Effect of Some Mineral Oils Alone and Their Mixtures with Cyhalothrin on The *In Vitro* Activity of Adenosine Triphosphatase Enzyme (ATPase) Extracted from *Spodoptera Littoralis* Larvae Brain

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ABSTRACT

The toxicity effects of three Mineral oils (Folic; Kemesol and National oil) alone and pretreated with Cyhalothrin (Pyrethroid) insecticide on the fourth larval instars of laboratory and field strains of *Spodoptera littoralis* have been evaluated. The results showed that Folic oil was the most potent against both two strains followed by Kemesol and National oil showed to have less toxic effect. The effect of LC_{50} of the tested oils and the interaction of Mineral oils with Cyhalothrin on the *in vivo* inhibition of ATPase from *Spodoptera littoralis* brain was investigated. Results proved that pretreated of Folic oil with Cyhalothrin incressed the percentage inhibition which found that to be 95.8 and 90.7% for lab and field strains respectively, while the percentage inhibition found by pretreated the Kemesol oil with Cyhalothrin were 90.5 and 88.6% for lab and field strains *Spodoptera* respectively, in the other hand the percentage inhibition found by pretreated the National oil with Cyhalothrin were 76.2 and 71.3% for lab and field strains *Spodoptera* respectively. The results emphasized that I_{50} and K_i values decreased when Folic and Kemesol oil pretreated with Cyhalothrin, so there were significant differences among the chemical combinations, which caused more reduction effect than signal treatment, and they affected enzyme activity by the same trend so results proved that ATPase was sensitive to the Folic and Kemesol oil. The present study open gate that physical toxicant can play important role to increase pesticide toxicity. Generally, Mineral oils pretreated with Cyhalothrin produce a new trend will lead to reduce the field dose of the Pyrethroid insecticides, enhance the role of beneficial insects and reduce the cost of pest control.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* is considered a very important pest in Egypt, which cause serios damage and losses in field crops. Several studies were carried out on chemical control (pesticide) of this insect and development of resistance was illustrated by Keddis *et al*., (1988); Ishaaya and Klein (1990); Martin *et al*., (2000) & El-Aw *et al*., (2002). In the other target to the extensive on target organisms uses of chemical toxicants for pest caused many problems, such as acute and chronic human and animal toxicity, development of insect resistance to chemicals and environmental pollution. So we need alternative effective and environmentally safe insecticides such as mineral oils (MARL, 1997). Local sprays of mineral oils are used for years against scale insects, mealybugs, thrips, aphids and mites on different crops and fruit trees, (Moursi, 1996 & El-Deeb *et al*., 2002). Oil sprays are used most commonly in horticulture to control scale insects and mites (Chapman *et al*., 1952: Micks and Berlin 1970 & El-Sebae *et al*., 1976) stated that resistance was not recorded for mineral oils which still have the advantage of being effective to resistant strains.

Therefore, the present work in an attempt to implement a new promising approach to suppress populations of this insect by using three Mineral oils (Folic; Kemesol and National oil) to evaluate alone and their pretreated Cyhalothrin (Pyrethroid) on *Spodoptera* larvae to decrease the environmental pollution and decrease development of resistance, due to the continous of chemical application of pesticides in the field. The study was directed to throw the effect of these chemicals on the activity of ATPase.

MATERIALS AND METHODS

Insect:

Laboratory strain of cotton leafworm, *Spodoptera littoralis* was chosen for bioassays and biochemical assessments. This strain start as field strain reared under laboratory conditions for several years in central lab. of pesticides, Agricultural Research Centre (ARC) Cairo, Egypt. Field strain of *Spodoptera littoralis* egg masses were collected from cotton fields at Abeis area in Alexandria Governorate, eggs masses let to be hatching in laboratory conditions of 27±2^{o}C and 65-70%RH. The 4th larval instar used in study.

Chemical:

Cyhalothrin (Pyrethroid) provided as technical grade insecticides from U.S.A. Environmental Protection Agency (EPA), USA. Mineral oils were used in this study namely: Folic oil 82% E.C was obtained from Kafer El-Ziat Pesticides and Chemicals Co. Kemesol oil 95% E.C and National oil 95% E.C were obtained from KEMEX Co. as commercial product from pesticide stores.

Bioassay tested:

Castor leaves disc (2cm^{2}) were dipped for 1min in each concentration of the tested Mineral oils. Control

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plants were dipped in distilled water. 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 4mM tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial 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 (-4^{o}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 was 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+} chloride, 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^{o}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).

