J.Pest Cont. & Environ. Sci. 8 (2) : 1 – 16 (2000).

Susceptibility of Indoor and Outdoor whiteflies to Certain Insecticides and Biochemical Characterization of their Acetylcholinesterase

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Recived 12/1/2000 , Accepted 18/3/2000.

ABSTRACT

Five insecticides were evaluated in the laboratory against the adult of two strains of whitefly, *Bemisia* spp collected from indoor and outdoor grown tomato plants. The toxicity data in terms of LC_{50} values showed that Actellic and Agrinate were the most toxic insecticides to indoor strain. Moreover, Decis, Salut and KZ oil showed moderate toxicity. In the meantime the previous insecticides were close to each other in their toxicity to the outdoor strain. The relative toxicity values reflected that Actellic had 1.01, 1.32, 1.43 and 1.44 fold as toxic as Agrinate, Decis, Salut and KZ oil, respectively against the indoor strain. While, the relative toxicity values revealed that Salut had 1.18, 1.21, 1.22 and 5.34 fold as toxic as Decis, Actellic, Agrinate and KZ oil, respectively, against the outdoor strain. In the meantime the data showed that the indoor strain was more tolerant to the all tested insecticides compared with the outdoor one.

The kinetic studies indicated that outdoor strain had lower Km and Vmax values of AChE compared with indoor strain. The values of Ki was 4.2 and 6.4 M for the AChE of indoor and outdoor strain using methomyl as inhibitor. Moreover, the Linewaver-Burk plots indicated that the type of inhibition of the enzyme from both strains was

competitive. These differences among the two strains of *Bemisia* spp were verified by electrophoretic analysis of proteins. SDS-polyacrylamide gel electrophoresis exibited that the outdoor strain possess two different bands at 104 KD and ~300 KD. However, the indoor strain had a single different band at 250 KD.

INTRODUCTION

In recent years whitefly has become an important pest of vegetables, cotton and ornamentals especially with the introduction of a new B-strain known as the silver leaf whiteflies, *Bemisia argentifolii*, (Bellows *et al* 1994). Silver leaf whitefly feeding can reduce yield directly due to its removal of plant sap. Moreover plants are commonly damaged by excretion of sticky honeydew, which supports the growth of black sooty mold. Feeding can additionally cause discoloration of certain crop plants, irregular ripening in tomato and silver leaf in squash. The whitefly also has the potential to cause serious losses through the transmission of viruses (Wisler *et al.* 1997).

Many conventional and biorational pesticides have been tested for control of whiteflies, but few give effective control (Tkachuk *et al.* 1986; Lopez and Rivera 1996). Moreover certain contact insecticide combinations especially pyrethroids plus organophosphates have provide excellent control in greenhouse and field studies as long as there was thorough coverage of the foliage (Horowitz *et al.* 1988). Other products with contact activity such as oils, soaps and K-salts of fatty acids can be very effective with thorough coverage. In the meantime resistance to soaps and oils is unlikely to ever develop, so these materials should be used as much as possible (Lindquist and Casey 1990; Butler and Hennbery 1991). Meanwhile, Hardee 1993; Horowitz and Ishaaya 1994; Patel and Patel 1997, mentioned that, neither chemical, biological nor cultural controls used alone have controlled these whiteflies, where it has become a predomenant pest in field crops. However, the integration of several contact tactics can be effective in reducing the overall impact of this pest and may lead to an acceptably low level of whitefly infestation.

Moreover judicious use of insecticides will also help to preserve populations of natural enemies of whiteflies and other pests. If economic losses occurred, alternating sprays of insecticides with different modes of action may help delay the development of resistance. Furthermore, there are lacks in the knowledge of biochemical characterization of the target enzymes for insecticides in this insect.

