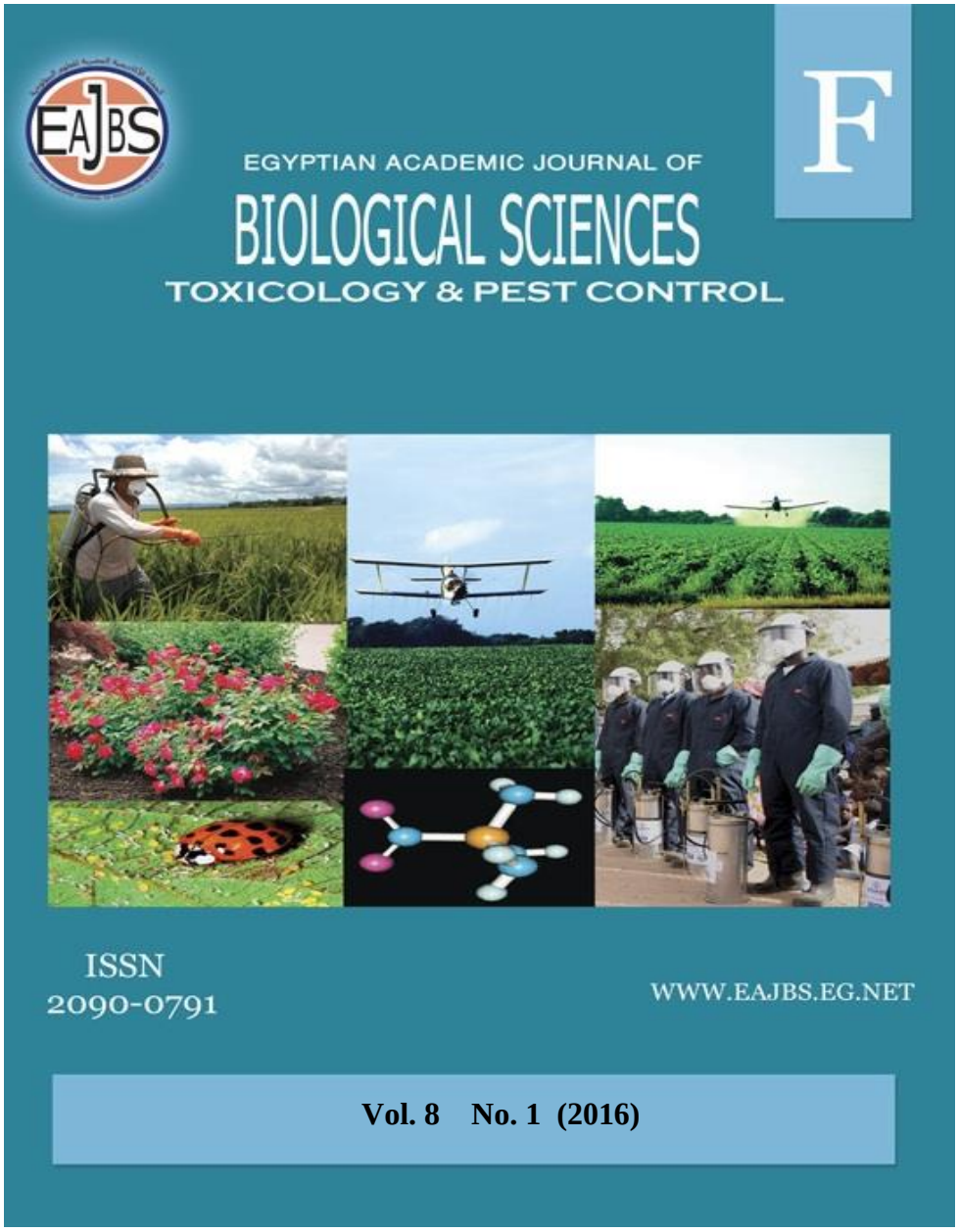

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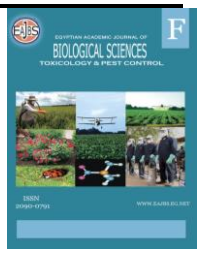

Monitoring Resistance in the Whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) to the Efficiency of Three Insecticides in Relation to Some Detoxification Enzymes.

