

## VARIATION IN SUSCEPTIBILITY BETWEEN WHITE FLY AND COTTON APHID INSECTS TO THE TOXIC EFFECT OF NEONICOTINOID INSECTICIDES

EMAN M.M.RADWAN , HALA M. ABOU YOUSEF AND M. SINGAB

Central Agricultural Pesticides Laboratory, ARC, Dokki , Giza, Egypt

### Abstract

Field Strains of the sweet potato white fly *Bemisia tabaci* (Genn.) and the cotton aphid , *Aphis gossypii* ( Glover ) from Beni-Suef, Menofia, Gharbia and Behera Governorates were tested with four neonicotinoides; three different formulations of Imidacloprid (Imidor 20%EC, Confidor 20% SL and Best 25%WP) and Thiamethoxam (Actara 25%WG) and three recommended insecticides; Carbosulfan (Marshal 25%WP) Diafenthiuron, (Polo 50%SC ) and Buprofezin (Applaud 25%SC). The four neonicotinoids were highly toxic to *B. tabaci* than *A. gossypii* collected from four Governorates. Cotton aphid was more susceptible to the effect of three other insecticides than white fly. The adult stage of whitefly was more susceptible to the toxic effect of all tested pesticides than the last nymphal instar. The total Protein content was highly concentrated in tissues of white fly than aphid tissues from the same localities. The electrophoretic separation of non specific esterases isozymes revealed A high activity of enzyme zones with  $\alpha$ -naphthyl acetate substrate in two pests tissues. The esterase isozymes of Aphid were classified as cholin and carboxylesterases so that the insect was susceptible to the effect of recommended insecticides. White fly esterases classified as cholin, carboxyl and arylesterase this may be illustrate the tolerant response of insect to the recommended insecticides

Key words: *Bemisia tabaci*, *Aphis gossypii*, neonicotinoid insecticides, proteins, nonspecific esterases

### INTRODUCTION

The White fly is a highly polyphagous insect, observed on more than 300 plant species, with a predilection for cotton, beans, sunflowers, aubergine, potato, capsicum, tobacco, tomato, citrus and ornamental plants (Greathead, 1986) Throughout tropical and subtropical regions of the world (Cock, 1986). *Bemisia tabaci* (Genn.) attacks many high value plant hosts of several different families in Egypt (Abd-Rabou, 1997). The Cotton aphid *Aphis gossypii* (Glover) constitutes one of the major and important economic pests of cotton plants in Egypt and it causes heavy losses in many years (Hassanein *et al.*, 1971). Whitefly and aphid have piercing-sucking type mouth parts, which they use to suck sap from the host plant. When the two insects populations are high the loss of sap can significantly affect plant growth and development, resulting in stunted plants. Because plant sap has a very high

carbohydrate content, but is relatively low in protein, insects must consume large amounts of sap more than they need to meet their protein need. The excess sugars without digestion were excreted as honey dew on plant leaves which supports the development of sooty mold fungi and reduce photosynthesis (Godfrey *et al.*, 1997). When honeydew is deposited on open lint it results in sticky cotton which discounts the price of cotton and causes difficulties in its milling (Brown *et al.*, 1995) In addition, whitefly is responsible for transmitting many devastating viruses to many plants (Hegab *et al.*, 1992).

Foliar applied insecticides are recommended when insect populations reach damaging levels but this is favorable to the development of insect resistance to many groups of insecticides. (Georghiou,1990). Aphids are difficult to be controlled because of their mobility, tremendous reproductive ability and resistance to many synthetic pesticides (Van Ientern, 1990), mean while, outbreaks have occurred in association with insecticidal use for controlling other pests (Slosser *et al.*,1989). The marked capacity of whiteflies to develop resistance to insecticides must therefore be viewed with grave concern and serious threat to the sustainability of current control programs. Resistance already extends to all established chemical groups (Cahill *et al.*, 1995 and Ayad *et al.*, 1999).

The neonicotinoids, the newest major class of insecticides have outstanding, potency and systemic action of crop protection against piercing sucking pests. They have low toxicity to mammals (acute and Chronic), birds and fishes. Biotransformation involve some activation reaction but largely detoxification mechanisms in these organisms (Tomizawa and Casida, 2005). The nicotinic acetylcholine receptor (nAChR) is an agonist regulated on channel complex responsible for rapid neurotransmission. The insect nAChR is the primary target site for the neonicotinoid insecticides (Tomizawa and Casida, 2001).

The objective of this study is to detect the sensitivity of *B.tabaci* and *A.gossypii* field strains collected from Beni-Suef, Behera, Menofia and Gharbia Governorates. toward neonicotinoid insecticides; Imidacloprid (Imidor 20% EC, Confidor 20% SL and Best 25% WP) and Thiamethoxam (Actara 25% WG) compared with Carbosulfan (Marshal 25% WP), Diafenthiuron (Polo 50% SC) and Buprofezin (Applaud 25% SC). Also, the difference in protein contents, and non-specific esterase isozymes between two insect species was evaluated.

## MATERIALS AND METHODS

### 1. Source of insects:

Samples of cotton leaves which infested with *Aphis gossypii* nymphs and adults were collected in paper bags from cotton plants grown in Beni-Suef, Behera, Menofia and Gharbia Governorates during April and May months of 2008 year. The adults of *Bemisia tabaci* were collected by using special aspirator from the same Governorates during June and July of 2008. The insects were released on cotton plants grown in plastic pots in separate rearing chambers under standard conditions ( $26\pm 2^{\circ}\text{C}$ ,  $70\pm 5\%$  R.H and photoperiod of 16:8 hrs L:D) for 24 hrs. The adults were collected to insecticides treatment and the infested plants with white fly were maintained under the laboratory condition until the immature stages reach to the last nymphal instar.