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 ATPase activity:

The inhibition of ATPase was determined in head capsoul fourth-instar larvae of *S. littoralis* using the LC_{50} value of each of the tested Mineral oils. To check whether these tested oils could enhance the inhibitory effect of the inhibitor insecticide (Cyhalothrin), the oil which produce the inhibition of the enzymatic activity was mixed with Cyhalothrin. 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 different concentrations of inhibitor for 30min.

RESULTS AND DISCUSSION

Toxicity of some Mineral oils:

The results of the toxicity of the Mineral 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 8.2; 12 and 15.1ppm for Folic; Kemesol and National oil respectively, against *Spodoptera* lab strain. For field strain LC_{50} values were 10.3; 14.4 and 17.1ppm for these three oils respectively. While LC_{50} values, after 48hr were 3.2; 4.8 and 7.9ppm for these three oils respectively, against lab strain. For field strain LC_{50} values were 5.2; 7.6 and 9.8ppm for these three oils respectively. The LC_{50} values after 72hr for lab strain were 1.6; 3.3 and 4.2ppm for these three oils respectively, against *Spodoptera,* while the LC_{50} values for field strain were 3.3; 5.1 and 7.4ppm for these three oils respectively. Folic oil was the most potent against both lab and field strains, followed by Kemesol oil while National oil was the least active, against *S. littoralis*. The tested oils exhibited more toxic effect on lab strain than field strains. These results are in agreement with many investigators. El-Attal *et al*., (1983) & Moustafa and El-Attal (1985) reported that Mineral oil more toxic against cotton leafworm. El-Halawany *et al*., (1987) who reported Mineral oil used successfully on citrus trees against both of tested insects. Amer *et al*., (2001) found that Kz oil more toxic against *Tetranychus urticae*. Also Hazzard *et al*., (2003) reported the Mineral oil alone provided better control of *Spodoptera fumgiperda* (Lepidoptera: Noctuidae) compared with corn oil and *B. thuringiensis*. Mohmoud *et al*., (2006) reported the Mineral oils were the most efficient compound for controlling *Russellaspis pustulans* on apple trees.

Table 1. LC_{50} values of some Mineral oils to 4th instar *S. littoralis* Larvae

Time (hr)	LC_{50} (ppm)					
	Folic oil		Kemesol oil		National oil	
	Lab strain	Field strain	Lab strain	Field strain	Lab strain	Field strain
24	8.2	10.3	12	14.4	15.1	17.1
48	3.2	5.2	4.8	7.6	7.9	9.8
72	1.6	3.3	3.3	5.1	4.2	7.4

Toxicity of Cyhalothrin alone or pretreated with the LC_{50} values of Mineral oils against *S. littoralis* larvae:

The LC_{50} values of Cyhalothrin after 24hr were 0.013 and 0.019ppm against *Spodoptera* lab and field strains respectively, the LC_{50} values after 48hr were 0.011 and 0.016ppm respectively, against *Spodoptera* lab and feld strains respectively,Table (2). The interaction of oils with Cyhalothrin against lab and field strains of *Spodoptera* larvae were studied, larvae were allowed to fed on castor oil leave discs treated with LC_{50} of the different Mineral oils.

The LC_{50} values of Cyhalothrin pretreated with the LC_{50} values of Folic; Kemesol and National oil on lab and field strains of *Spodoptera* larvae are presented in Table (2). The LC_{50} value of Cyhalothrin when pretreated with oils was lower than LC_{50} of Cyhalothrin 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 Folic; Kemesol and National oil are 3.25; 2.16 and 1.08 respectively after 24hr treatment, for lab strain, while the P.f. values of three Mineral oils are 2.71; 2.11 and 1.26 respectively after 24hr treatment, for field strain. In the other hand the P.f. values of three tested Mineral oils are 5.50; 3.66 and 1.10 respectively after 48hr treatment, for lab strain. Also the P.f. values of three Mineral oils are 3.20; 2.28 and 1.23 respectively after 48hr treatment, for field strain. This resultes are agreement with finding of Rodriguez *et al*., (1966) showed that Mineral oil plus insecticide under studied were effective against the coffee leaf-miner. Hopkins *et al*., (1982) reported that diflubenzuron combined with Mineral oil significantly reduced the number of the adult weevil *Anthonomus grandis* Boheman. Moustafa and El-Attal (1985) were found to increase the potency of insecticide against the cotton leafworm and thrips. Omar *et al*., (1987) showed that Mineral oils improved the efficiency of the insecticide used against both of tested insects. Abo-Shanab (2005) who reported that IGRs increased activity when mixed with Mineral oils. Ismail *et al*., (2006) reported the Cypermethrin and Mineral oil (Supermasrona) have a moderate toxicity against *Tetranychus urticae*.

