The Interaction of some Plant Essential Oils on the Toxicity and Biochemical Effects of Phenothrin on *Spodoptera littoralis* Larvae.

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ABSTRACT

Toxicity effect of five plant essential oils : Jojoba, Neem, Garlic, Peppermint and Ginger were determined against *Spodoptera littoralis*. The results showed that Jojoba oil was the most potent followed by Neem , Garlic and Peppermint Ginger oil showed to have less toxic effect. The effect of LC_{50} of the tested oils on the *in vivo* inhibition of Na^+,K^+-ATPase from *Spodoptera littoralis* brain was assayed. The interaction of plant essential oils with Phenothrin on the *in vivo* inhibition of Na^+,K^+-ATPase was investigated. Results proved that pretreated of Jojoba oil with Phenothrin incressed the percentage inhibition which found that to be 96.7% and 92.6% for lab and field strain respectively, while the percentage inhibition found by pretreated the Neem oil with Phenothrin were 90.5% and 86.7% for lab and field strain *Spodoptera* respectively. In the other hand Garlic, Peppermint and Ginger showed very weak inhibitory effect (less than 50%) on the Na^+,K^+-ATPase activity. The results emphasized that I_{50} and K_i values decreased when Jojoba and Neem oil pretreated with Phenothrin, so there were significant differences among the chemical combinations, which caused more reduction effect than single treatment, and they affected enzyme activity by the same trend so results proved that Na^+,K^+-ATPase was sensitive to the Jojoba and Neem oil. Generally of essential oils pretreated with Phenothrin will produce a new trend so as reduce the field dose of the Pyrethroid insecticides, enhance the role of beneficial insects and reduce the cost of pest control.

INTRODUCTION

Conventional pesticides are used to control pests of both agriculture and public health, but their use have been not welcomed many decades ago because they destroy the beneficial organisms they have adverse effects on man and animal health, they are stable in the environment for long periods and pests develop resistance against these synthetic pesticides. These disadvantages directed us to search for alternative safe and less stable pesticides, with no or minimum hazard effects on man and his environment. Many sources for alternative biopesticides and natural pesticides were found however, the main source of natural pesticides was from plants among all natural sources volatile or essential oils are very promising and deserve more efforts to be introduced in the field of pest control. They have the requirements of ideal pesticides: they perform their effects in very short time and disappear from the environment quickly, with no residues to threat the components of the environment, they have broad spectrum of pesticidal effects. They are able to control many pests of different species and classes, they have no or minimum effect on man and non target organisms (EPA,1993), they have several modes of action on the target pest, since they contain many compounds with different chemical structures and different chemical groups, which prevents or postpones the development of pest resistance (Trombetta *et al*., 2005 & Salvelev *et al*., 2003). *S. littoralis* is notable for its ability to develop resistance to chemical pesticides quickly (Keddis *et al*. 1988 & Ishaaya and Klein 1990). Therefore naturally occurring insecticides have been used in pest control for centuries (Ebeling 1971 & Coats 1994). Many of these compoundes are secondary plant substances (Raven *et al*. 1992), including alkaloids, quinines, essential oils (such as terpenoids), glycosides, and flavonoids. Monoterpenoids such as d-limonene in citrus and l-menthol and menthone in mint add distinctive aromatic characteristics to plants. These compounds are often used in cosmetics, foods, and pharmacological additives where they provide flavors and fragrances. Monoterpenoids induce a variety of responses by insects. For example, several monoterpenoids (Inazuka 1982 and Appel *et al.* 2001) and cedar oils (Appel and Mack 1989) are repellent to American, *Periplaneta americana* (L.), and German, *Blattella germanica* (L.), cockroaches, affect insect growth and development (Karr and Coats 1992, & Hink and Fee 1986) or are acutely toxic to insects (Smith 1965, Coyne and Lott 1976, Coats *et al.* 1991, Rice and Coats 1994, & Appel *et al* 2001).

The aim of the present work is to evaluate the toxicity of some essential oils as a natural product alone and their pretreated with Phenothrin on *Spodoptera* larvae. Also the study was directed to throw the light on the effect of these chemicals on the activity of Na^+,K^+-ATPase.