### 2. Treatments:

Aqueous emulsion of recommended concentration of each insecticide was prepared and then diluted to nine serial concentrations as follows:

- Actara (25% WG Thiamethoxame neonicotinoid insecticide agonist the nicotinic acetylcholine receptor) used at concentrations 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ppm.
- Best (25% WP Imidacloprid neonicotinoid insecticide, binds the postsynaptic nicotinic receptors in insect central nervous system) used at concentrations; 187.5, 93.75, 46.88, 23.44, 11.72, 5.86, 2.93, 1.47, 0.73 and 0.37 ppm.
- Confidor (20% SL Imidacloprid) used at concentrations; 100 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 ppm.
- Imidor (20% EC Imidacloprid) used at the same concentrations of Confidor.
- Marshal (25% WP Carbosulfan carbamate insecticide cholinesterase inhibitor) used at concentrations; 375, 187.5, 93.75, 46.88, 23.44, 11.72, 5.86, 2.93, 1.47 and 0.73 ppm.
- Polo (50% SC Diafenthiuron insecticide and acaricide is an inhibitor of mitochondrial respiration) used at concentrations; 375, 187.5, 93.75, 46. 88, 23.44, 11.72, 5.86, 2.93, 1.47 and 0.73 ppm.
- Applaud (25% SC Buprofezin insecticide and acaricide has hormone disturbing effect) used at the same concentrations of Polo.

The leaf-dip-bioassay (O'Brien *et al.*, 1992) was used to evaluated *the toxicity of all tested pesticides to A. gossypii* females and *B.tabaci* adults. The last nymphal instar of whitefly on cotton plants were sprayed with pesticide solutions by using hand

glass atomizer. Mortality was recorded after 48 hrs. of treatment and corrected according to Abbott (1925).  $LC_{50}$  and slope value of each pesticide were determined as Finney (1971) and the toxicity index was calculated as Sun (1950).

### 3. Biochemical techniques:

Hundred milligrams of cotton aphid females and whitefly adults of different field strains were homogenized in two milliliters of 0.9% sodium chloride solution in ice jacket. The homogenized tissues were centrifuged at 6000 r.p.m for 15 min. then the supernatant used in determination of total protein content by Biuret reaction (Henry, 1964).

Esterase isozyme patterns of each aphid and whitefly sample were separated on non denaturing polyacrylamide gel electrophoresis into groups based on their relative mobility (Davis, 1964). Thirty microliters of each supernatant were mixed with equal volume of 5% sucrose solution. After preparation of separating (11%) and stacking (4%) gels, the samples were loaded on wells of stacking gel. The fractionation process was carried out at 4°C, 60mA constant current and terminated after sample tracking dye reached to the end of the gel. The gel was stained for esterolytic activity by 0.1 gm  $\alpha$ -naphthyl acetate as a substrate in 3 ml acetone and 0.1 gm fast blue RR salt in 200ml of 0.1 M phosphate buffer pH 6.5 (Sell *et al.*, 1974). Esterases were classified on the basis of inhibition by esterase inhibitors (Azodrin, Dursban, Eserine and Paraoxon) examined on the gel before staining. The gel was scanned and analyzed on Gel pro program.

## RESULTS AND DISCUSSION

Efficiency of tested insecticides against field strains of the cotton aphid, *Aphis gossypii* was shown in Table 1. The results indicated that the Imidacloprid Imidor (20% EC) was the most potent insecticide on cotton aphid collected from Behera, Menofia and Gharbia Governorates ( $LC_{50}$ =4.84, 7.08 and 6.21, ppm respectively and 100% toxicity index) while Confidor (20% SL) was the most effective on Beni-Suef insects ( $LC_{50}$  = 6.75 ppm and 100% toxicity index). Following toxicity descending order was Actara (6.75 ppm and 71.70%), Best (7.40 ppm and 65.41%) Confidor (7.72 ppm and 62.69%), Marshal (12.92 ppm and 37.46%), Polo (17.46 ppm and 27.72%) and Applaud (24.94 ppm and 19.41%) in Behera, Best (8.09 ppm and 87.52%), Confidor (10.00 ppm and 70.80%), Actara (11.68 ppm and 60.62%), Marshal (13.09 ppm and 54.09%) Polo (19.95 ppm and 35.49%) and Applaud (25.79 ppm and 27.45%) in Menofia, Confidor (6.85 ppm and 90.66%), Marshal (7.77 ppm and 79.92%), Actara (9.04 ppm and 68.70%) Best (11.98 ppm and 51.84%), Polo

(17.78 ppm and 34.93%) and Applaud (21.57 ppm and 28.79%) in Gharbia and Best (9.73 ppm and 69.37%), Imidor (11.81 ppm and 57.15%), Actara (13.10 ppm and 51.53%), Marshal (13.28 ppm and 50.83%) Polo (18.32 ppm and 36.85%) and Applaud (26.04 ppm and 29.92%) in Beni-Suef. These results revealed that the neonicotinoids insecticides have highly toxic action on cotton aphid than other tested insecticides. Wiesner and Kayser (2002) mentioned that Thiamethoxam was more effective on *Aphis craccivora* than *Myzus persicae* and *Locusta migratoria*, because it was highly active on Aphis nicotinic acetylcholine receptors in radioligand binding assays. The carbamate insecticide (Marshal 25% WP) has high toxic effect on Gharbia, Menofia and Beni-Suef aphids. The insect growth regulator Applaud (25% SC) had the lowest toxic effect on four Governorates aphid.

