

Toxic and biochemical impact of certain plant essential oils on *Bemisia tabaci* Genn. (Hom., Aleyrodidae)

El-Meniawi, Fatma A. and Ismail, Seham M. *

Department of Economic Entomology, Faculty of Agriculture, University of Alexandria.

* Central Laboratory of Pesticides, Sabahia, Alexandria, A.R.C.

ABSTRACT

Insecticidal effect of five plant essential oils namely: garlic, neem, ginger, peppermint and jojoba were determined against *Bemisia tabaci* female and male adults. The results showed that jojoba oil was the most potent against both sexes of the subject whitefly followed by peppermint and neem oil. On the contrary, ginger oil showed to have less toxic effect followed by garlic oil. Generally, all tested oils exhibited a superior toxic effect on males rather than females of *B. tabaci*. In addition, effects of LC_{50} of the tested oils on the *in vitro* inhibition of ATPase, chitinase and AChE were determined in comparison with three specific inhibitors pesticides (cyhalothrin, diuron and malathion). All essential oils showed very weak inhibitory effect (less than 50%) on the three tested enzymes. Effect of mixing plant essential oils with specific inhibitors on the *in vitro* inhibition of ATPase, chitinase and AChE was also investigated. Results proved that mixing of jojoba oil with cyhalothrin induced non-significant differences in the percentages of inhibition against ATPase. While, mixing of garlic oil with diuron and mixing of peppermint oil with malathion significantly increased the percentages of inhibition against chitinase and AChE, respectively. The results emphasized that I_{50} and K_i values decreased when oils mixed with pesticides. The highest reduction percentage of I_{50} was recorded for the mixture of peppermint oil with malathion, as the reduction were 38.00 and 37.14% for females and males followed by the mixture of garlic oil with diuron which caused 31.48 and 35.14% for females and males, respectively. While, the mixture of jojoba oil with cyhalothrin caused 12.50 and 6.25% reduction in I_{50} value for females and males, respectively. The same trend was also shown for K_i values. The present study suggest that the toxicity of the tested oils make them potential materials for use in a comprehensive integrated pest management program for the subject pest. In addition, results declared that the inhibitory effect of organophosphorus insecticide, malathion on AChE and phenylurea herbicide, diuron on

chitinase can be enhanced by mixing with peppermint and garlic oil, respectively.

INTRODUCTION

The whitefly, *Bemisia tabaci* Genn. causes great concern among agricultural producers throughout the world. This pest damages plants in several ways including direct damage from feeding individuals, production of massive quantities of honeydew upon which sootymold fungus can grow and transmission of geminiviruses (Costa *et al.*, 1991; Brown, 1992; Costa *et al.*, 1993). The combination of these effects has promoted this species to one of the most damaging pests in agricultural production.

B. tabaci is notable for its ability to develop resistance to chemical pesticides quickly (Costa and Brown, 1991; Cahill *et al.*, 1995, 1996). Therefore, it is extremely urgent and important that alternative control methods should be developed and unconventional materials, should be investigated. In recent years, there has been increased interest in natural plant-derived oils as alternative pesticides to conventional and broad-spectrum toxicants. The relative low cost of these oils compared to most insecticides and the low environment hazard make them attractive candidates for use in pest control strategy. Generally, previous studies about the effects of plant derived oils were focused on the repellency effect, feeding or oviposition deterrence, toxicity, sterility, and growth regulatory-activity (Shmutterer, 1990, 1997; Butler *et al.*, 1993; Flint *et al.* 1995; Thibout and Auger, 1997; Hiisaar *et al.*, 2000; Tripathi *et al.* 2000; Choi *et al.*, 2003).

The aim of the present research was to decrease the applied pesticides concentrations in order to reduce the cost of chemical control strategy and minimize hazards of human, animals and natural enemies of pests as well as reduce the environmental pollution. To achieve this aim, the present study was conducted to evaluate the potency of five plant essential oils namely: garlic, neem, ginger, peppermint and jojoba, on both sexes of *B. tabaci* adults. Besides, this study attempted to investigate the effect of LC₅₀ values of the tested plant oils on the activity of three important biological targets; ATPase, chitinase and acetylcholinesterase in comparison with the effect of three specific inhibitor pesticides. In addition, it was thought profitable to investigate the combined effects of mixture between plant oils and the chosen pesticides on the *in vitro* inhibition of the tested

three enzymes in order to check whether these oils enhance the inhibitory effect of the pesticides.

