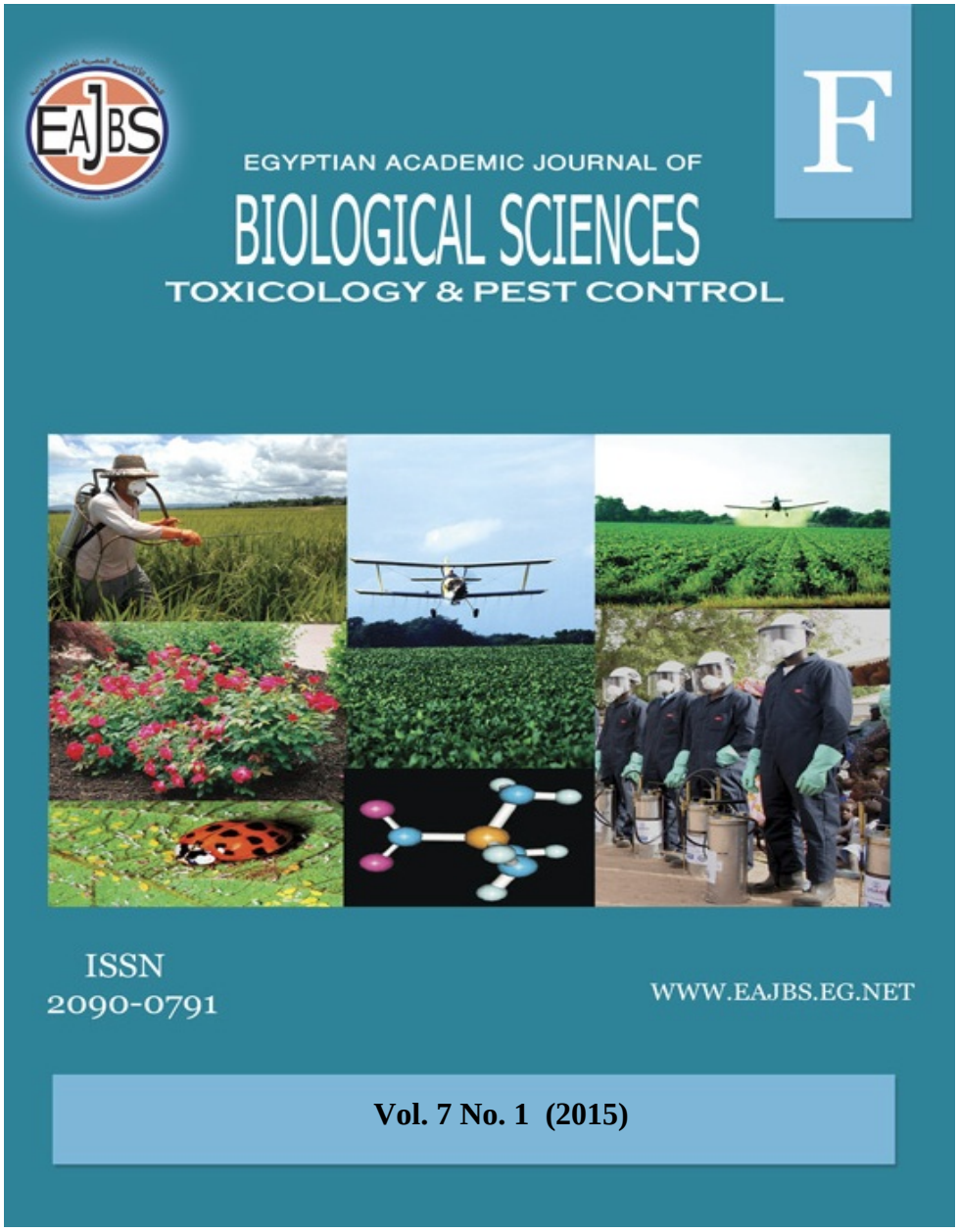

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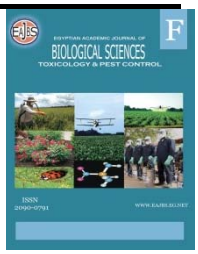

