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EVALUATION OF ENZYMES ROLE IN INSECTICIDES RESISTANCE MECHANISM OF *BEMISIA TABACI* (GENNADIUS) FROM SEVEN GOVERNORATES OF EGYPT

Raghda M. Hassan[1]*, Hala M. AbouYousef[1], Karima H. E. Haggag[2] and Bahia Y. Riad[3]

[1*]Bioassay Department, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt.
[2]Pest Rearing Department, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt.
[3]Organic Department, Faculty of science, Cairo University, Giza, Egypt.

Abstract

Seven field populations of whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) were collected from Qalubia, Sharkia, Dakahlia, Gharbia, Monofia, Behira and Kafr El- Sheikh Governorates, Egypt, during season 2018. Development of resistance in insect populations against insecticides [pyrethroids (α-cypermethrin and etofenprox), organophosphates (malathion and primiphos-methyl), carbamates (carbosulfan and methomyl) and neonicotinoids (acetamiprid and thiamethoxam)] were evaluated for determining the slope response line, LC50 values and resistance ratio (RR) in laboratory tests. The RR in different governorates population were in ranges from 1.42 to 185.78 fold for pyrethroids, 0.80 to 9.63 fold for organophosphates, 0.38 to 44.98 fold for carbamates and 0.19 to 166.66 fold for neonicotinoids, respectively, comparing with Gharbia which showing the lowest LC_{50} values. The activity levels of detoxifying enzymes *viz*, acetylcholinesterase (AChE), glutathione -S-transferase (GST) and Carboxylesterase (CarE) were determined in insect populations of different governorates, compared to Gharbia as well as the control.

Key words: *Bemisia tabaci*, detoxifying enzymes, insecticides, resistance ratio, susceptibility

Introduction

Bemisia tabaci Genn. (Hemiptera: Aleyrodidae) is classified as one of the most destructive agricultural pest worldwide owing to its ability to feed on hundreds of important plant species (Dinsdale *et al*., 2010). It causes damages directly through phloem feeding as sap sucking pest and indirectly through the transmission of plant viral diseases (Navas-Castillo *et al*., 2011; Gnankine *et al*., 2018). Different groups of insecticides such as organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, thiourea and insect growth regulators etc. were applied by farmers for managing of whitefly over the world, where the insecticides still to be key weapons for the controlling insect pests (Horowitz *et al*., 2007). In conventional crop management systems, the excessive insecticides application cause development high resistance (Vassiliou *et al*., 2011 and Kontsedalov *et al*., 2012), increase the reliance upon chemicals and dramatically multiply crop production costs (Naranjo and Ellsworth, 2009). Therefore, introduction of neonicotinoids *viz*. imidacloprid was the first insecticide of this group which gave good results on the carbamates, organophosphate, pyrethroids resistance whitefly strains (Cox, 2001). Understanding of the resistance mechanisms is essential for improving control efficacy, especially the control of whitefly mostly depended on synthetic insecticides for decades (Zhang *et al*., 2012). The population of *B. tabaci* collected in Turkey and Crete showed 20- to 310-fold resistance to Ops; 30- to 600 fold resistance to α-cypermethrinand 38- to 1958-fold resistance to imidacloprid (Erdogan *et al*., 2008; Roditakis *et al*., 2009). In China, *B. tabaci* resistance to neonicotinoids is a serious problem. Adult *B. tabaci* collected from south eastern China exhibited 28- to 1900-fold resistance to imidacloprid and 29- to 1200-fold resistance to thiamethoxam (Wang *et al*., 2010).

