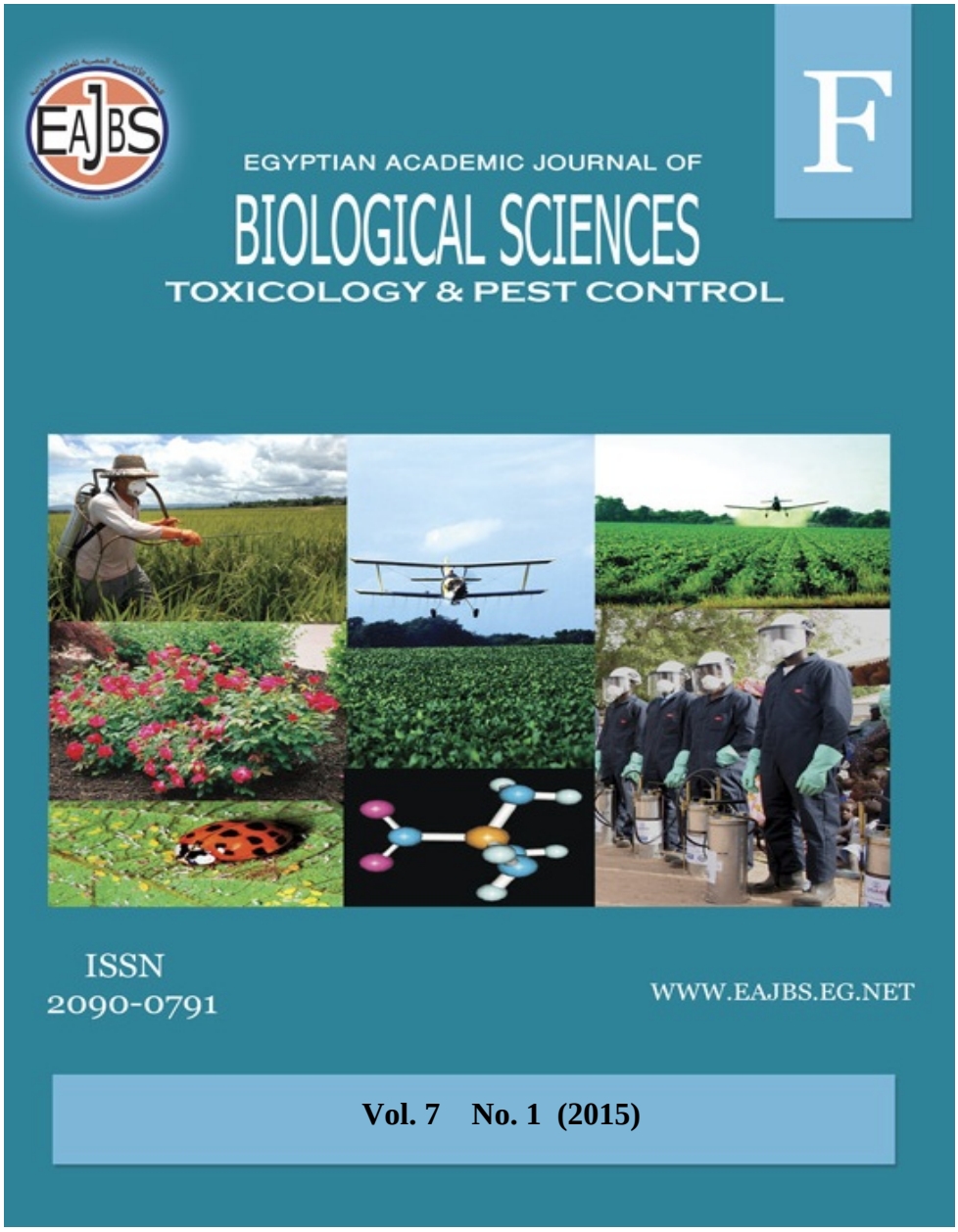

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Toxicity and Biochemical Studies on The Cotton Leaf-worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) From Some Governorates in Egypt.

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ABSTRACT

In order to investigate the comparative effectiveness of some insecticides including pyrethroids (Alpha cypermethrin and Fenvalerate) , organophosphates (Chlorpyriofos, Cyanophos and Profenofos) and carbamates (Methomyl) bioassay experiments were performed on the 4th larval instars of *Spodoptera littoralis*. Surveys or monitoring level of toxicity in some governorates of Egypt (Beheira, kalubia, Dakahlia, Fayum, Beni suef) and laboratory strains, were determined . According to LC_{50} and LC_{90} values the data revealed that pesticides were highly effective on laboratory strains The other strains or governorates populations tested with insecticides were less affected and behaved differently according to the strain location . Resistance ratios , analyzed protein of all governorate population tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic patterns of esterase isozyme during the larval stage were determined.