Insecticides Resistance in the Cotton Aphid, *Aphis gossypii*(Glover) in Egypt

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ABSTRACT

The cotton aphid, *Aphis gossypii* Glover (Hemiptera-Aphididae), is one of the most important sap sucking pests in many cotton-growing areas world-wide. In this study, the efficacies of eight insecticides organophosphate (Dimethoate, Malathion and Pirimiphos-methyl), carbamate (Pirmicarb and Methomyl), pyrethroid (Etofenprox, Lambda-cyhalothrin and Cypermethern) were determined against four strains of *A. gossypii* (Glover) collected from three Governorates in 2013 and four Governorates in 2014 (Sharkia, Gharbia, Behera and Fayum). Collected strains were bioassay and compared with a reference Laboratory strain.

Results indicated that the carbamate Pirmicarb and Methomyl were the most effective insecticides and recorded the least resistance levels and the highest toxic action when compared with the other tested organophosphates insecticides. A similar results were obtained in the case of the organophosphate (Dimethoate, Malathion and Pirimiphos-methyl), while pyrethroid insecticides were more resistance levels and least toxic action, also analyzed protein of all field strains tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic patterns of esterase isozyme during the adult stage were determined.

INTRODUCTION

Cotton aphid, *A. gossypii* Glover (Hemiptera-Aphididae) is the main aphid pest of cotton throughout the world causing significant problems due to honeydew contamination of the open boll lint (Schepers, 1989). The importance of *A. gossypii* as a cotton pest is increasing throughout the cotton-producing regions of the world (Leclant and Deguine, 1994). In Egypt, *A. gossypii* has recently emerged as one of the most serious pests, *A. gossypii* damage affects the yield of cotton seeds as well as the fiber quality, also these aphids can transmit viral diseases. As such applications are frequent, the role of most of the abundant natural enemies is eliminated particularly after the aphid develops resistance to these insecticides (Godfrey *et al.* 2009) thus making subsequent treatments inefficient and leading to increase in aphid population levels (Godfrey and Fuson 2001). There has been a general evolution of resistance in *A. gossypii* to most insecticides. First reported insecticide resistance was by Melander (1914). Resistance to insecticides has been found in at least 20 aphid species (Georghiou, 1981).

In regards to *A. gossypii*, resistance to organophosphorious products was first reported by (Kung *et al.*, 1964). Subsequently, resistance to carbamates (Furk*et al*., 1980) and pyrethroids (Zil'bermints and Zhuravleva, 1984) were reported until now. Resistance to OP and carbamate insecticides had been reported by several authors in many countries (Li *et al.,* 2003). Pyrethroid resistance in *A. gossypii* has been previously documented in some other countries (Ahmad *et al.,* 2003) in Pakistan, (Herron *et al.,* 2001).

Apparently, limited work had been done on monitoring the resistance to the different insecticides in cotton aphid in Egypt. In that respect, reference may be made to the studies of *A. gossypii*, Singab (2007 a and b), Ahmad *et*. *al*., (2003a), El-Kady (2007),Tabacian*etal*., (2011), Jam *et al.*,(2014) Abdelmotaleb (2015). The present work focuses on a survey of the resistance to certain insecticides commonly used in Egypt for the control of the population of the cotton aphid, *A. gossypii* Glover collected from different governorates during the period 2013-2014 to determine levels of resistance of the insecticides, biochemical determ-inations were also study enzyme activities of total esterase's and total protein by Gel electrophoresis SDS were investigated in an attempt to clarify the correlation between the development of resistance and the activity of these enzymes.

