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BIOCHEMICAL MECHANISMS OF INSECTICIDE RESISTANCE IN SOME FIELD POPULATIONS OF *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

Zidan, Lobna T.[(1)]; Abd-Elaziz, M. H.[(2)]; Abouelghar, G. E.[(3)]; Elsheikh, A. E.[(3)] and Ammar, Hagar A.[(3)]

[(1)] Central Agricultural Pesticide Laboratory, Agricultural Research Center, Dokki, Egypt.

[(2)] Ministry of Agriculture and Land Reclamation, New Valley, Egypt.

[(3)] Department of Pesticides, Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt.

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ABSTRACT: Responses of whitefly, *Bemisia tabaci* (Gennadius), adults to several classes of insecticides were determined using two different bioassays. The toxicity data showed that adult-vial bioassay was more sensitive technique than leaf-residue bioassay. LC_{50} values of most insecticides tested were < 1.0 µg/vial. The neonicotinoid imidacloprid was the most toxic insecticide (0.11 µg/vial) followed by buprofezin, abamectin, thiamethoxam and pyriproxyfen, whereas methomyl had the lowest toxicity. Status of insecticide resistance levels in three field strains of whitefly was reported using leaf-residue technique. The resistance levels to most selected insecticides were lower especially in BNS-strain indicating RR < 2.1-fold compared to the Lab-SUS strain, whereas slight increases in resistance levels ranged from 3.0- to 5.4- fold for the conventional insecticides tested were detected in all three field strains. The acetylcholinesterase (AChE), non-specific esterases (EST), and phosphatase activities in the tested field strains and susceptible laboratory strain were examined to better understand biochemical mechanisms of resistance. Highest esterase activity was observed for the MNF and FYM strains in comparison with that for the Lab-SUS. AChE activity was also significantly higher in all field strains tested by ~ 5.3-7.6-fold more than that in the SUS strain. In addition, significant increases in the activities of both acid- and alkaline-phosphatase were reported for MNF and FYM strains than these in the SUS strain. These results provide baseline information for further research on the involvement of esterases in the resistance mechanisms of field populations of the whitefly.

Key words: Whitefly, Non-conventional insecticides, Resistance, Esterases.

INTRODUCTION

The sweet potato whitefly, *Bemisia tabaci* (Gennadius), has become one of the most damaging insect pests of vegetables, ornamental and field crops worldwide (Perring et al., 2018). Crops have been harmed either directly or indirectly as a result of whitefly feeding, which has resulted in significant crop damage and output losses worth millions of dollars (Ou et al. 2019). The adults and nymphs can cause severe damage to the host plants through inserting their mouthparts into the plant tissues during feeding and by transmitting a large number of pathogenic viruses (Polston and Capobianco 2013; Gangwar and Charu 2018; Fiallo-Olivé et al. 2020). Conventional insecticides are used primarily for controlling the whitefly, even though over-reliance on chemical control has resulted in resistance development, ecological problems, and higher costs to the farmers (Horowitz *et al.* 2007, 2020; Basit 2019). The extensive use of insecticides with the same active ingredient as well as application of extreme doses of such compounds during a particular cropping season has induced development of insecticide resistance in particular to conventional insecticides, organophosphates and pyrethroids, in field populations of whitefly. Among the popular cryptic *B.tabaci* species complex, it was found that the Mediterranean- biotype Q (MED) of *B. tabaci* is considered more resistant to insecticides, pyriproxyfen and neonicotinoids, than the Middle East-Asia Minor 1, biotype B (MEAM1) (Middle East-Asia Minor 1, biotype B) (Horowitz et al. 2005). MEAM1 (biotype B) is the most common *B. tabaci* species, and it is thought to have spread over the world mostly through international trade in ornamentals (Horowitz et al., 2020). The

*Corresponding author: ammar.hager@gmail.com

previous broad-spectrum insecticides (organophosphates and pyrethroids) have not been very effective against *B. tabaci*, except possibly when applied in combination (Perring et al., 2018). Since their introduction in the mid-1990s, systemic neonicotinoids have been major players, particularly those that are generally stable in the soil and effectively absorbed by the root system.

Many researchers from various countries have reported the development of resistance in whiteflies to even novel chemical substances (e.g. Basij et al., 2016; Ahmad and Khan, 2017; Wang et al., 2020). In Egypt, high levels of resistance in field populations of *B. tabaci* to organophosphate, carbamate and pyrethroid pesticides by 20–52-fold, 20–80-fold, 20–528-fold, respectively (Kady and Devine 2003; Farghaly 2010). Several field issues, including an inadequate chemical selection and poor application procedures, aggravated pesticide control failures against *B. tabaci* in India (Peshin and Zhang 2014). In India, over 650 cases of pesticide resistance have been reported in *Bemisia* genus with resistance moreover 60 active ingredients (Mota-Sanchez and Wise 2019). Other modes of action widely used as foliar sprays primarily against nymphs include the IGRs, pyriproxyfen and buprofezen, inhibitors of acetyl CoA carboxylase (e.g. spiromesifen, spirotetramat), the anthranilic diamides (e.g. cyantraniliprole, and chlorantraniliprole), as well as oils, soaps, and detergents also have been used widely for whitefly control (Insecticide Resistance Action Committee, 2020; Perring et al., 2018). Insecticide resistance has resulted in the efficacy of many earlier insecticides being lost, putting undue demand on discovering new compounds with novel mode of action (Nauen et al., 2015).

Resistance mechanisms in *B. tabaci* are comparable to those documented for other pest species, namely, metabolically driven by higher levels of detoxifying enzymes or target-site resistance caused by point mutations. The mechanisms of resistance to organophosphates (OPs) and carbamates in whitefly have been explored since the 1980s, but no new findings have been reported in the last decade. There have been numerous levels of resistance linked to variable numbers of altered sensitivity alleles of acetylcholinesterase (AChE), the target site for organophosphates (Byrne et al. 1994; Byrne and Devonshire 1993). Other mechanisms of insecticide resistance, such as enhanced esterase activity, have been related to insensitivity of OPs (Byrne and Devonshire 1991). Pyrethroids affect the central nervous system's para-type voltage-gated sodium channel (VGSC), causing paralysis and death. Pyrethroid resistance was linked to the two mutations L925I and T929V in the IIS4-5 linker of VGSC in *B. tabaci* (Alon et al. 2008; Farghaly 2010).