Table 2 show that Confidor ( $LC_{50}$ =1.68ppm and 100% toxicity index) was the most effective insecticide against Beni-Suef whitefly, followed by best (2.27 ppm and 74.40%), Actara (2.46 ppm and 68.29%) and Imidor (2.65 ppm and 63.40%). Actara (2.00 ppm and 100%) was the superior toxicant against Behera whitefly followed by Best (2.12 ppm and 94.34), Imidor (2.16 ppm and 92.59%) and Confidor (2.23 ppm and 89.69%). The same effect in Menofia with Best (1.88 ppm and 100%) followed by Actara (2.03 ppm and 92.61%), Confidor (2.12 ppm and 88.68%) and Imidor (5.95 ppm and 31.60%) and in Gharbia with Best (1.71 ppm and 100%) followed by Imidor (2.07 ppm and 82.61%) Actara (2.18 ppm and 78.44%) and Confidor (2.51 ppm and 68.13%). Marshal, Polo and Applaud had very low toxic effect on whitefly from four tested Governorates. Radwan and Zidan (2003) reported that Imidacloprid was effective on adults of *B.tabaci* than Thiamethoxam, Diafenthiuron and Carbosulfan when sprayed on upper and both surface of plant leaves of cotton seedlings

Data in Table 3 indicated that Confidor was the most effective insecticides on the last instar of *B.tabaci* nymphs followed by Best, Actara and Imidor, while Marshal, Polo and Applaud had low toxic effect on nymphs of four tested Governorates. Imidacloprid is taken up systemically through the plant which reduced feeding of whitefly nymphs and produced high mortality of them (Nauen *et al.*,1998). The last nymphal instar of whitefly was more tolerant to the effect of all tested pesticides than the adult stage. The newly hatched crawlers and the adult are more susceptible to chemicals, but the waxy covering on the larger immature makes them more difficult to cover thoroughly with spray material (James and Eizen, 2001).

Table 1. Efficiency of tested insecticides against field strains of the cotton aphid, *Aphis gossypii* (Glover)

Insecticide	Beni-Suef			Behera			Menofia			Gharbia		
	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)
Imidor (20% EC)	11.81 ± 1.34	1.46	57.15	4.84 ± 1.23	2.01	100.00	7.08 ± 1.16	1.43	100.00	6.21 ± 2.55	1.95	100.00
Confidor (20% SL)	6.75 ± 1.63	0.98	100.00	7.72 ± 1.94	1.70	62.69	10.00 ± 1.87	1.58	70.80	6.85 ± 1.72	1.19	90.66
Best (25% WP)	9.73 ± 0.81	1.26	69.37	7.40 ± 1.66	1.56	65.41	8.09 ± 2.22	1.54	87.52	11.98 ± 1.46	1.63	51.84
Actara (25% WG)	13.10 ± 2.11	1.05	51.53	6.75 ± 1.34	1.63	71.70	11.68 ± 2.63	1.56	60.62	9.04 ± 2.27	1.39	68.70
Marshal (25% WP)	13.28 ± 1.45	1.14	50.83	12.92 ± 2.84	1.41	37.46	13.09 ± 3.14	2.17	54.09	7.77 ± 1.04	1.57	79.92
Polo (50% SC)	18.32 ± 3.19	0.71	36.85	17.46 ± 2.65	1.49	27.72	19.95 ± 3.00	1.52	35-49	17.78 ± 6.41	0.87	34-93
Applaud (25% SC)	26.04 ± 1.09	0.83	25.92	24.94 ± 5.18	1.20	19.41	25.79	1.54	27.45	21.57 ± 4.33	1.49	28.79

S.E.=Standard error

Table 2. Efficiency of tested insecticides against field strains of the white fly, *Bemisia tabaci* (Genn.)

Insecticide	Beni-Suef			Behera			Menofia			Gharbia		
	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)
Imidor (20% EC)	2.65 ± 0.83	1.73	63.40	2.16 ± 0.33	1.48	92.59	5.95 ± 1.04	1.55	31.60	2.07 ± 0.67	1.64	82.61
Confidor (20% SL)	1.68 ± 0.27	1.65	100	2.23 ± 0.64	1.42	89.69	2.12 ± 0.29	1.69	88.68	2.51 ± 0.54	1.43	68.13
Best (25% WP)	2.27 ± 0.46	1.49	74.40	2.12 ± 0.93	1.28	94.34	1.88 ± 0.16	1.56	100	1.71 ± 0.08	1.61	100
Actara (25% WG)	2.46 ± 0.34	1.29	68.29	2.00 ± 0.49	1.46	100	2.03 ± 0.21	1.53	92.61	2.18 ± 0.44	1.32	78.44
Marshal (25% WP)	18.75 ± 4.14	1.38	8.96	29.55 ± 6.77	1.13	6.77	36.44 ± 7.14	1.17	5.16	49.25 ± 13.66	1.31	3.47
Polo (50% SC)	32.95 ± 11.17	1.17	5.10	48.22 ± 13.16	1.28	4.15	57.47 ± 6.17	1.29	3.27	61.29 ± 19.45	1.22	2.79
Applaud (25% SC)	118.35 ± 20.66	1.19	1.42	122.25 ± 17.10	1.28	1.64	167.19 ± 29.09	1.17	1.13	131.75 ± 26.22	1.13	1.30

S.E.=Standard error

Table 3. Efficiency of tested insecticides against last nymphal instar of field strains of the white fly, *Bemisia tabaci* (Genn.)