Azza I. Dawood
Central Agricultural Pesticides Laboratory Agricultural Research Center, Giza, Egypt

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ABSTRACT

Monitoring resistance in the whitefly *Bemisia tabaci* (Genn) in the three Egyptian Governorates, Beni-suif, Giza and Sharkia using three insecticides from the neonicotinoid compounds confidor (imidacloprid), actara (thiamethoxam) and chess (pymetrozin) were studied. To obtained results clearly indicated a great effect of these neonicotinoid with LC_{50}'s 0.02, 0.05 and 0.13 ppm forconfidor, actara and chess, respectively, against laboratory strain. The insecticide confidor was most potent one among the three tested insecticides with less LC_{50}'s 0.03, 0.35 and 0.49 for Beni-suif, Giza and Sharkia respectively. Beni-suif field population was the most susceptible one compared with the other populations, where LC_{50} values were 0.12, 0.03 and 0.25 ppm for actara, confidor and chess, respectively. Thus, the collected adults in different populations which treated with insecticides under this study compared with laboratory strain. Sharkia Governorate population displayed the highest resistant ratios at LC_{50} and LC_{90} levels were,14.8 and 13.8 fold for actara, 24.5 and 19.3 fold for confidor and 28.6&17.9 fold for chess insecticide, respectively. Also, quantifying the activity of the detoxification enzymes (MFOs, GST and α-esterases) were assessed, it was found that a correlation between the increasing in resistance and the activity of these enzymes. Beni-suif Governorate population which was susceptible one among all tested populations, it showed less levels in detoxifying enzymes activity, with values 32.2, 6.13 and 27.63 for α-esterase, GST and MFOs respectively.

INTRODUCTION

Since 1930s the whitefly *B. tabaci* became the first whitefly species to be implicated as a vector of the cotton leaf curl disease in Sudan and Nigeria. With the exception of cotton and few other crops, agriculturalists are much more concerned about the whitefly as a vector than a pest.

Many workers have pointed out the increase prevalence as well as expanded distribution of whitefly born viruses, during the last decade and the devastating impact yield losses range from 20-100% depending on the crop, season, vector prevalence and other factors (Sastry and Singh 1973; Horowitz *et al*.,1984; Brown and Bird 1992).

On the other hand, some authors recorded that *B. tabaci* damage a wide range of crops not as a vector but as a feeder. Mound (1965) experimentally demonstrated that general weakening of the plants due to whitefly infestation could cause serious reduction of cotton yield due to in part to a decreased number of bolls and in part to a decline in weight of seed and lint per boll. Heavy colonization of *B. tabaci* can lead to serious direct and indirect damage Basu1995 and Lemos *et al*.,(2003).

Neonicotinoid have established themselves world-wide, as key components in insect control programs, because of their unique chemical and biological properties, such as, broad-spectrum insecticidal activity, low application rates, excellent uptake and translocation in plants, new mode of action and favorable safety profile. As first neonicotinoid, confidor and the second generation is actara Nauen *et al*., (2003); Mainfisch *et al*., (2001 and Wakita *et al*., (2003).

Insects contain numerous enzymes with different substrate spectra. It is a fact that development of more active hydrolytic detoxification systems by an insect species is the most probable explanation of self resistance (Casida, 1958; Oppenoorth and Asperen,1960). Also, Loxdale and Lushai,1988; Al-Beltagy *et al*., 1993 and Zhang *et al*., 2015 reported that the resistance in insects to several insecticides was conferred by high carboxyl esterase activity.

MATERIALS AND METHODS

Insect populations

Field populations:

The whitefly adults of *Bemisia tabaci* used were collected from cabbage fields of Sharkia, Beni-suif and Giza Governorates in October which following the insecticidal applications by different types of insecticides.

Adults SPWF (sweet potato whitefly) were collected from field using custom made battery operated suction sampler (Dittrich*etal*., 1990). Adults were randomly collected across a representative collage growing area from two or more fields. Samples collected were pooled in wide mouth glass jars and kept in cool box during the transport from the field to the laboratory.

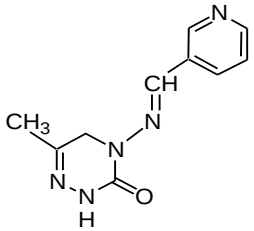
Laboratory strain

The laboratory reference strain of *B. tabaci* (Genn), originated from field collection over vegetables and ornamentals. This strain has been reared in laboratory culture for 30 generations under standard conditions at 26 ±1c^0 and 70 ±5 R.H, and a photoperiod of 16 :8 (light :dark) as described by Coudriet *et al*., (1985).