MATERIALS AND METHODS

1- The whitefly, *Bemisia tabaci* Genn. laboratory strain:

The subject insect has been taken from a susceptible laboratory culture of the cotton whitefly *B. tabaci* Gennadius, which has been reared on tomato plants since 1999 in greenhouses at $25\pm 7^\circ\text{C}$, 65 ± 5 RH and under natural light conditions in Economic Entomology Department, Faculty of Agriculture, Alexandria University.

2- A) ATPase preparation and activity assay:

ATPase was isolated from *B. tabaci* adults according to the method of Koch (1969). The obtained inorganic phosphate (Pi) was measured spectrophotometrically according to the method of Taussky and Shorr (1953) method. Absorbance was measured at 750nm. The specific activity of ATPase was measured as $\mu\text{mole Pi / mg protein per min}$.

2-B) Chitinase preparation and activity assay:

Chitinase was isolated from *B. tabaci* adults using the method of Deul *et al.* (1978). Chitinase activity was determined by the method of Reissig *et al.* (1955) which modified by Andrew *et al.* (1982). The optical densities were measured spectrophotometrically at 416nm.

2-C) AChE preparation and activity assay:

Separation and activity assay of AChE from *B. tabaci* adults was performed according to the method of Ellman *et al.* (1961). The rate of color production as function of enzyme activity is measured spectrophotometrically at 412nm. Specific activity was calculated as mg protein / hour . Protein content in prepared homogenates of *B. tabaci* adults was assayed spectrophotometrically by the method of Lowery *et al.* (1951) at 750nm using bovine serum albumin as a standard.

3- Optimal conditions of the tested enzymes:

According to the study of EL-Meniawi and Ismail, (2006) on the same *B. tabaci* strain; the activity of chitinase and AChE was determined at the optimal conditions of 37°C , 120 min, $1\mu\text{g}$ protein concentration and pH 4.5, while these parameters for AChE were at 35°C , 20 min, $2.2\mu\text{g}$ protein concentration and pH 8.0.

To determine the optimal conditions of ATPase activity, effect of temperature on ATPase activity was carried out from 25 to 45°C, the enzymatic reaction was carried out from 5 to 25 min, the effect of protein concentration was measured from 0.55 to 1.6 mg/ reaction. Effect of tris-HCl buffer pH was also tested in a range from pH 6.5 to 8.5. Effect of substrate concentration (ATP) on ATPase activity was investigated from 3 to 7 μ M. Besides ATPase kinetic parameters (K_m , V_{max}) were estimated from the Lineweaver-Burk regression lines (Lineweaver and Burk, 1934).

4) Tested plant essential oils:

The insecticidal activities of five plant essential oils were determined against *B. tabaci* male and female adults. The evaluated plant essential oils included:

- a- Garlic oil (*Allium sativum*, Fam.: Liliaceae), supplied by the Department of Pharmacology, Faculty of Pharmacy, Alex., University.
- b- Neem oil (pure oil 10%) (*Azadirachta indica*, Fam.: Miliaceae), Neemguard®, Gharda chemicals, Ahme, India.
- c- Ginger oil (*Zingiber officinale*, Fam.: Zingiberaceae). This oil was supplied by the Department of Pharmacology, Faculty of Pharmacy, Alex., University.
- d- Peppermint oil (*Mentha piperita*, Fam.: Labiatae), Egyptian natural Co., Egypt.
- e- Jojoba oil 96% E.C. (*Simmondsia chinensis*), Egyptian natural Co., Egypt.

Basic stock solution of each tested oil was made in distilled water containing 0.5% triton X-100 as an emulsifier. Series of at least five concentrations of each tested oils were prepared in distilled water.