Insecticide	Beni-Suef			Behera			Menofia			Gharbia		
	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)	LC50 (PPm) ±S.E.	Slope	Toxicity index (%)
Confidor (20% SL)	1.96 ± 0.31	1.33	100.00	3.91 ± 1.18	1.43	100.00	6.15 ±0.96	1.35	100.00	3.64 ± 1.28	1.67	100.00
Imidor (20% EC)	3.64 ± 1.11	1.25	53.85	5.11 ± 1.56	1.46	76.52	8.95 ±1.04	1.27	68.72	4.33 ± 1.12	1.43	84.07
Best (25% WP)	2.53 ± 0.98	1.43	77.47	4.18 ± 0.66	1.58	93.54	6.73 ±1.25	2.08	91.38	3.86 ± 0.79	1.74	94.30
Actara (25% WG)	3.00 ± 0.83	1.22	65.33	4.47 ± 1.00	1.17	87.47	7.82 ±1.37	1.31	78.65	4.17 ± 0.94	1.43	87.29
Marshal (25% WP)	27.44 ± 4.11	1.24	7.14	46.33 ± 6.22	1.42	8.44	39.25 ±5.11	1.21	15.67	54.50 ± 4.15	1.25	6.68
Polo (50% SC)	58.32 ±12.63	1.18	3.36	67.71 ±15.53	1.35	5.78	62.18 ±7.14	1.17	9.89	75.46 ± 5.33	1.35	4.82
Applaud (25% SC)	98.52 ±13.77	1.24	1.99	93.44 ±18.45	1.42	4.19	99.38 ±16.36	1.11	6.19	101.93 ± 13.24	1.23	3.57

S.E.=Standard error



Colorimetric determination of the total protein contents (Table 4) of *B.tabaci* whole body tissues revealed that the concentration of proteins reached to 0.861, 0.896, 0.882 and 0.952 (mg/100mg tissue of insect) in Beni-Suef, Behera, Menofia and Gharbia, resp. The lower protein level in *B.tabaci* tissues was recorded in Beni-Suef and the higher one was recorded in Gharbia but the difference in protein level between Beni-Suef and three other Governorates was insignificant. The same trend was present in aphid tissues, there was insignificant increase in total protein reached to 2.29, 3.82 and 8.40% for Gharbia, Menofia and Behera resp. than Beni-Suef aphid. The highly significant difference in protein level (71.85%) was recorded in Gharbia whitefly than aphid of the same Governorate. This difference reached to 68.30, 69.16 and 69.57% between whitefly and cotton aphid tissue from Behera, Menofia and Beni-Suef resp.

Enzymes are the most widely used protein markers, when enzymes of similar function are produced at different loci, they are referred to as isozymes. Isozymes are multiple molecular form with the same enzymatic specificity each molecule that is synthesized and controlled by the same gene (Loxdale and Lushai 1998). Non-specific esterases are formerly known to hydrolyze and also catalyze hydrolysis of a variety of diversified insecticidal esters such as benzilic, carbamate compounds and pyrethroids (Devonshire, 1991).

Nine esterase fractions were found in whole body tissues of *B.tabaci* and *A.gossypii*. Fig 1 and Table 5. Fraction no.1 ( relative mobility 0.021) was present in *B.tabaci* only, this fraction is the specific one for whitefly. Fraction no. 9 (relative mobility 0.31) is the specific enzyme for *A.gossypii*. Fractions no. 2, 3, 4, 5, 6, 7 and 8 (with relative mobility 0.048, 0.077, 0.10, 0.14, 0.18, 0.21 and 0.26) were common esterases in both insects. There were no differences in number and position of esterase isozymes between four field strains of *B.tabaci* and *A.gossypii*. Moreover, the esterase patterns of four strains of each insect were identical but different in their activity (intensity of bands). The densitometric scanning of esterase isozyme patterns in the whole body tissues of *A.gossypii* and *B.tabaci* was revealed that the activity of all esterase bands of Gharbia and Behera aphids was lower than those of Menofia and Beni-Suef aphids, but the activity of esterase bands no 1 and 2 in Behera and Beni-Suef whitefly was less than those of Gharbia and Menofia. These results may be illustrate the difference in response of *A.gossypii* and *B.tabaci* field strains to the toxic effect of tested pesticides.

Table 4. Total protein content (mg/100mg) of the whole body tissues of different field strains of *B.tabaci* and *A.gossypii*.

Governorate	<i>B.tabaci</i>		<i>A.gossypii</i>		Comparison between <i>B.tabaci</i> and <i>A.gossypii</i> protein content (%)
	Conc. of protein (mean $\pm$ S.E)	Change (%)	Conc. of protein (mean $\pm$ S.E)	Change (%)	
Beni-Suef	0.861 $\pm$ 0.032	0.0	0.262 $\pm$ 0.028	0.0	(-) 69.57
Behera	0.896 $\pm$ 0.053	(+) 4.07	0.284 $\pm$ 0.033	(+) 8.40	(-) 68.30
Menofia	0.882 $\pm$ 0.041	(+) 2.44	0.272 $\pm$ 0.047	(+) 3.82	(-) 69.16
Gharbia	0.952 $\pm$ 0.066	(+)10.60	0.268 $\pm$ 0.027	(+) 2.29	(-) 71.85

S.E.=Standard error

Table 5. Relative mobility of esterase isozyme patterns in the whole body tissues of different field strains of *A.gossypii*. and *B.tabaci*.