Many researchers have reported resistance to the ‘Insect Growth Regulators’ (IGRs), buprofezin and pyriproxyfen, in whitefly (e.g., Horowitz and Ishaaya 2014; Roy et al. 2019). Pyriproxyfen is a powerful juvenile hormone (JH) mimic that suppresses embryogenesis, metamorphosis, and adult creation in insects by disrupting their hormonal balance. Pyriproxyfen has long been regarded as a major insecticide for controlling whiteflies, particularly biotype B (Crowder et al. 2008; Castle et al. 2010). Naranjo et al. (2004) indicated that these selective IGRs may be utilised in a whitefly integrated control strategy. However, during the 1990s, its widespread use has led in the development of resistance in many countries (Horowitz et al. 1994; Horowitz et al. 2005). Neonicotinoids are one of the most effective insecticide classes for controlling whiteflies. They have systemic and translaminar characteristics, as well as a high level of residual activity (Takahashi et al. 1992; Horowitz et al. 1998). Because there have been a few overview papers describing the global issues of neonicotinoid resistance (e.g., Nauen and Denholm 2005; Bass et al. 2015), we will only discuss a few key recent findings below. Neonicotinoid resistance has been linked to the Q biotype in many cases (e.g., Nauen et al. 2002; Horowitz et al. 2004; Dennehy et al. 2010), while a few incidences of neonicotinoid resistance have been reported in B-biotype strains as well (Byrne et al. 2003; Wang et al. 2010). Diamide insecticides, the most current type of chemical brought to the market about 13 years ago, are

another major pesticide widely employed to combat whiteflies (Nauen and Steinbach 2016). Among these compounds, Chlorantraniliprole and Flubendiamide are effective against lepidopteran pests; whereas Cyantraniliprole is effective against sucking pests like whiteflies and aphids (Sattelle et al. 2008; Lahm et al. 2009). Additionally, Insect Ryanodine receptors, which are huge tetrameric calcium release channels found in neuromuscular tissues, are targeted by these chemicals.

Therefore, the main goal of the current research was to detect the resistance levels of some field populations of the whitefly, *B. tabaci*, from different locations to the major insecticide classes extensively used in pest management programs and to investigate the possible correlation between resistance levels and activities of certain specific enzymes to better understand biochemical mechanisms of resistance.

MATERIALS AND METHODS

Chemicals

Commercial formulations of different insecticides used in this study were purchased from the local market of pesticides: chlorpyrifos (Dursban® 48% EC), Profenofos (Actacron® 72% EC), Carbosulfan (Marshal 20% EC), methomyl (Lannate® 90% SP), pyriproxyfen (Admiral® 10% EC), lufenuron (Match® 5% EC), buprofezin (Applaud® 25%SG), abamectin (Avermectin® 1.8% EC), spinosad (Spintor® 24% SC), acetamiprid (Mospilan® 20% SP), imidacloprid (Best® 25% WP), thiamethoxam (Actara® 25% WG), Thimethoxam + λ- cyhalothrin (Engeo® 24.7% SC), and chlorantraniliprole (Coragen® 20% SP). All chemicals used in the biochemical analyses were of analytical grade-kits of purity and purchased locally from Biodiagnostic Co., Dokki- Giza.

Insect Strains

A laboratory-susceptible strain (Lab-SUS) was provided by Syngenta, Kaha Research Station, Egypt. This reference susceptible strain originated from a population maintained without insecticide treatments for >10 years. It was maintained primarily on cotton plants, at Syngenta-insect rearing laboratories without any exposure to insecticides.

Three field strains of *B. tabaci* were collected from commercial fields of cucumber, tomato and squash fields, during 2018/2019 season, in Menoufia (Saqiya-Abusharah valley), Fayoum (Yousef-Elsedeek valley), and Beni-Suef (Suds valley), with heavy infestation of whitefly. These fields were exposed to the regular schedule of pest control established by Agricultural pesticide committee recommendations, Ministry of Agriculture using different commercial insecticides including organophosphate, carbamate, pyrethroid and neonicotinoid insecticides. The adult collections were made in the early, colder morning hours, using a custom-made manual—operated suction aspirator when adults were inactive. The collected insects were pooled in 3 to 4 wide mouth glass jars (4.4 liter).

The insect was reared on untreated cotton plants (*Gossypium hirsutum* L.) as described by Coudriet et al. (1985) in plastic pots (15 cm diameter) under laboratory conditions at 26 ± 2 °C and 70 ± 5% R.H., and a photoperiod of 16:8 (L: D) h. All pots were grown in chambers with metallic stands (70 × 200 cm and 80 height) and provided by lamp-unit (12 fluorescent lamp, 40 Watt) and surrounded by muslin.

Bioassay Methods

Adult-Vial Test

The vial technique was used for evaluating the susceptibility of whitefly adults from Lab-strain to the different selected insecticides. The technique used was similar to that described by Plapp et al. (1987) and Staetz et al. (1992) with some minor modifications. In this technique, unsexed adult whiteflies were placed in a 120-mL glass vial coated previously with a residual film of tested insecticide. To estimate LC_{50}s, five to seven doses (30 adults per dose) were used in each adult vial test. Acetone was used for making stock solution and serial dilutions based on active ingredient (AI) by weight. The coating of chemical residues was achieved by pipetting a volume of 1 mL of the tested concentration into each vial and by rotating the vial until the acetone completely

evaporated, leaving the insecticide residues evenly distributed on the inside surface. Control vials were per-coated with acetone only. Each concentration was replicated three times. Mortality counts were determined 24 h after exposure. The morbid insects, which could not fly even at short distance (<3 m) were scored as dead. By this technique no more than 10% mortality occurred in the control at any time. The mortality was corrected for control mortality using Abbott's formula **(**Abbott, 1925**)**. The concentration-response analysis was used to estimate values of slope (b), LC_{50}, and 95 percent confidence limits (95% CL) using the Probit and Logit Analysis software program (Polo Plus, ver. 2.0, 2008), based on Finney analysis (Finney 1971). The LC_{50} results were given in g (AI) per vial.