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MATERIALS AND METHODS

Insects:

Laboratory strain of cotton leaf worm, *Spodoptera littoralis* was chosen for bioassays and biochemical assessments. This strain start as field strain reared under laboratory condition for several years in central lab. of Pesticides, Agricultural Research Center (ARC) Cairo , Egypt. Field strain of *Spodoptera littoralis* egg masses were collected from cotton fields at Abeis area. The 4th larval instar used for bioassay.

Tested Plant Essential Oils:

The insecticidal activities of five plant essential oils were determined against *S. littoralis.* The evaluated plant essential oils included:

a- Jojoba oil 96% E.C. (*Simmodsia chinensis*), Egyptian natural Co., Egypt..

b- Neem oil (pure oil 10%) (*Azadirachta indica*, Fam.: Miliaceae), Neemguard, Gharda chemicals, Ahme, India.

c- Garlic oil (*Allium sativum*, Fam.: Liliaceae), supplied by the Department of Pharmacology, Faculty of Pharmacy, Alex., University.

d- Peppermint oil (*Mentha piperita*, Fam.: Labiatae), Egyptian natural Co., Egypt.

e- Ginger oil (*Zingiber officinale*, Fam.: Zingiberaceae). This oil was supplied by the Department of Pharmacology, Faculty of Pharmacy, Alex., University.

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.

Chemicals:

Phenothrin (Pyrethroides) provided as technical grade insecticides from U.S.A .Environmental Protection Agency (EPA),USA. Ouabain is a cardiac glycoside which specifically inhibits the Na^+,K^+-ATPase (McIIwain, 1963). A pure sample was obtained from Sigma Chemical Co. ST. Loius.

Bioassay Tests:

Castor leaves disc (2 Cm^2) were dipped for 1min 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. Three replicates for each all treatments and control with ten larvae in each replicate. Number of alive and dead larvae per replicate was counted 24 and 48hr. after treatment. Concentration-mortality percentages were calculated and corrected for natural mortality according to Abbott equation (Abbott, 1925). LC_{50} values were calculated by using the probit- analysis method of Finney (1971).

Enzyme Preparation and Activity Assay :

Head capsoul from *S. littoralis* fourth-instar larvae was dissected and homogenized in a solution of 0.32M sucrose, 1mM EDTA and 40mM Tris buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. ATPase was prepared according to the method reported by Koch (1969), by differential centrifugation of the homogenate at 8000Xg for 10min. The supernatant was then centrifuged at 20000Xg for 30min. The formed pellets were then suspended in the buffer and stored at 20°C for use.

The ATPase activity measurements performed according to the method reported by Koch (1969), with slight modification by Morshedy (1980) using Tris-HCl buffer instead of imidiazol buffer. Absorbancy of Inorganic Phosphate (Pi) was measured at 750nm (Taussky and Shorr, 1953). The method was based on the spectrophotometric determination of the inorganic phosphate (Pi) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity wae measured in a total volume of 1ml. The homogenate preparation was mixed with a reaction mixture (700µl) containing 100mM Na^+, 20mM K^+, 5mM Mg^{2+} chlorides, 40mM Tris-HCl buffer(pH 7.4). and 5mM ATP. The volume was completed to 850µl with the buffer. The mixture was incubated for 15min. in a shaking water bath at 37 °C. The reaction was stopped by adding 150µl trichloroacetic acid (TCA, 30%). Hydrolyzed Pi was determined according to the method, described by Taussky and Shorr, (1953). The activity of Mg^{2+}-ATPase was measured after the addition of 1mM ouabain, a specific inhibitor for Na^+,K^+-ATPase (McIIwain, 1963), whereas the activity of Na^+,K^+-ATPase was calculated as the difference between the total ATPase and Mg^{2+}-ATPase activities.

Protein content in prepared homogenates of *S. littoralis* fourth-instar larvae was assayed spectrophotometrically by the method of Lowery *et al.(*1951), at 750nm using Bovine Serum Albumin (BSA) as a standard protein.

Inhibition of Na^+,K^+-ATPase Activity:

The inhibition of Na^+,K^+-ATPase was determined in head capsoul fourth-instar larvae of *S. littoralis* 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 inhibitor insecticide (Phenothrin), the oil which produce higher inhibition of the enzymatic activity was mixed with Phenothrin. 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. ATP (the substrate of ATPase) concentrations were 3.0 and 5.0mM. Estimation of I_{50} value was carried out by pre incubating the enzyme with the inhibitor for 30min.