MATERIALS AND METHODS

Cotton Aphid strains

The laboratory standard clone of *A. gossypii* (susceptible reference strain; SUS) maintained in culture throughout the study was obtained from Central Agricultural Pesticides Laboratory, where it had been maintained in the absence of insecticides. Four field strains of *A. gossypii* were collected fromdifferent cotton fields at the early cotton growing seasons 2013 and 2014 in Sharkia (SH), Gharbia (GH), Behera (BE) and Fayum (FAY) Governorate**s.**

Insecticides used

Formulations of the eight tested insecticides were used for bioassays:

- Organophosphates: Pirimiphos-methyl (Actellic 50% EC), Malathion (Malathion 57% EC), Dimethoate (Dimethoate 40% EC).
- Carbamates: Pirimicarb (Primer 50% WP) and Methomyl (Lannate 90% WP).
- Pyrethroids: Lambda-cyhalothrin (Karate 2.5 % EC), Cypermethern (Polytrin 20% EC) and Etofenprox (Trebon 30% EC).

Leaf-dip bioassay

The bioassay leaf disc as adapted by Sawicki and Rice (1978), discs (35 mm diameter each) were cut from cotton leaves and dipped in insecticide solution for 20s, placed abaxial surface upper most on an agar bed (25 mm in depth) in disposable plastic containers (30mm high), and allowed to air-dry. Adults of each strain (10 per container) were gently placed on the treated leaf surface. Leaf discs dipped in distilled water were used as controls. Bioassay containers were covered with a fine mesh lid and maintained at 25 ±1ºC and 65% RH under ambient daylight conditions. All bioassays were scored at 24 h intervals following initial exposure to insecticide. Insects were considered alive if they showed any sign of movement, each bioassay test used three replicates of five concentrations each.

Analysis

Mortality at the discriminating concentration and dose response regressions were corrected for control mortality (Abbott 1925), which did not exceed 10%. Dose-response regressions were computed on Probit 5 for Windows (Gillespie 1995) to estimate the LC_{50} (lethal concentration to kill 50% of the test population). Resistance ratios were calculated by dividing the LC_{50} of the

field collected strain by the LC_{50} of the laboratory susceptible strain.

Refractionation of protein bands by sodium dodecyl sulphate polyacrylamide gel electrophoresis SDS-PAGE

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) are 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 polyacrylamide 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 (0.05 M pH 6.8) 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., and 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 and resistance ratio (RR) of the tested insecticides against the cotton aphid, *A. gossypii* Glover during two seasons of cotton growth (2013 and 2014) are presented in Table (1). The LC_{50}, slope values and RR for the tested compounds against the adult or last nymph collecting from some governorates under laboratory conditions using leaf-dipping technique after 24 h from treatments were demonstrated. The data obtained in season 2013 (Table1) showed that Pirmicarb was the most effective insecticide in Gharbia and Behera strains equal Laboratory strain (LC_{50} =172.6 ppm) followed by Sharkia (LC_{50} = 199.55 ppm). Methomyl, the second Carbamate was the most effective insecticide in Behera (LC_{50} =15.9 ppm) followed by Sharkia (LC_{50} = 30.86 ppm), while it was less effective in Gharbia (LC_{50} = 35.77 ppm).

Lambda-cyhalothrin first pyrethroid was the most effective insecticide in Behera (LC_{50} = 1.23 ppm) followed by Gharbia (LC_{50} = 42.57 ppm), while it was less effective insecticide in Sharkia (LC_{50} = 272.40 ppm), Cypermethern second pyrethroid was the most effective insecticide in Gharbia (LC_{50} = 8.54 ppm) followed by Behera (LC_{50} =12.8 ppm), while it was less effective insecticide in Sharkia (LC_{50} = 23.85 ppm), Etofenprox third pyrethroid was the most effective insecticide in Behera (LC_{50} = 8.6 ppm) followed by Gharbia (LC_{50} = 33.7 ppm), while it was less effective insecticide in Sharkia (LC_{50} = 36.67 ppm).