The aim of this work is to compare the susceptibility between the indoor and outdoor whiteflies to certain insecticide. The biochemical characterization of their acetylcholinesterase is also studied

MATERIALS AND METHODS

Insects:

The adults of whiteflies *Bemisia* spp were collected early in the morning from tomato plants which cultivated either indoors or outdoors at the King Fiasal Agriculture Research Station, Al-Hasa, KSA. The adult insects were transferred to the laboratory in icebox .

Insecticides:

The following insecticides were purchased locally and used for the bioassay test

Agrinate (methomyl), 90 SP., belongs to the oxime carbamate insecticides, **Decis** (deltamethrin), 2.5% EC. Belongs to synthetic pyrethroids, **Actellic** (pirimiphos-methyl) 50% EC. and **Salut** (dimethoate (222g/L)+ chlorpyrifos-ethyl (278g/L))50% EC belong to organophosphorus compounds and KZ oil.

Reagents:

All reagents for acetylcholinesterase and electrophoresis were analytical grade. Acetylthiocholine iodide (ASChI), used as substrate, 5,5' -dithiobis-(2-nitrobenzoic acid (DTNB), Sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co.(St Louis, MO, USA). Bovine serum albumin (BSA) was purchased from Bio-Rad.

Susceptibility test.

Laboratory trials against the adults of whitefly were conducted in glass vials (250cc). Tomato leaves were dipped for 15 sec in increased series of prepared formulated insecticide dilutions with distilled water then left to dry at the room temperature. After drying, the treated leaves were placed in the glass vials. To reduce the movement activity of whitefly, the adult were put in the refrigerator for 3 min, then exposed to the treated leaves in the vials (20-30 adults in each vial). The vials were covered with muslin by the aid of rubber band. Similar pieces of tomato leaves were dipped in distilled water and used as control. Every insecticide concentration was replicated three times. To asses the effect of insecticides, the whiteflies were examined by the aid of binocular microscope after 24 hrs from exposure and the mortality percentages were recorded. An insect was considered dead if it neither moved nor responded by reflex movement when touched by fine brush. The concentration required for killing 50% (LC_{50}) was calculated according to Finney 1971. The relative toxicity was calculated from the following equation.

Relative toxicity = LC_{50} of the low toxic insecticide / LC_{50} of the high toxic insecticide

The fold of tolerance was also calculated from the following equation.

Fold of tolerance=LC_{50} of the more tolerant strain / LC_{50} of the more susceptible strain

Determination of acetylcholinesterase (AChE).

Preparation of AChE.

The adults of whitefly were homogenized in 50mM Tris-HCl buffer pH 7.4 for 2 min by an electric homogenizer. The homogenate was centrifuged at 10,000 xg for 10 min at 4°C. The supernatant was used as enzyme source.

Enzyme assay and its inhibition by methomyl.

The colorimetric method of Ellman *et al* 1961 was used for assaying AChE activity using acetylthiocholine iodide as a substrate. Specific activity is expressed as ΔOD at λ_{412} nm/mg protein per min. The AChE activity was assayed in total volume 1.5 ml contains 200 g protein of each strain, 50 mM Tris-HCl buffer pH 7.4, 10 mM DTNB. The reaction was initiated by adding the ASChI ranged from 0.5 to 20mM followed by incubation at 37°C for 15 min. For the inhibition study 10 M of methomyl was incubated with the enzyme for 10 min at the room temperature before adding the substrate. The absorbance was measured colorimetrically at 412 nm. Lineveaver-Burk plot of ASChI versus AChE activity in the presence and absence of inhibitor was plotted to figure out Km, Vmax and Ki for both strains.

SDS-Gel electrophoresis

Electrophoresis was performed in 12% polyacrylamide gel as described by Laemmli (1970).

Determination of protein

Protein concentrations were determined according to Bradford (1976) using BSA as standard.