Band No.	Relative mobility value (Rm)	<i>A.gossypii</i>				<i>B.tabaci</i>			
		G <sub>2</sub>	Mf <sub>2</sub>	B <sub>2</sub>	BS <sub>2</sub>	G <sub>1</sub>	Mf <sub>1</sub>	B <sub>1</sub>	BS <sub>1</sub>
1	0.021	-	-	-	-	+	+	+	+
2	0.048	+	+	+	+	+	+	+	+
3	0.077	+	+	+	+	+	+	+	+
4	0.10	+	+	+	+	+	+	+	+
5	0.14	+	+	+	+	+	+	+	+
6	0.18	+	+	+	+	+	+	+	+
7	0.21	+	+	+	+	+	+	+	+
8	0.26	+	+	+	+	+	+	+	+
9	0.31	+	+	+	+	-	-	-	-
Total band No.		8	8	8	8	8	8	8	8

( + ) Present ( - ) Absent G: Gharbia Mf: Menofia B: Behera Bs: Beni-Suef

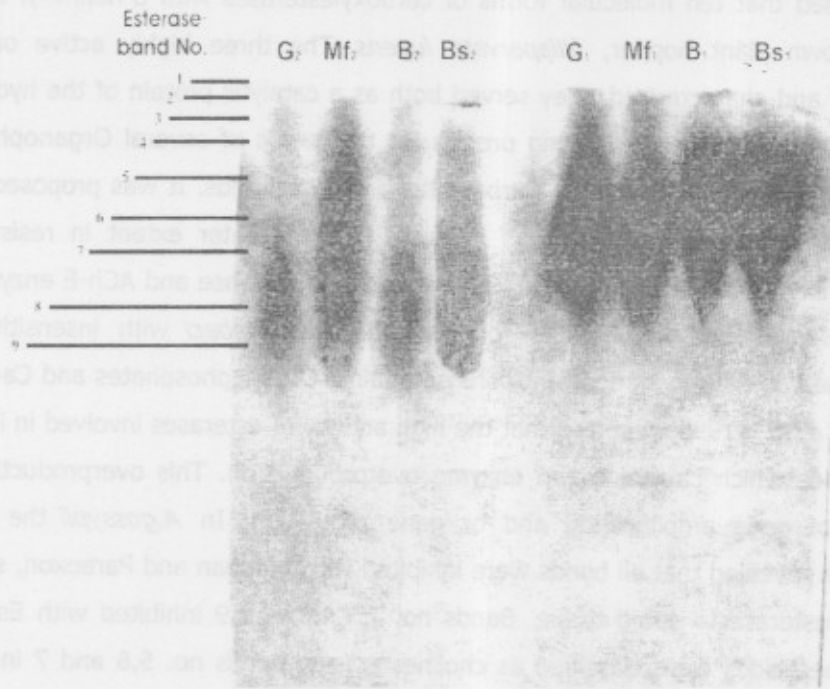


Fig. 1. Polyacrylamide gel zymogram of esterase isozyme patterns in whole body tissues of *Bemisia tabaci* and *Aphis gossypii* field strains stained with  $\alpha$ -naphthylacetate acetate as substrate. Lanes : G<sub>2</sub> , Mf<sub>2</sub> , B<sub>2</sub> and Bs<sub>2</sub> of *A. gossypii*. Lanes : G<sub>1</sub> , Mf<sub>1</sub> , B<sub>1</sub> and Bs<sub>1</sub> , of *B.tabaci* collected from Gharbia, Menofia, Behera and Beni-Suef governorates, respectively Esterase band numbers are indicated on the left side of the gel.

Esterases have been classified according to their reaction with various specific inhibitors. At least three classes of esterases can be identified based on the substrate specificity and the inhibition test, cholinesterases inhibited by carbamates and organophosphates, carboxyl esterases (aliesterases) inhibited by organophosphates only and aromatic esterases (arylesterases) not inhibited by carbamates or organophosphates (Bush *et al.*, 1970 and Augustinsson, 1961). Fig. 2 and Tables 6 and 7 illustrated the response of esterase bands to the specific inhibitors and their type with  $\alpha$ -naphthyl acetate substrate. Bands no. 4,5 and 6 in *B.tabaci* from four Governorates were highly capable for hydrolyzing  $\alpha$ -naphthyl acetate after the inhibition with Eserine, Azodrin, Dursban and Paraoxon so that they were classified as arylesterases. Bands no. 2, 3 and 7 were inhibited by Dursban and Paraoxon they were classified as carboxylesterases. Band no. 1 and 8 were inhibited by Eserin, Azodrin, Dursban and Paraoxon were classified as cholinesterases. These results may be explained the tolerance of whitefly from four Governorates to Marshal, Polo and Applaud pesticides. The results were agreed with those of Sun and Chen (1993) who

mentioned that ten molecular forms of carboxylesterases with  $\alpha$ -naphthyl acetate in rice brown plant hopper, *Nilaparvata lugens*. The three highly active ones were purified and characterized, they served both as a catalytic protein of the hydrolysis of some insecticides and a binding protein for the oxons of several Organophosphorus compounds and possibly some Carbamates and Pyrethroids. It was proposed that the gene encoding the enzymes was expressed to a greater extent in resistant than susceptible insects. Byrne *et al.*, (1994) stated that esterase and ACh-E enzymes have been widely used as markers for resistance in *B.tabaci* with insensitive ACh-E confirmed as the most important defense against Organophosphates and Carbamates. Rooker *et al.*, (1996) reported that the high activity of esterases involved in insecticide resistance which caused by an enzyme overproduction. This overproduction is the result of gene amplification and or gene regulation. In *A.gossypii* the inhibition reaction revealed that all bands were inhibited with Dursban and Paraoxon, so there is no arylesterase in aphid tissue. Bands no. 2,3,4,8 and 9 inhibited with Eserine and other pesticides were classified as cholinesterases. Bands no. 5,6 and 7 inhibited by Dursban and Paraoxon were classified as carboxylesterases. Owusu *et al.*, (1996) mentioned that the tissues of cotton aphid contained concentrated bands of carboxyl and cholin esterase isozymes.