Leaf-Residue Test

Another set of bioassay was carried out to evaluate the response of *B. tabaci* adults of the selected field strains to various insecticides compared to the laboratory-susceptible strain. The leaf–residue bioassay method described by Cahill et al. (1995) was adopted with slight modifications. Briefly, leaf discs (50 mm in diameter) were cut from cotton leaves and dipped in aqueous solutions of 5-7 series of diluted concentrations of an insecticide for 5 to 10 s. Three replicates were used for each concentration. The leaf discs were then dried in the ambient air in the laboratory. The bases of small Petri dishes (55 mm in diameter) were filled with 7 ml of agar gel (15 g/L). The leaf discs were placed on the agar with their upper side down. In order to facilitate insect's manipulation, we put the adults of *B. tabaci* in the freezer for 10 min in order to immobilize whiteflies instead anaesthetized with CO_2. Using a tube, 30 insects were removed onto the Petri dish with treated leaf discs. With thin mesh had been opened on the side wall of the dish to allow adequate ventilation. The dishes were inverted for the insects to orientate normally and placed in a large controlled environment room at 25 (±2) ˚C, 50–60%R.H. and 16:8 h Light: Dark photoperiod. We used the distilled water for a control leaf discs. Mortality was assessed after 48 h. In control dishes (with leaves treated with only water) 97-99% of the whiteflies survived at least 24 h. Mortality on all leaves was determined after an exposure period of 24 h. Insects were considered alive if any sign of movement was observed. As mentioned above, the mortality data were corrected using Abbott's (1925) formula. The data were analyzed in the same manner as that of the adult vial bioassay. Resistance ratios were determined by dividing the LC_{50} of each field strain by the LC_{50} of the laboratory-susceptible strain.

Biochemical Assays

Preparation of Enzymes

Samples from whitefly adults were collected from the selected field strains (MNF, BSW, FYM) and reference-susceptible strain (SUS) for enzyme preparations. The insects were homogenized in 0.1 mM phosphate buffer, pH 7.0, in cold tubes which coated previously with crystals of phenylthiourea to prevent melanization. The samples were centrifugated at 2500 rpm for 5 minutes under cooling (4°C), then, the supernatant fluid was collected and divided into small aliquots (0.5 ml) and stored at –20 °C until analysis where used as the enzyme source.

Measurement of Esterase (EST) Activity

In this assays, esterase activity (EST) was measured in samples of *B. tabaci* adults using the procedure described by Van Asperen (1962). In this technique, α - and β -esterases activities were determined using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. The reaction mixture consisted of 5 ml. substrate solution (3×10^{-4} M α -or β-naphthyl acetate, 1% acetone and 0.04 M phosphate buffer pH 7) and 20 µl of insect homogenate, The mixture was incubated for exactly 15 minutes at 27°C then 1 ml of color reagent, diazoblue sodium lauryl sulphate solution, which produces a strong blue color in case of α -naphthol or strong red color in the case of β-naphthol. The developed color was read colorimetrically (Milton Roy Spectronic model 1201 spectrophotometer) at 600 nm and 555 nm for α- and β-naphthol, respectively. The activity was expressed as µg α- or β-naphthol released /min/mL of homogenate samples.

Measurement of Acetylcolinesterase (AChE) Activity

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson et al. (1964) using acetylcholine bromide (AChBr) as a substrate at level of 6×10^{-3} M. The assay was done by adding 0.2 mL of insect homogenate to 0.5 mL 0.067 M phosphate buffer and 0.5 mL substrate (3 mM AChBr) in tube labeled [T]. Another tube labeled [TS] contains 0.7 ml phosphate buffer (0.067 mM) and 0.5 ml substrate. Control tube [C] contains 0.2 mL of insect homogenate in 1 mL phosphate buffer. All test tubes were incubated for 30 min at 37°C. After incubation period, 1 mL of alkaline hydroxylamine was added to all tubes. The tubes were shaken well and allowed to stand for 2 min, then 0.5 mL of HCl was added. The mixture was shaken vigorously and allowed to stand for 2 min, then 0.5 ml of ferric chloride solution (0.92 M $FeCl_3$ in 0.1 M HCl) was added and mixed well. The resulting mixture was centrifuged and the supernatant was measured at 515 nm.

The AChE activity in the homogenates was expressed as follows:

$$AChE\ activity = \frac{(TS\ +\ C)}{wt/mL\ homogenate} =$$

= µg substrate hydrolyzed/min/mL homogenate.

Where (T) = test, (TS) = substrate, (C) = control

Measurement of Acid- and Alkaline-Phosphatase Activities

Acid- and alkaline- phosphatase activities were measured according to the method described by Laufer and Schin (1971). For acid phosphatase (ACP) assay, 0.2 mL of insect homogenate was added in a test tube containing a mixture of 0.5 mL *p*-nitrophenyl phosphate substrate and 0.5 mL acid buffer (pH 4.8) that was previously incubated for 10 min at 27°C in a water bath. A blank tube contains all the reagents except the insect homogenate. The reaction mixture was incubated at 27°C for 20 min, then 0.1 N NaOH was added to terminate the reaction. The developed color was measured at 400 nm by using a spectrophotometer. For alkaline phosphatase (ALP) assay, similar technique to that described previously was followed except of adding alkaline buffer, pH 10.5, instead of acid buffer, pH 4.8. The ALP activity was measured specrophotometrically at 400 nm

Measurement of Total Protein Content

The total content of protein in insect homogenate was determined using Folin phenol reagent according to the method of Lowry *et al.* (1951). Insect homogenate was added to 1 ml of 5% trichloroacetic acid, and the precipitated protein was dissolved by boiling for 5 min in 2 mL of 1 N NaOH solution. Then 0.2 ml from this solution was placed in test tubes, each contained 1.0 mL of buffer cupper sulphate solution. After 10 min, 0.2 ml from Folin reagent was added to the mixture and the contents were heated for 2.5 min at 50°C. The tubes were allowed to stand for 10 min to cool at room temperature. The blank tube was similarly run using 0.2 ml NaOH instead of the insect homogenate sample. Reading was measured spectrophotometrically at 750 nm.