The detoxifying enzymes *viz*. acetylcholinesterase (AChE), glutathione S-transferase and total esterases play important roles in resistance development. The acetylcholinesterase (AChE) is a key enzyme in nerve transmission (Houndété *et al*., 2010 a); the GSTs belong

***Author for correspondence :** E-mail : raghda1983@yahoo.com

to a group of multifunctional proteins that represent a central role in detoxification of xenobiotic compounds (Hayes *et al*., 2005) and esterases hydrolyze ester and play a key role in the detoxification of many conventional groups of insecticides (Wheelock *et al*., 2005). Esterases along with glutathione-S-transferase are two major enzymes involved in detoxification of most conventionally used groups of insecticides in all the field populations of *B. tabaci,* when compared with laboratory strain. (Hanskumar, 2016). The Pyrs and OPs insecticides, for example, act on the insect nervous system by altering the normal functioning of the para-type voltage gated sodium channel and by inactivating the enzyme acetylcholine esterase, respectively. Insecticide resistance mechanisms against members of both classes mediated either by changes in the sensitivity of insecticide targets or by increases in the rate of insecticide detoxification, are clearly functional in *B. tabaci* (Byrne *et al.,* 2000; Alon *et al.,* 2008). Neonicotinoids target is the acetylcholine receptor of the insect nervous system. They are one of the world's most important chemicals groups owing to their high toxicity against an extensive range of arthropods (Nauen *et al.,* 2008; Jeschke *et al.,* 2011). Particularly well suited to the control of Hemipteran sucking pests, they became useful insecticides against *B. tabaci* (Bass *et al.,* 2015).

This work is aimed to study the susceptibility of field populations *B. tabaci* , collected from seven governorates covering Delta geographic area of Egypt, against eight insecticides include pyrethroids, organophosphates, carbamates and neonicotinoids well as the activity levels of detoxifying enzymes [acetylcholinesterase (AChE), glutathione -S- transferase (GST) and carboxylesterase (CarE)], in different insect field populations were investigated to clarify the mechanism of insecticides resistance.

Materials and methods

Whitefly strains

Seven field samples of *B. tabaci* collected from different Governorates of Egypt (Behira, Dakahlia, Kafr El- Sheikh, Gharbia, Monofia, Qalubia and Sharkia) during season 2018. Fig. 1. Whiteflies were collected in the early morning hours using a mouth aspirator into large pots with ventilated lids modified to fit the aspirator. The whiteflies were transported to the laboratory in a cool box and used within a few hours after collection for the toxicological tests (Roditakis *et al.,* 2005).

Insecticides

The insecticides, *i.e*. pyrethroids [α-cypermethrin (Bestox 15% EC) and etofenprox (Trebon 30% EC)], organophosphates [malathion (Malathion 57% EC) and primiphos-methyl (Actellic 50% EC)], carbamates [carbosulfan (Marshal 25% EC) and methomyl (Neomil 90%)] and neonicotinoids [acetamiprid (Acetamor 20% SP and thiamethoxam (Actara 25% WP)] were applied in this study.

Bioassay

The leaf-dip technique was applied for bioassay of tested insecticides against field populations of *B. tabaci* collected from different governorates. The cotton leaves firstly were washed by tap water, dried and then cut into discs (35 mm in diameter). The cotton leaf discs were separately dipped in aqueous solutions (10s) in serial of different concentrations of commercial formulations of each insecticide. After drying, the leaf discs were placed with their adaxial surface downward on agar in the base of a small plastic vial of appropriate size. Control leaf discs were dipped in distilled water only. Twenty adults of *B. tabaci* (mixed sex) were then added with a mouth aspirator and placed into a small plastic vial (40 mm diameter, 37 mm high). Each diluting concentration of each insecticide as well as the control had 3 replicate vials (Houndété *et al.,* 2010a). Mortality was recorded after incubation for 24 h at 26 ± 2ºC, 55 ± 5% R.H and with a photoperiod of 16:8 (L : D) h. Insect signs of movement were considered alive. The mortality data were corrected according to Abbott's formula (Abbott, 1925).

$$\text{Corrected percentage mortality} = \frac{T - C}{100 - C} \times 100$$

Where:

T = No. of dead insects adult in treated replicates and C = No. of dead insects adult in control replicates.

Data analysis

The LC_{50} values, 95% confidence limits and the slopes of the regression lines were estimated by LDP- Line statistical computer program. The resistance ratio for each insecticide was calculated with reference to a population with the lowest LC_{50} (Houndété *et al.,* 2010 b and Shadmany *et al.,* 2015).

