

MONITORING FOR RESISTANCE IN TWO FIELD STRAINS OF *Bemisia* spp. TROUGH BIOASSAY AND BIOCHEMICAL ASSAY

By

Fathia I. Moustafa*, Manal A. Attia , M. S. Shawir***

* Pesticide chemistry department, Faculty of Agriculture, Alexandria University

** Central Pesticide Laboratory, Agriculture Research Center, Alexandria

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ABSTRACT

Monitoring for resistance through bioassay: The susceptibility of two field strains of whiteflies *Bemisia* spp. to representatives of the main insecticidal groups were compared to study the development of resistance in *Bemisia* spp. field populations. The susceptibility tests were done according to (Prabhaker *et al.*, 1985). The results revealed that, El-Beheira field strain of *Bemisia* spp. was more tolerant to the tested organophosphate and carbamate insecticides than Abees one. The highest level of resistance was obtained for thiodicarb ($R/R = 10.53$) followed by profenofos, methomyl, pyrimiphos-methyl and chlorpyrifos. Concerning a laboratory-susceptible strain as a reference, the results revealed that Abees and El-Beheira field strains were tolerant or resistant to most of the tested insecticides except for deltamethrin. For the Abees strain the highest level of resistance was obtained for malathion ($R/R = 16.85$) followed by pirimiphos-methyl, profenofos, methomyl and cypermethrin, while for El-Beheira strain the highest level of resistance was obtained for profenofos ($R/R = 45.17$) followed by malathion, pirimiphos-methyl and methomyl. Moreover, the field strains of *Bemisia* spp. showed low or no level of tolerance to the tested synthetic pyrethroids. Generally, El-Beheira field strain was more tolerant or resistant to the tested organophosphates and carbamates compared with Abess field strain or the laboratory-susceptible one.

Monitoring for resistance through biochemical techniques: Biochemical techniques based on qualitative esterases activity were used for monitoring insecticide resistance, to establish the frequency and resistance levels in *Bemisia* spp. The non-specific esterases activity using tile with grooves technique for the two tested strains of *Bemisia* spp. showed that, the percent of resistant individuals (had high level of non-specific esterases activity) was higher compared with that of Abees one. This technique is useful in monitoring organophosphate resistance, acetylcholin-esterase activity was also determined by using tile with grooves technique. The results reflect that, El-Beheira field strain of *Bemisia* spp. had also higher percent of resistant individuals (had high level of acetylcholinesterase activity) compared with Abees one.

Complementary of bioassay and biochemical assay had important implementation in monitoring resistance phenomenon, and hence the postulation of resistance management recommendations.

INTRODUCTION

Whitefly, *Bemisia* spp. has become a serious pest of cotton and vegetable crops in Egypt. Simultaneity of several factors that may be responsible for increases in whitefly population include a viability of over wintering hosts, abundance of summer hosts, moderate winter temperature and rain fall, low population of natural enemies, and the development of resistance to insecticides as a result of varied selection pressure on cotton pests to which they are subjected (Prabhaker, *et al.*, 1985). Because of whitefly is an important vector of plant virus besides damaging plants directly when it present in significant numbers, various groups of insecticides are therefore widely applied for its control in glasshouse and outdoors. Extensive use of several classes of insecticides has occurred for insect control on cotton before and since 1981 build up *Bemisia* spp. Populations, and the possible development of resistance patterns in this insect. However, resistance to numbers of pesticides or pesticides groups has developed in whiteflies (Prabhaker, *et al.*, 1985,

Omer *et al.*, 1993, Williams, *et al.*, 1998). Development of resistance in *Bemisia* spp. highlighted the need for an effective resistance management strategy. Resistance monitoring techniques play an integral role in resistance management programs and if used before pesticide treatment can avoid ineffective applications (Brent 1986, Roush & Miller 1986, French-Constant & Roush 1990). The most commonly used resistance monitoring technique for whitefly is through a leaf dip assay. However, this technique is time consuming and need large numbers of insects in one assay. Dittrich and Ernst 1983, Dittrich *et al.*, 1985, Prabhaker *et al.*, 1988 used bioassay to monitor resistance in whitefly. However, in many insects a close association has been demonstrated between organophosphate resistance and esterases (Kasai & Ogita 1965, Needham & Sawicki 1971, Yasutomi 1971, Beranck 1974, Georghiou & Pasture 1978, Curtis & Pasture 1981, Dittrich *et al.*, 1990, Bloch & Wool 1994). A simple biochemical technique could be used for monitoring of resistance in such insects. Moustafa & Abd El-Rahman 1988 used three biochemical techniques to monitor resistance (especially to organophosphates) in cotton leafworm *Spodoptera littoralis*. However Georghiou & Saito 1983 used a simple biochemical technique to monitor resistance especially to carbamate. The objective of our work is to use bioassay and biochemical techniques to monitor for resistance in field populations of *Bemisia* spp.