RESULTS AND DISCUSSION

Toxicity of some Plant Essential Oils:

The results of the toxicity of the essential oils expressed in terms of LC_{50} are given in Table (1) for 4th instar larvae of *S. littoralis*. LC_{50} values after 24hr were 0.012, 0.015, 0.016, 0.017 and 0.019ppm for Jojoba, Neem, Garlic, Peppermint and Ginger oil respectively, against *Spodoptera* lab. strain. For field strain LC_{50} values were 0.17, 0.24, 0.51, 0.71 and 0.97ppm for these five oils respectively. While LC_{50} values, after 48hr were 0.006, 0.007, 0.012, 0.014 and 0.017ppm for these five oils respectively, against lab. strain. For field strain the LC_{50} were 0.085, 0.96, 0.38, 0.57 and 0.85ppm for the five oils respectively. Jojoba oil was the most potent against both lab. and field strain, followed by Neem and Garlic oil. Peppermint and Ginger oil were the least active, against *S. littoralis.* The tested oils exhibited more toxic effect on lab. strain than field strain. These results are in agreement with many investigators. El-Sayed (1982) proved that the Neem oil was the best of all evaluated plant oils tested against the cotton leafworm. Olkowski (1991) reported that horticulture oils are effective in controlling sawfly larvae and whiteflies. They flood insects breathing pores which lead to prompt asphyxiation and suffocation. Oils also kill an insect when it touches the outer body, or cuticle, of an insect leading to dehydration and death of the pest. Farrag and Zakzouk (1998) who reported highly toxic effect of Jojoba oil on *Bemisia tabaci* immature and adult stages. Bhargava and Meena (2002) found that Castor bean, Mustard, Groundnut, Sesame, Coconut and Sunflower oils caused significant mortality adults of pluse beetle, *Callosobruchus chinensis* (Linn.) on cowpea after three days of treatment. Mesbah *et al.*, (2004) reported that the plant oil-Neem caused 87% reduction while Sunflower oil gave the lowest reduction 52.5% against *S. littoralis.* Moustafa *et al*., (2006) reported that Jojoba oil high toxicity against *S. littoralis* 3rd instar.

Toxicity of Phenothrin Alone or Pretreated with the LC_{50} Values of Essential Oils Against *S. littoralis* Larvae:

The LC_{50} values of Phenothrin are 0.013 and 0.018 ppm against lab and field *Spodoptera* strains respectively (Table 2). The interaction of oils with Phenothrin against lab. and field strains of *Spodoptera* larvae were studied, larvae were allowed to fed on caster oil leave discs treated with LC_{50} of the different essential oils.

Table 1. LC_{50} Values of some Essential Oils to 4th Instar *S. littoralis* Larvae.

Compound	LC_{50}(ppm)			
	24hr		48hr	
	Lab	Field	Lab	Field
Jojoba oil	0.012	0.17	0.006	0.085
Neem oil	0.015	0.29	0.007	0.096
Garlic oil	0.016	0.51	0.012	0.38
Peppermint oil	0.017	0.71	0.014	0.57
Ginger oil	0.019	0.97	0.017	0.85

The LC_{50} values, of Phenothrin pretreated with the LC_{50} values of Jojoba, Neem, Garlic, Peppermint and Ginger on lab. and field strains of *Spodoptera* larvae are presented in (Table 2). The LC_{50} value of Phenothrin when pretreated with oils was lower than LC_{50} of Phenothrin alone in lab. or field *Spodoptera* strains. The enhancement of toxicity is calculated as a Potentiation Factor (P.F.) (Table 2). P.F. values for Jojoba, Neem, Garlic, Peppermint and Ginger oil are 2.6, 2.17, 1.3, 1.18 and 1.08 respectively after 24hr for lab strain, while the P.F. values of five essential oils are 2.57, 2.25, 1.5, 1.38 and 1.29 respectively after 24hr treatment, for field strain. This resultes are agreement with finding of Tripathy and Singh (2005) who reported that Cotton seed oil alone and Custard apple seed oil with Cypermethrin or Fenvalerate gave the highest larval mortality.

Table 2. Comparative Toxicities of Phenothrin Alone or Pretreated with some Essential Oils on *Spodoptera* Larvae.