Dimethoate first organophosphates was the most effective insecticide in Behera (LC_{50} = 55.87 ppm) followed by Sharkia (LC_{50} =173.63 ppm), while it was less effective insecticide in Gharbia (LC_{50} = 201.23 ppm), Malathion second organophosphates was the most effective insecticide in Gharbia (LC_{50} = 19.11 ppm) followed by Sharkia (LC_{50} = 78.67 ppm), while it was less effective insecticide in Behera Governorate (LC_{50} = 256.79 ppm), Primiphos-methyl third organophosphates was the most effective insecticide in Sharkia (LC_{50} = 16.46 ppm) followed by Behera (LC_{50} = 24.5 ppm), while it was less effective insecticide in Gharbia (LC_{50} = 32.8 ppm).

Table 1: Resistance of insecticides against *Aphis gossypii* in different Governorates during seasons 2013 and 2014.

Insecticide	Season	Lab. Strain		Fayoum strain			Sharkia strain			Gharbia strain			Behera strain		
		Slope ± S.E	LC_{50} ppm	Slope ± S.E	LC_{50} ppm	RR*	Slope ± S.E	LC_{50} ppm	RR*	Slope ± S.E	LC_{50} ppm	RR*	Slope ± S.E	LC_{50} ppm	RR*
Pirmicarb (Primer 50% WP)	2013	3.76±0.93	172.65	--------	--------	-------	1.76±0.25	199.55	1.15	1.53±0.77	172.6	1	1.02±0.76	172.6	1
	2014			1.26±0.23	172.65	1	1.45±0.27	288.29	1.66	2.53±0.72	172.55	1	1.55±0.26	621.08	3.59
Methomyl (Lannate 90% WP)	2013	2.27±0.53	12.9	--------	--------	-------	1.56±0.3	30.86	2.39	2.14±0.53	35.77	2.77	2.32±0.58	15.9	1.2
	2014			1.77±0.43	19.9	1.54	1.24±0.23	20.45	1.58	1.20±0.20	15.7	1.21	1.43±0.27	13.65	1.09
Lambda cyhalothrin (Karate 2.5% EC)	2013	2.33±0.46	1.23	--------	--------	-------	2.75±1.93	272.40	221.46	2.89±0.72	42.57	34.60	1.26±0.43	1.23	1
	2014			1.13±0.4	59.5	48.37	2.39±0.32	23.35	18.98	1.33±0.32	15.15	12.31	1.54±0.2	53.22	43.26
Cypermethern : (Polytrin 20% EC)	2013	1.59±0.36	3.2	--------	--------	-------	2.6±0.32	23.85	7.45	3.67±0.32	8.54	2.66	2.46±0.25	12.8	4
	2014			1.59±0.36	23.6	7.37	1.27±0.25	176.97	55.3	2.12±0.37	1257.1	392.84	1.26±0.21	1053.3	329.15
Etofenprox (Trebon 30%EC)	2013	1.62±0.27	7.2	--------	--------	-------	1.56±0.53	36.67	5.09	1.08±0.47	33.7	4.68	0.46±0.25	8.6	1.19
	2014			1.62±0.27	33.6	4.66	1.88±0.46	4889.3	679.06	1.80±0.29	3435	477.08	2.06±0.33	3592.3	498.93
Dimethoate (Dimethoate 40% EC)	2013	1.71±0.43	16.4	--------	--------	-------	2.76±0.23	173.63	10.58	2.28±0.65	201.23	12.27	2.1±0.57	55.87	3.4
	2014			1.91±0.43	95.77	5.83	1.54±0.24	430.6	26.25	2.98±0.65	293.53	17.89	2.09±0.36	635.4	38.74
Malathion (Malathion 57% EC)	2013	1.31±0.63	13.3	--------	--------	-------	2.36±0.53	78.67	5.9	2.98±0.45	19.11	1.43	1.09±0.57	256.79	19.3
	2014			1.31±0.63	39.9	3	1.66±0.27	149.44	4.5	2.67±0.92	118.7	8.92	2.7±0.34	239.55	18.01
Primiphos/methyl (Actellic 50% EC)	2013	1.81±0.46	8.2	--------	--------	-------	1.46±0.96	16.46	2	1.38±0.25	32.8	4	3.51±0.57	24,5	3
	2014			1.71±0.39	6.2	0.75	1.68±0.35	59.9	7.3	1.47±0.32	20.55	2.50	2.18±0.30	51.8	6.31