Activity of detoxifying enzymes

Acetylcholine esterase (AChE) activity

The acetylcholinesterase activity in field insect populations was colorimetric determined by using the method described by Ellman *et al.,* (1961). Fifty adults of *B. tabaci* from each population were homogenized in 350 µl of ice-cold Tris- HCl buffer (0.038 M and pH 8.5) containing 0.1% (V/V) Triton X-100. The homogenates

were centrifuged at 13000g for 15 min at 4^{0}C and the supernatant was transferred to new tube. About 850 µl of Tris- HCl buffer was placed in cuvette and then 100 µl of supernatant were added, followed by addition of 40 µl of 5'5-dithio-bis (2- nitro benzoic acid) [DTNB] solution (0.01 M) and 10 µl of substrate solution acetylthiocholine iodide (0.075 M) and mixed well. The absorption was measured at 412 nm using spectrophotometer for 5 min against the blank, which contained the entire reagent except the substrate. The acetylcholine activity was calculated as µ moles of acetylcholine iodide hydrolyzed per mg protein per minute.

Glutathione -S- transferase

Glutathione-S-transferase activity was measured in field populations of insect as indicated by Habig *et al.*, (1974). Fifty adults of *B. tabaci* from each population were homogenized in 350 µl of ice-cold Phosphate buffer (0.1M and pH 6.5). The homogenates were centrifuged at 13000g for 15 min at 4^{0}C and the supernatant was transferred to new tube. About 880 µl of phosphate buffer was placed in cuvette and then 100 µl of supernatant were added. Then, 10 µl of 1-chloro 2, 4-dinitrobenzene, 30 mM [CDNB] and 10 µl substrate solutions GSH (50 mM) were added, mixed well and incubated for 3 min. at room temperature. The absorption was measured at 340 nm using UV/Vis spectrophotometer (V-530) against the blank, which contained the entire reagent except the substrate.

Carboxyl esterase

The carboxyl esterase activity was determined in field populations of *B. tabaci* using á-naphthyl acetate (α-NA) as a substrate according to the method described by Van Asperen (1962). Fifty insect adults from each population were homogenized in 350µL of ice-cold phosphate buffer (0.1M, pH 7.2). The homogenates were centrifuged at 13000 g for 15 min at 4^{0}C and then the supernatant was transferred to new tube. About 850 µl of phosphate buffer was placed in cuvette , then 50 µl of supernatant was added followed by 50 µl substrate solution (α-naphthyl acetate 0.2 M), mixed well, and then incubated at 27ºC for 15 min. After incubation time, 50 µl of stop solution [two parts of solution of Fast Blue RR (1%) and five parts of sodium dodecyl sulfate (5%)] was added. The absorption was measured at 600 nm for the hydrolysis of α-naphthyl acetate using UV/Vis spectrophotometer (V-530) against the blank, which contained the entire reagent except the substrate. The esterase activity was calculated as mean levels of total esterase activity cited were based on protein content and α–naphthol standard curves.

Results

Bioassays

The bioassays of *B. tabaci* populations, collected from seven different Governorates *viz.*, Behira, Dakahlia, Kafr El- Sheikh, Gharbia, Monofia, Qalubia and Sharkia with tested insecticides are listed as the slope line, LC_{50}, Confidence limit (CL 95%) and RR values in Tables (1, 2, 3 and 4).

Pyrethroids

The tested *B. tabaci* populations, obtained from different governorates, (Table 1) showed the slope of response line ranged from 0.33 to 1.02 for α-cypermethrin, where the insect population of Qalubia revealed the highest slope line, followed by population of Dakahlia, Sharkia, Monofia, Kafr El-Sheikh, Behira and Gharbia, respectively. The slope of response line of etofenprox were in the ranges of 0.32 to 3.27, where the highest with Kafr El-Sheikh population, followed by Qalubia, Dakahlia, Sharkia, Behira, Monofia and Gharbia populations, respectively. While the LC_{50} values ranged from 44.94 to 570.76 mg/l for α-cypermethrin, while the values were in the ranges of 6.17 to 1146.26 mg/l for etofenprox, respectively. The highest LC_{50} value was recorded with population of Kafr El-Sheikh with two pyrethroids**,** followed by Sharkia, Qalubia, Dakahlia, Behira, Monofia and Gharbia, respectively. Details of 95% CL of two pyrethroids insecticides were shown in Table 1. The resistance values (RR) ranged from 1.42 to 12.70 fold for α-cypermethrin, while it's were in the ranges of 4.27 to 185.78 fold for etofenprox, where the highest with Kafr El-Sheikh population, while the lowest with Monofia population, respectively.

