mg protein.

Glutathione S-transferase assay

Glutathione S-transferase activity was measured in the 4th instar larvae using assay procedure of Grant *et al*. (1989). Activity was measured by catalysing the reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) to form S-(2,4-dinitrophenyl) glutathione which absorbs light at 340 *nm*.

For assay, 2 ml GST substrate buffer (prepared by mixing 75 ml glycerol and 6.82 g KH_2P0_4 per 500 ml of distilled water, pH 6.8) and 400 ml CDNB solution (prepared by dissolving 45.6 mg CDNB in 100 ml GST substrate buffer) were transferred to cuvette using a pipet. 50 µl of larvae homogenate were added and then 50 ml of GSH solution (pH 7.8) (prepared by dissolving 79.9 mg GSH + 2.42 mg Tris + 0.31 mg dithiothritol in 1 ml 15% glycerol) were added. The cuvette was equilibrated at room temperature for 15 min and the change in absorbance was measured at 340 *nm* by Jenway 6105 spectrophotometer for 10 minutes against blank prepared from substrate buffer, CDNB solution and GSH solution. Specific GST activity was determined as nMole/min/mg protein using the extinction coefficient for CDNB at 340 nm (9.6 mM/ml).

Statistical analysis

Data for biochemical analysis were performed to one way analysis of variance (ANOVA) by using Costat program (1998) and significant differences among the means values were determined according to (Duncan 1955) multiple range test at probability levels of P = 0.05. The correlation between the changes of enzymes activities and resistance ratio was calculated at 5% level. Relationship between enzyme activities and resistance ratio were estimated using the SigmaPlot (version 10.0) Software.

RESULTS AND DISCUSSION

Development of resistance

The development of resistance for parent strain of *S. littoralis* 4th instar larvae that exposed to the selection pressure with the tested insecticides: chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron at their LC_{30} values for several successive generations is shown in Tables (1 and 2).

Development of resistance to chlorpyrifos

The results listed in Table (1) clearly showed that, the resistance ratio for chlorpyrifos slightly increased from 1.31-fold in G_1 to 3.27-fold in G_4, followed by a decrease in G_5 and G_6 showing resistance ratio of 3.16- and 1.92-fold, respectively, then remained nearly constant until G_9. The resistance ratio value regained its increase in G_{10}, and then steadily shifted to higher levels until it reached 16.30-fold at the end of selection course in G_{14}. The slope values of regression lines between logarithm concentration and mortality probit value remained nearly similar from G_1 to G_9 which ranged from 2.50 to 4.26 indicating the homogeneity of the strain under investigation to selection with chlorpyrifos. With continuous selection, the slope values unexpectedly increased at G_{10}, G_{11}, and G_{12}, showing 6.62, 5.72 and 5.33, respectively, it means an increase in homogeneity of the strain. On the other hand, the slope of the last selected generation (G_{14}) markedly decreased showing 2.93.

Development of resistance to profenofos

Data shown in Table (1) indicated that resistance ratio (RR) values increased gradually from 1.71-fold (G_1) to 36.11-fold in G_8. Resistance ratios of G_9-G_{12} fluctuated between 34.38- to 37.92-fold then increased to 38.96- and 40.23-fold in G_{13} and G_{14}, respectively. The slope values of most generations ($G_{1, 2, 5, 7, 8, 9, 12, 13}$) fluctuated from 3.00 to 3.93. However, $G_{3, 4, 6, 11}$ exhibited differential slope values of 5.00, 4.33, 7.5 and 5.12, respectively. As resistance was progressed, the mortality line became steeper in G_{14} indicating more homogeneity toward resistance to profenofos at the end of the selection period.

Development of resistance to cypermethrin

As shown in Table (1), the values of resistance ratio (RR), increased gradually during the first eight generations to reach 227.58-fold in G_8, then increased rapidly recording 459.54- and 608.50-fold in G_9 and G_{12}, respectively. At the end of selection in G_{14} a sharp increase in RR (1070.40-fold) was observed. When parent strain was selected with cypermethrin, different slope values were recorded. The slope values slightly decreased to 3.15 and 2.67 during G_1 and G_2 but it further increased in G_3 and G_4 recording 3.64 and 3.92, respectively. During G_5-G_8 the slope values underwent a degree of shallowness and became steeper in G_9. With the completion of selection the slope values recording 3.33 at the end ofselection in G_{14}. This pattern of changes in slope is typical resource of mortality regression lines undergoing true resistance.

t1

Development of resistance to spinosad

Data shown in Table (2) revealed that, the resistance ratio (RR) value jumped from 2.92-fold in G_1 to 9.28-fold in G_2, then increased to be 12.53-fold in G_3. The ratio was slightly stable from G_4 to G_5. Staring from G_{10} the resistance ratio consecutively increased recording 29.10-fold in G_6 and 145.14-fold at the end of the selection course in G_{12}. The slope values of the mortality regression lines decreased a long the course of selection with the spinosad compared with the parent strain. The slope values of the plotted toxicity regression lines for spinosad was 4.17 for the parent strain and ranged between 1.36 (G_4) and 3.92 (G_{12}) when the parent strain was selected with spinosad. The low slope values of the log dose-probit lines in all generations compared with the parent strain indicated the heterogeneity of selection strain toward spinosad.

Table (2): Rate of development of resistance to spinosad and flufenoxuron in 4th instar larvae of *Spodoptera littoralis* during selection with spinosad and flufenoxuron for 12 successive generations.

Generations	spinosad			flufenoxuron		
	LC_{50} (ppm)	Slope (± SE)*	RR**	LC_{50} (ppm)	Slope (± SE)*	RR**
Parent-strain	6.73	4.17 ± 0.47	1.00	0.46	2.74 ± 0.31	1.00
G_1	19.65	2.57 ± 0.29	2.92	0.55	2.64 ± 0.30	1.20
G_2	62.43	2.75 ± 0.31	9.28	0.34	2.44 ± 0.28	0.74
G_3	84.35	3.22 ± 0.36	12.53	0.56	2.23 ± 0.25	1.21
G_4	137.50	1.36 ± 0.15	20.43	0.59	2.48 ± 0.28	1.28
G_5	143.15	2.90 ± 0.33	21.27	0.94	2.65 ± 0.30	2.04
G_6	195.85	1.75 ± 0.20	29.10	0.32	3.20 ± 0.36	0.70
G_7	266.33	3.08 ± 0.35	39.57	0.77	2.61 ± 0.29	1.68
G_8	343.11	3.39 ± 0.38	50.98	1.96	2.57 ± 0.29	4.26
G_9	413.68	2.83 ± 0.32	61.47	2.47	3.03 ± 0.34	5.37
G_{10}	562.25	2.50 ± 0.28	83.54	3.15	2.63 ± 0.30	6.85
G_{11}	751.65	3.87 ± 0.44	111.69	3.19	2.61 ± 0.29	6.93
G_{12}	976.78	3.92 ± 0.44	145.14	3.79	2.64 ± 0.30	8.24

*SE: Standard error **Resistance Ratio = LC_{50} of Selected strain / LC_{50} of Parent strain.