5) Bioassay tests:

Leavs of uninfested tomato seedlings, 15-20 cm height, were dipped for 1 min in each concentration of the tested oils. Control plants were dipped in distilled water containing 0.5% triton X-100. Treated and control plants were air-dried for 3hrs. Twenty newly emerged adults of each segregated sex were introduced onto treated and control plants covered with glass cages with muslin in the upper opened and then kept at $25 \pm 2^\circ\text{C}$. All treatments and controls were replicated three times. Number of alive and dead adults per replicate was counted 24 and 72 hr. after treatment. Concentration-mortality regressions were statistically analyzed with SPSS computer program for Probit analysis of Finney (1971).

6) *In vitro* inhibition and kinetics of ATPase, chitinase and AChE:

The inhibition of ATPase, chitinase and AChE were determined in both male and female adults using the LC_{50} value of each of the tested plant essential oils. To check whether these tested oils could enhance the inhibitory effect of the specific inhibitor pesticides, the oil which produce higher inhibition of the enzymatic activity was mixed with specific inhibitors for each three tested enzymes. The pesticides which chosen as specific inhibitors were:- cyhalothrin ((S)- α -cyano-3-phenoxybenzyl) as a specific inhibitor for ATPase; diuron (3-(3,4-Dichlorophenyl)-1,1-dimethylurea) as a specific inhibitor for chitinase and malathion (Diethyl (dimethoxyphosphinothioylthio) succinate) as a specific inhibitor for AChE. Kinetic parameters (I_{50} & K_i) were determined at the optimal conditions of each enzyme. The method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting $1/V$ versus concentrations of the inhibitor at two concentrations of the substrate. Chitin (the substrate of chitinase) concentrations were 200, 500 μ g, the substrate of ATPase (ATP) concentrations were 3.0 and 5.0 mM. While acetylthiocholine iodide (substrate of AChE) was used at concentrations of 10^{-3} and 10^{-4} M. Estimation of I_{50} value was carried out by pre incubating the enzyme with the inhibitor for 30 min at the optimum conditions of ATPase, chitinase and AChE.

Data were statistically analysed by "F" test. All probable comparison combinations were achieved by Duncan's new multiple range test Snedecor and Cochran (1976) at 0.01 probability level.

RESULTS AND DISCUSSION

1-Optimal conditions for ATPase activity assay:

The optimal conditions for measuring total ATPase activity in whole body homogenates of *B. tabaci* adults are summarized in Fig.1. The optimal temperature and pH at which maximum enzymatic velocity occurs was at 37°C and pH7.4. The obtained results emphasize that ATPase activity was increasing with the increase of substrate concentration with a linear proportional way fitting with 1^{st} order reaction kinetic model (Fig. 1, D). The same trend was also observed for the optimal incubation time as it was at 15 min. It is also clear from Fig. (1, E) that the initial hydrolysis velocity is directly proportional to the enzyme concentration. The protein

concentrations maintain linear up to 1 mg protein enzyme in the incubation reaction mixture.

The affinity of ATPase in whole body homogenate to the substrate (ATP) and the maximum enzymatic velocity were determined by the Lineweaver-Burk plots illustrated in Fig. 1, F.

The results revealed that K_m value of ATPase from *B. tabaci* females increased to 1.5 folds than that of males. This may reflect a relative lower affinity of ATPase from females to the substrate and in return lower activity than those of ATPase from males. In other words, the observed reduction in K_m value of ATPase from males indicated that the enzyme acquired more affinity to ATP. The same trend was also found in V_{max} values as V_{max} of ATPase from females exceeded 1.7 folds that of males.

2-Insecticidal effect of the tested plant essential oils:

Toxic effect of the five tested plant essential oils against *B. tabaci* male and female adults was evaluated. On the light of LC_{50} values (Table 1), it is obviously noticed that jojoba oil was the most potent against both sexes of the subject whitefly followed by peppermint and then by neem oil. On the contrary, ginger oil showed to have less toxic effect against *B. tabaci* adults followed by garlic oil. Generally, all tested oils exhibited a superior toxic effect on males rather than females. This may be due to the differences in fat bodies and protein contents between females and males.