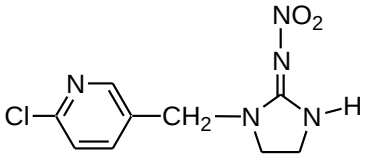
Insecticides used:

All the tested insecticides were from neonicotinoids group as follows:

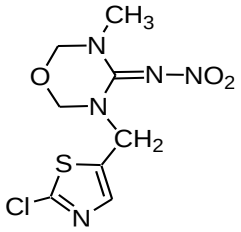
Pymetrozin (Chess) 25 % EC; (*E*)-4,5-dihydro-6-methyl-4-[(3-pyridinylmethylene)amino]-1,2,4-triazin-3(2*H*)-one



Imidacloprid (Confidor) 20 % SL;1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine



Thiamethoxam (Actara) 25 %WP; 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine



Biochemical assays:

α-esterase activity:

Alpha esterases (α-esterases) and Beta esterases (β-esterases) were determined according to Van Aspren (1962) using α-naphthyl acetate and β-naphthyl acetate, respectively.

The reaction mixture consisted of 5ml substrate solution ($3x10^{-4}$ α or β-naphthylactate, 1%acetone and 0.1 M phosphate buffer, pH7) and 20µl of larval homogenate.

The mixture was incubated for exactly 15 min. at 27º C, then 1 ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was reading at 600 or 555 nm for α- and β-naphthol produced from hydrolysis of the substrate, respectively.

α- and β-naphthol standard curves were prepared by dissolving 20mg α- or β-naphthol in 100 ml phosphate buffer, pH7 (stock solution). Ten milliliters of stock solution were diluted up to 100 ml by the buffer. Aliquots of 0.1, 0.2, 0.4, 0.8, and 1.6 ml of diluted solution (equal to 2.4, 8.16 and 32 µgnaphthol) were pippeted into test tubes and completed to 5 ml by phosphate buffer. One milliliter of diazoblue reagent s was added and the developed color was measured as mentioned before.

Glutothion-S-Transferase assay

GST activity was determined based on the technique of Habig *et al*. (1974) using 1-chloro-2,4-dinitrobenzene (2,4-CDNB) as a substrate. The reaction mixture comprised of 10 µL reduced glutathione (GSH) (10 mM) in sodium phosphate buffer (100 mM, pH 6.5) and 10 µL of the enzyme solution. The reaction was initiated by adding 10 µL of 2,4-CDNB (6 mM in methanol) resulting in a final volume of 30 µL. The plates were immediately transferred to absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The reactions were allowed to continue for 5 min and absorbance readings were taken at 340 nm automatically once per min against blanks (wells containing all reaction components except the enzyme solution). The increase in absorbance was linear throughout the 5 min reading interval. An extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ was used to calculate the amount of 2,4-CDNB conjugated.

Mixed Function Oxidases (MFOs) assay

MOs activity were detected through the transformation of *p*-nitroanisole to *p*-nitrophenol through *O*-

demethylation via the enzyme *p*-nitroanisole-*O*-demethylase based on the methods of Hansen and Hodgson (1971) with slight modifications. The standard incubation mixture contained 1 mL sodium phosphate buffer (0.1 M, pH 7.6), 1.5mL enzyme solution, 0.2 mL NADPH (final concentration 1 mM), 0.2 mL glucose-6-phosphate (final concentration 1 mM) and 50 µg glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of *p*-nitroanisole in 10 µL acetone to give a final concentration of 0.8 mM and was incubated for 30 min at 37°C. The incubation period was terminated by the addition of 1 mL HCl (1N), and *p*-nitrophenol was extracted with $CHCl_3$ and NaOH (0.5 N). The absorbance of NaOH solution was measured at 405 nm. An extinction coefficient of 14.28 $mM^{-1}cm^{-1}$ was used to calculate the concentration of 4-nitrophenol.

Total protein:

Total protein content was determined according to Bradford (1976).

Statistical analysis:

The percentage mortality of treated larvae was corrected against that of the control using Abbott's formula (Abbott, 1925). Then, the corrected mortality was subjected to Probit analysis (Finney, 1971). Data of the biochemical assays were analyzed using one-way analysis of variance (ANOVA). When the ANOVA statistics were significant ($p< 0.05$), the means were compared by Duncan's multiple range test. All the analyses were computed by IBM® SPSS® Statistics 21.0 (IBM Corp., Armonk, NY, USA.

RESULTS AND DISCUSSION

Baseline data from susceptible strains are a prerequisite for understanding the development of resistance in the field insecticides in populations to the tested insecticides. Because resistance is a genetically based shift in population response, resistance monitoring is added by the initial quantification of responses to toxic substances by susceptible strain (Robertson *et al*., 2007).