INTRODUCTION

Cotton is one of the most important crops and a major source of the national economy of Egypt. The Egyptian cotton leaf-worm, *Spodoptera littoralis* (Boisd) is a major polyphagous pest, widely distributed throughout Africa, Mediterranean Europe, and several parts of Asia. The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most harmful insect cotton pests in Egypt. It infests >87economically important plant species belonging to 40 families (Brown and Dewhurst, 1975). Due to its economic importance, it causes considerable damage to cotton plants as well as more than 29 hosts from other crops and vegetables. The environmental hazards of conventional insecticides necessitate introduce of other new insecticides that are effective, safer for human and negligible effects on ecosystem.

The mechanisms of action of the examined compounds are studied previously. The effect organophosphates on acetylcholinesterase., chemical–bioinsecticides combination provides several distinct advantages for Insect Pest Management programs (IPMs), including the potential effect for reducing the amounts of each agent used. Such reduction would mean potentially lower costs, lower environmental pollution, less damage to beneficial organisms and reduced selection pressure leading to the development of resistance to each agent (Harper, 1987and Temerak, 2005).

One of most accurate ways to monitor resistance in the Egyptian cotton leaf worm, *Spodoptera littoralis* for insecticides is through bioassays, many of these methods such as dipping was used extensively in determining resistance level in the laboratory (Moustafa *et al.*, 1987). Many investigators developed and used techniques based on biochemical determination of esterase in monitoring resistance phenomenon for organophosphates or carbamate insecticides. Miyata *et al.*, (1981) and Georghiou and Saito (1983) described a simple method to detect resistance to carbamate and organophosphate in green rice leaf hopper by examining its esterase activity. In general the best approach is an integrated pest management strategy that principally relies on cultural and biological control methods and the use of chemical control only when needed .The environmental hazards of conventional insecticides necessitate introduce of other new insecticides that are effective ,safer for human and negligible effects on ecosystem. In the present work, the toxicity of each insecticide used was determined according Sun equation(1950) on *Spodoptera littoralis* 4th larval instars in some governorates of Egypt. In addition resistance ratios, protein analysis of all governorate populations tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic patterns of esterase isozyme during the 4th larval stage of *Spodoptera littoralis* (Boised) were determined.

MATERIALS AND METHODS

Rearing technique

Laboratory strain

The strain of *S. littoralis* (Boisd.) in this study originally was obtained from the Central Agriculture Pesticide Laboratory, Agriculture Research Center, Dokki, Giza, Egypt. This strain was not previously exposed to any insecticides. The colony was reared under constant conditions; at 25 ± 2 ºC, 65 ± 5% R.H. and photoperiod 12:12 L:D for six successive generations as described by El-Defrawi *et al*. (1964). Egg masses were kept in glass jars (500 ml) covered with muslin cloth and provided daily with fresh castor bean leaves (*Ricinus communis*) as a source of food for the larvae. Third instar larvae (6-days old) were transferred to glass jars (1 L) provided with the same food. The prepupae were allowed to pupate in clean jars containing 2 cm high dry sawdust. The resulting pupae were transferred to glass jars containing filter papers and were kept in suitable cages (35 × 35 × 35 cm) for mating of the emerged moths. Emerged moths were fed on a piece of cotton dipped in 10% sugar solution. The cages were supplied with fresh leaves of *Nerium oleander* (L.) that served as an oviposition site.

Field strain

Samples of egg –masses were collected from different cotton fields of each governorate studied (Beni Suif , Fayum, Dakahlia, Kalubia and Behera). The samples were collected during two years 2012 and 2013 .The larvae were reared in laboratory as the laboratory strain .A series of experiments was conducted using the leaf dipping technique and the 4th-instar larvae ,the most destructive instar under field conditions.

All pesticides were used as formulated materials .the six pesticides chosen included the pyrethroids (Alpha cypermethrin 25% E.C and Fenvalerate 20%) organophosphates (Chlorpyriofos 50% E.C , Cyanophos 50% E.C and Profenofos 72% E.C) and carbamates (Methomyl 90% S. P) .A stock solution or emulsion of each tested insecticide was prepared freshly, on the basis of active ingredient, by diluting the

formulated compounds with water (w/v). Subsequently, serial aqueous dilutions were prepared to obtain the concentrations causing mortality ranging from 70% to 80%. Six fresh castor bean leaves (*Ricinus communis*) were dipped for 5 sec .in each concentration and then air-dried for one h. The larvae from each governorate were provided with treated leaves, making three replicates of 10 larvae for each concentration .Mortality was recorded at 24h post-treatment and corrected for control mortality by Abbott´s formula (abbott,1925). The LC_{50} and LC_{90} values were computed by probit analysis (Finney, 1971) based on the corrected data.

Refractionation of protein bands by SDS-PAGE (LKB Application Note 306, 1977):

In a solution of SDS and β-mercaptoethanol, proteins dissociate into subunits (polypeptide chains)in which the diameter of the rods is although to be constant, while the long axis varies in proportion with the molecular weight (MW). The latter value can be determined by comparing the relative electrophoretic mobility of unknown proteins with the mobility of known protein markers.