Compounds	LC_{50} (ppm)			
	Lab. Strain		Field Strain	
Phenothrin	0.013	P.F.*	0.018	P.F.
Phenothrin + Jojoba	0.005	2.6	0.007	2.57
Phenothrin + Neem	0.006	2.17	0.008	2.25
Phenothrin + Garlic	0.010	1.3	0.012	1.5
Phenothrin+ Peppermint	0.011	1.18	0.013	1.38
Phenothrin + Ginger	0.012	1.08	0.04	1.29

*Potentiation Factor (P.F.) = LC_{50} Insecticide Alone / LC_{50} Insecticide + Oil

In Vivo Inhibition of Brain *S. littoralis* Na^+,K^+-ATPase Activity:

The *in vivo* inhibitory effect of the LC_{50} values of five essential oils against to the *Spodoptera* 4th instar lab. and field strain larval Na^+,K^+-ATPase is shown in the data given in Table (3). The data declared that Jojoba and Neem oil exhibited the highest percentages of reduction of Na^+,K^+-ATPase activity as values were 64.8% and 56.5% respectively for lab. strain, while values were 58.5% and 53.6% respectively, for field strain. On the other hand, Garlic, Peppermint and Ginger oil not active as inhibitor on Na^+,K^+-ATPase, activity.

Data in Table (3) summarize the interaction of Jojoba and Neem oil on the inhibitory effect of Phenothrin on the activity of Na^+,K^+-ATPase. The resultes proved that pretreated of Jojoba and Neem oil with Phenothrin induce increase the inhibition of enzyme activity. The inhibition of Na^+,K^+-ATPase by Phenothrin alone were 85.2% and 80.2% for lab. and field strain respectively. While the inhibition increased to be 96.7% and 92.6% for lab. and field strain respectively when Jojoba oil pretreated with Phenothrin. Morever the inhibition of enzyme activity increased to be 90.5% and 86.7 % for lab. and field strain respectively when Neem oil pretreated with Phenothrin. This results aggrement with Coats *et al.*, (1991) who reported that monoterpenoids such as menthone in Mint oil are considered neurotoxic because of their speed of action and their effects on neurotransmitters.

Table 3. *In Vivo* Inhibition of *Spodoptera* Larvae 4th Instar Na^+,K^+-ATPase Activity by some Compounds (LC_{50}).

Compounds	% Inhibition	
	Lab. Strain	Field Strain
Phenothrin	85.2	80.1
Jojoba oil	64.2	58.5
Neem oil	56.5	53.6
Garlic oil	18.1	13.4
Peppermint oil	14.2	11.4
Ginger oil	12.1	10.3
Phenothrin+ Jojoba oil	96.7	92.6
Phenothrin+ Neem oil	90.5	86.7

The *In Vitro* Inhibition of Brain *S. littoralis* Na^+,K^+-ATPase Activity:

Table (4) show the *in vitro* interaction of Phenothrin and the two essential oils on Na^+,K^+-ATPase activity of *S. littoralis* 4th instar brain. The I_{50} values of Phenothrin for lab. and field strain larval brain Na^+,K^+-ATPase are 0.60 and 0.70µM respectively. We have shown that the efficacy of Jojoba and Neem oil has a very good additive toxicity for Phenothrin in lab. and field *Spodoptera* strain (Table 2), because for the enhancement toxicity of the Jojoba and Neem, we study the *in vitro* biochemical interaction of them with the Na^+,K^+-ATPase activity and compare with the Phenothrin *in vitro* effects. The I_{50} values for Jojoba were 0.77 and 0.85µM against both lab. and field strain enzyme, respectively. While The I_{50} values for Neem were 0.86 and 0.93µM against lab. and field strain enzyme. It is quite clear that Phenothrin at I_{50} concentration acts as potential inhibitors for *Spodoptera* larvae Na^+,K^+-ATPase activity. These results are in agreement with many investigators. Desaiah *et al.*, (1975), Saleh *et al.*, (1984) and Korkor *et al.*, (1995) reported that synthetic Pyrethroids were the most effective insecticides against Na^+,K^+-ATPase of Cockroaches, Fish and Bollworms.