Data presented in Table (1) showed that Sharkia field population was the most resistance one to the novel compounds, followed by Giza field population. While Beni-suif field population was the most susceptible population with LC_{50} 0.12, 0.03 and 0.25 for actara, confidor and pymetrozin, respectively. Confidor was the most potent one among the tested compounds, this means that the confidor has a great effect. Afzal and Basit (2002) reported that confidor was the most effective insecticide moong four insecticides. Also, actara exhibits exceptional systemic characteristic and provides excellent control of a broad range of commercially important pests, such as aphids, jassids and whiteflies (Mainfisch *et al*., 2001). Although neonicotinoids proved high efficacy, it is important to bear in mind that the probability of build up a resistance to them (Olson *et al*., 1996; Zhou *et al*., 2004; Mota-Sanchez *et al*., 2006). Qi-AiMing *et al*., 2004 reported that confidor was the most effective insecticide for both whitefly and black thrips, also Dewar *et al*., 2004 reported that the confidor gave good control of aphids and whitefly.

In general Sharkia population exhibited no wide range of resistance ratio to tested insecticides Table (1). In other words, chess insecticide indicated increase in resistance to be ranged from 1.9, 12.6 and 28.6 folds for Beni-suif, Giza and Sharkia populations, respectively while RR values were doubled or tripled for actara with values 2.4, 7.2 and 14.8 folds for Beni-suif, Giza and Sharkia respectively. For confidor, 1.5, 17.5 and 24.5 folds for Beni-suif, Giza and Sharkia, respectively.

Table 1: LC_{50}, LC_{90} values and Resistance ratios for *Bemisia tabaci* strains collected from three Egyptian Governorates to three neonicotinoid compounds compared to the susceptible laboratory strain.

Strains / Insecti-cide	Laboratory			Beni-suif					Giza					Sharkia				
	Slope± S.E.	Lc_{50} (F.L.)	LC_{90} (F.L.)	Slope±S.E	Lc_{50} (F.L.)	LC_{90} (F.L.)	RR Based on LC_{50}	RR Based on LC_{90}	Slope±S.E	Lc_{50} (F.L.)	LC_{90} (F.L.)	RR Based on LC_{50}	RR Based on LC_{90}	Slope ±S.E	Lc_{50} (F.L.)	LC_{90} (F.L.)	RR Based on LC_{50}	RR Based on LC_{90}
Actara	1.12±0.20	0.05 (0.08-0.13)	0.53 (0.18-1.92)	1.20±0.21	0.12 (0.06-0.18)	1.39 (0.73-4.55)	2.4	2.62	1.74±0.34	0.36 (0.23-0.51)	1.95 (1.18-5.33)	7.2	3.68	1.28±0.25	0.74 (0.46-1.18)	7.31 (3.49-33.44)	14.8	13.8
confidor	0.92±0.13	0.02 (0.002-0.16)	0.31 (0.18-1.52)	1.03±0.26	0.03 (0.005-0.05)	0.49 (0.24-2.77)	1.5	1.59	1.21±0.27	0.35 (0.16-0.57)	3.98 (1.94-20.21)	17.5	12.8	1.18±0.25	0.49 (0.27-0.79)	5.99 (2.78-32.36)	24.5	19.3
chess	2.81±0.18	0.13 (0.06-2.14)	2.16 (0.87-16.73)	1.01±0.21	0.25 (0.09-0.44)	4.73 (2.32-20.53)	1.9	2.18	1.43±0.26	1.64 (0.99-2.43)	12.82 (7.35-36.4)	12.6	5.9	1.26±0.32	3.73 (2.16-6.35)	38.72 (16.27-450.74)	28.6	17.9

$$\text{Resistance Ratio (RR)} = \frac{\text{LC50 or LC90 of field strain}}{\text{LC50 or LC90 of laboratory strarin}}$$

F.L: Fiducial limits.S.E: Standard error.

Results obtained indicated that resistance of these insecticides were built up slowly. Such finding completely agree with Wang *et al*., 2002; Prabhaker *et al*.1997; Basit*etal*., 2011; Gorman *et al.* 2010 and Chen Xiaokun *et al*., 2013.

Detoxifying enzymes activity:

α-esterase activity:

Treatment of *B. tabaci* with the LC_{50} neonicotinoides revealed a significant decrease ($p< 0.05$) in α - esterase activity of the samples collected from Giza, Beni- suif and Sharkia governorates compared to the susceptible laboratory strain (Table2).

Table 2: Activity of α-esterase, glutathione S-transferase (GST) and mixed-function oxidases (MOs)in *Bemisia tabaci* strains collected from different Egyptian Governorates compared to the laboratory strain.