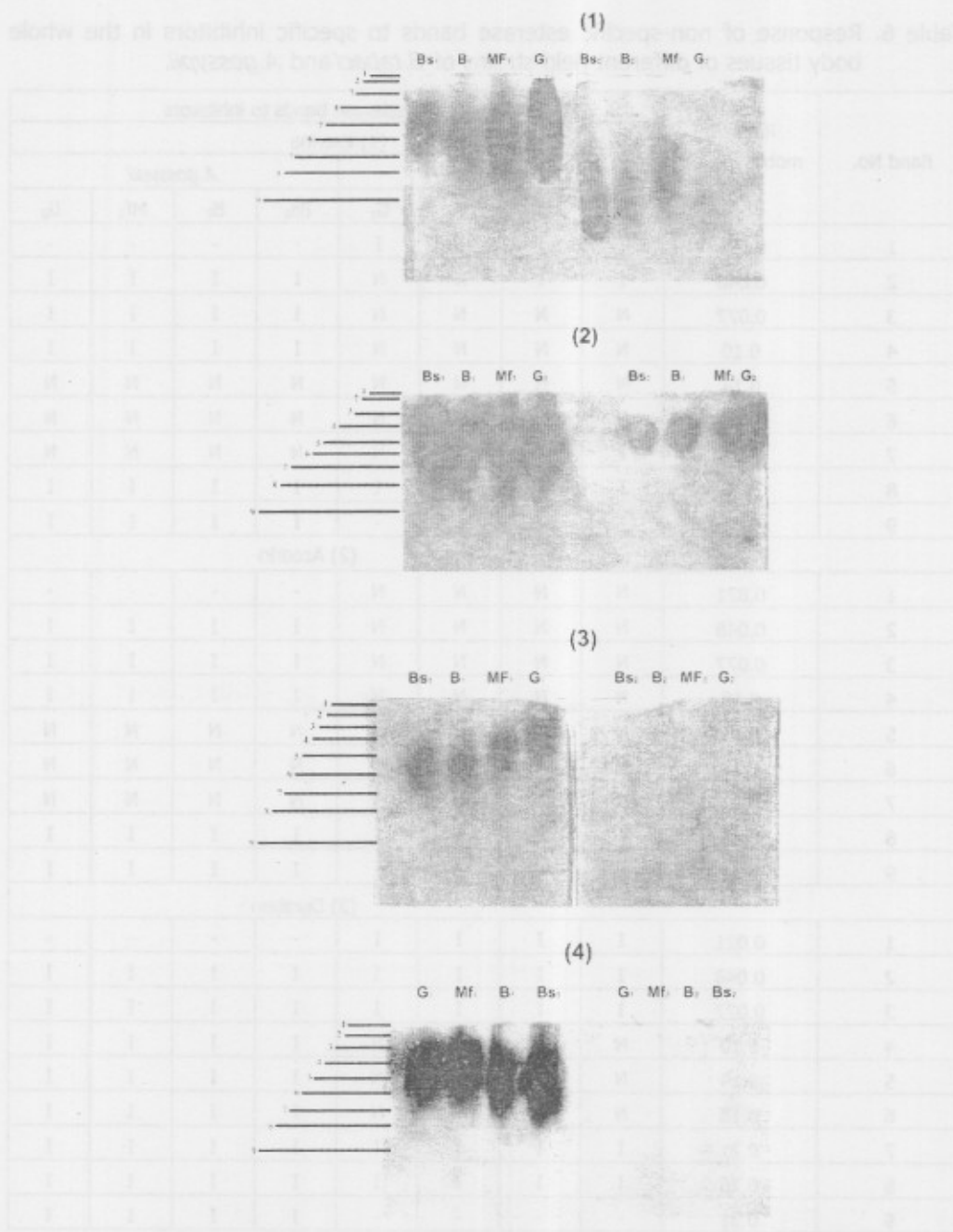


Fig. 2. Polyacrylamide gel zymogram of esterase isozyme patterns in whole body tissues of *Bemisia tabaci* and *Aphis gossypii* field strains stained with  $\alpha$ -naphthylacetate as substrate after using (1) Eserine, (2) Azodrine, (3) Dursban and (4) paraoxon as inhibitors. Lanes: Bs<sub>1</sub>, B<sub>1</sub>, Mf<sub>1</sub>, and G<sub>1</sub>, of *B.tabaci*. Lanes: Bs<sub>2</sub>, B<sub>2</sub>, Mf<sub>2</sub> and G<sub>2</sub> of *A. gossypii* collected from Beni-suef, Behera, Menofia and Gharbia respectively. Esterase band numbers are indicated on the left side of the gel .

Table 6. Response of non-specific esterase bands to specific inhibitors in the whole body tissues of different field strains of *B. tabaci* and *A. gossypii*.

Band No.	Relative mobility value (R <sub>m</sub> )	Response of esterase bands to inhibitors							
		(1) Eserine							
		<i>B. tabaci</i>				<i>A. gossypii</i>			
		BS <sub>1</sub>	B <sub>1</sub>	Mf <sub>1</sub>	G <sub>1</sub>	BS <sub>2</sub>	B <sub>2</sub>	Mf <sub>2</sub>	G <sub>2</sub>
1	0.021	I	I	I	I	-	-	-	-
2	0.048	I	I	N	N	I	I	I	I
3	0.077	N	N	N	N	I	I	I	I
4	0.10	N	N	N	N	I	I	I	I
5	0.14	N	N	N	N	N	N	N	N
6	0.18	N	N	N	N	N	N	N	N
7	0.21	I	N	N	N	N	N	N	N
8	0.26	I	I	I	I	I	I	I	I
9	0.31	-	-	-	-	I	I	I	I
(2) Azodrin									
1	0.021	N	N	N	N	-	-	-	-
2	0.048	N	N	N	N	I	I	I	I
3	0.077	N	N	N	N	I	I	I	I
4	0.10	N	N	N	N	I	I	I	I
5	0.14	N	N	N	N	N	N	N	N
6	0.18	N	N	N	N	N	N	N	N
7	0.21	N	N	N	N	N	N	N	N
8	0.26	I	I	I	I	I	I	I	I
9	0.31	-	-	-	-	I	I	I	I
(3) Dursban									
1	0.021	I	I	I	I	-	-	-	-
2	0.048	I	I	I	I	I	I	I	I
3	0.077	I	I	I	I	I	I	I	I
4	0.10	N	N	N	N	I	I	I	I
5	0.14	N	N	N	N	I	I	I	I
6	0.18	N	N	N	N	I	I	I	I
7	0.21	I	I	I	I	I	I	I	I
8	0.26	I	I	I	I	I	I	I	I
9	0.31	-	-	-	-	I	I	I	I
(4) Paraoxon									
1	0.021	I	I	I	I	-	-	-	-
2	0.048	I	I	I	I	I	I	I	I
3	0.077	I	I	I	I	I	I	I	I
4	0.10	N	N	N	N	I	I	I	I
5	0.14	N	N	N	N	I	I	I	I
6	0.18	N	N	N	N	I	I	I	I
7	0.21	I	I	I	I	I	I	I	I
8	0.26	I	I	I	I	I	I	I	I
9	0.31	-	-	-	-	I	I	I	I