Determination of molecular weights of proteins:

Molecular weights (MW) is a property often used in the identification of organic compounds such as protein. SDS-PAGE had been carried out for the determination of MW of proteins in the presence of a standard protein marker. We used gel pro documentation for analysis of data.

Electrophoretic analysis of protein:

The electrophoretic analysis aimed to identify the general protein pattern in insect test.The protein extraction samples were identified by SDS polyacrylamtride gel electrophoresis(SDS-PAGE) according to Laemmlia (1970).

Detection of protein:

Detection of protein was made by silver nitrate staining (Sammans *et al* 1981).

Sample preparation for esterase isozymes:

Tris buffer (0.05M pH6.8) containing 10 % glycerol was used to extract native protein to study the esterase isozymes.

Detection of esterase isozymes:

Gel was incubated in 0.1M phosphate buffer (pH 7.0) for 10 min .,then transferred into the reaction mixture containing 0.03g Fast Blue RR-salt (stabilizer diazonium) as the coupler, 0.5ml of 1%(w/v) alpha naphthyl acetate in a 50% acetone as the substrate and 24.5 ml DW. Incubation was carried out at room temperature for 20 min ., after which the reaction was halted by a 7% acetic acid solution. Computer program gel pro documentation for analysis of data was used

RESULTS

Toxicity of the tested insecticides against the cotton leaf worm *S. littoralis* during the summer two years, 2012 and 2013 are presented in Tables (1, 2). The LC_{50}, LC_{90}, slope values and significant or non significant for the tested compounds against the 4^{th} larval instars collecting from some governorates under laboratory conditions using leaf –dipping technique after 24 h from treatments were demonstrated . The data obtained in 2012 (Table1)showed that Chlorpyriofos was the most effective insecticide in Fayum governorate (LC_{50} =59.04 ppm) followed by Beni suif (LC_{50}= 82.67ppm), while less effective in Behera governorate (LC_{50}=750.67 ppm).

Cyanophos., the second organophosphates was the most effective insecticide in Fayum governorate (LC_{50} = 2154.4 ppm) followed by Dakahlia governorate (LC_{50} =2216.69), while it was less effective in Kalubia Governorate (LC_{50}=3632.74 ppm). Profenofos., the third organophosphates was the most effective insecticides in

Fayum governorate (LC_{50} = 74.33ppm) followed by kalubia Governorate (LC_{50}=172.57 ppm) ,while it was less effective in Behera governorate (LC_{50}=554.97ppm). Alpha cypermethrin., one of pyrethroids was the most effective insecticide in Beni suif governorate (LC_{50}= 153.97ppm) followed by Fayum governorate (LC_{50}=431.35ppm). Fenvalerate (the second of pyrethroids) was the most effective insecticide in Beni suif governorate (LC_{50}=212.27ppm), while less effective in Dakahlia governorate (LC_{50}=2327.07ppm).

Table 1: Comparative toxicity of insecticides tested against *Spodoptera littoralis* in different governorates during the year 2012.

Insecticide	Beni suif Governorates				Fayum Governorates				Dakahlia Governorates				Kalubia Governorates				Behera Governorates			
	Slope ± S.E	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90	P	Slope ±SE	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90 ppm	P
Chlorpyriofos	3.66 ±0.96	82.67	185.21	0.3	2.35 ±0.5	59.04	207.77	0.69	2.53 ±0.72	156.06	501.3	0.01	1.71 ±0.44	307.67	1723.2	0.59	3.02 ±0.76	750.67	1993.59	1.81
Cyanophos	2.67 ±0.63	2644.57	7994.3	0.2	3.03 ±0.60	2154.89	5701.4	0.9	3.14 ±0.53	2216.69	5674.5	0.96	4.41 ±1.14	3632.74	7095.33	1.26	2.82 ±0.58	2684.73	7644.1	1.04
Profenofos	2.13 ±0.36	359.57	1431.5	0.8	2.65 ±0.66	74.33	226.16	0.58	2.99 ±0.72	226.93	6091.69	0.05	1.31 ±0.24	172.57	1684.9	0.74	2.26 ±0.41	554.97	2052.25	6.17
Alpha cypermethrin	1.39 ±0.31	153.97	1288.9	0.89	2.82 ±0.48	431.35	1230.01	1.17	1.67 ±0.32	468.81	2753.1	1.44	1.94 ±0.43	581.45	2663.0	1.67	1.46 ±0.25	574.61	4332.8	0.5
Fenvalerate	1.92 ±0.37	212.27	982.46	0.41	-	-	-	-	3.08 ±0.47	2327.07	6065.6	1.55	1.92 ±0.47	613.73	2864.7	0.87	1.46 ±0.25	574.61	4332.8	0.5
Methomyl	1.61 ±0.33	467.38	2925.3	0.76	1.84 ±0.46	206.44	1022.81	0.06	2.98 ±0.65	983.19	2650.91	0.18	3.99 ±0.74	312.65	655.05	1.36	3.9 ±0.57	886.81	1890.43	0.34

Methomyl (one of carbamate insecticides) was the most effective insecticide in Fayum governorate (LC_{50}=206.44ppm), while it was less effective in Dakahlia Governorate (LC_{50} = 983.19ppm). The results of the slope values indicated that ,the insect population was relatively heterogeneous in their susceptibility toward the tested insecticides by leaf-dip method (Slope values in the same table were more than 1.0 for most tested insecticides). The data obtained during 2013 (Table 2) cleared that Chlorpyriofos was the most effective insecticide on laboratory strain (LC_{50}=15.07 ppm).