Development of resistance to flufenoxuron

With the exception of G_5 the results listed in Table (2) clearly revealed that, the resistance ratio remained nearly constant through the first 7 generations of selection. The RR value regained its increase in G_8, and continuous this increase until it reached 8.24-fold at the end of the selection. The slope values of all the selected generations ranged from 2.23 to 2.65 excepting G_6 and G_9 which exhibited slope values of 3.20 and 3.03, respectively. These findings indicated of most selected generations are parallel, and that the strain under selection exhibited a homogenous pattern towards selection with flufenoxuron.

Biochemical analysis

Alpha- and Beta-esterases activities

The specific activities of both α- and β-esterases determined in different generations of 4th instar larvae of chlorpyrifos-, profenofos-, cypermethrin-, spinosad and flufenoxuron-resistant strains of *S. littoralis* are shown in Tables (3 and 4).

3-4

For chlorpyrifos-resistant strain, a significant increase in α-esterase activity was found after the second generation of selection as compared with the parent-strain as they exhibited activity levels of 124.46 and 81.30 nMole/min/mg protein, respectively. A slight increase was observed in G_5, G_7 and G_9 then significant increase to 230.19, 283.11, 322.65 and 377.70 nMole/min/mg protein in G_{11}, G_{12}, G_{13} and G_{14} were obtained, respectively (Table, 3). The β-esterase activity was significantly increased starting from G_5 (288.37 nMole/min/mg protein) as compared with the parent strain (134.30 nMole/min/mg protein). The activity was also increased significantly at G_9, G_{11}, G_{12}, G_{13} and G_{14} to reach 559.31 nMole/min/mg protein in G_{14} (Table, 4).

In the profenofos-resistant strain, no significant difference in α-esterase activity was detected during selected G_1 and G_2 (96.38 nMole/min/mg protein and 98.56 nMole/min/mg protein, respectively) as compared with that of the parent-strain (81.30 nMole/min/mg protein) (Table, 3). With the exception of G_{11} (216.80 nMole/min/mg protein), significant increases in activities of all the tested generations starting from G_5 (125.46 nMole/min/mg protein) until G_{14} (379.42 nMole/min/mg protein) were recorded. For β-esterase activity, similar results were recorded as no significant difference was detected in G_1 and G_2 of selection as compared with the parent-strain (134.30 nMole/min/mg protein). From G_5 a significant increase was observed in the enzyme activity, the levels of activity were 91.67, 227.89 and 326.25 nMole/min/mg protein in G_5, G_7 and G_9, respectively. In G_{11} the activity decrease to reach 306.20 nMole/min/mg protein, then increased significantly to 412.88, 470.15 and 546.21 nMole/min/mg protein at G_{12}, G_{13} and G_{14}, respectively (Table, 4).

For cypermethrin-resistant strain, α-esterase activity decreased in G_1 and G_2, showing 77.58 and 80.62 nMole/min/mg protein, respectively, comparing with that of the parent-strain (83.30 nMole/min/mg protein). A significant increase in enzyme activity was found after the 5^{th} generation of selection as compared with the parent strain. For example, the levels of activity were 104.56, 245.40 and 252.45 nMole/min/mg protein in G_5, G_{13} and G_{14}, respectively (Table, 3). For β-esterase activity, it increased in G_1 and G_2 showing 155.47 and 143.17 nMole/min/mg protein compared with the parent-strain (134.30 nMole/min/mg protein) with no significant differences between them (Table, 4). The activity of β-esterase significantly increased starting from G_5 (178.94 nMole/min/mg protein) and gave the maximum value of 417.88 nMole/min/mg protein at the end selection in G_{14}.

In the spinosad-resistant strain, the activity of α-esterase significantly decreased in G_1 and G_2 which reached 58.81 and 44.24 nMole/min/mg protein, respectively, compared with the parent-strain (81.30 nMole/min/mg protein) (Table, 3). Starting from G_9, the activity increased significantly recording a maximum value in G_{12} (276.52 nMole/min/mg protein). For β-esterase activity, results showed non significant decrease during the first five generations compared with the parent-strain. Then, significant increase in such activity was observed in the remained generations G_7, G_9, G_{11} and G_{12} showing 224.20, 207.79, 389.82 and 489.25 nMole/min/mg protein, respectively (Table, 4).

For flufenoxuron, the α-esterase activity of G_1 and G_2 did not differ significantly when compared with that of the parent strain. With further selection, such activity increased significantly ranging from 92.91 nMole/min/mg protein for G_7 to 100.74 nMole/min/mg protein for G_{12} (Table, 3). On the other hand, the β-esterase activity of flufenoxuron-resistant strain exhibited significant increase during the four generations G_1, G_2, G_5 and G_7 showing 151.2, 159.61, 151.50 and 146.53 nMole/min/mg protein, respectively. On the contrary, such activity significantly decreased in G_{11} and G_{12} showing 126.65 and 129.13 nMole/min/mg protein, respectively (Table,4).

The relationship levels between α-and β-esterases activities and resistance ratio for chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron are shown in Figs (1 and 2). In the case of α-esterase, the correlation coefficient values were 0.967, 0.864, 0.981, 0.954 and 0.864 for these insecticides respectively, Fig. (1). The corresponding correlation coefficient values for β-esterase were 0.957, 0.852, 0.986, 0.980 and -0.777 Fig. (2).

Glutathione-S-transferase (GST) activity

The specific activity of glutathione S-transferase in the 4th instar larvae of *S. littoralis* parent as well as chlorpyrifos-, profenofos-, cypermethrin-, spinosad- and flufenoxuron-resistant strains are shown Table (5).

In the chlorpyrifos-resistant strain, the activity of GST slightly increased during G_1 and G_2 (22.13 and 23.73 nMole/min/mg protein, respectively) comparing with the parent strain (15.23 nMole/min/mg protein) with no significant differences between them. Thereafter, the remained generations exhibited significant GST activities ranging between 39.41 nMole/min/mg protein for G_7 and 100.87 nMole/min/mg protein for G_{14} (Table, 5).

For profenofos-resistant strain, no significant differences were detected between GST activity of both selected G_1 and G_2 and that of the parent-strain. Starting from G_5 of selection, GST activity recorded significant increase ranging from 25.19 nMole/min/mg protein for G_5 to 97.90 nMole/min/mg protein for G_{14} (Table, 5).

In case of cypermethrin-resistant strain, the GST activity decreased significantly at G_1 showing 10.37 nMole/min/mg protein comparing with the parent-strain (15.23 nMole/min/mg protein). During G_2, G_5 and G_7 the GST slightly increased and remained nearly similar to that of the parent strain.
With further selection, the activity increased significantly during the other selected generations ranging from 23.64 nMole/min/mg protein for G_9 to 34.00 nMole/min/mg protein for G_{13} (Table, 5).

Based on the obtained results related to spinosad-resistance strain, the GST activity during the 1st two generation was nearly similar to that of the parent strain. A significant increase in the level of activity was proved at G_5 reaching 23.23 nMole/min/mg protein. Then a slight increase to 27.46 and 31.27 nMole/min/mg protein in G_7 and G_9 were obtained, respectively. At G_{12} the level of GST activity dramatically increased to 71.45 nMole/min/mg protein (Table, 5).