RESULTS AND DISCUSSION

Toxicity of the tested insecticides

Five insecticides with different modes of action were evaluated in the laboratory against *Bemisia* spp adults. The regression equation of normal equivalent deviate, Chi^2, LC_{50} and its 95% confidence limits of the tested insecticides against the indoor whitefly are presented in Table (1). The toxicity data in terms of LC_{50} showed that Actellic and Agrinate were the most toxic insecticides, whereas the LC_{50} values were 105.5 and 107.0 ppm after 24 hr of exposure to the residual film of tested insecticides. Moreover, Decis, Salut and KZ oil were moderately toxic, whereas the LC_{50} values were 139.0, 150.4 and 152.0 ppm, respectively.

The toxicity data of the previous insecticides against the outdoor *Bemisia spp* is shown in Table (2). The data showed that, all the tested insecticides were close to each other in their toxicity. The LC_{50} values were 29.7, 35.1, 35.9 and 36.3 for Salut, Decis, Actellic and Agrinate, respectively. In the meantime the toxicity of KZ oil (LC_{50}=158.5) was

Table (1).Toxicity of certain insecticides to indoor *Bemisia* spp adult after 24 hr of exposure.

Insecticide	LC_{50} (95% fid. Limits) (ppm)	Regression of N.E.D (Y) on log dose (x)	X^2
Actellic	105.5 (86.8 –128.1)	Y= -5.0 + 2.5x	13.4
Salut	150.4 (2.5 – 758.9)	Y= -3.0 + 1.4x	17.8
Decis	139.0 (21.3 – 1036.7)	Y= -1.4 + 0.7x	2.3
Agrinate	107.0 (24.0 – 468.9)	Y= -3.7 + 1.8x	2.0
KZ oil	152.0 (25.3 – 364.2)	Y= -2.4 + 1.1x	2.8

Table (2).Toxicity of certain insecticides to outdoor *Bemisia* spp adult after 24 hr of exposure.

Insecticide	LC_{50} (95% fid. Limits) ppm	Regression of N.E.D (Y) on log dose (x)	X^2
Actellic	35.9 (31.9 –40.3)	Y= -5.1 + 3.3x	10.9
Salut	29.7 (26.3 – 33.6)	Y= -5.4 + 3.7x	10.7
Decis	35.1 (4.2 – 294.8)	Y= -4.3 + 2.7x	6.8
Agrinate	36.3 (1.2 – 294.8)	Y= -1.4+ 0.9x	22.0
KZ oil	158.5 (1.3 – 369.7)	Y= -2.2 + 1.0x	22.4

less than that of organophosphate, carbamate and/ or synthetic pyrethroid insecticides. The results also reflect a poor variation between the two strains in their susceptibility to KZ oil. However, there was a quite clear variation in the susceptibility between both strains to the tested compounds. That might be due to the unique mode of action of oil which kill insect by suffocation mechanism and the insect can not resist or tolerate oil compared with the other insecticides.

Table (3).Comparative toxicity of certain insecticides to indoor and outdoor *Bemisia* spp adult at the LC_{50} levels.

Insecticide	Strain	Relative toxicity*	Fold of tolerance**
Actellic	Indoor	1.00	2.90
	Outdoor	1.21	
Salut	Indoor	1.43	5.10
	Outdoor	1.00	
Decis	Indoor	1.32	3.05
	Outdoor	1.18	
Agrinate	Indoor	1.01	2.95
	Outdoor	1.22	
KZ oil	Indoor	1.44	1.04
	Outdoor	5.34	

***Relative toxicity = LC_{50} of the low toxic insecticide/ LC_{50} of the high toxic insecticide**

Comparative toxicity

The comparative toxicity of the tested insecticides at the LC_{50} level is shown in Table (3). The relative toxicity values reflect that