The resistance ratios (RR) were calculated using the LC_{50} of the field strains relative to those of the laboratory strain (Table1). The current study revealed that all strains showed varied degrees of resistance to the 8 insecticides studied. The highest resistance was recorded at LC_{50}, in general, pronounced levels of resistance to carbamate insecticides different site to site in all field strains. This is indicated by the averages of the calculated RRs, were 1.15, 1 and 1fold in Sharkia, Gharbia and Behera, respectively, to Pirmicarb, but rate of resistance increase in Methomyl were 2.39, 2.77 and 1.2 fold Sharkia, Gharbia and Behera, respectively.

Resistance ratio (RR) of pyrethroid insecticides tested against field strains of the cotton aphid, *A. gossypii* Glover during 2013are shown in Table (1). Resistance to Lambda-cyhalothrin were 221.46, 34.60 and 1fold in Sharkia, Gharbia and Behera, respectively, but Cypermethern were 7.45, 2.66 and 4 fold in Sharkia, Gharbia and Behera, respectively, while Etofenprox were 5.09, 4.68 and 1.19 fold in Sharkia, Gharbiaand Behera, respectively. In general, pronounced levels of resistance to organophosphates insecticides in all field strains were noticed,this is indicated by the averages of the calculated RRs where LC_{50} were 10.58, 12.27 and 3.4 fold in Sharkia, Gharbia and Behera governorates respectively with Dimethoate, but RRs of Malathion were 5.9, 1.43 and 19.3 fold in Sharkia, Gharbia and Behera, respectively, while RRs of Primiphos-methyl were 2, 4 and 3

fold in Sharkia, Gharbia and Behera, respectively.

Toxicity and resistance ratio (RR) of the tested insecticides against the cotton aphid, *A. gossypii*Glover during season 2014 shown in table (1), the field strain was highly resistance to all the insecticides tested in season 2014. In general, pronounced levels of resistance to organophosphates, pyrethroids and carbamates insecticides in all field strains were noticed in Table (1).

The data of electrophoretic proteins in the *A.gossypii*(Table2) and Fig. (1) showed clear differences between the governorate populations and laboratory strains tested. The data obtained showed specific protein bands for each governorate population tested. Also, as shown from the results, the number of protein bands in laboratory strains were nearly.

Table 2: Protein patterns 0f*Aphisgossypii*isolate by (SDS-PAGE)

	Mol.w	1	2	3	4	5	6	7	8
No	K.daltons	SH13	GH13	BE13	FA14	SH14	GH14	BE14	Lab.strain
1	99.25	+	+	+	+	+	+	+	+
2	95.25	+	+	+	+	-	-	-	-
3	92.250	+	-	-	-	-	-	+	-
4	88.750	+	+	+	-	+	-	+	+
5	85.833	+	-	-	-	-	-	-	-
6	82.917	+	+	+	+	+	+	-	-
7	80.583	+	+	+	+	+	+	+	-
8	75.333	+	-	-	-	-	-	-	-
9	73.583	+	+	+	-	-	-	-	-
10	70.667	-	+	+	+	+	+	-	-
11	65.417	+	+	+	+	+	+	-	+
12	61.333	+	+	+	+	+	+		+
13	55.50	-	-	+	+	+	-	+	+
14	53.027	+	+	+	+	+	+	+	+
15	49.083	+	+	+	+	+	+	-	+
16	46.750	-	+	+	+	+	+	-	-
17	45.583	-	-	-	-	-	+	+	-
18	44.246	+	-	-	+	-	-	-	+
19	42.738	-	+	+	-	+	+	-	+
20	39.721	+	+	+	+	+	-	+	-
21	37.459	+	+	+	+	+	+	+	+
22	34.820	+	+	+	-	-	-	-	+
23	31.426	+	-	-	-	-	-	-	+
24	29.918	+	+	+	+	+	+	-	+
25	27.279	+	+	+	+	+	+	+	+
26	25.016	+	+	+	-	+	-	+	
27	21.246	+			+	+	+	+	+
28	19.738	+	+	+	+	+	+	-	
29	17.852	-	+	+	+	+	+	-	+
30	13.328	-	+	-	-	-	-	-	-
31	12.197	+	-	-	-	-	-	-	-
32	10.311	-	+	-	+	+	+	-	-
33	9.180	+	+	-	-	-	-	+	+
34	6.918	+	+	-	-	-	-	-	+
35	4.655	-	-	+	+	+	+	+	+
36	3.524	+	+	+	+	+	+	+	+