( - ) Absent

( I ) Inhibition

( N ) Noninhibition

Table 7. Type of esterase enzyme bands in the whole body tissues of *B.tabaci* and *A.gossypii*.

Band No.	Relative mobility value (Rm)	Type of esterase band	
		<i>B.tabaci</i>	<i>A.gossypii</i>
1	0.021	Cholinesterase	Absent
2	0.048	Carboxylesterase	Cholinesterase
3	0.077	Carboxylesterase	Cholinesterase
4	0.10	Arylesterase	Cholinesterase
5	0.14	Arylesterase	Carboxylesterase
6	0.18	Arylesterase	Carboxylesterase
7	0.21	Carboxylesterase	Carboxylesterase
8	0.26	Cholinesterase	Cholinesterase
9	0.31	Absent	Cholinesterase

## REFERENCES

1. Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. *J.Econ. Entomol.*, 18:265-267.
2. Abd-Rabou, S. 1997. Hosts, distribution and vernacular names of white flies (Homoptera: Aleyrodidae) in Egypt. *Annals of Agric. Sci., Moshtohor*, 35: 1029-1048.
3. Augustinsson, K.B. 1961. Multiple forms of esterases in vertebrate blood plasma. *Ann. N.Y. Acad. Sci.*, 94:844-860.
4. Ayad, F.A., N.M. Bakry, M.M.Abdel-Hafez, M.E Keddiss, Y.F. Ghoreium and M.S. Khalid. 1999. Monitoring of resistance in Egyptian field strains of the whitefly *Bemisia tabaci* Genn. For the season. 1998. Strategy for Safe Agricultural Production Conference. Cairo, Egypt:677-693.
5. Brown, J.K, D.R. Frohlich and R.C. Rosell. 1995. The sweet potato or silver leaf whiteflies : biotype *Bemisia tabaci* or a species complex. *Annu. Rev. Entomol.*, 40:54-534.

6. Bush, F.M., J.R. price and J.I. Townsend. 1970. Plasma esterases, their definitions and status as isozymes in the house sparrow. *Int. J.Biochem.* 1:85-107.
7. Byrne, F.J., M. Cahill, I.Denholm and A.L. Devonshire. 1994. A biochemical and toxicological study of the role of insensitivity acetylcholinesterase in organophosphorus resistant *Bemisia tabaci* (Homoptera: Aleyrodidae) from Israel. *Bull. Entomol. Res.*, 84:179-185.
8. Cahill, M., F.J.Byrne, K.Gorman, I.Denholm and A.L. Devonshire. 1995. Pyrethroid and organophosphate resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Of Entomol., Res.*, 85:181-187.
9. Cock, M.J.W. 1986. *Bemisia tabaci*, A literature survey on the cotton whitefly with an annotated bibliography. Food and Agriculture Organization of the U.N., C.A.B. *Int. Inst. Bio. Cont* 121 p.
10. Davis, B.J. 1964. Disc electrophoresis. 2 method and application to human serum proteins. *Ann. N.Y. Acad. Sci.*, 121: 404-427.
11. Devonshire, A.L.K. 1991. Role of esterases in resistance of insects to insecticides. *Biochem. Soc. Trans.*, 19:755-759.
12. Finney, D.J. 1971. *Probit Analysis*. (3<sup>rd</sup> Ed.) Cambridge University Press, London pp:318.
13. Georghiou, G.P. 1990. Overview of insecticide resistance. pp:18-41 in Green, M.B., H.M. Le Baron and W.K. Moberg (Eds). *Managing resistance to agrochemicals from fundamental research to practical strategies*. Washington DC, American Chemical Society.
14. Godfrey, L.D., K.J. Fuson, J.P. Wood and S.D. Wright. 1997. Physiological and yield responses of cotton to mid-season cotton aphid infestations in California. *Proc. Beltwide Cotton Conf.* : 1048-1051.
15. Greathead, A.H. 1986. Host plants. Chapter 3, pp:17-25. (In: *Bemisia tabaci* – a literature survey on the cotton whitefly with annotated bibliography (Ed. M.J.W. Cock). CAB International Institute of biological Control, Ascot, UK.121pp.
16. Hassanein, M.H., A.H.El-Sebae, F.M.Khalil and F.A. Mouftah. 1971. Studies on *Aphis gossypii* and *Aphis Craccivora* in the cotton fields of upper Egypt. *Bull. Soc. Ent. Egypt.* 55:163-169.