MATERIALS & METHODS

Insects : Two field strains of whitefly *Bemisia* spp. were collected from cotton fields. Alexandria strain (Abees) was collected from Abees Station for Research at Alexandria Governorate. Another field strain El-Beheira was collected from El-Beheira governorate. Both strains collected as adult insects. Field populations of whitefly adults were collected by the aid of suction apparatus, early in the morning according to (Dittrich *et al.*, 1990). After collection, the insects were transferred to glass jars (one liter), then kept in ice box during transportation to the laboratory. Before bioassay tests, jars were taken out of the ice box and

inverted up side down on table, so that the healthy individuals would move to the top due to their negative geotactic and positive phototropic behavior (El-Helaly *et al.*, 1971). Weak and dead individuals were discarded.

Insecticides Organophosphorus: Chlorpyrifos [0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl) Phosphorothiate], as a formulation of 48% EC., Chlorpyrifos-methyl [0,0-dimethyl-0-(3,5,6-trichloro-2-pyridinyl)] phosphorothioate, as a formulation of 60% EC., Malathion. [0,0-dimethyl-0-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], as a formulation of 57% EC, Pirimiphos-methyl [0-2-diethylamino-6-methylpyrimidin-4-yl 0,0-dimethyl phosphorothioate], as a formulation of 50% EC, and Profenofos 0-(4-bromo-2-chlorophenyl)-0-ethyl-S-propyl-phosphorothioate, as a formulation of 72% EC, Carbamates: Methomyl [methyl N-((methylamino carbonyl) oxy) ethanimidothioate], as a formulation of 90% SP., and Thiodicarb [dimethyl N-N-[thiobis (methylimino carbonyloxy)] bis [ethanimidothioate], as a formulation of 80% EC Synthetic Pyrethroids; Cypermethrin RS)-(-cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, as a formulation of 25% EC, Deltamethrin [1R-[1 ((S*), 3)] cyano (3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-ethylcyclopropane) carboxylate, as a formulation of 2.5% EC and Fenvalerate: (RS)-(-cyano-3-phenoxy benzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate. as a formulation of 20% EC. All of the tested insecticides were local purchased.

Monitoring of resistance through bioassay:

The healthy insects were used for the bioassay according to (Prabhaker *et al.*, 1985). Castor bean leaves were washed, air dried, then cut into discs about (9 cm in diameter). Insecticide (formulated) solutions were prepared into tap water. A series of 6-8 concentrations for each insecticide were used, each concentration was replicated four times. Leaf discs were dipped in the insecticide solution for ten seconds, then air dried for 30 min. After drying the treated discs were confined to Petri-dishes (9 cm.) contained a thin layer of 2% agar to keep the discs wetted

and fresh for 24 hours or more. The insects were carefully immobilized with carbon dioxide, then placed on Petri-dishes contained the treated leaves with the aid of black and waxy paper. About 25-40 adults were used for each replicate. The exact number of healthy adults in each petri-dish was recorded within 10 min, when they recovered from anesthesia. The insects were maintained at 27°C, 70-85% RH and photo period 12 hours light during bioassay experiments. Mortality counts were recorded after 24 hours by the aid of dissecting microscope. A computer program for probit analysis (Finney 1971) was used to estimate the LC_{50} and slope values. Mortality were corrected in relation control using Abbott's formula (Abbott 1925).

Monitoring of resistance through biochemical techniques :

Qualitative determination of non-specific esterases activity (Tile with groove technique). This technique based on the qualitative biochemical determination of non-specific esterases activity. Five mg of *B*-naphthyl acetate was dissolved in 2 ml acetone, then 50 ml of 0.02 M phosphate buffer (pH 7.4) was added. The volume was completed to 100 ml immediately before the determination. An individual of *Bemisia* spp. adult was crushed in the tile groove in 10 µl distilled water. The tiles were kept in refrigerator at 4-5°C for 30 min. After that 10 µl of *B*-naphthyl acetate solution was added to each groove, then incubated for 15 min at 37°C. and 300 µl 0.4% fast blue B salt was dropped on each groove. Ten µl of 25% trichloroacetic acid were added to stop the reaction. A hundred to three hundred insects were assayed in each determination. The two field strains of *Bemisia* spp. were tested at the same time. Individuals whose reaction color is pink considered as resistant, while individuals, whose reaction color is not apparent, considered susceptible. This technique is used also to monitor for organophosphate resistance.