These results observed of new trend will lead to reduce the field dose of the Pyrethroid insecticides, enhance the role of beneficial insects and reduce the cost of pest control. So the additive effect was obtained in three tested Mineral oils with Cyhalothrin, while the potentiation effect was observed in Folic; Kemesol and National oil. The addition of Mineral oil may be prolonged the residual activity of candidate. The facilitate foliar absorption of pesticides. The evaporation from a falling oil emulsion dropet is shower than from a water dropet, and this is useful in increasing foliar retention and reducing drift.

***In vivo* inhibition of brain *S. littoralis* ATPase activity:**

The *in vivo* inhibitory of the LC_{50} values of three Mineral oils against to the *Spodoptera* 4^{th} instar lab and field strains larval ATPase are shown in the data given in Table (3). The data declared that Folic and Kemesol oil exhibited the highest percentages of reduction of ATPase activity as values were 67.5 and 58.6% respectively for lab strain, while values were 64.8 and 55.9% respectively, for field strain. On the other hand, National oil not active as ATPase inhibitor, as percentages of reduction on ATPase activity as values were 53.4 and 50.2% respectively, for lab strain and field strain.

Data in Table (3) summarize the interaction of Folic; Kemesol and National oil inhibitory effect of Cyhalothrin on activity of ATPase. The results proved that pretreated of Folic; Kemesol and National oil with Cyhalothrin induce increase the inhibition of enzyme activity. The inhibition of ATPase by Cyhalothrin alone were 75.7 and 70.1% for lab and field strains respectively. While the inhibition increased to be 95.8 and 90.7% for lab and field strains respectively, when Folic oil pretreated with Cyhalothrin. Morever the inhibition of enzyme activity increased to be 90.5 and 88.6% for lab and field strains respectively, when Kemesol oil pretreated with Cyhalothrin. Also the

Table 2. Comparative toxicities of Cyhalothrin alone or pretreated with some Mineral oils on *Spodoptera* larvae

Compounds	LC_{50} (ppm)							
	24hr				48hr			
	Lab strain		Field strain		Lab strain		Field strain	
		P.f.		P.f.		P.f.		P.f.
Cyhalothrin	0.013		0.019		0.011		0.016	
Folic oil+Cyhalothrin	0.004	3.25	0.007	2.71	0.002	5.50	0.005	3.20
Kemesol oil+Cyhalothrin	0.006	2.16	0.009	2.11	0.003	3.66	0.007	2.28
National oil+Cyhalothrin	0.012	1.08	0.015	1.26	0.010	1.10	0.013	1.23

Potentietion factor (P.f.)= LC_{50} insecticide alone/LC_{50} insecticide+Mineral oil.

Table 3. *In vivo* inhibition of *Spodoptera* larvae 4th instar ATPase activity by some compounds (LC_{50})

Compounds	%Inhibition	
	Lab strain	Field strain
Cyhalothrin	75.7	70.1
Folic oil	67.5	64.8
Kemesol oil	58.6	55.9
National oil	53.4	50.2
Folic oil+ Cyhalothrin	95.8	90.7
Kemesol oil+ Cyhalothrin	90.5	88.6
National oil+ Cyhalothrin	76.2	71.3

inhibition increased to be 76.2 and 71.3% for lab and field strains respectively, when National oil pretreated with Cyhalothrin. Generally, the results proved that mixing of Folic and Kemesol oil with Cyhalothrin significantly induce increase in the inhibition percentages, but National oil had weak inhibitory effect on the ATPase. This results fully agree with Kaygisiz (1980) & McDonald (1981) reported that synthetic Pyrethroids were the most effective against 4th instar larvae of *S. littoralis*. Saleh *et al*., (1984) & Korkor *et al*., (1995) reported that synthetic Pyrethroids were the most effective insecticides against Bollworm.

The potentiation of insecticides as a result of some tested Mineral oils may be attributred to affinity of some of their components and/or their fast reaction with detoxifying enzyme, forming a relatively stable substrate enzyme complex that block the detoxifying enzyme.