STRAIN	A-ESTERASES (MGA-NAPHTHOL/ MIN/MG PROTEIN)MIN?	GST (NMOL 2.4- CDNB CONGUGATED/MIN/G ROTEIN)	MFOS (NMOL SUB.OXIDIZED/ MIN/MG PROTEIN)
Susceptible	23.13±0.83a	6.13±0.20a	11.11±0.007a
Beni-suif	32.20±3.50c	6.06±0.13b	27.63±0.41a
Giza	55.80±3.90b	6.11±0.11b	28.30±1.50a
Sharkia	176.10±11.6a	7.68±0.22a	25.10±0.90a

Means followed by different letters in the same column are significantly different ($p< 0.05$) using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for comparison among the means.

GST activity:

B. tabaci collected from Giza, Beni-suif and Sharkia Governorates showed a significant increase ($P< 0.05$) in GST activity compared to the laboratory strain.

MFOs activity:

MOs activity compared to the laboratory strain by contrast, showed that MFOs activity of the strains collected from Giza, Beni- suif and Sharkia Governorates was significantly increased ($p< 0.05$) (Table 2).

Typically, enhanced metabolic detoxification among insects may contribute to insecticide resistance (Low *et al*. 2007), the metabolic detoxification system in insects consists of three major groups of enzymes. The phase I detoxification enzymes, acting on a broad range of substrates directly to reduce their toxicity, are represented by cytochrome P450. The phase II enzymes, including GST, UDP-glucuronosy-ltransferases (UGTs), and α - esterase, facilitate the excretion of hydrophobic

toxic compounds by improving their hydrophilicity. Several studies indicated that increased levels of detoxification gene expression are known to result in increased levels of detoxifying enzymes that are responsible for insecticide resistance (Karunker *et al*. 2008; Liu *et al*. 2011; Schuler, 2011; Gong *et al*. 2013,; Zhang *et al*. 2015).

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ARABIC SUMMERY

مقاومة الذبابة البيضاء (Homoptera: Aleyrodidae) لفاعلية ثلاث مبيدات حشرية وعلاقتها ببعض انزيمات إزالة السموم

عزة ا .داود
المعمل المركزي للمبيدات مركز البحوث الزراعية، الجيزة، مصر.

تم رصد مقاومة الذبابة البيضاء *B. tabaci* (Genn) التي جمعت من ثلاث محافظات بمصر تشمل بني سويف والجيزة والشرقية وأيضا تقييم ثلاثة من المبيدات الحشرية من مركبات (neonicotinoid) ايميداكلوبريد (كونفيدور)، ثيامثوكسام (اكتارا) وبيمتروزين (تشيس) لدراسة معايير المقاومة في هذه العشائر وأشارت النتائج التي تم الحصول عليها الي تأثير مرتفع من هذه المركبات بناءا علي التركيز القاتل لنصف العشيرة بقيم 0.02, 0.05 , 0.13 جزء في المليون لايميداكلوبريد (كونفيدور)، ثيامثوكسام (اكتارا) و بيمتروزين (تشيس) على التوالي، لسلالة المختبر. كان مبيدايميداكلوبريد (كونفيدور) أقوى تأثيرا من بين الثلاثة مبيدات الحشرية المختبرة مع أقل LC_{50}'s 0.03,0.35و 0.49 لبني سويف والجيزة والشرقية على التوالي. كانت السلالة المجمعة من محافظة بني سويف الأكثر حساسية مقارنة مع غيرهم من السلالات، مع LC_{50} 0.12,0.03 و 0.25 جزء في المليون لثيامثوكسام (اكتارا)، ايميداكلوبريد (كونفيدور) وبيمتروزين (تشيس)، على التوالي. وهكذا، تمت مقارنة الحشرة الكاملة المجمعة من المحافظات المختلفة التي تعامل بالمبيدات مع سلالة المختبر لدراسة و تحديد نسبة المقاومة (RR′S) .

وقد اظهرت السلالة المجمعة من محافظة الشرقية أعلى مقاومة مع التركيز القاتل لنصف العشيرة والتركيز القاتل ل 90 % من العشيرة ، 14.8و 13.8 أضعاف لثيامثوكسان (اكتارا) ، 24.5 و 19.3 أضعاف لايميداكلوبريد (كونفيدور) 28 و17.9ضعف لبيمتروزين (تشيس) ، على التوالي.

تم أيضا قياس نشاط إنزيمات إزالة السموم (α- GSTاستريز)، فقد وجد وجود علاقة موجبة بين الزيادة في المقاومة ونشاط هذه الأنزيمات. كانت عشيرة محافظة بني سويف و التي كانت اقل السلالات مقاومة لفعل المبيدات من بين جميع العشائر المختبرة ، كانت أيضا أقل نشاط انزيمي في إزالة السموم، مع قيم 32.2، 6.13 و 27.63 لل-α استريز، GST , P450 على التوالي.