Qualitative determination of acetylcholinesterase activity was determined according to (Georghiou & Saito 1983). Acetyl thiocholine iodide (ATChI) 4×10^{-2} M) was used as a substrate. Dithiobisnitrobenzoic acid (DTNB) 2×10^{-2} M) in phosphate buffer 0.02 M (pH 7.0) was used as a reagent. An individual of *Bemisia* spp. adult was crushed in a tile groove

in 200 µl buffer, then 100 µl of the resultant insect homogenate was transferred to each groove, ten µl of DTNB and 10 µl of ATChI. Were added to each groove. The tiles were incubated for 30 min. at 37°C. After that 10 µl of eserine sulfate solution (10^{-3} M) was added to stop the reaction. A hundred to three hundred insects were assayed in each determination. The two field strains of *Bemisia* spp. were tested at the same time. Individuals whose reaction color is dark yellow considered as resistant, while individuals, whose reaction color is pale yellow considered as susceptible. This technique is used to monitor carbamate resistance.

RESULTS

Monitoring for resistance through bioassay results :

The toxicity data of four organophosphates, two carbamates and three pyrethroids insecticides against Abees and El-Beheira field strains of *Bemisia* spp. are presented in Tables (1 & 2). The LC_{50} with their confidence limits, slope and R/R values of Abees field strain are shown in Table (1). The data show that, deltamethrin was the most effective insecticide against this strain compared with the other tested insecticides, while fenvalerate was the least toxic one. The efficacy in descending order concerning the LC_{50} values of the tested insecticides was deltamethrin, chlorpyrifos-methyl, cypermethrin, profenofos, thiodicarb, pirimiphos-methyl, malathion and fenvalerate. The toxicity data for organophosphates revealed that, chlorpyrifos-methyl was the most toxic followed by profenofos, pirimiphos-methyl and malathion. For carbamate insecticides the range of toxicity was very narrow between thiodicarb and methomyl. While pyrethroids insecticides showed wide range of toxicity.

The LC_{50} s with their confidence limits at 95% level, slope, and R/R value of El-Beheira field strain are shown in Table (2). The obtained data show that, deltamethrin was the most toxic insecticide, while thiodicarb was the least toxic one. The other tested insecticides showed different toxicity levels. The efficacy in descending order concerning the

Table (1): Toxicological data of certain insecticides for Abees field strain of *Bemisia* spp.

Insecticides	LC ₅₀ mg / liter	Confidence limits at 95% level	Regression of N. S. D response (Y) on log dose (X)	R/R*
Chlorpyrifos-methyl	19.880	13.69 – 28.040	$Y = -0.87 + 0.67 X$	-
Malathion	171.83	104.1 – 320.80	$Y = -0.99 + 0.44 X$	16.85
Pirimiphos – methyl	125.89	90.49 – 134.32	$Y = -2.52 + 1.20 X$	5.860
Profenofos	34.010	23.25 – 41.790	$Y = -1.21 + 0.79 X$	4.790
Methomyl	103.20	73.21 – 140.38	$Y = -1.47 + 0.73 X$	3.610
Thiodicarb	100.00	67.69 – 147.91	$Y = -2.86 + 1.43 X$	-
Cypermethrin	32.290	20.96 – 51.980	$Y = -0.75 + 0.497 X$	2.340
Deltamethrin	2.5000	1.450 – 3.1900	$Y = -0.29 + 0.73 X$	0.480
Fenvalerate	346.48	239.3 – 551.10	$Y = -1.60 + 6.63 X$	-

$$R/R^* \text{ (Resistance Ratio)} = \frac{LC_{50} \text{ of Abees strain}}{LC_{50} \text{ of laboratory – susceptible strain}}$$

Table (2): Toxicological data of certain insecticides for El-Beheira field strain of *Bemisia* spp.