Statistical Analysis

The obtained data was statistically analyzed using analysis of variance (ANOVA) at 5% probability. The means of different treatments were separated by L.S.D. (Least Significant Difference) test (LSD) through CoStat software program (Version 6.400).

RESULTS

Evaluation of Adult-Vial and Leaf-Residue Bioassays

The responses of *B. tabaci* adults exposed to various insecticides in the vial technique are shown in Table 1. The susceptibility of whitefly adults to tested insecticides varied in relation to the bioassay techniques used. Based on LC_{50} and slope values of tested insecticides after 24 h of exposure, it was obvious that the response of adult whiteflies to most insecticides was higher in the vial-residue bioassay compared to the leaf-residue technique. The toxicity data, in the adult-vial technique, showed that LC_{50} values of most insecticides tested were < 1.0 µg/vial. The neonicotinoid imidacloprid was the most toxic insecticide (0.11 µg/vial) followed by buprofezin (0.21 µg/vial), abamectin (0.29 µg/vial),

thiamethoxam (0.41 µg/vial) and pyriproxyfen (0.88 µg/vial), whereas methomyl had the lowest toxicity (7.62 µg/vial). In the leaf-residue bioassay, the toxicity data showed that abamectin was the most toxic insecticide (LC_{50} = 0.95 µg/vial) followed by the neonicotinoids, acetamiprid (1.03 µg/vial) and thiamethoxam (1.70 µg/vial) and flufenuron (1.92 µg/vial). Similarly, methomyl had the lowest toxicity (4.73 µg/vial) against the insect.

Insecticide Resistance Status in Field Strains of *B. tabaci*

The responses of the whitefly, *B. tabaci*, adult populations collected from different cucumber, tomato and squash fields to various insecticides are determined in comparison to the reference susceptible strain (Lab-SUS), using leaf-residue technique (Table 2). Based on 24 h-LC_{50} data, it was obvious that the all three field strains, MNF, BNS and FAY, have developed slightly resistance spectrum to the organophosphate, profenofos, and carbamates, carbosulfan and methomyl, insecticides, with resistance ratios (RR) ranged from 3.0 to 5.4- fold, compared to the SUS strain.

Table 1. Response of whitefly, *Bemicia tabaci,* adults of laboratory insecticide- susceptible strain (Lab-SUS) to different insecticides using leaf-residue and glass vial bioassays.

Insecticides	Adult-vial technique			Leaf-residue technique		
	Slope ± SE	LC_{50} (95% FL)[¥]	TI[¥]	Slope ± SE	LC_{50} (95% FL)[¥]	TI[¶]
Chlorpyrifos	0.74 ± 0.15	3.59 (1.69 - 6.16)	3.06	1.62 ± 0.12	2.32 (1.73 – 10.62)	40.9
Profenofos	-*	-	-	1.36 ± 0.47	3.51 (1.98–10.11)	27.1
Carbosulfan	-	-	-	1.14 ± 0.33	4.75 (3.75–13.62)	20
Methomyl	0.95 ± 0.23	7.62 (3.5.-11.82)	1.44	1.24 ± 0.09	4.73 (2.98 – 12.44)	20.1
Pyriproxyfen	1.39 ± 0.18	0.88 (0.58 - 1.18)	12.5	1.72 ± 0.23	3.65 (2.11 – 12.39)	26.0
Lufenuron	0.16 ± 0.10	1.16 (0.58 - 2.16)	9.48	1.39 ± 0.22	1.92 (0.43 – 7.64)	49.5
Buprofezin	0.71 ± 0.09	0.21 (0.12 - 0.35)	52.4	1.52 ± 0.10	3.45 (1.99 – 9.49)	27.5
Abamectin	0.85 ± 0.15	0.29 (0.15 - 0.46)	37.9	1.12 ± 0.31	0.95 (0.12 – 2.17)	100.0
Spinosad	0.85 ± 0.02	4.86 (2.59 - 8.67)	2.26	1.28 ± 0.39	3.22 (1.53 – 9.32)	29.5
Acetamiprid	0.61 ± 0.15	0.62 (0.10 - 1.53)	17.7	1.25 ± 0.80	1.03 (0.70 – 5.13)	92.2
Imidacloprid	0.58 ± 0.09	0.11 (0.04 - 0.26)	100	1.81 ± 0.24	3.30 (2.33 – 13.52)	28.8
Thiamethoxam	0.74 ± 0.12	0.41 (0.20 - 0.82)	26.8	1.19 ± 0.42	1.70 (0.92 – 6.97)	55.9
Thimethoxam + λ- cyhalothrin	-	-	-	1.32 ± 0.26	3.91 (2.02–13.95)	24.3
Chlorantraniliprole	0.88 ± 0.18	3.36 (1.57 - 6.02)	3.27	1.17 ± 0.21	3.17 (1.41 – 12.04)	30.0

[¥]Concentrations are expressed in µg AI/vial
[¶]TI, Toxicity index, calculated by dividing LC_{50} of the most toxic insecticide/ LC_{50} of the other one × 100 (Sun, 1950).
* Data not available.

Table 2. Comparative toxicity of selected insecticides against laboratory insecticide- susceptible strain (Lab-SUS) and field-collected strains of whitefly, *Bemicia tabaci*, adults in leaf-residue bioassay.