Table 2: Comparative toxicity of insecticides tested against Spodoptera littoralis in different governorates during the year 2013.

Insecticide	Laboratory strain				Fayum Governorates				Dakahlia Governorates				kalubia Governorates				Behera Governorates			
	Slope ±S.E	LC50 ppm	LC90 ppm	P	Slope ± S.E	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90 ppm	P	Slope ±S.E	LC50 ppm	LC90 ppm	P
Chlorpyriofos	2.65 ±0.49	15.07	52.98	0.69	3.7 ±0.6	283.1	622.4	2.9	1.93 ±0.4	291.25	1412.98	0.6	2.6 ±0.5	122.4	373.4	2.1	1.6 ±0.4	364.8	1636.8	0.5
Cyanophos	1.88 ±0.32	615.86	2969.3	1.98	3.7 ±0.9	10714	23553	0.6	2.4 ±0.4	1901.1	6585.8	0.6	3.9 ±0.7	3989.2	8508.0	0.9	1.4 ±0.4	1801.9	14907	0.01
Profenofos	2.0 ±0.41	18.79	81.68	2.73	2,4 ±0.5	579.5	2031.7	1.5	2.1 ±0.3	100.57	401.96	0.3	2.6 ±0.0.5	218.9	673.3	0.5	1.6 ±0.4	236.14	1464.3	0.2
Alpha cypermethrin	2.68 ±0.42	2.43	7.31	0.27	1.5 ±0.3	537.1	4012.9	0.1	1.8 ±0.6	717.35	3835.6	0.8	1.8 ±0.4	562.5	3001.7	4.3	2 ±0.4	364.8	1636.8	0.5
Fenvalerate	4.58 ±0.59	4.08	7.8	0.06	4.1 ±0.8	3360.6	6921.3	0.2	2.0 ±0.3	1119.7	4832.8	0.6	2.3 ±0.6	1208.1	4278.5	3.6	2.3 ±0.5	1096.2	4022	0.8
Methomyl	7.83 ±1.18	30.51	44.47	0.69	3.0 ±0.6	1442.4	3897.4	2	2.4 ±0.4	250.96	853.73	0.1	3.1 ±0.7	564.4	1474.2	0.5	25 ±0.4	510.8	1676.0	1.0

Cyanophos was most effective insecticides in laboratory strain, showed (LC_{50}= 615.86 ppm) followed by Behera governorate (LC_{50}=1801. 9ppm), while it was less effective in Kalubia Governorate (LC_{50}=3989.2 ppm). Profenofos was the most effective insecticides on laboratory strain (LC_{50}= 18.79ppm) followed by Dakahlia Governorate (LC_{50}=100.57 ppm), while it was less effective in Fayum governorate (LC_{50}=579.5ppm). Alpha cypermethrin was the most effective insecticides on laboratory strain

(LC_{50}=2.43ppm) followed by Behera governorate (LC_{50}=364.8ppm). Fenvalerate was the most effective insecticide on laboratory strain (LC_{50}=4.08 ppm) followed by Behera (LC_{50}=1096.2 ppm), while it was less effective in Fayum governorate (LC_{50}=.3360.6 ppm). Methomyl insecticide was the most effective insecticide on susceptible strain or laboratory strain (LC_{50}=30.51ppm) followed by Dakahlia Governorate (LC_{50}=250.96ppm) where the LC_{50} and LC_{90} in Dakahlia and Kalubia governorates were significant but all governorates tested were non-significant ($p > 0.5$). The resistance ratios (RR) were calculated using the LC_{50} or LC_{90} of the field strains relative to those of the laboratory strain .The data revealed ,in general ,pronounced levels of resistance to organophosphate insecticides in all field strains This is indicated by the averages of the calculated RRs LC_{50}, where was 49.81, 20.41,10.3, 5.48, 3.91 fold in Beheira, kalubia, Dakahlia, Beni Suef and fayum governorates with Chlorpyriofos, respectively in 2012. But RRs of Cyanophos were 5.89, 4.35, 4.29, 3.59, 3.49 fold in Kalubia, Beheira, Beni suef, Dakahlia and Fayum governorates, respectively. RRs of Profenofos were 29.53, 19.13, 12.07, 9.18, 3.95 fold in Beheira, Beni suef , Dakahlia ,Kalubia and Fayum governorates, respectively.

The resistance ratios of the pyrethroids tested against field strains of *S. littoralis*, during 2012 are shown in Table (3). Resistance to Alpha cypermethrin were 239.27, 236.46, 192.92, 177.51, 63.36 fold in Kalubia, Beheira, Dakahlia, Fayum, and Beni suif governorates, respectively, while they were 570.36, 150.42, 140.83, 52.02 fold for Fenvalerate.