Insecticides	LC ₅₀ mg / liter	Confidence limits at 95% level	Regression of N. S. D response (Y) on log dose (X)	R/R*	R/R**
Chlorpyrifos-methyl	76.910	54.450 – 108.64	Y = 2.150 + 1.140 X	-	3.870
Malathion	334.34	263.40 – 441.65	Y = -3.130 + 1.240 X	32.48	1.950
Pirimiphos – methyl	662.33	505.90 – 867.80	Y = -3.660 + 1.298 X	30.81	5.260
Profenofos	320.69	248.73 – 412.61	Y = -2.679 + 1.069 X	45.17	9.430
Methomyl	837.52	824.19 – 850.69	Y = -55.00 + 18.83 X	29.28	8.120
Thiodicarb	1052.5	79.43 – 12589.3	Y = -1.360 + 0.450 X	-	10.53
Cypermethrin	11.170	5.0000 – 24.600	Y = -1.761 + 1.680 X	0.810	0.350
Deltamethrin	3.7500	2.7500 – 5.0100	Y = -1.360 + 2.370 X	0.720	1.500
Fenvalerate	265.40	220.09 – 315.38	Y = -4.220 + 1.744 X	-	0.770

$$R/R^* = \text{Resistance Ratio} = \frac{\text{LC}_{50} \text{ of El-Beheira field strain}}{\text{LC}_{50} \text{ of laboratory – susceptible strain}}$$

$$R/R^{**} = \text{Resistance Ratio} = \frac{\text{LC}_{50} \text{ of El-Beheira field strain}}{\text{LC}_{50} \text{ of Abees field strain}}$$

LC₅₀ values of the tested insecticides was deltamethrin, cypermethrin, chlorpyrifos-methyl, fenvalerate, profenofos, malathion, pirimiphos-methyl, methomyl, and thiodicarb. For organophosphate insecticides chlorpyrifos-methyl was significantly the most toxic insecticide. Malathion and profenofos had moderate toxicity, while pirimiphos-methyl was the least toxic organophosphate. The toxicity data of carbamate insecticides revealed that, methomyl was more effective than thiodicarb. The tested pyrethroid insecticides showed wide range of toxicity, however, deltamethrin was significantly the most toxic pyrethroid insecticide, while fenvalerate was significantly the least toxic one.

The susceptibility data of Abees and El-Beheira field strains of *Bemisia* spp. to the tested insecticides are shown in Tables 1 & 2) the results revealed that, El-Beheira field strain was more tolerant or resistant than Abees field one (as a reference) to all the tested organophosphate and carbamate insecticides. The highest level of resistance was obtained for thiodicarb followed by profenofos, methomyl, pirimiphos-methyl and chlorpyrifos-methyl. El-Beheira strain was about two times more tolerant than Abees to malathion. For pyrethroid insecticides El-Beheira strain showed slight susceptibility than Abees strain for both cypermethrin and fenvalerate, while the same strain was slightly changed toward tolerance for deltamethrin compared with Abees strain. Concerning a laboratory-susceptible strain as a reference one (Sherif 1994). Abees and El-Beheira field strains showed tolerance or resistance to the most tested insecticides except deltamethrin. For Abees field strain the highest level of resistance obtained for malathion followed by pirimiphos-methyl, profenofos, methomyl and cypermethrin. While, for El-Beheira field strain the highest resistance level was obtained for profenofos, followed by malathion, pirimiphos-methyl and methomyl. However, El-Beheira field strain was more susceptible to cypermethrin and deltamethrin. Generally, El-Beheira field strain was more tolerant or resistant to organophosphates and carbamates compared with Abees field strain or the laboratory-susceptible one.

Table (3): The qualitative activity of nonspecific esterases in Abees and El-Beheira field strains of *Bemisia* spp. (Tile with grooves technique)

Criteria Strain	Reaction group						% of individuals which had R Gene
	A (RR) resistant individuals		B (RS) heterozygous individuals		C (SS) susceptible individuals		
	Number	%	Number	%	Number	%	
Abees	0.0	0.0	100	33.3	200	66.6	33.33
El-Beheira	78	22	222	63.8	48.0	13.8	86.21

Table (4): The qualitative activity of acetylcholinesterase in Abees and El-Beheira field strains of *Bemisia* spp.