Insecticides	Lab-SUS		MNF			BNS			FAY		
	Slope ± SE	LC_{50} (95% FL)¥	Slope ± SE	LC_{50} (95% FL)¥	RR¶	Slope ± SE	LC_{50} (95% FL)¥	RR¶	Slope ± SE	LC_{50} (95% FL)¥	RR¶
Chlorpyrifos	1.62 ± 0.12	2.32 (1.73–10.62)	1.28 ± 0.23	5.44 (3.16–8.62)	2.34	1.63 ± 0.19	4.81 (2.13–10.69)	2.07	0.72 ± 0.01	3.56 (1.94–5.80)	1.53
Profenofos	1.36 ± 0.47	3.51 (1.98–10.11)	1.23 ± 0.38	15.02 (14.52–31.41)	4.27	1.21 ± 0.87	19.02 (17.9–32.4)	5.41	1.81 ± 0.54	15.75 (10.22–46.82)	4.48
Carbosulfan	1.14 ± 0.33	4.75 (3.75–13.62)	1.49 ± 0.25	19.32 (17.26- 32.66)	4.06	1.18 ± 0.19	26.36 (13.91- 43.13)	4.94	0.83 ± 0.13	14.26 (11.60- 25.94)	3.00
Methomyl	1.24 ± 0.09	4.73 (2.98–12.44)	1.13 ± 0.18	21.47 (18.69–36.64)	4.53	1.50 ± 0.29	25.46 (19.27–49.67)	5.38	1.27 ± 0.21	17.70 (13.46–31.59)	3.74
Pyriproxyfen	1.72 ± 0.23	3.65 (2.11–12.39)	1.81 ± 0.34	6.37 (2.22–7.21)	1.74	0.85 ± 0.18	8.77 (4.96–15.74)	2.40	0.83 ± 0.21	8.07 (6.34–30.13)	2.21
Lufenuron	1.39 ± 0.22	1.92 (0.43–7.64)	0.95 ± 0.14	5.24 (3.27–8.27)	2.72	1.21 ± 0.20	5.62 (4.14–9.70)	2.92	0.94 ± 0.19	3.22 (1.51–5.24)	1.67
Buprofezin	1.52 ± 0.10	3.45 (1.99–9.49)	0.88 ± 0.14	4.37 (3.48–15.12)	1.26	0.67 ± 0.14	15.39 (7.27–35.45)	1.56	0.76 ± 0.16	10.10 (6.87 - 27.85)	2.92
Abamectin	1.12 ± 0.31	0.95 (0.12–2.17)	1.45 ± 0.25	2.47 (1.58–3.54)	2.6	0.85 ± 0.24	3.12 (0.98–5.49)	3.28	2.05 ± 0.29	1.98 (1.43– 2.53)	2.08
Spinosad	1.28 ± 0.39	3.22 (1.53–9.32)	1.86 ± 0.37	4.36 (2.01–6.43)	1.35	1.34 ± 0.21	12.40 (10.88–34.47)	3.85	1.31 ± 0.19	15.00 (13.18–31.37)	4.65
Acetamipird	1.25 ± 0.80	1.03 (0.70–5.13)	1.37 ± 0.22	3.29 (2.97–7.56)	3.19	0.75 ± 0.20	0.60 (0.01–1.72)	0.58	1.23 ± 0.24	3.12 (1.54–4.95)	3.02
Thiamethoxam	1.19 ± 0.42	1.70 (0.92–6.97)	1.08 ± 0.27	4.13 (1.49-7.56)	2.42	0.70 ± 0.22	2.94 (0.77-6.44)	1.72	1.38 ± 0.23	5.03 (3.19-8.01)	2.95
Thimethoxam + λ- cyhalothrin	1.32 ± 0.26	3.91 (2.02–13.95)	1.03 ± 0.18	6.80 (4.88–7.56)	1.73	1.00 ± 0.19	15.05 (11.97–35.20)	3.84	0.95 ± 0.19	10.92 (7.19–24.17)	2.79
Imidacloprid	1.81 ± 0.24	3.30 (2.33–13.52)	0.87 ± 0.13	10.04 (5.89–15.87)	3.04	1.38 ± 0.14	7.21 (0.10–8.24)	2.18	1.21 ± 0.12	13.72 (11.82–24.36)	4.15
Chlorantraniliprole	1.17 ± 0.21	3.17 (1.41–12.04)	1.06 ± 0.20	6.93 (2.94–13.32)	2.18	1.05 ± 0.22	8.06 (4.73–23.93)	2.54	0.83 ± 0.24	7.77 (1.69–14.43)	2.45

¥Concentrations are expressed in ppm.
¶RR = Resistance ratios= LC_{50} of the field population divided by LC_{50} of the Lab-SUS insecticide-susceptible strain.

Low resistance levels (RR) of the tested non-conventional insecticides, pyriproxyfen (1.74), buprofezin (1.26), spinosad (1.35) and thiamethoxam/ cyhalothrin mixture (1.73) were detected in MNF-field strain, compared to Lab-SUS strain (Table 2). Similarly, the whitefly adults of BNS-field strain also showed lower resistance levels to the selected non-conventional insecticides with resistance ratios of 0.58 (acetamiprid), buprofezin (1.56), and 1.72 (thiamethoxam) compared the SUS-strain. However, the whitefly adults from FAY-field strain in part showed slight increase in the resistance levels to spinosad, thiamethoxam/ lambdacyhalothrina, cetamiprid, acetamiprid, buprofezin and thiamethoxam with 4.65-, 4.15-, 3.02-, 2.95-, and 2.92-fold, respectively.

The data in Table 2 showed also low slope values, in general, for all the three field strains indicating a genetic heterogeneity that may allow the development of resistance should selection take place.

Enzyme Activity

Data on spectrophotometric analysis of esterases indicated a higher enzyme activity in the three field strains compared with the susceptible strain (Table 3). The activity of α-naphthyl acetate esterase significantly increased in MNF, FYM and BNS-field strains by 5.03-, 4.33- and 1.73-fold than that in the Lab- SUS strain. Similarly, the β-naphthyl acetate esterase significantly increased in these field strains by 5.12-, 6.05- and 3.32- fold, respectively, compared to the Lab-SUS strain.

The activity of acetylcholinesterase (AChE) was also significantly increased in the field strains, MNF, FYM and BNS by 7.63-, 7.14- and 5.31-fold compared to that in the SUS-strain (Table 4). Also, there is a significant difference in enzyme activities in field strains with each other.