Table 3: Comparative resistance ratio (LC50, LC90) to insecticides tested against *Spodoptera littoralis* between strains of different governorates during the year 2012

Insecticide	Beni suif Governorates		Fayum Governorates		Dakahlia Governorates		Kalubia Governorates		Behera Governorates	
	RRLC 50	RRLC 90	RRLC 50	RR LC 90	RRLC 50	RR LC 90	RRLC 50	RRLC 90	RRLC 50	RR LC 90
Chlorpyriofos	5.48	3.49	3.91	3.92	10.355	9.4	20.41	32.52	49.81	37.61
Cyanophos	4.29	2.69	3.49	1.92	3.59	1.91	5.89	2.38	4.35	2.57
Profenofos	19.13	17.52	3.95	2.76	12.07	74.57	9.18	20.62	29.53	25.12
Alpha cypermethrin	63.36	176.32	177.51	168.26	192.92	376.62	239.27	364.29	236.46	592.72
Fenvalerate	52.02	125.95	-	-	570.36	777.64	150.42	367.26	140.83	555.48
Methomyl	15.31	65.78	6.76	23	32.22	59.61	10.24	14.73	29.06	42.51

Dakahlia, Kalubia, Beheira and Beni Suef governorates, respectively. Methomyl was the only carbamates tested in 2012, it is clear from the results that Dakahlia strain was the most resistance to this chemical (32.22 fold) followed by Beheira (29.06 fold),15.31, Beni suif, (10.24 fold), Kalubia and Fayum (6.76 fold), respectively. As shown in Table (4), the field strain was highly resistance to all the insecticides tested in season 2013.

Table 4: Comparative resistance ratio (LC_{50}, LC_{90}) to insecticides tested against Spodoptera littoralis between strains of different governorates during the year 2013.

Insecticide	Fayum Governorates		Dakahlia Governorates		Kalubia Governorates		Behera Governorates	
	RR LC 50	RR LC90	RR LC50	RR LC90	RR LC50	RR LC90	RR LC50	RR LC90
Chlorpyriofos	18.78	11.74	19.32	26.67	8.12	7.04	24.2	30.89
Cyanophos	17.39	7.93	3.08	2.21	6.47	2.86	2.92	5.02
Profenofos	24.87	24.87	5.35	4.92	11.64	8.24	12.56	200.3
Alpha cypermethrin	221.02	548.96	295.2	524.7	231.48	410.6	150.12	223.9
Fenvalerate	823.67	887.34	274.43	619.58	296.1	548.52	268.67	515.64
Methomyl	47.27	87.64	8.22	19.19	18.49	33.15	16.74	37.68

In general ,pronounced levels of resistance to organophosphates, pyrethroids and carbamates insecticides in all field strains was noticed. This is indicated by the averages of the calculated RRs where LC50 was 18.78, 19.32, 8.12, 24.2 fold in Fayum, Dakahlia, kalubia, and Beheira governorates with Chlorpyriofos, respectively.

But RRs of Cyanophos were 17.39, 3.08, 6.47,2.92 fold in Fayum, Dakahlia, kalubia, and Beheira governorates, respectively. while RRs of Profenofos were 24.87, 5.35, 11.64, 12.56 fold in Fayum, Dakahlia, kalubia, and Beheira governorates, respectively. The resistance ratios, the pyrethroids tested against field strains of *S. littoralis* during the 2013 are shown in Table (4). Resistance to Alpha cypermethrin was 221.02, 295.2, 231.48,150.12 fold., in Fayum, Dakahlia, kalubia, and Beheira governorates, respectively. While it was for Fenvalerate 823.67, 274.43, 296.1, 268.67 fold in Fayum, Dakahlia, kalubia, and Beheira governorates, respectively. Meanwhile it was 47.27, 8.22, 18.49, 16.74 fold in Fayum, Dakahlia, kalubia, and Beheira governorates for Methomyl, respectively.

The data of electrophoretic proteins in the 4th larval instars of *Spodoptera littoralis* (Table 5) and Fig. (1) showed clear differences between the governorate populations and laboratory strains tested. The data obtained showed specific protein bands for each govrnorate population tested .Also, as shown from the results, the number of protein bands in laboratory strains were less than in field strains.

Table 5: Protein patterns of *Spodoptera littoralis* isolate by (SDS-PAGE).