Organophosphates

The slope of response line of field populations of *B. tabaci* (Table 2) ranged from 0.80 to 1.44 for primiphos-methyl, where the insect population of Qalubia revealed the highest slope line, followed by Kafr El-Sheikh, Dakahlia, Sharkia, Monofia, Gharbia and Behira populations, respectively. The slope response line of *B. tabaci* population to malathion was in the ranges of 0.69 to 1.79, where the highest with Dakahlia populations, followed by Qalubia, Gharbia, Kafr El-Sheikh, Sharkia, Behira and Monofia, respectively. The LC_{50} values of primiphos- methyl ranged from 18.22 to 175.49 mg/l, while the values ranged from 420.97 to 4223.37 mg/l for malathion, respectively. The highest LC_{50} value was recorded with population of Kafr El-Sheikh for two organophosphates, while the lowest one was recorded with Gharbia population for primiphos- methyl and Behira population for malathion, respectively. Details of 95%

CL of two organophosphours were shown in Table 2. The RR values for primiphos- methyl ranged from 1.96 to 9.63 fold, while the resistance ratio was in the ranges of 0.80 to 8.07 fold with malathion, respectively. The highest ratio was recorded in Kafr El-Sheikh population for two organophosphours, while the lowest was recorded in Monofia and Behira populations in two organophosphours, respectively.

Carbamates

The slope of response line of tested *B. tabaci* populations for carbosulfan (Table 3) ranged from 0.39 to 2.54, where the highest slope line was obtained with insect population of Qalubia, followed by populations of Monofia, Behira, Dakahlia, Kafr El-Sheikh, Sharkia and Gharbia, respectively. The slope of response line of methomyl was in the ranges of 0.64 to 2.72, where the population of Gharbia showed the highest slope line, followed by Qalubia, Sharkia, Behira, Dakahlia, Monofia and Kafr El-Sheikh, respectively. The LC_{50} values ranged from 18.26 to 821.33 mg/l for carbosulfan, where the highest value was recorded Qalubia population, followed by population of Kafr El-Sheikh, Behira, Dakahlia, Sharkia, Monofia and Gharbia, respectively. The LC_{50} values of methomyl were in the ranges of 36.39 to 437.11 mg/l, where the highest in Qalubia population also, followed by Kafr El-Sheikh, Monofia, Gharbia, Behira, Dakahlia and Sharkia, respectively. Details of 95% CL of two Carbamates were shown in Table 3. The RR values for carbosulfan ranged from 1.29 to 44.98 fold, where the highest in Qalubia population and the lowest in Monofia population, respectively. The RR values were in the ranges of 0.38 to 4.56 fold with methomyl.

Table 1: Susceptibility of field populations *Bemisia tabaci* from seven governorates of Egypt to pyrethroids insecticides.

Insect -icides	Govern -orates	Slope ±SE	LC_{50} (mg/l)	Confidence lim -it 95% (mg/l)	Resistance Ratio
α-Cyper-methrin	Gharbia	0.33±0.05	44.94	19.91-141.17	1
	Behira	0.35±0.05	97.41	47.92-260.14	2.17
	Dakahlia	0.87±0.23	133.27	76.11-196.37	2.96
	Monofia	0.56±0.10	63.98	33.30-200.31	1.42
	Sharkia	0.70±0.20	277.06	171.92-615.55	6.16
	Qalubia	1.02±0.11	135.96	53.30-403.58	3.02
	Kafr El-Sheikh	0.50±0.11	570.76	320.32-1451.68	12.70
Etofe-nprox	Gharbia	0.32±0.07	6.17	2.75-21.88	1
	Behira	0.69±0.16	349.521	196.97-1132.98	56.65
	Dakahlia	1.37±0.32	513.26	327.54-1045.58	83.19
	Monofia	0.45±.07	26.35	12.78-77.13	4.27
	Sharkia	0.89±0.08	409.59	316.30-544.79	66.38
	Qalubia	2.19±0.36	1096.07	953.43-1336.41	177.64
	Kafr El-Sheikh	3.27±0.31	1146.26	1037.53-1265.71	185.78

Table 2: Susceptibility of field populations *Bemisia tabaci* from seven governorates of Egypt to organophosphours insecticides.