Table 3. Non-specific esterases activity in whitefly, *Bemicia tabaci,* adults of laboratory insecticide-susceptible strain (Lab-SUS) and field-collected strains.

Strain	Mean Esterase (EST) Activity			
	α-Esterase[a]	%[b]	β- Esterase[a]	%[b]
Susceptible (SUS)	99.73 ± 1.3d	100	88.53 ±2.2 d	100
Menoufia (MNF)	502.18 ± 5.4a	503.6	453.75 ± 3.9b	512.5
Fayoum (FYM)	432.65 ± 3.4b	433.8	535.61 ± 4.4a	605.2
Beni-Suef (BNS)	172.31 ± 1.8c	172.8	293.51 ± 2.7c	331.6

Values followed by the same letter are not significantly different (LSD test, $P = 0.05$).
[a]Mean esterase activities (±SD) expressed as µg of α-naphthol or β-naphthol formed/ mL/ min.
[b]Percentage change equals the EST activity in field strain divided by the EST activity in susceptible strain multiplied by 100.

Table 4. Acetylcholinesterase (AChE) activity in whitefly, *Bemicia tabaci,* adults of laboratory insecticide- susceptible strain (Lab-SUS) and field-collected strains.

Strain	Mean Acetylcholinesterase (AChE) Activity	
	AChE[a]	%[b]
Susceptible (SUS)	197.8 ±1.5c	100
Menoufia (MNF)	1509.5 ± 5.6a	763.1
Fayoum (FYM)	1412.5 ± 4.8ab	714.1
Beni-Suef (BNS)	1050.4 ± 3.6b	531.0

Values followed by the same letter are not significantly different (LSD test, $P = 0.05$).
[a]Mean AChE activities (±SD) expressed as µg acetylcholine bromide/ min/ml .
[b]Percentage change equals the enzyme activity in field strain divided by the enzyme activity in susceptible strain multiplied by 100.

The data of acid- and alkaline-phosphatase activities in the tested field strains compared to that in the laboratory-susceptible strain are summarized in Table 5. The acid-phosphatase activity in both MNF- and FYM-field strains was significantly higher by 2.53- and 3.49-fold than that in the Lab SUS-strain. Likewise, the alkaline phosphatase indicated a higher activity in all the three field strains, MNF, FYM and BNS than that of the Lab-SUS strain by 2.08-, 4.28- and 2.42-times, respectively. strain.

The estimated total protein content in the homogenates of whitefly adult insect samples from both field and Lab-SUS strains are summarized in Table 6. A slight significant increase in the total protein content was reported in the MNF- and BNS- field strains by 1.50- and 1.66-times than that of the Lab-SUS strain.

Discussion

The combined results from two different bioassays indicated that the response of adult whiteflies to most tested insecticides was higher in the vial-residue bioassay compared to the leaf-residue technique. Application of insecticides weekly, every four days or even once or twice a day will not provide the proper control of *Bemisia tabaci* adults, the vector of tomato yellow leaf curl virus (TYLCV) where the actual economic threshold is zero for the whitefly adults that be able to infect host plants with TYLCV within 4 hours of inoculative feeding (Sharaf, 1986). Therefore, the vial technique, based on contact exposure, was used in the present bioassay for screening the conventional and novel available insecticides to select the most quick and efficient adulticides to be recommended in areas of tomato plantations where TYLCV was prevalent. Plapp et al. (1987) developed a glass-vial technique for detecting resistance in tobacco budworm, *Heliothis virescence* (F.), adult moths in cotton. A similar procedure was adapted for detecting insecticide resistance in field populations of *Bemisia spp.* adults (Prabphaker et al. 1996; Sivasupramaniam et al. 1992, 1997). Also, glass vial with the interior coated with varying levels of technical insecticides has been used to determine their contact toxicity on the adults of laboratory-reared and field-collected cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (LÓPEZ, et al. 2008). The traditional field tests that evaluate formulated insecticides for control of cotton fleahopper in small plots can be expensive and require spatial and temporal validation of the data to obtain meaningful results. Thus, the glass-vial bioassay is a simple, inexpensive, and an effective technique for evaluating the insecticidal activity and speed of killing of various insecticides against whitefly adults. This technique is also valuable for monitoring and determining resistance in field populations of whitefly adults. In addition, this method provides the user with results quickly, does not require the insects to be reared, and could be used by producers, consultants, and extension personnel to make informed decisions on adequate control means (Sivasupramaniam et al. 1997).

Table 5. Acid- and Alkaline-Phosphatase activities in whitefly, *Bemicia tabaci,* adults of laboratory insecticide- susceptible strain (Lab-SUS) and field-collected strains.

Strain	Mean Acid phosphatase (ACP) and Alkaline-phosphatase (ALP) Activities			
	Acid phosphatase[a]	%[b]	Alkaline phosphatase[a]	%[b]
Susceptible (SUS)	12.11 ± 1.1d	100	11.34 ± 0.8c	100
Menoufia (MNF)	28.49 ± 1.8b	235.3	23.59 ± 1.2b	208.0
Fayoum (FYM)	42.27 ± 2.2a	349.1	48.56 ± 3.3a	428.2
Beni-Suef (BNS)	20.34 ± 1.7bc	167.9	27.45 ± 1.7b	242.1

Values followed by the same letter are not significantly different (LSD test, $P = 0.05$).
[a]Mean ACP and ALP activities (±SD) expressed as µg phosphate/min/ml.
[b]Percentage change equals the enzyme activity in field strain divided by the enzyme activity in susceptible strain multiplied by 100.

Table 6. Total protein content in in whitefly, *Bemicia tabaci,* adults of laboratory insecticide-susceptible strain (Lab-SUS) and field-collected strains.

Strain	Mean Total Protein Content	
	Total protein[a]	%[b]
Susceptible (SUS)	24.50 ± 2.2c	100
Menoufia (MNF)	37.18 ± 1.7ab	151.8
Fayoum (FYM)	29.48 ± 1.9bc	120.3
Beni-Suef (BNS)	40.87 ± 2.4a	166.8

Values followed by the same letter are not significantly different (LSD test, $P = 0.05$).
[a]Mean values of total protein content (±SD) expressed as mg/ml.
[b]Percentage change equals the protein content in field strain divided by the protein content in susceptible strain multiplied by 100.