Band	Mol.w	1	2	3	4	5	6	7	8	9	10
No	K.daltons	Lab. strain	F13	k13	D13	B13	K12	D12	Be12	F12	B12
1	141.97	+	+	-	-	-	-	-	+	-	+
2	129.26	-	+	+	-	+	-	-	-	-	-
3	121.43	-	+	-	+	+	+	+	+	+	+
4	113.18	-	-	-	-	-	-	-	-	-	+
5	109.70	-	-	-	-	-	-	-	-	-	+
6	105.49	-	-	-	-	-	-	-	-	-	+
7	99.874	-	+	+	+	+	+	+	+	-	+
8	91.646	+	+	+	+	+	+	+	+	+	+
9	82.147	-	+	+	-	+	+	+	+	+	+
10	75.379	+	+	+	+	+	+	+	+	+	+
11	66	-	-	-	-	-	-	-	-	-	+
12	62.976	+	+	+	+	+	+	+	+	+	+
13	57.338	+	+	+	+	+	+	+	-	-	+
14	53.027	-	+	+	+	+	+	+	+	+	+
15	45	+	+	-	+	+	+	+	+	+	+
16	44.320	+	+	+	+	+	+	+	+	+	+
17	37.485	-	+	+	-	-	-	-	-	+	+
18	35.812	-	-	-	+	+	+	+	+	-	+
19	31.226	+	+	+	+	+	+	+	+	+	+
20	26.410	+	+	+	+	+	+	+	+	-	+
21	22	-	-	-	-	+	-	-	-	-	+
22	20.70	+	+	+	+	+	-	-	-	-	+
23	19.183	+	+	-	+	-	+	+	+	+	+
24	18.049	-	-	+	+	-	+	+	+	+	+
25	16.726	-	+	+	+	+	+	+	-	+	+
26	15.033	-	+	+	-	+	-	+	+	+	+
27	13.515	-	+	+	+	-	+	-	-	-	+

Lab.strain: Laboratory strain F13:Fayum 2013 F12:Fayum2012 K13 :Kalubia2013
K12:Kalubia 2012 B13:Behiera 2013 B12 Behiera 2012 D13 Dakahlia 2013
D12:Dakahlia 2012 Be12 :Beni suif 2012

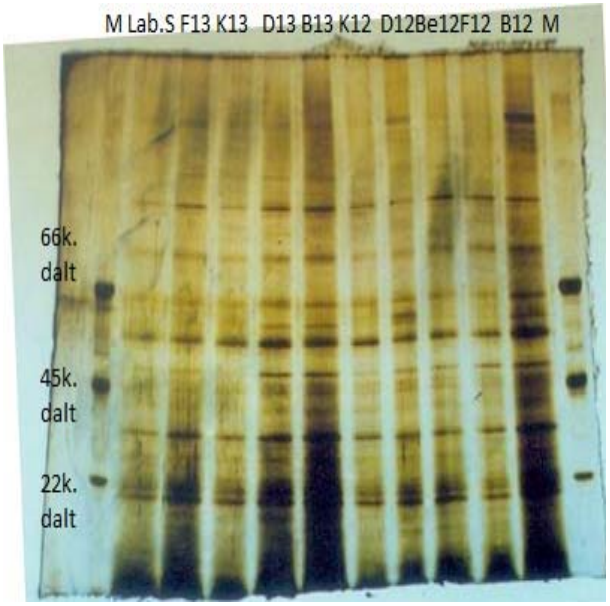

Fig. 1: SDS Polyacrylamide gel of denatured protein patterns in ten samples of the 4th larval instar of *Spodoptera littoralis*

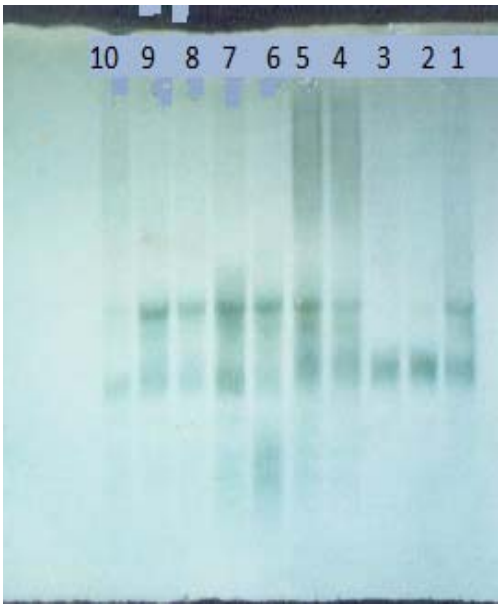

Fig. 2: Polyacrylamide gel zymogram of esterase isozyme patterns in different population governorates.

Esterase have been classified according to their action with various specific enzyme inhibitors (Bush *et al*., 1970) Three classes of esterases Could be identified as cholinesterase, carboxyesterase and arylesterase based on the substrate specificity and inhibition tested in 4th larvae instars (Augustinsson 1961) As shown from data given in Table (6) the number of protein bands were different from one governorate to another. Also the enzymatic activity in some population governorates was detected or activated after using specific enzyme inhibitor.