Insect -icides	Govern -orates	Slope ±SE	LC_{50} (mg/l)	Confidence lim -it 95% (mg/l)	Resistance Ratio
Primi phos-methyl	Gharbia	0.84± 0.06	18.22	6.94-49.54	1
	Behira	0.80±0.08	54.34	15.19-146.63	2.98
	Dakahlia	1.24±0.15	64.46	49.66-81.98	3.54
	Monofia	0.89±0.08	35.71	26.02-49.26	1.96
	Sharkia	1.12±0.16	52.38	38.40-76.62	2.87
	Qalubia	1.44±0.11	160.74	95.09-248.19	8.82
	Kafr El-Sheikh	1.31±0.12	175.491	136.41-225.26	9.63
Mala thion	Gharbia	1.70±0.21	523.23	438.42-639.91	1
	Behira	0.87±0.10	420.97	289.16-596.57	0.80
	Dakahlia	1.79±0.17	759.59	609.93-916.62	1.45
	Monofia	0.69±0.15	478.26	272.49-1443.13	0.91
	Sharkia	1.18±0.17	578.65	425.56-860.82	1.10
	Qalubia	1.77±0.29	2939.76	2493.55-3567.41	5.62
	Kafr El-Sheikh	1.50±0.20	4223.37	3465.64-5319.69	8.07

Neonicotinoids

The slope of response line of tested *B. tabaci* populations (Table 4) ranged from 0.62 to 1.41 for acetamiprid, where the insect population of Qalubia revealed the highest slope line, while the lowest one was recorded with Kafr El-Sheikh population. The slope of response line of thiamethoxam was in the range of 0.43 to 2.15, where the populations of Gharbia and Monofia were the highest and the lowest values, respectively. The LC_{50} values ranged from 0.71 to 118.33 mg/l for acetamiprid, while the values were in the range of 13.98 to 534.33 mg/l for thiamethoxam, respectively. The highest LC_{50} value was recorded with population of Kafr El-Sheikh for two neonicotinoids, while the lowest one was recorded with populations of Gharbia and Behira for acetamiprid and thiamethoxam, respectively. Details of 95% CL of two neonicotinoids were shown in table 4. The RR values for acetamiprid ranged from 6.59 to 166.66 fold, respectively, while the values ranged from 0.19 to 7.38 fold with thiamethoxam, respectively. In Behira and Kafr El-Sheikh population showed the highest value with two neonicotinoids, while the lowest one was recorded with populations of Monofia and Behira with

acetamiprid and thiamethoxam, respectively.

Enzymatic activity

Acetylcholinesterase (AChE)

Results revealed that mean values of AChE activity 3.52 to 10.28 nmol/min./mg protein (Table 5 and Fig. 2) decrease in activity in all populations except Qalubia population, compared with the control (Gharbia). Significant reduction in the enzyme activity was occurred in Dakahlia and Behira (3.59 and 3.52 nmol/min./mg protein, respectively). While, significant increases in activity of AChE were observed in Qalubia (10.28 nmol/min./mg protein). While, the percentage of change reached to (+76.33%) in Qalubia, compared to control (5.83 nmol/min./mg protein).

Glutathione-S-transferase (GST)

In case of GST activity, data in Table 5 and Fig. 3 indicated that all tested populations caused various significant increases in enzyme activity except Dakahlia population compared with control (Gharbia). Significant increase in the enzyme activity was occurred in Sharkia, Behira, Monofia and Kafr El Sheikh (953.11, 1211.01, 762.27 and 815.82 nmol/min./mg protein, respectively), but Dakahlia Population showed significant decrease (188.79nmol/min./mg protein) compared to control (639.59 nmol/min./mg protein).