The present results are in agreement with Farrag and Zakzouk (1998) who reported highly toxic effect of jojoba oil on *B. tabaci* immature and adult stages. Also, EL-Bessomy (2003) found that jojoba oil at rate of 1L/100L water could be used instead of imidachloprid against the immature stages of *B. tabaci* on tomato plants. Monoterpenoids such as menthone in mint oil induce a variety of responses by insects. For example, several monoterpenoids are repellents for *Periplaneta americana* and *Blatta germanica* (Appel *et al.* 2001, 2004), affect growth and development (Karr and Coats, 1992) and acutely toxic to insects (Coats *et al.* 1991, Rice and Coats 1994; Appel *et al.* 2001). Prabhaker *et al.* (1989) recorded that neem was generally the least toxic when compared with conventional insecticides to all stages of *B. tabaci*. However, more effectiveness of *Azadirachta indica* have been recorded against aphid species (Pavela *et al.* 2002, Baspinar *et al.* 2002). The present results agreed with those of Liu and Stanley (1995) and Flint *et al.* (1995), which proved that the silver leaf

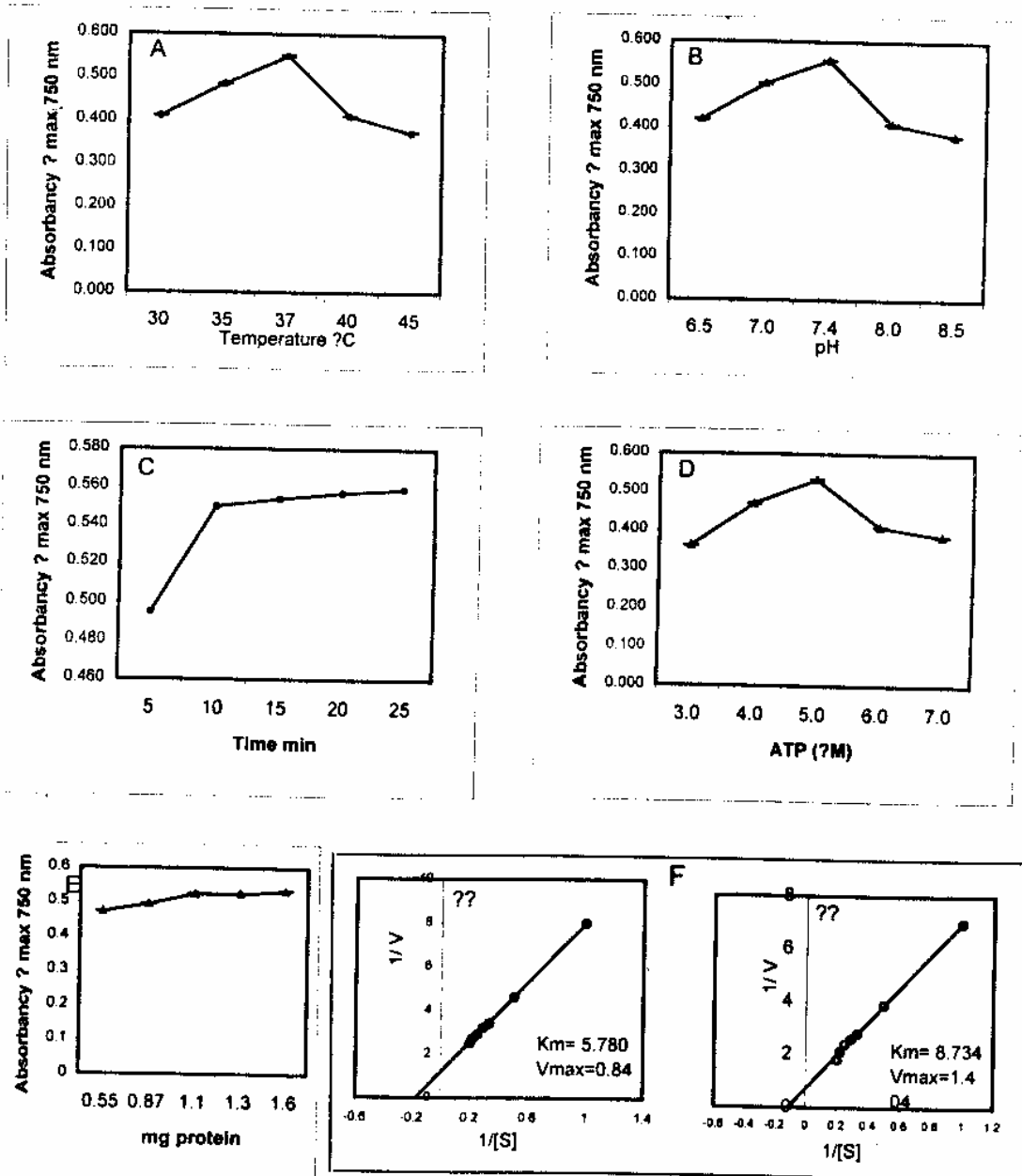


Fig. 1: Optimal conditions for *B. tabaci* ATPase activity assay as a function of incubation temperature (A), buffer pH (B), incubation time (C), ATP concs in μ M (D), protein concs (E) and Lineweaver-Burk plots of ATPase activity from *B. tabaci* male and female adults (F). Each point represents of at least three determinations.

Table (1): Toxicity parameters of some essential oils against *B. tabaci* female and male adults at 24 and 72 hr after treatment.

Tested oil		24hr after treatment					72hr after treatment				
		LC ₅₀ (p.p.m.)	95% C.L.*		Slope±S.E	Chi ²	LC ₅₀ (p.p.m.)	95% C.L.*		Slope ± S.E	Chi ²
			Lower	Upper				Lower	Upper		
Garlic	♀	537	291	958	1.132±0.113	11.669	537	291	958	2.006±0.195	11.344
	♂	410	222	666	1.101±0.113	9.143	410	222	666	1.215±0.120	8.861
Neem	♀	311	262	363	1.797±0.177	1.617	311	262	363	1.578±0.147	1.592
	♂	265	225	306	2.028±0.183	1.117	265	225	306	1.747±0.152	0.567
Ginger	♀	646	499	815	1.358±0.173	2.770	646	499	815	1.585±0.172	2.596
	♂	572	436	720	1.153±0.149	2.748	572	436	720	1.407±0.169	2.867
Peppermint	♀	265	109	443	2.221±0.188	16.288	265	109	443	1.718±0.157	4.612
	♂	237	105	378	2.342±0.193	14.872	237	105	378	1.610±0.188	0.936