Table 6: Esterase patterns in field and laboratory strains of *Spodoptera littoralis*

Band		1	2	3	4	5	6	7	8	9	10
No	RF	B12	F12	Be12	D12	K12	B13	D13	k13	F13	Lab.strain
1	0.13	-	-	-	-	-	-	-	-	-	-
2	0.29	-	-	-	-	-	-	-	-	-	-
3	0.32	-	-	-	-	-	-	-	-	-	-
4	0.51	-	-	-	-	-	-	-	-	-	-
5	0.52	+	+	-	+	+	+	+	+	+	-
6	0.53	-	-	-	-	-	-	-	-	-	-
7	0.55	+	-	-	+	+	+	-	-	+	-
8	0.62	+	-	-	-	-	-	-	-	-	-
9	0.63	-	+	+	+	+	+	+	+	+	+
10	0.64	-	-	-	-	-	-	-	+	-	-
11	0.66	-	-	-	-	-	-	-	-	-	-
12	0.78	-	-	-	-	-	+	+	-	-	-
13	0.81	-	-	-	-	-	+	+	-	-	-

DISCUSSION

Toxicity of the insecticides tested against the cotton leaf worm , *S. littoralis.* in Tables (1 and 2) demonstrated the LC_{50}, LC_{90}, slope value and toxicity index values for the tested compounds against the 4th instar larvae under laboratory conditions .The toxicity of the different insecticides to laboratory strain was established .The LC_{50} values

indicated that carbamate and organophosphate insecticides were less effective against this strain, while synthetic pyrethroids were more effective (Alpha cypermethrin 2.43 ppm) The results obtained can be discussed in light of our past experience with resistance to in field strains of *S. littoralis* and also ,the accumulated knowledge on resistance development to different groups of insecticides El-Guindy *et al.* (1978-1979) reported low, moderate and high levels of resistance to chlorpyrifos and methomyl in field strains of the cotton leaf-worm .Resistance to sulprofos was high and stable while to methamidophos it was low to moderate . Chlormonstrated thapyrifos and methomyl however were used in practice on large scale over a period of 12 years. Therefore ,resistance observed to the former compounds can be attributed to cross-resistance while that detected to the latterones was due to continuous selection in subsequent generations. Moreover, El-Guindy *et al.* (1983) clearly demonstrated that laboratory selection for diflubenzuron (IGR) resistance in the cotton leaf-worm induced 300fold resistance compared with the original strain. The resistant strain was also characterized by high levels of cross –resistance to organochlorines, organophosphates, carbamates and particularly to pyrethroids. It is clear from the results indicated in Tables (3,4) that the resistance levels pyrethroids or carbamates and organophosphates fluctuated from one year to another .This could be correlated to the population size of the pest in each year .It is well known that the more the population increases in number ,the more genetic variability necessary for effective selection is available .In 2012 ,the population of the cotton leaf worm was relatively high, accordingly this was accompanied with high resistance ratios of most insecticidal tested .Apart from some fluctuations observed in the resistance ratios of the other successive year 2013.Our results are in agreement with those obtained in cotton field of Arizona and California(Twine and Reynolds, 1980 shekeban *et al.*, 2010). Resistance ratios (RRs) documented for organophosphates were 24- to 116-fold for profenofos and 22- to 87-fold for chlorpyrifos. For pyrethroids, RRs were 3- to 69-fold for cypermethrin and 3- to 27-fold for deltamethrin. resistance levels were moderate to very high against organophosphates, very low to high against pyrethroids, and very low to low against the newer-chemical insecticides (Qayyum *et al.*, 2015).

The first report of resistance to organophosphate and pyrethroid insecticides in Pakistani populations of *Phenacoccus solenopsis*. Regular insecticide resistance monitoring programs are needed to prevent field control failures. Moreover, integrated approaches including the judicious use of insecticides and rotation of insecticides with different modes of action are needed to delay the development of insecticide resistance in *P. solenopsis*. (Saddiq, *et al.*, 2014 and Saleem, *et al.*, 2015).

This was in harmony with the low population densities from the pest observed in those years .However, it should be pointed out that some of the Governorates which are densely cultivated with cotton and are characterized by favourable environmental conditions for the propagation of the insect.

Qualitative biochemical determination of esterases showed a good discrimination between Susceptible and field strains. Esterase play an important role in insecticide resistance .Insect esterases have been intensively studied because they are target sites for organophosphate and carbamate insecticide (Abdel-Hafez *et al.* 1982 and Mostafa 1988). Studies on the insecticidal action of organophosphorus

(oP) compounds raised great interest in insect esterases, since OP-compounds were found to cause strong inhibition of several esterases in a number of insects. It seems well established that their insecticidal action is due to inhibition of the cholinesterase present in nervous system and genetically modifyed esterases are capable of hydrolyzing OP-compounds and carbamate. Our results are in agreement with those obtained on esterase activity compared to susceptible populations., where resistant *Helicoverpa armigera* has additional esterase bands which are not detected in susceptible individuals (Gunning *et al*., 1996).

REFERENCES

Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Entomol 18:265-267.

Abdel-Hafez, M. M; Saleh, W .S; Hafez, M. A. and M. G. Abbass, M. (1982): Some biochemical studies on the Egyptian cotton leafworm *Spodotera littoralis (Boisd)*. II. Changes in esterase enzymes activity during metamorphosis. Proc. Egypts. National Conf. Ent., 1:401.