Carboxylesterase (CarE)

The carboxylesterase activity showed in Table 5 and Fig. 4. The results indicated significant increase in activity in Kafr El- Sheikh only (0.94 mol/min./mg protein) compared with Gharbia (control) (0.69 mol/min./mg protein). Reduction in the enzyme activity was occurred in all populations except Qalubia has increase in activity (0.61, 0.61, 0.60, 0.50 and 0.77 mol/min./mg protein) for Sharkia, Dakahlia, Behira, Monofia and Qalubia, respectively.

Table 3: Susceptibility of field populations *Bemisia tabaci* from seven governorates of Egypt to carbamates insecticides

Insect -icides	Govern -orates	Slope ±SE	LC_{50} (mg/l)	Confidence lim -it 95% (mg/l)	Resistance Ratio
Carbos-ulfan	Gharbia	0.39±0.07	18.26	7.49-88.02	1
	Behira	1.03±0.01	116.32	83.45-156.95	6.37
	Dakahlia	1.01±0.15	86.52	61.13-115.70	4.74
	Monofia	1.04±0.16	23.49	16.53-38.50	1.29
	Sharkia	0.74±0.06	46.99	12.04-201.32	2.57
	Qalubia	2.54±0.26	821.33	725.57-942.91	44.98
	Kafr El-Sheikh	0.85±0.10	224.35	156.21-324.42	12.29
Meth omyl	Gharbia	2.72±0.25	95.94	85.24-106.77	1
	Behira	0.81±0.10	89.42	61.16-130.64	0.93
	Dakahlia	0.70±0.10	36.39	23.86-59.47	0.38
	Monofia	0.67±0.10	120.75	71.38-271.39	1.26
	Sharkia	1.30±0.16	44.14	34.22-55.63	0.46
	Qalubia	1.42±0.12	437.114	232.92-791.65	4.56
	Kafr El-Sheikh	0.64±0.10	126.31	79.71-212.33	1.32

Table 4: Susceptibility of field populations *Bemisia tabaci* from seven governorates of Egypt to neonicotinoids insecticides.

Insect -icides	Govern -orates	Slope ±SE	LC_{50} (mg/l)	Confidence lim -it 95% (mg/l)	Resistance Ratio
Acetam -iprid	Gharbia	0.66±0.07	0.71	0.41-1.08	1
	Behira	0.80±0.08	12.50	8.53-17.69	17.60
	Dakahlia	1.03±0.15	12.75	9.50-17.20	17.96
	Monofia	0.81±0.10	4.68	3.19-6.81	6.59
	Sharkia	1.12±0.11	11.54	8.65-16.08	16.25
	Qalubia	1.41±0.15	65.58	53.69-79.40	92.37
	Kafr El-Sheikh	0.62±0.10	118.33	73.36-224.18	166.66
Thiamet hoxam	Gharbia	2.15±0.25	72.44	60.94-84.07	1
	Behira	0.74±0.06	13.98	3.70-42.78	0.19
	Dakahlia	0.58±0.08	40.88	26.06-67.21	0.56
	Monofia	0.43±0.07	30.66	16.30-75.43	0.42
	Sharkia	0.79±0.08	26.71	18.80-37.77	0.37
	Qalubia	1.05±0.11	203.88	160.53-257.52	2.81
	Kafr El-Sheikh	1.88±0.17	534.33	300.89-831.12	7.38

Discussion

In present study, populations of *B. tabaci* were collected from different seven Governorates of Egypt were evaluated their susceptibility towards eight insecticides as well as detoxifying enzymes activity, compared to Gharbia population as a reference strain because it produced the lowest LC_{50} values for the tested insecticides. The present data showed that the *B. tabaci* populations revealed less than thirteen fold resistance ratio to α-cypermethrin, compared to etofenprox, where all insect populations were more than fifty fold resistance ratio, except Monofia population. These results agreement with the field strains in eastern China that showed resistance to cypermethrin were less than nine fold (Yuan *et al.*, 2012). All five strains exhibited significant resistance to the cypermethrin, where the RF (Resistance Factor) ranged from 20 to 246 fold (Luo *et al.*, 2010). On the other hand, high resistance levels to etofenprox were observed in all whitefly populations (Marasinghe *et al.*, 2017).