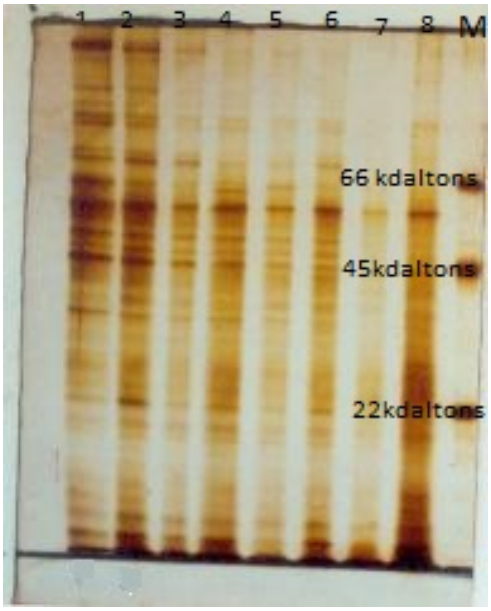

Fig.1:SDS Polyacrylamide gel of denatured Protein patterns in ten samples of the *Aphisgossypii*

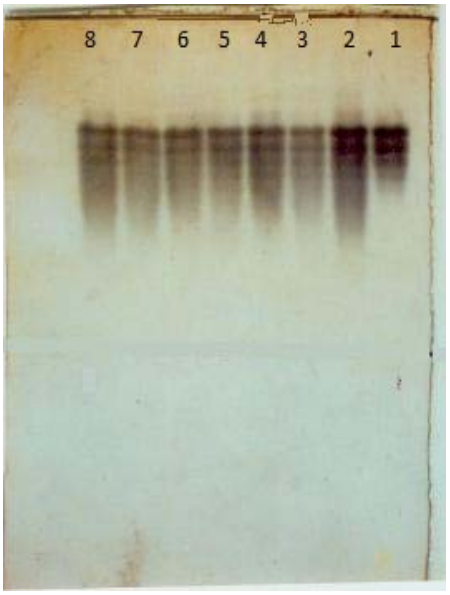

Fig. 2: Polyacrylamide gel zymogram of esterase isozyme patterns in different population governorates

Esterase have been classified according to their action with various specific enzyme inhibitors, three classes of esterases could be identified as cholinesterase, carboxyesterase and arylesterase based on the substrate specificity and inhibition tested in last nymphal instars or adult. As shown from data given in table(3) 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 3: Esterase patterns in field and laboratory strains of *Aphisgossypii*

		1	2	3	4	5	6	7	8
No	RM	SH13	GH13	BE13	FA14	SH14	GH14	BE14	Lab.strain
1	0.20	+	+	+	+	+	+	+	+
2	0.23	+	+	+	+	+	+	+	+
3	0.25	+	+	+	+	+	+	+	+
4	0.28	-	-	-	+	+	+	+	+
5	0.30	-	-	-	+	+	-	-	-