Actellic had 1.01,1.32,1.43 and 1.44 fold as toxic as Agrinate, Decis , Salut and KZ oil, respectively against the indoor adults of *Bemisia spp*. The comparative toxicity of the tested insecticides against the outdoor *Bemisia spp* at the LC_{50} is also shown in Table (3). The relative toxicity values revealed that Salut had 1.18,1.21,1.22 and 5.34 fold as toxic as Decis, Actellic, Agrinate and KZ oil, respectively. A quite variation of relative toxicity was observed between the indoor and outdoor strain of *Bemisia* spp for the tested insecticides. The variation might be due to the effect of intrinsic or the extrinsic factors. The fold of tolerance is also represented in Table (3). The data reflect that the indoor *Bemisia* spp was tolerant to all the tested insecticides compared with the outdoor strain. The level of tolerance in the indoor strain might due to the extensive use of insecticides inside the greenhouses, besides the dispersal factors; emigration or the immigration (the movement out or into the population). This close system will help to build up the R gene inside the indoor population. In contrast, the movement of outdoor *Bemisia* spp will dilute the R genes, depending on the existence of refugia or the susceptible insects in the surrounding ecosystem. The lower frequencies of resistant phenotypes in some of the locations to certain insecticides may be caused by the presence of untreated fields around, which may provide an effective refugia, thereby conserving susceptible genotypes (Georghiou, 1980). However, many studies have shown that factors such as lack of refugia, heavy selection pressure, limited immigration, other ecological and genetic factors contribute to the rapid development of resistance (Tylor,, 1983, Georghiou and Tylor, 1986 and Prabhaker *et al*, 1996). In addition the annual multiple generations of whiteflies combined with the intensive insecticide use patterns will likely accelerate the development of insecticide resistance. In the meantime limited tolerance to certain insecticide may be closely associated with treatment history of this insecticide against other agricultural pests in the region. These results indicated that resistance to insecticides in *Bemisia* spp adults continues to be a significant problem and requires continous evaluation and management.

Biochemical characterization of AChE of indoor and outdoor Bemisia spp.

Acetylcholinesterase as a target enzyme for organophosphate and carbamate insecticides was determined in both strains as well as its inhibition by methomyl. The kinetic parameters of AChE inhibition (Km, Vmax and Ki) were estimated. The lineweaver-Burk plot for the indoor and outdoor whiteflies acetylcholinesterase are represented in (Table 4 and Fig. 1). Kinetic studies indicated that the activity of AChE in indoor strain was slightly different from the outdoor one. The outdoor strain presented slightly lower Km value (higher affinity) compared with indoor strain. These results suggest that there was a slight difference in AChE activity toward ASChI. The Vmax values in indoor strain was about 1.3 fold. The difference in the Vmax value suggest that the AChE in these two different strains were qualitatively different. The inhibition constant (Ki) was deduced from Linwaver-Burk plots, which indicated that the type of inhibition is competitive. The values of Ki were 4.2 and 6.4 M for indoor and outdoor AChE. These differences among the two strains of *Bemisia* spp, were verified by electrophoresis (PAGE).

Table(4). Kinetic constant estimates from Lineweaver-Burk plot for whiteflies AChE inhibition by (10 M) methomyl.

Strain	Km (mM)	Km app (mM)	Vmax (Od_{412}/mg protein .min.)	Vmax app (OD_{412}/mg protein .min.)	Ki ±SD M
Outdoor	2.00	3.85	0.101	0.101	6.4 ± 0.65
Indoor	2.63	4.16	0.133	0.133	4.2 ± 0.27