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For flufenoxuron-resistant strain, a significant increase in GST activity was found during G_1 (16.86 nMole/min/mg protein) as compared with the parent strain (15.23 nMole/min/mg protein). A slight decrease in the GST activity was observed in G_2 showing 14.98 nMole/min/mg protein. With further selection, GST activity significantly increased recording 16.43, 18.44, 19.65, 19.75 and 20.09 nMole/min/mg protein in G_5, G_7, G_9, G_{11} and G_{12}, respectively (Table, 5).

The correlation coefficient values for the tested insecticides chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron were 0.965, 0.791, 0.946, 0.981 and 0.881, respectively Fig (3).

The results of the present study clearly demonstrated that the development of resistance to flufenoxuron was rather slow and did not exceed 8.24-fold after 12 generations of selection. On the other hand, cypermethrin when used as a selective agent induced high level of resistance (1070.42-fold) after 14 generations of selection, while the spinosad induced (145.14-fold) in G_{12} at the end selection. The chlorpyrifos showed low level of resistance (16.30-fold) after 14 generations, whereas profenofos exhibited a medium level of resistance (40.23-fold) at the end of selection in G_{14} (Tables, 1 and 2). The results were in agreement with previous reports Allam *et al*. (1994) found that resistance ratio of *S. littoralis* was 9.7-fold after selection with chlorpyrifos for 12 generations. El-Sherif (1996) mentioned that selection of *S. littoralis* with profenofos induced high level of resistance (97.09-fold) compared with susceptible strain. After 23 generations of selection on 4^{th} instar larvae of *S. littoralis* with spinosad, the resistance ratio was 86.85-fold (Ezz El-Din *et al*. 2009). Wang *et al.* (2006) found that resistance to spinosad increased 345-fold compared with the susceptible strain after 5 generations of selection in *S. exigua*. The field strain of *S. littoralis* obtained from Gharbia governorate in Egypt in 2010 showed very high level of tolerance to esfenvalerate (168.1-fold) and profenofos (25.8-fold), slightly high level of tolerance to chlorpyrifos (14.8-fold), very low levels of tolerance for all the tested IGRs (ranged between 1.9- to 3.2-fold) except with flufenoxuron and triflumuron which exhibited moderate tolerance (5.5- and 6.0-fold, respectively) (Ghoneim *et al.* 2012). After 14 generations of selection with profenofos, Abass *et al*. (2014) indicated 52-fold resistance in *S. litura*. Kim *et al*. (1998) showed that resistance level in *S. litura* to cypermethrin, chlorpyrifos, chlorpyrifos-methyl were 2200-, 2- and 32-fold, respectively.

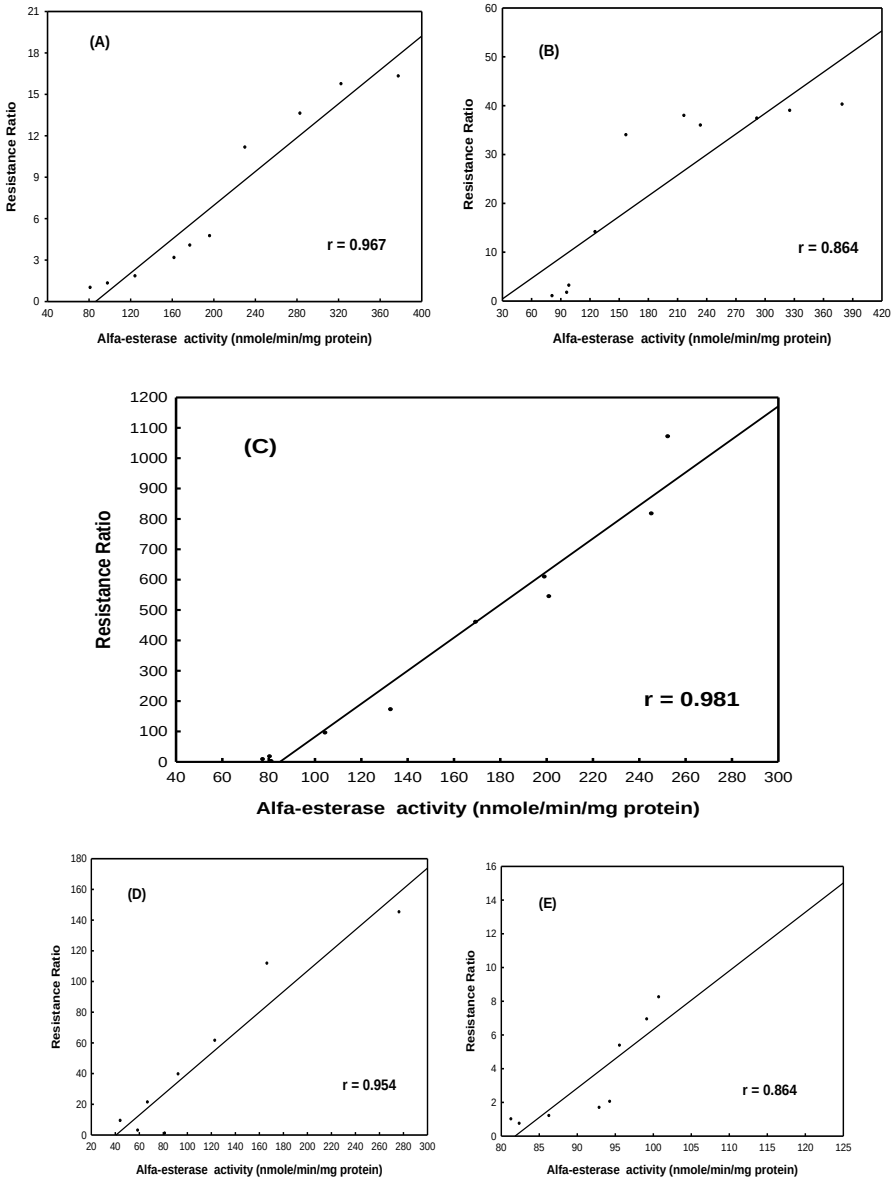

Fig. (1): The relationship between the α-esterase activity and level of resistance to chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron in parent strain and the selected generations of chlorpyrifos (A), profenofos (B), cypermethrin (C), spinosad (D) and flufenoxuron (E) resistant strains.
r: Correlation Coefficient Value