Jojoba ₁	♀	153	124	185	1.430±0.125	2.528	153	124	185	1.269±0.188	0.271
	♂	139	111	168	1.438±0.145	2.082	139	111	168	1.315±0.192	0.204

* C.L.: Confidence limits.

whitefly *B. argentifolii* was not susceptible to the toxic properties of some garlic oils in adult and immature stages. Volatile compounds of garlic oil are thought to cause insect mortality but the exact modes of actions are unclear (Olkowski *et al.*, 1991).

3- *In vitro* inhibition of *B. tabaci* aATPase, chitinase and AChE:

Effects of the obtained LC₅₀ values of each of the five tested plant oils on the *In vitro* inhibition of the three tested enzymes from each sex of *B. tabaci* were investigated. The obtained data (Fig. 2) declared that jojoba oil exhibited the highest percentages of reduction on ATPase activity as these values were 46.7 and 39.0 % for male and females, respectively. On the other hand in case of chitinase, the most obvious reduction in its activity was recorded for garlic oil. The percentages of chitinase inhibition were 46.5 and 40.7% for males and females, respectively. In addition, peppermint caused the highest percentage of AChE inhibition as these values were 45 and 40.4% for male and females, respectively.

When comparing the reduction of enzymatic activity due to the tested oils with the inhibitory activity of the specific inhibitor pesticides (Fig. 2), it is obviously observed that plant essential oils had a very weak inhibitory effect on the three tested enzymes, as percentage of inhibition always decreased than 50%.

Present findings suggest that tested plant oils may prevent the binding of substrate with the active site of the enzyme or change the reaction media between the substrate and the enzyme. Such latter suggestions needs further investigations.

To check the combined effects of both plant essential oils and chosen pesticides on the enzymatic activity inhibition, the LC₅₀ of the oil, which produce higher reduction in the enzymatic activity, was mixed with the pesticides. Data represented in Fig. 3 summarize the inhibition percentages in ATPase, chitinase and AChE due to the combinations between oils and specific inhibitor pesticides. To declare these combined effects, Fig. (3) also demonstrates the percentages of enzymatic activity due to the *in vitro* treatment with oil or pesticides alone.