*Correlation coefficient

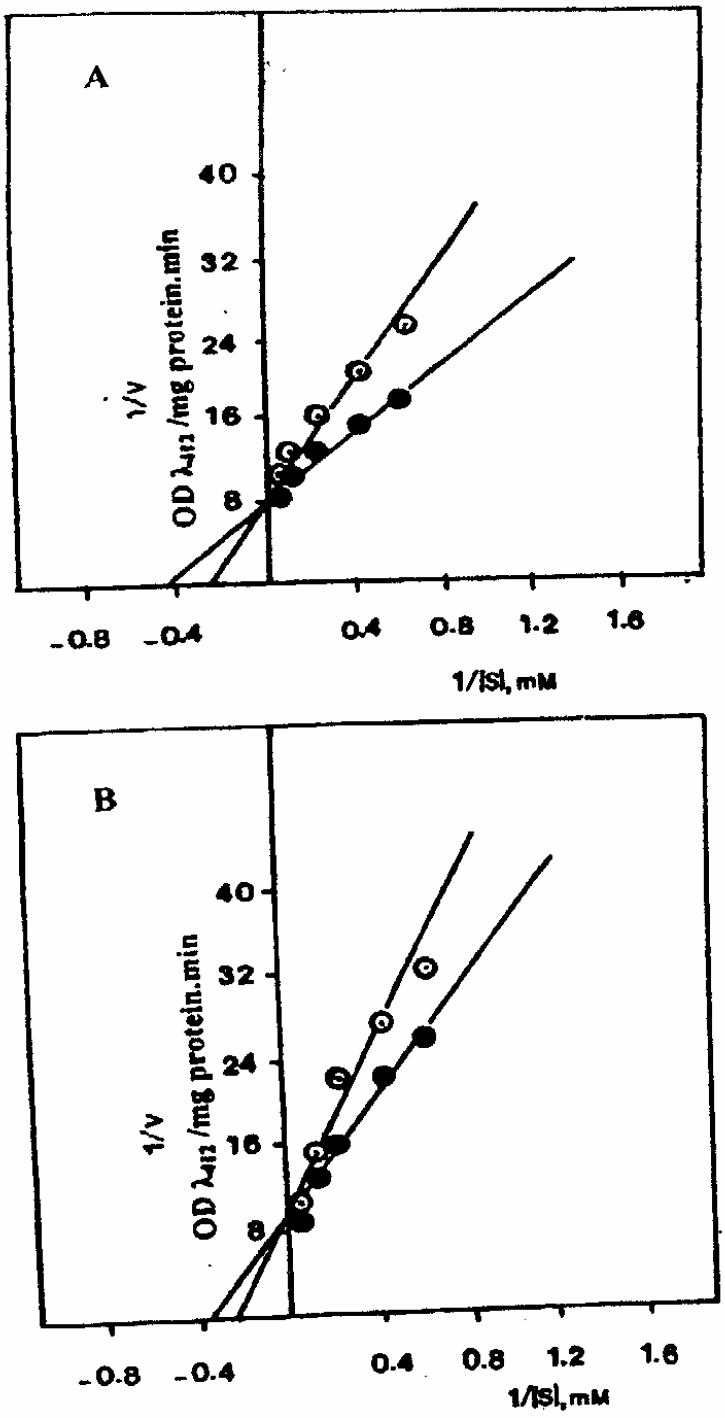

Fig (1) : Double reciprocal plots of I/V versus I/[S] for indoor (A) and outdoor (B) whitefly AChE in presence (o) and absence (•) of 10 µM mettomyl

Electrophoretic analysis of indoor and outdoor *Bemisia spp* proteins

SDS-gel electrophoresis of proteins isolated from indoor and outdoor *Bemisia* spp is shown in Fig (2). The SDS-polyacrylamide gel electrophoresis revealed that there were some differences in the pattern profile in both strains. In case of outdoor strain, two observed bands were

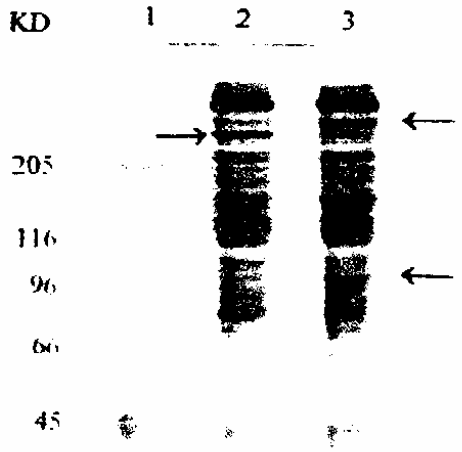

Fig. (2). SDS-polyacrylamide gel electrophoresis of indoor and outdoor whitefly protein. Lane (1) protein marker, lane (2) indoor and lane (3) outdoor.