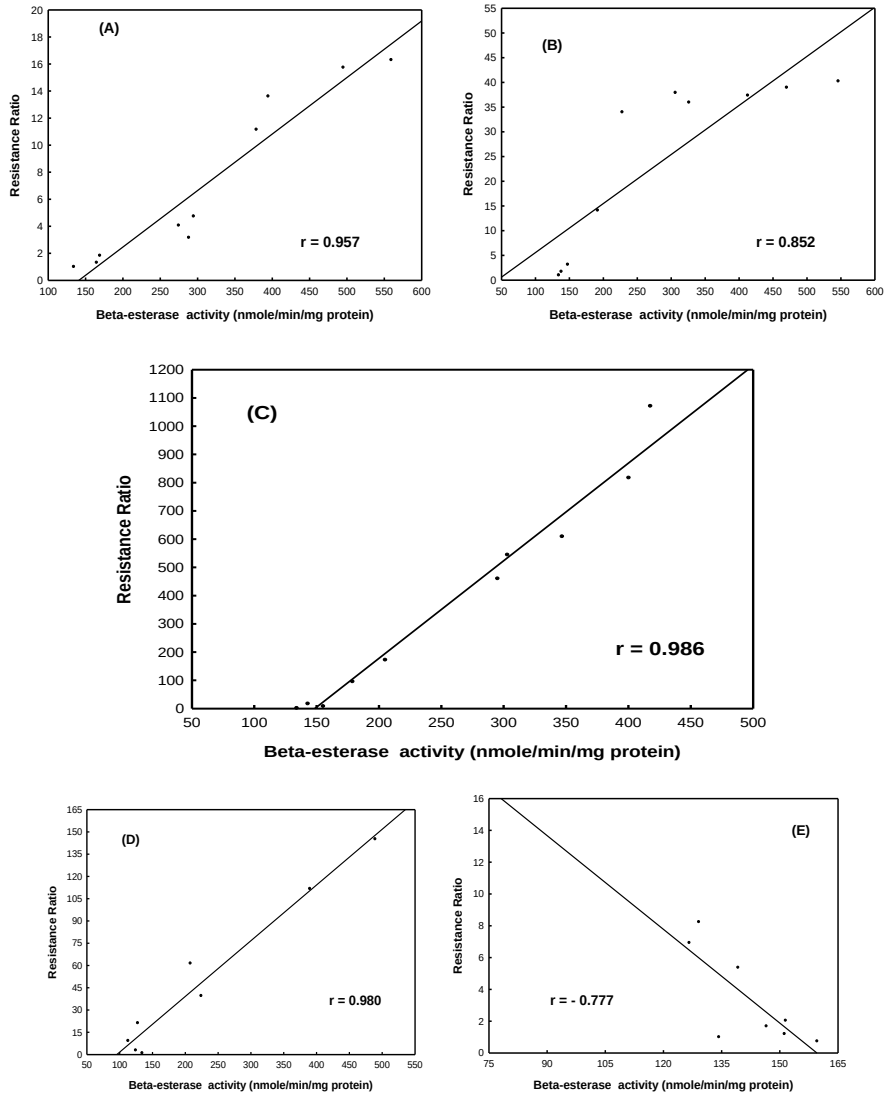

Fig. (2): The relationship between the β-esterase activity and level of resistance to chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron in parent strain and the selected generations of chlorpyrifos (A), profenofos (B), cypermethrin (C), spinosad (D) and flufenoxuron (E) resistant strains.
r : Correlation Coefficient Value

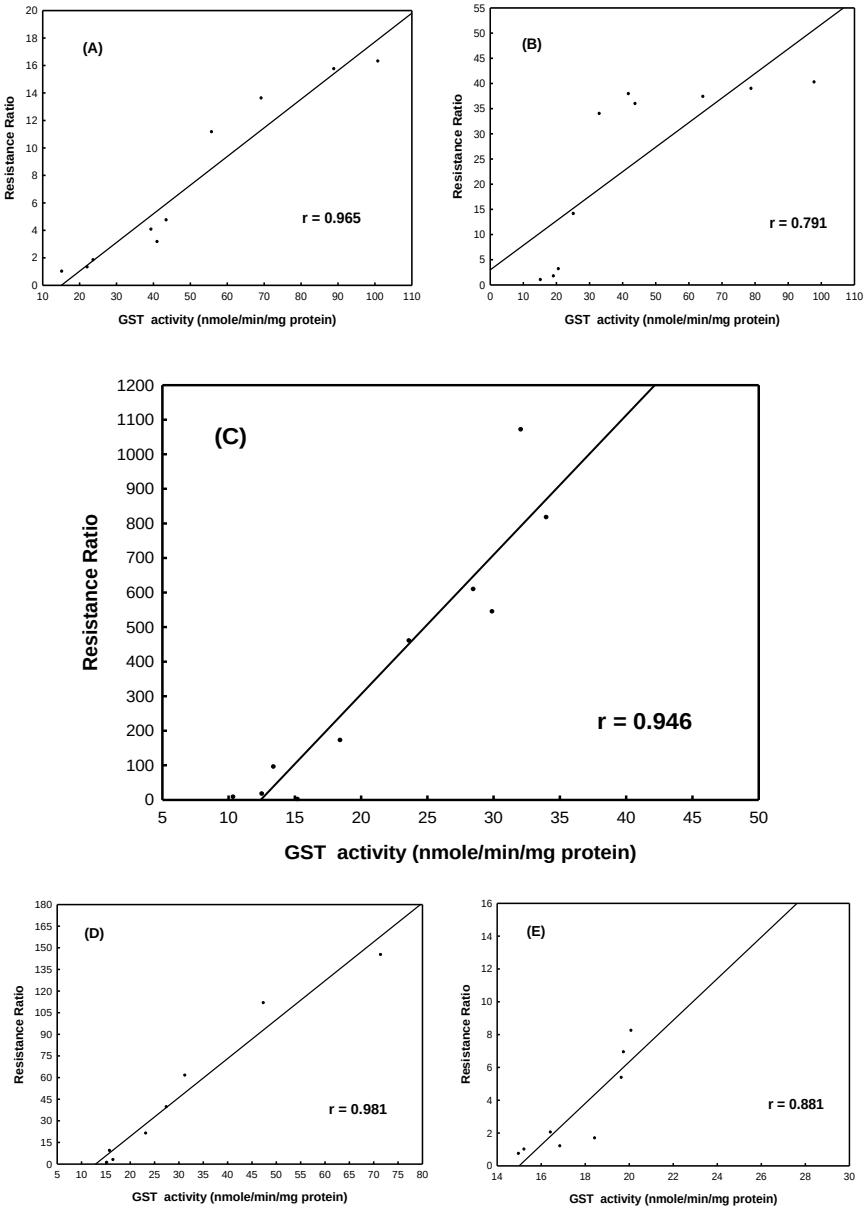

Fig. (3): The relationship between the glutathione S-transferase (GST) activity and level of resistance to chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron in parent strain and the selected generations of chlorpyrifos (A), profenofos (B), cypermethrin (C), spinosad (D) and flufenoxuron (E) resistant strains.
r: Correlation Coefficient Value

At LC_{50}, the field-collected strain of *S. littoralis,* showed mild tolerance to chlorpyrifos (8.3-fold), and was slightly tolerance to profenofos and flufenoxuron compared with the susceptible laboratory strain (Betana *et al*. 2000). These results indicated that multi-resistance factors caused by various groups of insecticides may be associated with biological constraints. These constraints may play a major role in field resistance management programs which are based on using a good alternative to highly resistance insecticides, having no or low resistance in field application.