To characterize more details about the *in vitro* inhibition of Na^+,K^+-ATPase by the inhibitor, the I_{50} and K_i values of each inhibitor were estimated from the graphical method of Dixon and Weeb, 1964 (Table 4). The obtained data proved that compounds competitive inhibition of Na^+,K^+-ATPase activity and the K_i values were 26, 56 and 66µM for Phenothrin, Jojoba and Neem, respectively.

In conclusion, Jojoba and Neem oil were proved to inhibit the Na^+,K^+-ATPase activity thus, causing a decrease in the unidirectional transport of Na^+ and K^+ through cell membranes.

Essential oils of many medicinal plants possess compounds with pesticidal properties as well as antioxidant activity and therefore can be used as natural pesticides and preservative ingredients in food and/or pharmaceutical industry, some oils contain individual active compounds up to or more than 80% of its composition; however, most of essential oils compose of a blend of compounds which are chemically different and have different mode of action. They are safe, degradable and do not accumulate in the food chain. These properties render essential oils-based pesticides as candidates to be used in IPM programs for such deleterious subject pest.

Table 4. *In Vitro* Inhibition of Brain *Spodoptera* Larvae Na^+,K^+-ATPase Activity by Certain Compounds.

Compounds	I_{50} (µM)		Ki (µM)
	Lab. Strain	Field Strain	Lab. Strain
Phenothrin	0.60	0.70	26
Jojoba oil	0.77	0.85	56
Neem oil	0.86	0.93	66

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الملخص العربي

تداخل بعض الزيوت النباتية على سمية الفعل البيوكيماوى لمبيد الفينوثرين على دودة ورق القطن

سهام منصور اسماعيل و محمود مرشدى

الهدف من البحث هو تقييم التأثير الأبادى لخمسة من الزيوت الطبيعية وهى الجوجوبا والنيم و الثوم و النعناع الفلفلى و الزنجبيل، على يرقات العمر الرابع لدودة ورق القطن للسلالة المعملية والحقلية بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة. أوضحت النتائج أن أكثر الزيوت تأثيرا الجوجوبا والنيم والثوم وزيت النعناع الفلفلى أما زيت الزنجبيل فقد أظهر أقل سمية على الحشرة وبصفة عامة أظهرت السلالة المعملية حساسية أعلى من السلالة الحقلية بالنسبة لجميع الزيوت النباتية المختبرة. أيضا تم دراسة تأثير المعاملة بتلك الزيوت مع تركيزات منخفضة من المبيد المختبر الفينوثرين وقد زادت الفعالية نتيجة هذه المعاملة زيادة ملحوظة خاصة فى حالة الجوجوبا والنيم ويتضح ذلك من قيم معامل التنشيط (P.F.) التى تم حسابها وكذلك تم دراسة المقدرة التثبيطية للزيوت المختبرة على النشاط الأنزيمى لأنزيم هام وحيوى بالنسبة للحشرة و هو Na^+,K^+-ATPase ولقد أوضحت النتائج أن فى حالة الجوجوبا بعد المعاملة بالفينوثرين كانت النسبة المئوية للتثبيط هى 96.7% و92.6% للسلالة المعملية و الحقلية على الترتيب، بينما فى حالة النيم بعد المعاملة بالفينوثرين كانت النسبة المئوية للتثبيط هى90.5 % و86.7 % لكل من السلالة المعملية و الحقلية على التوالى. ولقد كانت النسبة المئوية للتثبيط بواسطة الثوم و النعناع الفلفلى و الزنجبيل أقل من50 %. وكذلك تم دراسة تأثير الزيوت النباتية المختبرة بعد المعاملة مع المبيد المختبر الفينوثرين على قيم I_{50} ولقد أثبتت النتائج حدوث أنخفاض فى تلك القيم وكانت أعلى نسبة للأنخفاض عند معاملة زيت الجوجوبا و زيت النيم مع الفينوثرين على الترتيب وقد وجد أن هذه المركبات أظهرت تثبيط تنافسى على نشاط أنزيم Na^+,K^+-ATPase ومن هذه النتائج أتضح أن يمكن أستخدام هذه الزيوت كمبيدات طبيعية بديلة وأكثر أمانا للأنسان والبيئة وخاصة مع المبيدات البيرثرويدية وبذلك يمكن الأستعانة بها فى برامج المكافحة.