found as shown in Lane 3, Fig (2). These two bands were estimated in the range of ~ 104 KD and 300 KD as indicated by arrow on the gel. However, a single protein band of indoor strain was observed in the range of ~250 KD (lane 2) but not found in the outdoor strain. In

addition to above, similar profile pattern of proteins for both strains was observed. The different bands of proteins from indoor and outdoor strains of *Bemisia* spp could interpret the susceptibility differences between the two strains. All these results supported that the marginal tolerance in indoor strain might be due to altered AChE or the insensitivity of AChE in the tolerance of *Bemisia* spp. The present results are consistent with numerous studies on OP resistance which suggested that increased esterase activity is a major resistance mechanism (El-Sebae *et al* 1973, Shawir *et al* 1991 and Zhu & Gao 1998), whereas reduced sensitivity of AChE also plays an important role in conferring overall OP resistance in many species (Moustafa *et al* 1983 , Abo-El-Saad *et al* 1998 and Siegfried and Ono, 1993). So, this study may be helpful in identifying the best approach for managing insecticide tolerance or resistance in whitefly populations.

Aknowledgement
The author would like to thank Dr. .M. M Abo El-Saad for his valuable help during this
work.

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الملخص العربي

حساسية الذباب الأبيض من البيوت المحمية والحقل المكشوف لبعض المبيدات والخواص البيوكيميائية لأنزيم الأسيتيل كولين استيريز لتلك السلالات

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قسم كيمياء المبيدات
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تم تقييم خمس مبيدات معملياً ضد الطور الكامل لسلالتين من الذباب الأبيض إحداهما جمعت من الحقل المكشوف والأخرى من البيوت المحمية. حيث أظهرت نتائج السمية من خلال مصطلح التركيز القاتل لـ ٥٠% من الحشرات أن مبيدي الأكتليك والأجرينيت كانا أكثر المبيدات سمية لسلالة البيوت المحمية بينما أظهر كل من الديسيز والسالوت والزيت المعدني سمية متوسطة. كما أظهرت النتائج تقارب سمية تلك المبيدات على سلالة الحقل المكشوف ما عدا الزيت المعدني والذي أظهر سمية أقل. ومن خلال مصطلح السمية النسبية كان مبيد الأكتليك حوالى ١,٠١، ١,٣٢، ١,٤٣، ١,٤٤ ضعف سمية كل من الأجرينيت و الديسيز والسالوت والزيت المعدني على الترتيب ضد سلالة البيوت المحمية. ومن ناحية أخرى كان مبيد السالوت حوالي ١,١٨، ١,٢١، ١,٢٢، ٥,٣٤ ضعف سمية كل من الديسيز والأكتليك والأجرينيت والزيت المعدني على الترتيب ضد سلالة الحقل المكشوف. كما أظهرت النتائج أن سلالة البيوت المحمية كانت أكثر تحملا للمبيدات السابقة من سلالة الحقل المكشوف. كما أشارت دراسة حركية إنزيم الأسيتايل كولين أستيريز في السلالتين إلي وجود اختلافات بين السلالتين حيث اختلفت قيم كل من السرعة القصوى لنشاط الأنزيم و قيمة ثابت ميكائيلس وثابت التثبيط. وكان التثبيط من النوع التنافسي عند استخدام تركيز ١٠ ميكرومولر من الميثوميل كمثبط. كما أكدت نتائج التحليل بالهجرة الكهربائية وجود تلك الاختلافات بين السلالتين حيث كان هناك اختلافات في وجود بعض حزم البروتين في سلالة الحقل المكشوف وغير موجودة في مثيلتها في البيوت المحمية.