Biochemical studies revealed that, the levels of α-esterase activity at the end of selection by chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron were 4.64, 4.67, 3.10, 3.40 and 1.24 times, respectively, higher than in the parent strain (Table 3). Similarly, the levels of β-esterase activity at the end of selection were 4.16, 4.07, 3.11 and 3.64 times for chlorpyrifos, profenofos, cypermethrin, and spinosad, respectively, higher than in the parent strain, while the activity level at the end of selection with flufenoxuron was 0.96 time lower than in the parent strain (Table 4). This indicated that α- and β-esterases may play a role in resistance to such chemicals in *S. littoralis*. We found that good correlation between α- and β-esterases activities and resistance to tested insecticide (Figs 1 and 2). Moreover, the corresponding activity levels of GST at the end of selection with the tested insecticides were 6.62, 6.43, 2.11, 4.69 and 1.32 times, respectively, higher than in the parent strain (Table 5). From these results, it could be suggested that the enzyme GST may play a role in resistance to chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron. Fig. (3) show a correlation between GST activity and resistance to these insecticides. Generally, detoxifying enzyme assays revealed that activities of esterases and glutathione S-transferase were high in selected strains. Since both enzymes are known to be effectively involved in the metabolism of insecticides and in resistance (Dauterman 1985; Soderlund and Bioomquist 1990), it is highly likely that increased activities of these detoxifying enzymes in the field populations play important roles in the observed resistance.

Resistance to profenofos in field strain of *H. virescens* was highly correlated with esterases activity toward α-naphthyl acetate (Harold and Ottea, 1997). Zhu and Gao (1998) revealed that α- esterase activities in two organophosphate-resistant strains, of the *S. graminum* were 1.9- and 2.4-fold higher than that of the susceptible strain. Also, they found that a good correlation between the α-Naphthyl acetate hydrolyzing esterase level and parathion resistance among two strains.

Biochemical analyses of detoxification enzyme levels indicated that esterases were important metabolic mechanisms medicating cypermethrin and fenvalerate resistance (Kranthi *et al*. 1999). The increase in α- and β-esterase activity in the Menofia field strain was higher than that of the laboratory strain Abd El-Mgeed *et al*. (2000). Farag (2005) found that most of the tested *S. littoralis* field strains tissues showed a high activity of esterase than laboratory strain with exception haemolymph. When larvae were treated with organophosphorus, pyrethroids or IGRs insecticides, Taha (2001) recorded that, the level of esterase activity at the end of selection by fenitrothion and profenofos in potato tuber moth, *phthorimaea operculell* were1.8 and 2.3 time higher than that of the parent strain.

Glutathione S-transferase enzymes were found to play a major role in resistance to organophosphorus insecticides (Motoyama and Dauterman 1980). The involvement of GST in OP resistance by catalyzing of conjugation of GST to

electrophilic sites on these compounds has been found for parathion (Oppenoorth *et al*. 1972) methyl parathion (Clark *et al*. 1986). Taha (2001) found that, GST activity of profenofos and fenitrothion selected strain were 1.8 and 2.14 time, respectively, higher than in the parent strain of potato moth, *P. operculella*. Martin *et al*. (2002) found that the glutathione S-transferase activity of deltamethrin selected strain was 2.7-fold higher than in the susceptible strain of *H. armigera*. Likewise, Yu *et al*. (2003) reported that GST activities toward CDNB and DCNB were 1.3- to 8.0-fold higher in the field strain than in susceptible strain of *S. frgiperda*. Moreover, Farag (2005) reported that there was a significant increase in GST's activity in field-strain of *S. littoralis* especially that collected at late season (after spraying season).

In general, the present study has provided some basic information on the non-specific esterases and glutathione S-transferase of *S. littoralis*. This will contribute to the complete understanding of the mechanisms of insecticide resistance of *S. littoralis* in the future.

REFERENCES

Abbas, N.; Samiullah; S. A. Shad; M. Razaq; A. Waheed and M. Aslam (2014).Resistance of *S. litura* (Lepidoptera: Noctuidae) to profenofos: Relative fitness and cross resistance. Crop Protection, 58: 49–54.

Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 256-267.

Abd El-Megeed, M. I.; M. G. Abbas; A. I. Gadallah and A. Hanafy (2000). Non specific esterases activities of susceptible and field strains of the cotton leafworm *S. littoralis* (Boisd.) as affected by certain chitin synthesis inhibitors. Annals Agric. Sci. Cairo. 4 (Special): 1585-1595.

Abo-Elghar, G. E. and M. H. Hussein (1992). Monitoring for resistance in the cotton leafworm, *S. littoralis* (Boisd.) against insecticide mixtures with benzoylphenyl ureas. Bul. Ento. Soc. Egypt, Eco. Ser., 19: 249-259.

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

Allam, A. M.; E. I. Mourad; Y. F. Ghoneim; A. Mohanna; F. A. Ayad and M. A. El-Guindy (1994). Relative susceptibility of Egyptian cotton leafworm, *S. littoralis* (Boisd.) larvae to chlorpyrifos in relation to cross-resistance pattern and esterase activity. J. Product Dev. 2: 169-183.

Betana, M. D.; A. M. A. El-Kadi and M. A. Hussein (2000). Toxicological responses and biological studies of susceptible laboratory and resistant field strains of *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae) to selected insecticides. Annals of Agricultural Science, Moshtohor, 38: 1279-1286.

Clark, A. G.; N. A. Shamaan; M. D. Sinclair and W. C. Dauterman (1986). Insecticide metabolism by multiple fly, *Musca domestica* glutathione S-transferase in two strains of the house (L.) Pestic. Biochem. Physiol., 25:169.

Dauterman, W. C. (1985). Insect metabolism: extramicrosomal, PP. 713-730. In G. a. Kerkut and L. I. Gilbert EDS, comprehensive insect physiology biochemistry and pharmacology, vol., 12. Pergamon, Oxford.

Duncan's, D. B.(1955).Multiple range and multiple F test. Bionetrics, 11: 1-41.

Eldefrawi, M. E.; A. T. Toppozada; A. Salama and S. A. El-Kishen (1964). Toxicological studies on the Egyptian cotton leafworm, *Prodenia litura* F.. II. Reversion of Toxaphene resistance in the Egyptian cotton leafworm. J. Econ. Entomol., 57: 593-595.

El-Guindy, M. A.; A. M. El-Refai and M. M. Abd-Elsattar (1983). The pattern of cross-resistance to insecticides and juvenile hormone analogues in a diflubenzuron-resistant strain of the cotton leafworm, *S. littoralis* (Boisd.). Pestic. Sci., 14: 235.

El-Guindy, M. A.; A. M. El-Refai; A. M. El-Samadesy and Y. F. Ghoneim (1989). The impact of several selection procedures on resistance to monocrotophos, diflubenzuron and methoprene in the cotton leafworm, *S. littoralis* (Boisd.). Int. Pest. Cont., 32: 72-76.

El-Guindy, M. A.; S. M. Madi; M. E. Keddis; H. I. Yehia; and M. M. Abdel-Satter (1982). Development of resistance to pyrethroids in field population of the Egyptian cotton leafworm, *S. littoralis* (Boisd.). Inter. Pest Contr., 24: 6-10.