Generally, it is noticed that tested oils enhanced the inhibitory effect of the three pesticides. Concerning the ATPase, the results proved that mixing of jojoba oil with cyhalothrin insignificantly induce increase in the

inhibition percentages (Fig 3). The percentages of ATPase inhibition by cyhalothrin alone were 83.4 and 87.7% for *B. tabaci* females and males, respectively. While these percentages increased to be 86.2 and 89.4% when the LC₅₀ of jojoba oil mixed with cyhalothrin.

On the other hand, mixing of garlic oil with diuron and peppermint with malathion significantly increased the percentages of chitinase and AChE, respectively.

The present findings suggest that increase sensitivity of the tested enzymes to inhibition by mixture with plant essential oils might be associated with decrease catalytic activity to substrate. Unfortunately, no available references in the literature about the enhancing effect of plant derived oils on the inhibition potency of pesticides. However, particular consideration should be paid to the results of Coats *et al.* (1991), which reported that monoterpenoids such as menthone in mint oil are considered neurotoxic because of their speed of action and their effects on neurotransmitters.

4- Effect of mixing plant essential oils with pesticides on the inhibition kinetic parameters:

Table (2) summarizes the inhibition kinetic parameters (I_{50} and K_i) of each pesticide alone and the mixture between plant oil and pesticide against the three tested enzymes that isolated from *B. tabaci* male and female adults. The percentages of reduction in I_{50} and K_i values due to mixing oil with pesticide were calculated.

Generally, it was noticed that mixing of plant-oil with the pesticide clearly decreases values of I_{50} and K_i . The lowest recorded I_{50} values of the mixture between oil and pesticide reflect that the plant-oil may activate the pesticide to inhibit the enzyme. In other words, adding the plant-oil to the pesticide increased its inhibition potency. In case of *in vitro* inhibition of ATPase, the results proved that mixing jojoba oil with cyhalothrin decrease I_{50} value to 12.5 and 6.25% in both female and male adults, respectively. While, the mixture of garlic oil with diuron resulted in a 31.48 and 35.14% reduction in the inhibitory effect of diuron against female and male adults, respectively. Likewise, mixing peppermint oil with malathion caused 38.0 and 37.14% reduction in the inhibition of AChE from females and males, respectively.