17. Hegab, M.F., A.M. Saber, A.M. Taha and H.M. Mazed. 1992. Evaluation of protective programs to control the cotton whitefly *B.tabaci* (Genn.) and reduce spread of tomato yellow leaf curl virus in tomato nurseries and in the open field. Al-Azhar J.Agric. Res., 16.
18. Henry, R.J. 1964. Clinical chemistry, Harper and Row Publishers. New York pp:181.
19. James, R.R. and G.W. Eizen. 2001. Antagomism between *Beauveria bassiana* and Imidacloprid when combined for *Bemisia argentifolii* (Homoptera : Aleyrodidae) control. J. Econ. Entomol., 94 (2): 357-361.
20. Loxadale, H.D. and G. Lushai. 1998. Molecular markers in Entomology (Review article). Bull. Entomol. Res; 88:577-600.
21. Nauen, R., B.Koob and A.Elbert. 1998. Antifeedant effects of Sublethal dosages of imidacloprid on *Bemisia tabaci*. Entomol. Exp. Appl., 88: 287-293.
22. O'Brien, P.J., Y.A. Abdel. Aai, J.A. Ottea and J.B. Gravers. 1992. Relationship of insecticide resistance to carboxylesterases in *Aphis gossypii* (Homoptera: Aphididae) from midsouth cotton. J. Econ. Entomol., 85:651-677.
23. Owusu, E.O., M. Horiike and C. Hirano. 1996. Polyacrylamide gel electrophoretic assessments of esterases in cotton aphid (Homoptera: Aphidae) resistance to Dichlorvos. J. Econ. Entomols 89 (2) : 302-306.
24. Radwan, H.S.A. and L.T.M. Zidan. 2003. Short-term translaminar bioavailability of certain insecticides against the sweet potato whitefly *Bemisia tabasi* (Genn.) on cotton. Bull. Ent. Soc. Egypt., Econ. Ser., 29:125-135.
25. Rooker, S., T. Guillemand, J. Berge, N. Pasteur and M. Raymodn. 1996. Coamplification of esterase A and B genes as a single unite in *Culex pipiens* mosquitoes. Heredity, 77: 555-561.
26. Sell, D.K., G.S. Whitt, R.L. Metcalf and L.P. Kan Lee. 1974. Enzyme polymorphism in the corn earworm *Heliothis zea* (Lepidoptera: Noctuidae). Haemolymph esterase polymorphism. Can. Entomol. 106: 701-709.
27. Slosser, J.E., W.E. Pinckok and D.R Rammel. 1989. A review of know and potential factors affecting the population dynamics of the cotton aphid. Southwest. Entomol, 14:302-313.
28. Sun, C. N. and W. L. Chen. 1993. Carboxylesterases of susceptible and resistant brown plant hopper. Resist. Pest. Manag., 5:9-10.

29. Sun, Y.P. 1950. Toxicity index on improved method of comparing the relative toxicity of insecticide. J. Econ. Entomol., 43:45-53.
30. Tomizawa, M. and J.E. Casida. 2001. Neura Targets: Structure and diversity of insect nicotinic acetylcholine receptors. Pest Manag. Sci, 57 (10) : 914-922.
31. Tomizawa, M. and J.E. Casida. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol., 45: 247-268.
32. Van Lenteren, J.C. 1990. Biological control in antitrophic system approach pp.3-28. In D.C. Peters, J.A. Webster and C.S. Chlouber (Eds.). Aphid plant interactions. Population of Molecules USDA-ARS, Oklahoma State Univ.
33. Wesner, P. and H. Kayser. 2002. Characterization of nicotinic acetylcholine receptors from the insects *Aphis craccivora*, *Myzus persicae* and *Locusta migratoria* by radioligand and binding assays: Relation to thiamethoxam action. J. Biochem. Toxicol., 14:221-230.

## الاختلاف في الحساسية بين حشرتي الذبابة البيضاء ومن القطن للتأثير الإبادي للمبيدات النيونيكوتينية

إيمان محمد مصطفى رضوان ، هالة محمد أبو يوسف ، محمد سنجاب

المعمل المركزي للمبيدات – مركز البحوث الزراعية – دقي – جيزة – مصر

تم اختبار حساسية سلالات حقلية لحشرتي الذبابة البيضاء ومن القطن من محافظات بني سويف والمنوفية والغربية والبحيرة لمستحضرات مختلفة لمبيد الأميداكلوبريد (أميدور ٢٠% EC ، كونفيدور ٢٠% SL وبست ٢٥% WP) ومبيد الثاميثوكزام بالمقارنة بثلاثة مبيدات موصى بها وهي كربوسلفين (مارشال ٢٥% WP) ودافنيوثيرون (بولو ٥٠% SC) وبيروفيزين (البلود ٢٥% SC). وكانت المبيدات النيونيكوتينية الأربعة الأكثر سمية لحشرة الذبابة البيضاء بيميزيا تاباسي عن حشرة من القطن أفيز جوسيبياي المجمعة من المحافظات الأربعة. وكان من القطن الأكثر حساسية لتأثير المبيدات الثلاثة الأخرى من الذبابة البيضاء كما كانت الحشرة الكاملة للذبابة البيضاء أكثر حساسية للمبيدات المختبرة من حوريات العمر الأخير. كما كان المحتوى الكلي لبروتين جسم الحشرة الكاملة للذبابة البيضاء أكثر تركيزاً من بروتين إناث المن من نفس المحافظات وأوضح التفريد الكهربائي للاستيرازات غير المتخصصة نشاط عالي لمناطق الإنزيمات مع وسط ألفا نفييل اسينات لأنسجة جسم الأفتين. وقد صنفت حزم الأنزيمات على أساس التنشيط بالمبيدات المتخصصة إلى كولين وكربوكسيل استريزات في المن (وهذا يفسر تأثير هذه الحشرة بالمبيد الكاربوماتي مارشال) وصنفت استيرازات الذبابة البيضاء إلى كولين وكربوكسيل وإيريل استريزات وهذا ربما يفسر تحمل هذه الحشرة للمبيدات التقليدية.