El-Sherif, S. A. (1996). The effect of use of some insect growth regulators on controlling the cotton leafworm, *S. littoralis* (Boisd.). M.Sc. Thesis, Institute of Environmental Studies and Research, Ain Shams Univ., Cairo, Egypt.

Enayati, A. A.; Ranson, H. and Hemingway, J. (2005). Insect glutathione transferases and insecticide resistance. Insect Mol. Biol., 14: 3-8.

Ezz El-Din, H.; A. M. El-Gahreeb; A. M. K. El-Sayed and G. A. M. Abdu-Allah (2009). The ability of cotton leafworm, *S. littoralis* (Boisd.) field strain to develop resistance toward some conventional and bio-insecticides. J. Agric. Sci. Mansoura Univ., 34: 5231-5241.

Farag, A. M. A. (2005). Biochemical studies on insecticide resistance mechanisms of field and laboratory cotton leafworm strains. Ph. D. Thesis. Cairo Univ., Egypt.

Finney, D. J. (1971). Probit Analysis. 3rd ed. Cambridge Univ. Press, London. 333 pp.

Gamal, A.; E. Abdel-Raof and E. Hossam (2009). Resistance stability to spinosad and abamectin in the cotton leafworm, *S. littoralis* (Bosid.). Resistant Pest Management Newsletter, 19: 21-26.

Ghoneim, Y. F.; A. M. Allam; M. E. Keddis; M. F. Rofail; F. A. Ayad and M. A. El-Guindy (1994). Comparative toxicity of certain insecticides to filed strains of the cotton leafworm *S. littoralis* (Boisd), and the role of esterases in insecticides resistance. Egypt J. Appl. Sci., 9: 64-80.

Ghoneim, Y. F.; M. Singab; M. M. Abdel-Sattar and M. A. El-Guindy (2002). Monitoring of resistance to insecticides, IGR's and interaction of their mixtures in field strains of the cotton leafworm *S. littoralis* (Boisd). Annals of Agricultural Science, Moshtohor, 40: 1321-1332.

Ghoneim, Y. F.; M. Singab; H. M. Abou-Yousef and N. S. Abd-El-Hai (2012). Efficacy of certain insecticides and their mixtures with the tested IGRs against a field strain of the cotton leafworm, *S. littoralis* (Boisd.) under laboratory conditions. Australian Journal of Basic and Applied Sciences, 6: 300-304.

Grant, D. F.; D. M. Bender and B. D. Hammock (1989). Quantitative kinetic assay for glutathione S-transferase and general esterase in individual mosquitoes using EIA reader. Insect Biochem., 19: 741-751.

Harold, J. and J. A. Ottea (1997). Enzyme activities in organophosphorus-resistant tobacco budworms *Heliothis virescens*. Proceedings Beltwide Cotton Conferences, New Orleans, LA, USA, Vol. 2: 1013-1018.

Ishaaya, I.(1993).Insect detoxifying enzymes: their importance in pesticide synergism and resistance.Arch. Insect Biochem. Physiol.,22:263-276.

Keddis, M. E.; M. E. Omar and M. A. El-Guindy (1988). Monitoring insecticides resistance in field strains of the cotton leafworm, *S. littoralis* (Boisd.)during 1986 cotton season.Agric.Res. Rev.,66:25-31.

Kim, Y. G.; J. R. Cho; J. N. Lee; S. Y. Kang; S. C. Han; K. J. Hong; H. S. Kim; J. K. Yoo and O. Lee (1998). Insecticide resistance in the tobacco cutworm, *S. litura* (Fabricius) (Lepidoptera: Noctuidae). J. Asia Pacific Entomol., 1: 115-122.

Kranthi, K. R.; N. J. Armes; N. G. V. Rao; S. Raj and V. T. Sundaramurthy (1999). Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in Central India. Pesti. Sci., 50: 91-98.

Li, F. and Z. Han (2004). Mutation in acetylcholinesterase associated with insecticide resistance in the cotton aphid, *Aphis gossypii* Glover. Insect Biochem. Mol. Biol., 34: 397-405.

Magd El-Din and S. E. El-Gengaihi (2000). Joint action of some botanical extracts against the Egyptian cotton leafworm *S. littoralis* Bosid (Lepidoptera: Noctuidae). Egypt. J. Biol. P. Cont., 10: 51-56.

Martin, T.; F. Chandre; O. G. Ochou; M. Vaissayre and D. Fournier (2002). Pyrethroid resistance mechanisms in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) from west Africa. Pesti. Biochem. Physiol., 74: 17-26.

Motoyama, N. and W. C. Dauterman (1980). Glutathioe S-transferase: Their role in the metabolism of organophoshours insecticides. Rev. Biochem. Toxicol., 2: 49-69.

Oppenoorth, F. J.; V. Rupes; S. Eibashir; N. W. H. Houx and S. Voerman (1972). Gulatathione S-trnsferases dependent degradation of parathion and its significance for resistance in the house fly. Pestic. Biochem. Physiol., 2: 262.

Soderlund, D. M. and J. R. Bioomquist (1990). Molecular mechanisms of insecticide resistance. PP. 58-96, In Pesticide resistance in arthropods. Eds. R. T. Roush and B. E. Tabashnik. Chapman and Hall, New York. Historical records of field cotton leafworm *S. littoralis* resistance to conventional insecticides as influenced by the resistance programs in Egypt from 1950-2002. Resistant Pest Management Newsletter,12:7-9.

Soderlund, D. M. and D. C. Knipple (2003). The molecular biology of knock-down resistance to pyrethroid insecticides. Insect Biochem. Mol. Biol., 33: 563-577.

Taha, T. A. K. (2001). Studies on development of resistance to some insecticides in potato tuber moth, *Phthorimaea operculella* (zeller.). M.Sc. Thesis, Fac. Agric. Moshtohor, Zagazig Univ., Benha Branch.

Temerak, S. A. (2002). Historical records of field cotton leafworm *S. littoralis* resistance to conventional insecticides as influenced by the resistance programs in Egypt from 1950-2002. Resistant Pest Management Newsletter, 12: 7-9.

Van Asperen, K. (1962). A study of housefly esterases by means of a sensitive colorimetric method. J. Insect. Physiol., 8: 401-416.

Wang, W.; J. C. Mo; J. Cheng; P. J. Zhuang and Z. H. Tang (2006). Selection and characterization of spinosad resistance in *S. exigua* (Hubner) (Lepidoptera: Noctuidae). Pesticide Biochemistry and Physiology, 84: 180-187.

Young, D. S. (2001). Effects of disease on clinical lab. Tests, 4th ed AACC.

Yu, S. J. and S. N. Nguyen (1996). Insecticide susceptibility and detoxication enzyme activities in permethrin selected diamondback moths. Pesti. Biochem. Physiol., 56: 69-77.

Yu, S. J.; S. N. Nguyen and G. E. Abo-Elghar (2003). Biochemical characteristics of insecticide resistance in the fall armyworm *S. frugiperda* (J.E. Simth). Pesticide Biochemistry and Physiology, 77: 1-11.