Criteria Field strain	Reaction group			
	A* Individuals had high AChE activity (dark yellow)		B** Individuals had low AChE activity (yellow)	
	Number	%	Number	%
Abees	248	68.84	112	31.16
El-Beheira	300	85.71	50.0	14.27

* Susceptible individuals (SS)

** Resistant individuals (RR + SS)

Monitoring for resistance through biochemical technique results :

Qualitative determination of non specific esterase activity data are shown in Tables 3 & 4 for Abees and El-Beheira field strains. This activity was classified into three categories (A, B, C) according to the degree of reaction taking part between enzyme system and substrates. The data showed that, the percent of individuals which had reaction group "A" (RR = resistant) was zero and 78 for Abees and El-Beheira strains, respectively. While the percent of individuals had reaction group "B" (RS = heterogeneous) was 33.33 and 63.8 for the respective strains. Moreover the percent of individuals had no reaction (SS = susceptible) was 66.67 and 13.8 for Abees and El-Beheira field strains, respectively. The percent of individuals which expected to had R gene responsible for resistance in El-Beheira strain was higher than that in Abees one 86.21 and 33.33, respectively. The data is in coincidence with the previous finding, that Abees and El-Beheira field strains were resistant to organophosphates. The obtained data for the two tested strains support the results of bioassay confirming the successful ability of using this technique not only for monitor resistance to organaophosphates but also for the pyrethroid resistance. The technique showed good discrimination between susceptible and field strains. The level of resistance developed was coincident with the increase in the individuals which had high level of esterases.

Qualitative determination of acetylcholinesterase data for Abees and El-Beheira field strains of *Bemisia* spp. are presented in Table (4). The obtained data showed that, the percentages of individuals which had high level of AChE was higher in El-Beheira strain than in Abees one, since, the percentage were 85.71 and 68.84, respectively. In other words El-Beheira field strain possessed more AChE compared with Abees one. Generally, the result reflects the possibility of using AChE activity through the qualitative determination technique as an indicator for monitoring carbamate resistance in the tested insects, since a relation between the R/R and level of AChE activity was observed.

DISCUSSION

The results revealed that, El-Beheira field strain of *Bemisia* spp. was more tolerant to the most tested organophosphate and carbamate insecticides than Abees one. The highest level of resistance was obtained for thiodicarb followed by profenofos, methomyl, pirimiphos-methyl and chlorpyrifos. Concerning a laboratory-susceptible strain as a reference strain, the results revealed that Abees and El-Beheira field strains of *Bemisia* spp. were tolerant or resistant to most of the tested insecticides except for deltamethrin. For Abees strain, the highest level of resistance was obtained for malathion followed by pirimiphos-methyl, profenofos, methomyl and cypermethrin, while for El-Beheira strain, the highest level of resistance was obtained for profenofos followed by malathion, pirimiphos-methyl and methomyl. These results are in agreement with that reported by Prabhaker *et al.*, (1985), (1988), Simmons & Dennehy (1990), Sanderson & Roush (1992), Cahill *et al.*, (1995), and Prabhaker *et al.*, (1998). Moreover, the field strains of *Bemisia* spp. showed low or no level of tolerance to synthetic pyrethroids. This finding is in agreement with that reported by Dittrich & Ernst (1983), Prabhaker *et al.*, (1985) and Horowitz *et al.*, (1988). In the meantime a high level of resistance was observed for malathion, that is in consistent with that reported by El-Hag & Horn (1983), Prabhaker *et al.*, (1988) and (1989).

Monitoring of resistance through biochemical techniques was achieved by using tile with grooves one. The data of non-specific esterases activity using tile technique for the two tested strains of which *Bemisia* spp showed that, El-Beheira field strain had high percent of resistant individuals compared with that for Abees field strain. The results confirmed the data of monitoring resistance through bioassay, in which El-Beheira field strain of *Bemisia* spp. was resistant to organophosphates. In the meantime these strains had high number of individuals with high activity of non-specific esterases. This finding is in agreement with that reported by Georgiou & Saito (1983) in which this technique could be used for monitoring resistance to organophosphates.

The results of acetylcholinesterase qualitative activity (using tile with grooves technique) showed that, El-Beheira field strain of *Bemisia* spp. had high percent of resistant individuals which had high level of AChE activity compared with Abees one. These results again confirmed the data of monitoring resistance through bioassay technique in which El-Beheira field strain so the tested insect had high level of resistance to carbamate insecticides. In the meantime these strains had high activity level of AChE. This finding is in agreement with that reported by Geourghiou & Saito (1983) in which this technique could be used for monitoring carbamate resistance.