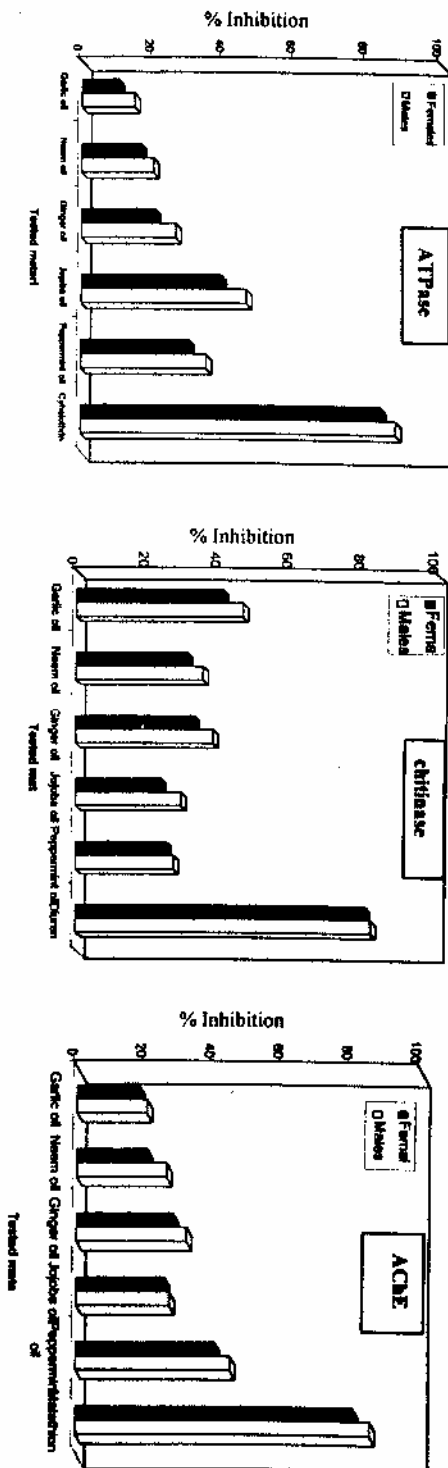


Fig. 2: *In vitro* inhibition of the three tested enzymes by the LC₅₀ value of each tested plant essential oils in comparison with pesticides chosen as specific inhibitors (cyhalothrin, diuron and malathion are inhibitors for ATPase, chitinase and AChE, respectively). (cyhalothrin concentration were 0.155, 0.088µM; diuron concentrations were 0.989, 0.368µM; malathion concentrations were 0.444, 1.88µM for females and males, respectively).

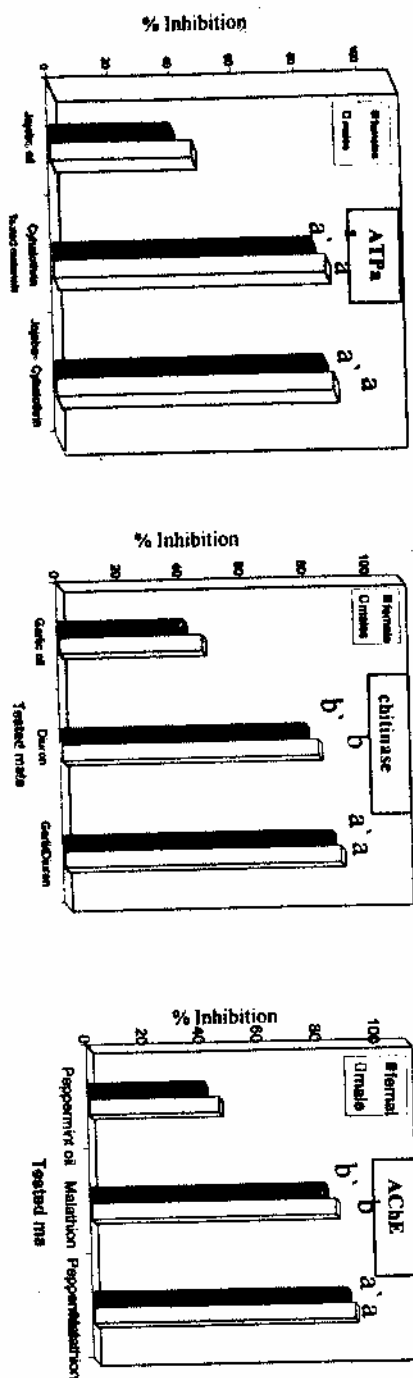


Fig. 3: *In vitro* inhibition of the three tested enzymes by the LC₅₀ of oil, pesticide and mixture of oil & pesticide. (cyhalothrin concentrations were 0.155, 0.088µM; diazinon concentrations were 0.989, 0.368µM; malathion concentrations were 0.444, 1.88µM for females and males, respectively). Columns followed by the same letter are not significantly different.

Table 2: Effect of mixing LC₅₀ of the plant essential oil with the pesticide on the I₅₀ and Ki of ATPase, chitinase and AChE from *B. tabaci* female and male adults.

Enzyme	Treatment	Females				Males			
		I ₅₀ (μ M)	% reduction	Ki (μ M)	% reduction	I ₅₀ (μ M)	% reduction	Ki (μ M)	% reduction
ATPase	Cyhalothrin	0.48		27.0		0.16		14.0	
	Cyhalothrin+ Jojoba oil	0.42	12.50	23.0	14.82	0.15	6.25	12.0	14.29
Chitinase	Diuron	0.54		53.0		0.37		38.0	
	Diuron+ Garlic oil	0.37	31.48	47.0	11.32	0.24	35.14	31.0	18.42
AChE	Malathion	0.50		45.0		0.35		30.0	
	Malathion+ Peppermint oil	0.31	38.00	34.0	24.44	0.22	37.14	27.0	10.00

Concentrations of each pesticide ranged from 0.1 to 100 μ M.