Zhu, K. Y. and J. R. Gao (1998). Kinetic properties and variability of esterase in organophosphate susceptible and resistant Green bugs, *Schizaphis graminum* (Homoptera:Aphididae).Pestic.Biochem.Physiol.,62:135-145.

تطور المقاومة لبعض المبيدات الحشرية وعلاقتها ببعض التغيرات البيوكيميائية في دودة ورق القطن المصرية

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تم دراسة تطور مقاومة دودة ورق القطن لخمس مبيدات حشرية هي (الكلوربيريفوس، البروفينوفوس، السيبرمثرين، الاسبينوساد، الفلوفينوكسيرون) وقد تم انتخاب السلالات المقاومة لمدة أربعة عشر جيلاً متتالياً لكل من مبيد الكلوربيريفوس والبروفينوفوس والسيبرمثرين ولمدة اثني عشر جيلاً متتالياً لكل من مبيدي الاسبينوساد والفلوفينوكسيرون، وذلك بتغذية يرقات العمر اليرقي الرابع على ورق الخروع المعامل بالتركيز الذي يقتل 30٪ من الأفراد المعاملة. كما تم تقدير بعض الأنظمة الإنزيمية والتي قد تكون مسئولة عن ظاهرة مقاومة تلك الآفة لهذه المبيدات. وقد أوضحت النتائج أن مستوى المقاومة بالنسبة لمبيد الكلوربيريفوس والبروفينوفوس والسيبرمثرين بعد أربعة عشر جيلاً من الانتخاب كانت 16.30، 40.23 و 1070.42 ضعف علي التوالي مقارنة بسلالة الآباء، بينما كان معدل المقاومة لمبيدي الاسبينوساد والفلوفينوكسيرون 145.14 و 8.24 ضعف علي التوالي وذلك بعد اثني عشر جيلاً من الانتخاب.

كما تشير التقديرات البيوكيميائية إلي زيادة معنوية في مستوى نشاط إنزيمات الإستيريز الغير متخصصة (الألفا - البيتا) وذلك في كل السلالات فيما عدا سلالة الفلوفينوكسيرون التي أظهرت انخفاض في مستوى البيتا إستيريز، كما أظهرت النتائج أيضا ارتفاع في مستوى نشاط جلوتاثيون اس-ترانسفيريز في جميع السلالات المنتخبة مقارنة بسلالة الآباء، وتشير هذه النتائج إلى ارتباط بين مستوى المقاومة وزيادة مستوى نشاط تلك الإنزيمات في تلك الآفة وبالتالي فان مقاومة هذه الآفة لهذه المبيدات تعود إلي آليات متعددة والتي منها إنزيمات الإستيريز والجلوتاثيون اس-ترانسفيريز. وفي النهاية فان سرعة تقدير نشاط تلك الإنزيمات ربما تساعد في مراقبة مقاومة تلك الآفة للمبيدات، كذلك يمكن استخدام هذه التحاليل البيوكيميائية في تقدير نسبة مقاومة هذه الآفة للمبيدات السابقة في مراحلها المبكرة في الحقل.

Table (1): Rate of development of resistance to chlorpyrifos, profenofos and cypermethrin in 4th instar larvae of *Spodoptera littoralis* during selection with chlorpyrifos, profenofos and cypermethrin for 14 successive generations.

Generations	chlorpyrifos			profenofos			cypermethrin		
	LC_{50} (ppm)	Slope (± SE)*	RR**	LC_{50} (ppm)	Slope (± SE)*	RR**	LC_{50} (ppm)	Slope (± SE)*	RR**
Parent-strain	2.09	6.20 ± 0.70	1.00	1.96	5.75 ± 0.65	1.00	0.240	3.75 ± 0.42	1.00
G_1	2.75	3.93 ± 0.44	1.31	3.36	3.93 ± 0.44	1.71	1.75	3.15 ± 0.36	7.29
G_2	3.82	4.26 ± 0.48	1.83	6.17	3.63 ± 0.41	3.15	3.96	2.67 ± 0.30	16.50
G_3	4.39	3.94 ± 0.45	2.10	19.86	5.00 ± 0.57	10.13	7.95	3.64 ± 0.41	33.12
G_4	6.84	3.09 ± 0.35	3.27	22.80	4.33 ± 0.49	11.63	13.73	3.92 ± 0.44	57.21
G_5	6.61	2.50 ± 0.28	3.16	27.68	3.37 ± 0.38	14.12	22.76	1.56 ± 0.18	94.83
G_6	4.01	3.18 ± 0.36	1.92	49.66	7.50 ± 0.85	25.34	27.44	1.91 ± 0.22	114.33
G_7	8.49	3.67 ± 0.41	4.06	66.58	3.00 ± 0.34	33.97	41.16	1.85 ± 0.21	171.50
G_8	10.20	3.80 ± 0.43	4.88	70.77	3.33 ± 0.38	36.11	54.62	2.19 ± 0.25	227.58
G_9	9.91	2.72 ± 0.31	4.74	70.47	3.75 ± 0.42	35.95	110.29	4.57 ± 0.52	459.54
G_{10}	15.07	6.62 ± 0.75	7.21	67.39	1.75 ± 0.20	34.38	143.52	2.44 ± 0.28	598.00
G_{11}	23.30	5.72 ± 0.64	11.15	74.32	5.12 ± 0.58	37.92	130.48	3.86 ± 0.44	543.67
G_{12}	28.45	5.33 ± 0.60	13.61	73.22	3.25 ± 0.37	37.36	146.02	1.68 ± 0.19	608.42
G_{13}	32.90	3.79 ± 0.43	15.74	76.36	3.33 ± 0.38	38.96	195.96	2.20 ± 0.25	816.50
G_{14}	34.07	2.93 ± 0.33	16.30	78.85	7.17 ± 0.81	40.23	256.90	3.33 ± 0.38	1070.42

*SE: Standard error

** Resistance Ratio = LC_{50} of Selected strain / LC_{50} of Parent strain.

Table (3): Specific activity of α-esterase in parent strain and chlorpyrifos-, profenofos-, cypermethrin-, spinosad- and flufenoxuron-resistant strains of *Spodoptera littoralis* during generations of selection.