It could be concluded that, there is no one monitoring technique that can cover all the needed informations about resistance. But it is necessary to complimentary between bioassay and biochemical techniques. This is useful for development and implementation of effective and accurate resistance monitoring system and potentially provides a novel means for pest control and management of resistance.

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الملخص العربى

رصد المقاومة فى سلالتين حقليتين من الذبابة البيضاء من خلال التقييم الحيوى والبيوكيميائى

فتحية إسماعيل مصطفى* ، منال أحمد عطيه** ، محمد سالم شعوير*
• كلية الزراعة - جامعة الأسكندرية ،
** المعمل المركزى للمبيدات - مركز البحوث الزراعية - الأسكندرية

أولاً : رصد المقاومة من خلال التقييم الحيوى

تم مقارنة حساسية سلالتين حقليتين من الذبابة البيضاء لمبيدات تمثل المجاميع الرئيسية (الفسفورية العضوية ، الكاربامات ، البيروثرويدات المخلفة) وذلك لدراسة تطور المقاومة فى السلالات الحقلية - وقد أجريت إختبارات الحساسية تبعاً لطريقة (Prabhaker et al., 1985)

أوضحت النتائج أن سلالة البحيرة كانت أكثر تحمل لمعظم المبيدات المختبرة من مجموعتى الفسفورية العضوية والكاربامات مقارنة بسلالة أبيس حيث كان أعلى مستوى من المقاومة تم تسجيله لمبيد الثيوديكارب (RR - ١٠٥٣) يليه البروفينوفوس ثم الميثوميل ثم البريميوفوس - ميثايل وأخيراً الكلوربيريفوس ، وباعتبار السلالة الحساسة العملية سلالة مرجعية نجد أن كل من سلالتى أبيس والبحيرة أظهرت تحملاً أو مقاومة لكل المبيدات المختبرة فيما عدا الدلتامثرين ، فبالنسبة لسلالة أبيس كان أعلى مستوى مقاومة تم تسجيله للملاثيون (RR - ١٦٨٥) يليه البريميوفوس - ميثايل ثم البروفينوفوس ثم الميثوميل وأخيراً السبيرميثرن ، أما بالنسبة لسلالة البحيرة فقد كان أعلى مستوى مقاومة تم تسجيله لمبيد البروفينوفوس (RR - ٤٥١٧) يليه الملاثيون ثم البريميوفوس - ميثايل وأخيراً الميثوميل . علاوة على ذلك أظهرت سلالتا الحقل (أبيس - البحيرة) من الذبابة البيضاء مستوى بسيط من التحمل أو لم تظهر تحمل للمبيدات المختبرة من مجموعة البيروثرويدات المخلفة وعموما كانت سلالة البحيرة أكثر تحمل أو مقاومة للمبيدات المختبرة من مجموعتى المبيدات الفسفورية العضوية والكاربامات مقارنة بسلالة أبيس أو السلالة الحساسة العملية .

ثانيا : رصد المقاومة من خلال التقييم البيوكيميائي

التقدير الوصفي لنشاط الأستيريز الغير متخصصة :

تم أيضا تقدير نشاط إنزيمات الأستيريز الغير متخصصة وصفا باستخدام طريقة Tile with grooves وقد كانت نتائج هذا الاختبار على السلالتين المختبرتين من الذبابة البيضاء تشير إلى أن نسبة الأفراد المقاومة (التي تحتوى على نشاط عالي لهذه الإنزيمات) كانت أعلى في سلالة البحيرة مقارنة بسلالة أبيس . هذه الطريقة السابقة من الممكن إستخدامها لرصد المقاومة للمبيدات الفوسفورية العضوية .

التقدير الوصفي لنشاط الأستاييل كولين أستيريز (إنزيم محلل أستر الأستاييل كولين)

تم أيضا تقدير نشاط إنزيم الإستاييل كولين إستيريز وصفا باستخدام طريقة Tile with grooves حيث أوضحت النتائج أن سلالة البحيرة من الذبابة البيضاء كان بها نسبة أعلى من الأفراد المقاومة (التي تحتوى على نشاط عالي من هذا الإنزيم) مقارنة بتلك النسبة في سلالة أبيس .

من النتائج السابقة فإن تكامل طرق التقييم الحيوى والطرق الكيميائية الحيوية يعتبر عنصر هام فى رصد ظاهرة المقاومة ، كما أن هذا التكامل من شأنه أن يزيد من تطور وإنجاح التوصيات الخاصة بالمكافحة أو إدارة المقاومة .