The present study suggest that the toxicity of the tested plant essential oils make them potential materials for use in a comprehensive integrated pest management program for the subject pest. In addition, results declared that the inhibitory effect of organophosphorous insecticide, malathion on AChE and phenylurea herbicide, diuron on chitinase can be enhanced by mixing with peppermint and garlic oil, respectively.

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السمية والفعل البيوكيماوى لبعض الزيوت النباتية الطيارة على ذبابة القطن الببيضاء *Bemisia tabaci*

فاطمة أحمد المنياوي و سهام منصور اسماعيل*

قسم علم الحشرات الإقتصادية، كلية الزراعة، جامعة الإسكندرية. *المعمل المركزي للمبيدات، الصباحية،
مركز البحوث الزراعية

تم دراسة التأثير الإبادى لخمسة من الزيوت النباتية الطيارة وهى: - الثوم، النيم، الزنجبيل، النعناع
الفلفلى والجوجوبا على إناث وذكور الحشرات الكاملة لذبابة القطن الببيضاء. أوضحت النتائج أن زيت
الجوجوبا كان أكثر الزيوت المختبرة سمية على الحشرة موضوع الدراسة، يليه تنازلياً زيت النعناع الفلفلى
ثم النيم. أما زيت الزنجبيل فقد أظهر أقل سمية على الحشرة يليه تصاعدياً زيت الثوم، وبصفة عامة
أظهرت ذكور الحشرة حساسية أعلى من الإناث بالنسبة لجميع الزيوت النباتية المختبرة. تم دراسة تأثير
التركيز القاتل لـ 50% من الأفراد (LC_{50}) لجميع الزيوت المختبرة على النشاط الإنزيمى لثلاثة إنزيمات
هامة حيويًا للحشرة وهى الـ $ATPase$, $chitinase$, $AChE$. وبصفة عامة فقد كانت النسب المنوية
لتثبيط الثلاث إنزيمات بواسطة الزيوت المختبرة أقل من 50%. تم دراسة تأثير خلط الزيوت النباتية التى
أعطت أعلى نسب للتثبيط الإنزيمى وذلك بقيم LC_{50} لها مع مثبطات متخصصة للإنزيمات موضوع
الدراسة. وعموماً أظهرت النتائج أن خلط زيت الجوجوبا مع مبيد الـ $cyhalothrin$ سبب زيادة غير
معنوية فى النسبة المنوية لتثبيط الإنزيمات موضوع الدراسة بينما وجد أن خلط زيت الثوم مع الـ
 $diuron$ و خلط زيت النعناع الفلفلى مع الـ $malathion$ نتج عنه زيادة معنوية فى النسبة المنوية لتثبيط
الإنزيمات موضوع الدراسة. كذلك تم دراسة تأثير خلط الزيوت النباتية بالمبيدات المختبرة على قيم الـ
 K_i ، I_{50} . ولقد أثبتت النتائج حدوث انخفاض فى تلك القيم نتيجة خلط الزيوت مع المبيدات وكانت أعلى
نسبة انخفاض للـ I_{50} عند خلط زيت النعناع الفلفلى مع الـ $malathion$ حيث كانت نسبة الانخفاض
38.37% لكل من إناث وذكور الحشرة على الترتيب يليها نسب الـ I_{50} عند خلط زيت الثوم مع الـ
 $diuron$ حيث كانت نسبة الانخفاض 31.48، 35.14% لكل من إناث وذكور الحشرة على الترتيب.
كذلك فقد أظهرت قيم الـ K_i نفس الاستجابة. تقترح الدراسة الحالية أن سمية الزيوت النباتية المختبرة
ضد الأطوار الكاملة لحشرة ذبابة القطن الببيضاء تجعل هذه المواد ملائمة لإضافتها إلى برامج لمكافحة
المتكاملة لهذه الآفة. بالإضافة إلى ذلك، فإن نتائج الدراسة الحالية توضح أن التأثير التثبيطى لمبيدات
الفوسفور العضوية على إنزيم الـ $AChE$ وكذلك لمركبات الفينيل يوريا على إنزيم الـ $chitinase$
يمكن تشجيعه بإضافة زيوت النعناع الفلفلى وزيت الثوم، على الترتيب.