The current results explained the resistance ratio to pirimiphos-methyl and malathion less than ten fold for both, where the Egyptian populations of *B. tabaci* showed no resistance to primiphos-methyl as recorded by El-Kady and Devine (2003). These results are agreement with those recorded by (Ahmed *et al.*, 2010). He showed that no resistance to malathion was detected during 1994 to 1998 and in 2000 seasons, while a very low level resistance was found during 1999 and 2001–2007 seasons in the field populations of *B. tabaci*.

Table 5: Activity of acetylcholinesterase, glutathione-S-transferase and esterase in *B. tabaci* field populations.

Govern -orates	AChE nmol/min./ mg protein	GST nmol/min./ mg protein	Esterase mol/min./ mg protein
	Mean±S.E	Mean±S.E	Mean±S.E
Gharbia	5.83±0 .27	639.59±13.45	0.69±0.03
Qalubia	10.28±0.56*	729.81±26.11	0.77±0.02
Sharkia	5.11±0.14	953.11±19.56*	0.61±0.02
Dakahlia	3.59±0.18*	188.79±15.63*	0.61±0.004
Behira	3.52±0.34*	1211.01±28.47*	0.60±0.13
Monofia	4.49±0.15	762.27±7.56*	0.50±0.01
Kafr El-Sheikh	4.67±0.09	815.82±33.84*	0.94±0.04*

Fig. 1: The map shows the survey locations and distributions of *B. tabaci* populations in Egypt.

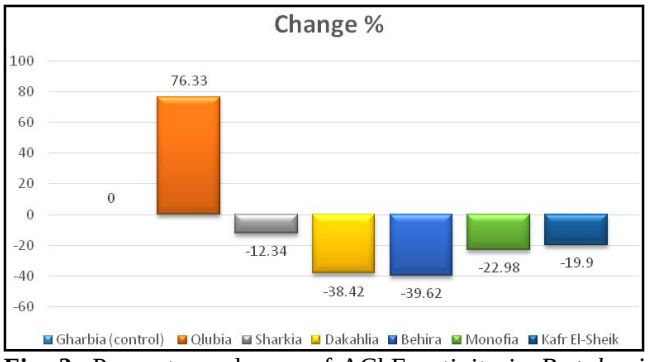

Fig. 2: Percentage change of AChE activity in *B. tabaci* populations.

The results also showed that *B. tabaci* population has low resistance ratio in all populations to methomyl (RR<5 fold). The obtained results are agreement with the resistance levels in *B. Tabaci* populations to methomyl ranged from 3- to 55-fold (Fernández *et al.*, 2009). There was a moderate resistance to methomyl in the Khanewal-1 and Multan-3 populations of *B. tabaci* tested in 1994 and 1995, respectively (RF = 17 and 20). The resistance reduced to very low levels during 1996–2002. Methomyl resistance again increased to low levels during 2003–2007 (RF= 10 and 20) (Ahmed *et al.*, 2010). A moderate to high level of methomyl resistance was also recorded in Indian populations of *B. tabaci* (Kranthi *et al.*, 2001). Results showed that the same insect populations showing resistance ratio to carbosulfan where RR was < 45 fold. El-Kady and Devine (2003) found that the Egyptian populations of *B. tabaci* had a moderate to high resistance to carbosulfan (RF= 22-50), while Yuan *et al.*, (2012) showed that all field strains consistently displayed no resistance to carbosulfan (RF 0.20- 0.79).

The current results showed that the tested populations exhibited resistance levels for thiamethoxam and acetamiprid (RR<8 fold and RR>18 fold), respectively, where strains collected in Israel showed up to1000-fold resistance to thiamethoxam and less than 74 fold for acetamiprid (Kontsedalov *et al.*, 2012). Adult *B. tabaci* collected from southeastern China exhibited resistance to thiamethoxam (Wang *et al.*, 2010). High levels of resistance were detected for thiamethoxam, while low resistance levels were observed for acetamiprid, where the insect population exhibited levels of RF was >50 and <12, respectively (Vassiliou *et al.*, 2011).