RM: RATE MIGRATION

DISCUSSION

A.gossypii are important pests in cotton fields and outdoor crops, and are increasingly difficult to mange deposit heavy application of insecticides, resistance of these been e insects has suggested (Ayad *et al*., 1992, Hardee and Answorth 1993) and has been demonstrated to organochlorine, organophosphate, carbamate, pyrethroids insecticides in Alabama and Texas (Kerns and Gaylor 1992). In Egypt, extensive use of several classes of insecticides has occurred for insect control on cotton before and since the 1949 build up of *A. gossypii* populations thus, possible development of resistance patterns in this insects. This study represents the results of laboratory tests conducted on field population of last nymphal instar as well as adult stage of *A.gossypii* to determine the relative resistance to insecticides and cross-resistance. The results of resistance

monitoring indicated that all field strains of (Fayum, Gharbia, Behera and Sharkia) exhibited extremely high levels of resistance (ranged between 0.75 to 679.06 fold).

Resistance ratio (RR) of carbamate insecticides adopted in Gharbia, Behera and Sharkia strains in 2013 but in 2014 season it was applied on Fayum, Gharbia, Behera and Sharkia strains. Pirimicarb was the most effective insecticide against *A. gossypii* for all tested strains in 2013, the (RR) in Gharbia and Behera were 1 fold, but Sharkia was 1.15 fold, however all strains were susceptible. During 2014 resistance ratio in Fayum and Gharbia were 1 fold but Sharkia was 1.66 fold while Behera was 3.59 fold. The results of Methomyl revealed tolerance for all strains in both seasons. The carbamate high effect on *A.gossypii* and no cross resistance in all strains, this results agree with that obtained by Holtkamp *etal*.,1992 ,Silver *etal*., 1995, EL-Kady (2oo7) and Abdelmotaleb (2015).

Resistance ratio (RR)of Pyrethroid Lambda-cyhalothrin in 2013 season showed (221.46, 34.6 and 1 fold) in Sharkia, Gharbia and Behera, respectively, but in season 2014 Lambda-cyhalothrin (RR) were (48.37, 18.98, 12.31 and 43.26 fold) in Fayum, Sharkia, Gharbia and Behera, respectively. As regards Cypermethrin, low resistance was observed in all strains in 2013 (ranged between 2.66 - 7.45 fold) but in 2014 increase low to high resistance ranged between 7.37-392.84 fold. Among pyrethroids Etofenprox as revealed that use in 2013 in Sharkia, Gharbia and Behera RR were (5.09, 4.68, 1.19 fold) respectively, but in 2014 Fayum, Sharkia, Gharbia and Behera were (4.66, 679.06, 477.08, and 498.93 fold) respectively. These results different to tolerance to high resistance and also this result agreement with that obtained by Singab (2007 a and b).

Resistance ratios of organophosphate Dimethoate indicate moderate levels in 2013 season ranged between (3.4- 12.27 fold) but in 2014 season showed high level ranged between (5.83 - 38.74 fold). Among the (OP) compounds Malathion showed susceptible or tolerant in all strains in seasons 2013 and 2014 seasons except Behera strain in 2013 RR was 19.3 fold. Also, Primiphos-methyl was the most effective insecticide against *A.gosspii* for all tested strains in both seasons except Sharkia strain (11.1 fold) Similar observations have been made by Silver *etal*., (1995) , Ahmad *et al*. (2003), Abdelmotaleb, (2015) and Saleem*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, *etal*., 2014 and Saleem, *etal*., 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 esterase's showed a good discrimination between Susceptible and field strains. Esterase plays an important role in insecticide resistance. Insect esterase's have been intensively studied because they are target sites for organophosphate and carbamate insecticide (Abdel-Hafez *etal* .1982). Studies on the insecticidal action of organophosphorus (OP) compounds

raised great interest in insect esterase's, since OP-compounds were found to cause strong inhibition of several esterase 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 modified esterase's are capable of hydrolyzing OP-compounds and carbamate. Our results are in agreement with those obtained on esterase's activity compared to susceptible populations, where resistant *Helicoverpa armigera* has additional esterase's bands which are not detected in susceptible individuals (Gunning *etal*., 1996).

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