These results indicated, in general, that slight levels of resistance were found in the three field strains toward most selected insecticides. For neonicotinoids, BNS- strain seemed to be more sensitive toward neonicotinods, acetamiprid, thiamethoxam and imidacloprid) than the other field strains (MNF and FYM). Horowitz et al. (2004) found that acetamiprid was more effective than imidacloprid in controlling *B. tabaci* by foliar application, however imidacloprid was found more effective than acetamiprid through soil application (Ishaaya and Horowitz 1998). It was also reported that resistance of *B. tabaci* populations in cotton fields in Israel to acetamiprid developed relatively slowly in spite of intense application of this compound (Horowitz et al. 2004). A similar occurrence was observed with imidacloprid, a neonicotinoid used in the southwestern United States, where a variety of agroecosystems may explain imidacloprid's continued efficiency against whiteflies in desert cropping systems (Palumbo et al. 2001). In greenhouses in Spain and Germany, however, very high imidacloprid resistance was reported, which was connected to *B. tabaci* biotype Q. (Rauch and Nauen 2003). Nauen et al. (2003) explained the reasons for the absence of cross-resistance to acetamiprid in thiamethoxam-selected *B. tabaci* because of these two neonicotinoids have two different modes of action. It was reported that thiamethoxam is a precursor that is converted to another neonicotinoid, clothianidin in both insects and plants. Furthermore, resistance in thiamethoxam-selected whiteflies may be linked to the activation mechanism, whereas in acetamiprid-selected whiteflies, the active ingredient is the primary target for detoxification, resulting in wide neonicotinoids cross-resistance (Nauen et al. 2003). The resistance to neonicotinoids in *B. tabaci* appears to be less severe than that to other conventional pesticides employed against this pest. Despite a few cases of resistance to the neonicotinoids have been detected, it is of critical importance to consider strategies for management of resistance for this important class (Elbert et al. 1996). Accordingly, because this effective group of insecticides is used largely against whiteflies and other sucking pests, the use of the neonicotinoids should be taken to reduce to the minimum needed, either by alternating with other insecticides that are still effective against whiteflies, or by using nonchemical methods.

A slight increase of approximately 5.0-fold resistance was detected in two field strains (MNF and BNS) toward the tested conventional insecticides, profenofos, carbosulfan and methomyl. One of the reasons for development of resistance may be the repeated applications of several insecticides on a broad scale to tomatoes pest complex, including whiteflies. However, slight resistance levels in all field strains to the IGRs tested, i.e. pyriproxfen, lufenuron and buprofezin, which ranged from 1.26- to 2.9- fold. The low levels of resistance to insecticides maybe because that's the adults were collected from fields at the early seasons, since it is well know that, in the absence of selection pressure, a reduction in the insecticide tolerance can occur following laboratory rearing for several generations (Needham and Sawicki 1971;

Ranasinghe and Georghiou 1979). That low levels of resistance to such insecticides might develop into higher levels of resistance under such selection pressures should not be overlooked. Therefore, periodic monitoring of resistance levels in the field should be continued (Prabhaker 1985). In addition to selection pressure, other biological and behavioural characteristics influence the rate and density of development of resistance (Georghiou and Taylor 1977).

The short generation time of whiteflies, the high rate of fecundity, the stage-specificity, and the greater mobility of adults are advantageous to the insect and thus enhance the evolution of insecticide resistance (Prabhaker et al. 1985). Recently, the novel insecticides such as neonicotinoids (imidacloprid, acetamiprid, thiamethoxam, thiacloprid, nitenpyram), insect growth regulators (buprofezin, pyriproxyfen), diamides (chlorantraniliprole), diacylhydrazines (methoxyfenozide,) and diafenthiuron (diafenthiuron) have been introduced with great success. Judicious rotation of old and new insecticides can therefore prevent or delay the onset of resistance in whiteflies (Ahmed et al. 2010). Recently, further modern methods have been performed to better understand of resistance mechanisms in the whitefly at which based on the molecular as well as gene sequence data from both resistant and susceptible field-strains (Horowitz et al. 2020). These findings led to a better understanding of mechanisms of insecticide resistance in this pest (Horowitz1 et al. 2020). Among the countermeasures used for management of insecticide resistance in this pest, there are different components of IPM-IRM (Integrated Pest Management-Insecticide Resistance Management) programs, e.g., using biorational and selective insecticides, rotation of different insecticides with different mechanisms of action as well as physical and nonchemical control methods.

Biochemical assays, including those with model substrates, can be utilized successfully for detecting and monitoring insecticide resistance in insect populations (Brown and Brogdon, 1987; Devonshire, 1987). Such knowledge of the nature of resistance mechanisms may serve as a foundation for the development of field kits to diagnose metabolic resistance in field populations of this pest. The basis for the present study is a probably correlation reported between esterases' activities and resistance or tolerance in field populations of whitefly, *B. tabaci.* The present study showed highly significant increases in the activities of non-specific esterases (α- and β-EST) in whitefly adults collected from tomato in field strains, MNF and FYM, by ~ 4.0 to 6.0- fold, relative to these of the susceptible strain (Lab-SUS). Additionally, acetylcholinesterase (AChE) activity was highly increased in all three field strains, MNF, FYM and BNS, by ~ 5.3 - 7.6-times compared to that in the Lab-SUS strain. These results provide indirect evidence for the role of ESTs in tolerance of *B. tabaci* field populations and illustrate the utility of EST activity toward α-NA β- NA or as a biochemical marker for insecticide resistance in this insect. Elevated EST activities toward model substrates have been associated with OP resistance in a number of insect pests (Abdel-Aal et al. 1993).