The results indicated decrease in AChE activity in all populations, except Qalubia population, where AChE play important role in degrading the neurotransmitter acetylcholine in the insect synapse which its primary target site for OP and carbamates insecticides (Ffrench–Constant, 1998). Acetylcholinesterase hydrolyzes acetylcholine to prevent its accumulation at nerve synapses since its accumulation leads to death (Das, 2013). The activity of GSTs was significantly greater in the all field populations, except Dakahlia population than in Gharbia population. Together, these results indicated that increased GST activity contributes to thiamethoxam resistance in the field *B. tabaci* THQR strain. The overexpression of several GSTs has been associated with insecticide resistance (Pavlidi *et al.*, 2015). The obtained results indicated that esterase activity decreased in all populations except Qalubia and Kafr El-sheikh showed

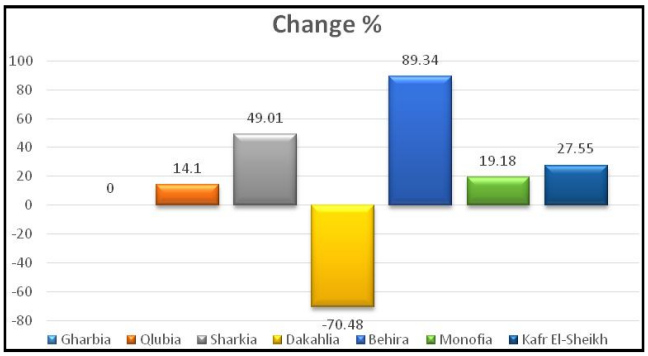

Fig. 3: Percentage change of GST activity in *B. tabaci* populations.

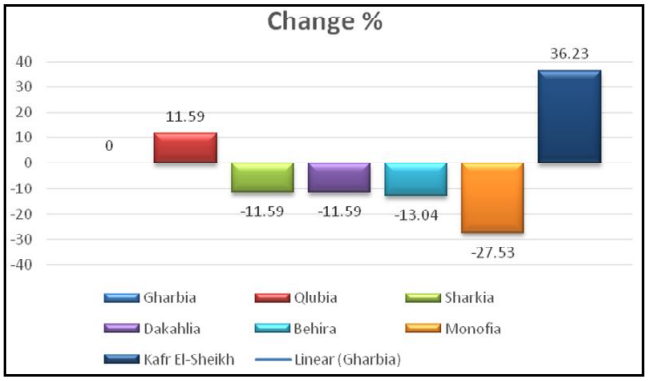

Fig. 4: Percentages change of Esterase activity in *B. tabaci* populations.

increase in activity compared with Gharbia population. Changes in CarE activity have been associated with resistance to OP insecticides in certain species of mosquitoes, aphids, lepidoptera and higher Diptera (Li *et al*., 2007). The results are agreement with GST activity has been found to increase in insects resistant to insecticides (Papadopoulos *et al*., 2000) and nonspecific esterases and GST were found to participate in metabolism and detoxification of organophosphates, pyrethroids, carbamates and juvenoids (Pasteur *et al*., 2001). The involvement of elevated esterase activity in OPs and pyrethroids resistance had been clearly demonstrated in *B. tabaci* species complex (Alon *et al*., 2008). It has been associated with different mechanisms of resistance such as detoxification by microsomal monooxygenases and esterase's, as well as target site modifications, e.g. insensitive acetylcholinesterase reducing the efficacy of organophosphates and carbamates (Rauch and Nauen, 2003) and Resistance to several classes of insecticides such as organophosphates, carbamates and pyrethroids is widespread in *B. tabaci* (Horowitz *et al*., 1999).

In conclusion, the results of this study showed that the use of insecticides to control *B. tabaci* resulted in development of resistance in field populations against different groups of insecticides while the detoxifying enzymes role can help to understanding the resistance mechanism.

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