Generations	Specific activity of α-esterase (nMole/min/mg protein)									
	chlorpyrifos-resistant strain	R/P*	profenofos-resistant strain	R/P*	cypermethrin-resistant strain	R/P*	spinosad-resistant strain	R/P*	flufenoxuron-resistant strain	R/P*
Parent-strain	81.30 ± 1.94 g	-----	81.30±1.94 g	-----	81.30 ± 1.94 f	-----	81.30 ±1.94 de	-----	81.30 ± 1.94 d	-----
G_1	97.94 ± 2.30 fg	1.20	96.38 ± 2.77 g	1.18	77.58 ± 2.45 f	0.95	58.81 ± 2.68 fg	0.72	86.30 ± 1.43 d	1.06
G_2	124.46 ± 4.12 f	1.53	98.56 ± 1.29 g	1.21	80.62 ± 3.30 f	0.99	44.24 ± 1.97 g	0.54	82.39 ± 0.95 d	1.01
G_5	162.00 ± 3.66 e	1.99	125.46 ±1.87 f	1.54	104.56±1.67 e	1.29	66.97 ± 1.00 ef	0.82	94.30 ±1.80 bc	1.16
G_7	177.19 ± 2.41 e	2.18	157.26±6.68 e	1.93	132.82± 8.06 d	1.63	92.51 ± 3.32 d	1.14	92.91 ± 1.80 c	1.14
G_9	196.22±8.45 de	2.41	233.70±4.62 d	2.87	169.52± 2.97 c	2.08	123.09±11.17 c	1.51	95.59±1.86 abc	1.17
G_{11}	230.19±21.22 d	2.83	216.80±6.64 d	2.67	201.19 ±2.52 b	2.47	166.49 ± 6.32 b	2.05	99.17 ±1.79 ab	1.22
G_{12}	283.11 ± 1.71 c	3.48	291.73±8.60 c	3.59	199.2±10.14 b	2.45	276.52 ± 10.33 a	3.40	100.74 ± 1.26 a	1.24
G_{13}	322.65±10.96 b	3.97	325.6±22.33 b	4.00	245.40± 6.77 a	3.02	------	----	------	----
G_{14}	377.70±25.43 a	4.64	379.42±5.58 a	4.67	252.45 ±6.40 a	3.10	------	----	------	----

*R/P, Ratios of α-esterase activity between selected strain and parent strain.
-Each value represents the mean of three replicates ± Stander error
-Means in the same column followed by the same letters are not significantly different at the 5 % level of probability (Duncan's test).

Table (4): Specific activity of β-esterase in parent strain and chlorpyrifos-, profenofos-, cypermethrin-, spinosad- and flufenoxuron-resistant strains of *Spodoptera littoralis* during generations of selection.

Generations	Specific activity of β-esterase (nMole/min/mg protein)									
	chlorpyrifos-resistant strain	R/P*	profenofos-resistant strain	R/P*	cypermethrin-resistant strain	R/P*	spinosad-resistant strain	R/P*	flufenoxuron-resistant strain	R/P*
Parent-strain	134.30±0.98 e	-----	134.30 0.98 f	-----	134.30 ± 0.98 f	-----	134.30 ± 0.98 d	-----	134.30 ± 0.98 cd	-----
G_1	164.88±3.07 e	1.23	138.09 ±4.51 f	1.03	155.47 ± 5.03 ef	1.16	124.41 ± 4.87 d	0.93	151.2 ± 1.53 b	1.12
G_2	169.20±4.07 e	1.26	147.61 ±2.44 f	1.10	143.17 ± 6.62 ef	1.07	112.81 ± 2.08 d	0.84	159.61 ± 3.01 a	1.19
G_5	288.37±11.8 d	2.15	191.67±1.13 e	1.43	178.94 ± 3.56 de	1.33	127.59 ± 5.12 d	0.95	151.50 ± 2.05 b	1.13
G_7	274.70±3.30 d	2.04	227.89±7.60 e	1.70	205.19 ± 12.82 d	1.53	224.20 ± 5.74 c	1.67	146.53 ± 2.04 b	1.09
G_9	294.75±17.57 d	2.19	326.25±6.42 d	2.43	295.32 ± 4.47 c	2.20	207.79 ± 13.75 c	1.55	139.24 ± 1.28 c	1.04
G_{11}	378.7 ±14.32 c	2.82	306.20±9.1 d	2.28	303.21 ± 8.09 c	2.26	389.82 ± 19.35 b	2.90	126.65 ± 1.81 e	0.94
G_{12}	394.58±19.66 c	2.94	412.8±13.15 c	3.07	347.03 ± 17.54 b	2.58	489.25 ± 34.36 a	3.64	129.13 ± 1.11 de	0.96
G_{13}	495.12±31.75 b	3.69	470.15±36.7 b	3.50	400.42 ± 29.94 a	2.98	------	----	------	----
G_{14}	559.31±23.03 a	4.16	546.2 ± 8.13 a	4.07	417.88 ± 8.47 a	3.11	------	----	------	----

*R/P, Ratios of β-esterase activity between selected strain and parent strain.
-Each value represents the mean of three replicates ± Stander error
-Means in the same column followed by the same letters are not significantly different at the 5 % level of probability (Duncan's test).

Table (5): Specific activity of glutathione S-transferase (GST) in parent strain and chlorpyrifos-, profenofos-, cypermethrin-, spinosad- and flufenoxuron-resistant strains of *Spodoptera littoralis* during generations of selection.

Generations	Specific activity of GST (nMole/min/mg protein)									
	chlorpyrifos-resistant strain	R/P*	profenofos-resistant strain	R/P*	cypermethrin-resistant strain	R/P*	spinosad-resistant strain	R/P*	flufenoxuron-resistant strain	R/P*
Parent-strain	15.23 ± 0.23 f	----	15.23 ± 0.23 g	----	15.23 ± 0.23 de	----	15.23 ± 0.23 f	----	15.23 ± 0.23 d	----
G_1	22.13 ± 0.42 f	1.45	19.16 ± 0.63 fg	1.26	10.37 ± 0.31 f	0.68	16.52 ± 0.62 ef	1.08	16.86 ± 0.14 c	1.11
G_2	23.73 ± 0.56 f	1.56	20.66 ± 0.23 fg	1.36	12.54 ± 0.50 ef	0.82	15.80 ± 0.31 f	1.04	14.98 ± 0.31 d	0.99
G_5	41.07 ± 1.74 e	2.70	25.19 ± 0.32 f	1.65	13.42 ± 0.31 ef	0.88	23.23 ± 0.40 de	1.52	16.43 ± 0.30 c	1.08
G_7	39.41 ± 0.57 e	2.59	33.00 ± 1.13 e	2.17	18.45 ± 1.18 d	1.21	27.46 ± 0.85 cd	1.80	18.44 ± 0.28 b	1.21
G_9	43.51 ± 2.55 e	2.86	43.82 ± 0.78 d	2.88	23.64 ± 1.69 c	1.55	31.27 ± 2.45 c	2.05	19.65 ± 0.25 a	1.29
G_{11}	55.82 ± 2.75 d	3.67	41.82 ± 1.43 d	2.75	29.91 ± 0.87 b	1.96	47.37 ± 1.88 b	3.11	19.75 ± 0.26 a	1.30
G_{12}	69.24 ± 4.29 c	4.55	64.29 ± 2.12 c	4.22	28.50 ± 1.46 b	1.87	71.45 ± 5.56 a	4.69	20.09 ± 0.20 a	1.32
G_{13}	88.93 ± 5.98 b	5.84	78.85 ± 6.70 b	5.18	34.00 ± 2.31 a	2.23	------	-----	------	-----
G_{14}	100.87 ± 4.06 a	6.62	97.90 ± 1.41 a	6.43	32.08 ± 0.76 ab	2.11	------	-----	------	-----

* **R/P, Ratios of GST activity between selected strain and parent strain.**

-Each value represents the mean of three replicates ± Stander Error.

-Means in the same column followed by the same letters are not significantly different at the 5 % level of probability (Duncan's test).