Asperen, K. van and Oppenoorth, F. J. (1959) Organophosphate resistance and esterase activity in houseflies. *Ent. exp. Appl.,* 2: 48-57.

Brown, E. S. and Dewhurst, C. F. (1975): The genus *Spodoptera* (Lepidoptera: Noctuidae) in Africa and the Near East. Bull. Entomol. Res., 65: 221-262

El-Defrawi, M. E.; Toppoyada, A.; Salama, A. and Elkishen, S. A. (1964): Toxicological studies on the Egyptian cotton leafworm *prodenia litura* II. Reverion of toxphene resistance in the Egyptian cotton leaf-worm. J. Econ. Entomol., 57:593-595.

El-Guindy, M. A.; Madi, S. M.; Elsayed, G. N.; Farrag, A. M. I.; Dogheim, S. M. A; Keddis, M. E. and Issa, Y. H. (1978-1979): Further studies on resistance spectrum and subgrouping of certain insecticides in field populations on the Egyptian cotton leaf-worm, *Spodotera littoralis* Boisd.during the cotton season. Bull. Ent. Egypt, Econ. Ser.,11:243-258.

Finney , D. J. (1971): Probit Analysis, 3rd ed., (Cambridge Uni. Press, London ,UK).

Georghiou, G. J. and Sito , T. (1983): Resistance to pesticides ,Plenum Press, New Yourk A division of Plenum Publishing Corporation 233 spring street, new Yourk ,N.Y.10013.pp809.

Gunning, R. V.; Moores, G. D.; Alan, L. and Devonshire, A. L. (1996): Esterases and Esfenvalerate Resistance in Australian *Helicoverpa*. *Armigera* (Hübner) Lepidoptera: Noctuidae Pesticide Biochemistry and Physiology., 54(1): 12-23.

Harper, D. J. (1987): Interaction between baculoviruses and other entomopa-thogenens, chemical pesticides and parasitoids in the Biology of Baculoviruses. In: Granados, R. R., Federici, A.B. (Eds.). CRC Press, Inc. p. 178 (1986, Chapter. 5).

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head bacteriophage T4.Nature 222:680-685

Miyata,T.; Saito, T.; Hama, H.; iuata, T. and Ozaki, K. (1981): A new and simple detection method for carbamate resistance in green rice leaf hopper *Nephottix cincticeps* Uhler. Applied Entomology and Zoology, 15: 351-352.

Mostafa, S. A. (1988): Effect of some insect growth regulators on some cotton pests .M.SC. Thesis Fac. Agric ., Al-Azhar univ.,Egypt.

Moustafa, F. I.; Abdel-Rahman, S. M.; Abd-El-Hamed, M. M. and El-sebae, A. H. (1987): Monitoring of resistance in the Egyptian cotton

leaf-worm, *Spodotera littoralis* from different locaitilies of El-Beheira Governrate. Alex. Sci. Exch., Vol 3

Qayyum, A.; Wakil, M.; Jalal Arif, W.; Sahi, M.; Abid Saeed, N. S. T. and Russell D. A. (2015): Multiple Resistances Against Formulated Organophosphates, Pyrethroids, and Newer-Chemistry Insecticides in Populations of Helicoverpa armigera (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. DOI: http://dx.doi.org/10.1093/jee/tou037 tou037 First published online.

Saddiq, B.; Sarfraz Ali Shad, S. A.; Ali Khan, H. A.; Aslam, M.; Ejaz, M. and Afzal, M. B. S. (2014): Resistance in the mealybug *Phenacoccus solenopsis Tinsley* (Homoptera: Pseudococcidae) in Pakistan to selected organoph-osphate and pyrethroid insecticides Crop Protection 66:29-33

Saleem, M.; Hussain, D.; Ghouse, G.; Abbas, M. and Fisher, S. W. (2015): Monitoring of insecticide resistance in *Spodoptera litura* (Lepidoptera: Noctuidae) from four districts of Punjab, Pakistan to conventional and new chemistry insecticides. Crop Protection http://dx.doi. 10.1016/j. cropore.2015.08.024.

Sammons, D. W.; Adams, L. D. and Nishizawa, E. E. (1981): Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels Electrophoresis, 2(3): 135–141.

Shekeban, M. M. K., Saad, A. S. A., Zaher, M. A. and Mehareb, S. R. (2010): The latent effect of certain insecticides on pinkbollworm, *P. gosszpiella* (Saund). J. Adv. Agric. Res. (Fac. Ag. Saba Basha Alexandria) 15(1): 57-75.

Sun, Y. P. (1950): Toxicity index-An improved method of comparingthe relative toxicity of insecticides. J. Econ. Entomol, 43:45–53.

Temerak, S. A. (2005): Spinosad, anew natural organic bio-insecticide to combat cotton leaf-worm and bollworms in Egypt. J. Agric. Res., 83(1): 387–389.

Twine, P. H. and Reymolds, H. T. (1980): Relative susceptibility and resistance of the tobacco budworm to methyl parathion and synthetic pyrethroids in Southern California. J. Econ. Entomol., 73:239-242