Resistance to organophosphates (OP) and carbamates (CAR) involves enhanced detoxification of the insecticides by non-specific carboxylesterases (COE) and cytochrome P450-dependent mono-oxygenases (Dittrich et al. 1990; Prabhaker et al. 1988; Bloch et al. 1994) and target site modifications (insensitivity of the synapse target enzyme acetyl cholinesterase, iAChE) (Anthony et al. 1998; Alon et al. 2006; Byrne et al. 1997). In *B. tabaci* populations from Crete, Greece, Roditakis *et al*. (2008) found substantial relationships between carboxylesterases (COE) and P450-dependent monoxygenase activity and resistance to -cypermethrin and imidacloprid, respectively. A moderate correlation between COE activity (α-naphthyl acetate) and resistance levels to imidacloprid was observed in whitefly populations, whereas no correlation was observed between imidacloprid resistance and COE activity (β-naphthyl acetate) and COE (α-NPA) (Roditakis et al. 2009). Synergism studies indicate that both oxidative and hydrolytic detoxifications are responsible for partial resistance to OPs and pyrethroids in the Pakistani whiteflies (Ahmad et al. 2002). Additionally, the

mechanisms of OP- and carbamate-resistance in *B. tabaci* from different regions of the world have been found to be due to insensitive AChE (Byrne et al. 1994; Byrne and Devonshire 1997; Anthony et al. 1998; Erdogan et al. 2008) and metabolic detoxification by esterases (Prabhaker et al. 1988; Dittrich et al. 1990; Cahill et al. 1995; Ahmad 2007), monooxygenases (Prabhaker et al. 1988; Dittrich et al. 1990; Kang et al. 2012) and glutathione *S*-transferases (Kang et al. 2006). Furthermore, it was found that over-expression of two acetylcholinesterase (AChE) genes, Ace1 and Ace2, as well as two carboxylesterase genes Coe1 and Coe2 was responsible for resistance in an OP-resistant strain of *B. tabaci* (Alon et al. 2008). For pyrethroids, it was reported that resistance of the whitefly to pyrethroid insecticides has been related with both enhanced detoxification of insecticidal compounds and target-site modifications (Byrne et al. 2000; Dittrich et al. 1990). Also, the hydrolytic and oxidative pathways related with higher activities of cytochrome P450-dependent-monoxygenase and carboxylesterases (COE) were reported by Shchukin and Wool (1994) and Byrne et al. (2000).

From the aforementioned results in the current study, it is concluded that the populations of whitefly adults from the selected fields vary in their responses to different insecticides. We found that the resistance levels for neonicotinoids tested were lower especially in BNS-strain indicating RR < 2.1-fold compared to the Lab-SUS strain, whereas slight increases in resistance levels ranged from 3.0- to 5.4- fold for the conventional insecticides tested were detected in all three field strains. We also found that the adult-vial bioassay seemed to be more sensitive technique for insecticide bioassay compared to the leaf-residue bioassay. It is also concluded that non-specific EST and AChE assays can be utilized as complementary tools for detecting and monitoring mechanisms of insecticide resistance in the field populations of whitefly adults. Accordingly, as resistance management programs become more refined, the importance of accurate and timely assessment of resistance will become increasingly critical.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publishing of this paper.

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ميكانيكيات المقاومة للمبيدات في بعض السلالات الحقلية لحشرة الذبابة البيضاء *Bemisia tabaci* (Hemiptera: Aleyrodidae)

لبنى زيدان[١] ــ محمد عبدالعزيز[٢] ــ جمال أبو أبوالغار[٢] ــ أنور الشيخ[٣] ــ هاجر عمار[٣]

[١] المعمل المركز للمبيدات ، مركز البحوث الزراعية ، الدقى ، مصر

[٢] وزارة الزراعة واستصلاح الأراضى، الوادى الجديد، مصر

[٣] قسم المبيدات الآفات، كلية الزراعة، جامعة المنوفية ، مصر

الملخص العربى

تم دراسة استجابة الحشرة الكاملة للذبابة البيضاء لبعض المبيدات من مجاميع مختلفة باستخدام طريقتين مختلفتين من طرق التقييم الحيوي. أوضحت نتائج الاختبارات أن التقييم الحيوي باستخدام طريقة الانبوبة adult-vial كانت أكثر حساسية من استخدام التقييم الحيوي عن طريق متبقي المبيد على الأوراق leaf-residue. وكانت قيمة التركيز النصفي القاتل لأكثر المبيدات المختبرة كفاءة < ١ ميكروجرام/أنبوبة وكان المبيد النيونيكوتينويد اميداكلوبراید أكثر المبيدات سمية (0.11 µg/vial) يليه مبيد بيبروفيزين، ابامكتين، ثياميثوكسام وبايريبروكسفين، على الترتيب. بينما كان مبيد ميثوميل اقل المبيدات سمية وقد تم دراسة حالة المقاومة لثلاثة عشائر حقلية من حشرة الذبابة البيضاء باستخدام طريقة متبقي المبيدات على الأوراق وأوضحت النتائج أن مستويات المقاومة لمعظم المبيدات المستخدمة كانت منخفضة خاصة في سلالة بني سويف (نسبة مقاومة < ٢,١ ضعف) بالمقارنة بالسلالة المعملية.. بينما تراوحت درجة ما بين ٣-٥,٤ للمبيدات التقليدية المختبرة في الثلاثة سلالات الحقلية. تم تقدير نشاط انزيم اسيتيل كولين استيريز، الاستيريز غير المحدد والفوسفاتيز في السلالة المعملية والسلالات الحقلية لمعرفة ميكانيكية المقاومة الكيماوية للمبيدات. أظهرت النتائج زيادة نشاط انزيم الاستيريز في السلالات الحقلية من المنوفية والفيوم بالمقارنة بالسلالة المعملية وأظهر نشاط انزيم الاستيل كولين أيضاً زيادة معنوية في كل السلالات الحقلية بمعدل يتراوح بين ٥,٣-٧,٦ أكثر مما هو في السلالة الحساسة. بالإضافة إلى أن نتائج كلٍ من الفوسفاتيز الحامضي والقاعدي أظهرت فروقاً معنوية في سلالتي المنوفية والفيوم أكثر من السلالة الحساسة.