The *in vitro* inhibition of brain *S. littoralis* ATPase activity:

Table (4) show the *in vitro* interaction of Cyhalothrin and the three Mineral oils on ATPase activity of *S. littoralis* 4th instar brain. The I_{50} values of Cyhalothrin for lab and field strains larval brain ATPase are o.64 and 0.76ppm respectively. We have shown that the efficacy of Folic oil and Kemesol oil has a very good additive toxicity for Cyhalothrin in lab and field *Spodoptera* strains Table (4), because for the enhancement toxicity of the Folic oil and Kemesol oil, we study the *in vitro* biochemical interaction of them with the ATPase activity and compare with the Cyhalothrin *in vitro* effects. The I_{50} values for Folic oil 4.4 and 5.6ppm against both lab and field strains enzyme respectively, while when Folic oil mixed with Cyhalothrin the I_{50} values were 0.28 and 0.47ppm for lab and field strains respectively. On the other hand the I_{50} values for Kemesol oil were 6.2 and 7.4ppm against lab and field strains enzyme respectively, the I_{50} values were 0.45 and 0.61ppm for lab and field strains respectively, when Kemesol oil mixed with Cyhalothrin. It is quite clear that Cyhalothrin at I_{50} concentration acts as potential inhibitor for *Spodoptera* larvae 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 ATPase of different insects.

To characterize more details about the *in vitro* inhibition of ATPase by the inhibitor, the I_{50} and Ki values of each inhibitor were estimated from the graphical method of Dixon and Weep, (1964) Table (4). The obtained data proved that compounds competitive inhibition of ATPase activity and the Ki values were 38 and 45ppm for Cyhalothrin alone, lab and field strains respectively. Ki values were 57 and 20ppm for Folic oil alone and mixed with Cyhalothrin for lab strain respectively, while, Kemesol oil exhibited competitive inhibition of activity as Ki values were 64 and 28ppm for Kemesol oil alone and mixed with Cyhalothrin for lab strain respectively. In the case of field strain the Ki values were 66 and 26ppm for Folic oil alone and mixed with Cyhalothrin respectively. Ki values were 72 and 34ppm for Kemesol oil alone and mixed with Cyhalothrin for field strain respectively. These results are in accordance with Hazzard *et al*., (2003) found that the combination of *Bacillus thuringiensis* and Mineral oil provided the largest and most consistent reduction in numbers of larvae.

Table 4. *In vitro* inhibition of brain *Spodoptera* larvae ATPase activity by certain compounds

Compounds	I_{50} (ppm)		Ki (ppm)	
	Lab strain	Field strain	Lab strain	Field strain
Cyhalothrin	0.64	0.76	38	45
Folic oil	4.4	5.6	57	66
Kemesol oil	6.2	7.4	64	72
Folic oil+Cyhalothrin	0.28	0.47	20	26
Kemesol oil+Cyhalothrin	0.45	0.61	28	34

Generally, it could be concluded that use of Pyrethroids and mixtures with Mineral oils instead of conventional hazardous insecticides; were efficient as inhibitors for ATPase and this may reduce the environmental pollution and hazard effects on human health our data supported that, Mineral oils may play an important role in future insect pest management programs especially when mixed with Pyrethroids.

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الملخص العربي

كفاءة بعض الزيوت المعدنية ومبيدات البيرثرويد ومخاليطهم على نشاط أنزيم أدينوسين تراى فوسفاتيز خارجياً فى يرقات دودة ورق القطن

سهام منصور إسماعيل، نادر شاكر

تم دراسة سمية ثلاثة من الزيوت المعدنية وهى (فوليك–كيمسول–ناشيونال) على يرقات العمر الرابع للسلالة المعملية والسلالة الحقلية لدودة ورق القطن. أوضحت النتائج أن زيت الفوليك كان أكثر الزيوت المختبرة سمية على الحشرة موضوع الدراسة يليه زيت الكيمسول أمازيت الناشيونال فقد أظهر أقل سمية على الحشرة وذلك بالنسبة للسلالتين المعملية والحقلية.أيضا تم دراسة تأثير المعاملة بتلك الزيوت مع المبيد المختبر السيهالوثرين وقد زادت الفعالية نتيجة هذه المعاملة زيادة ملحوظة خاصة فى حالة الفوليك والكيمسول 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 وهو أنزيم أدينوسين تراى فوسفاتيز ولقد أوضحت النتائج أن فى حالة الفوليك بعد المعاملة بالسيهالوثرين كانت النسبة المئوية للتثبيط هى 95.8%و90.7% للسلالة المعملية والحقلية على الترتيب، بينما فى حالة الكيمسول بعد المعاملة بالسيهالوثرين كانت النسبة المئوية للتثبيط هى90.5%و88.6 % لكل من السلالة المعملية والحقلية على التوالى، ولقد كانت النسبة المئوية للتثبيط بواسطة الناشيونال بعد المعاملةبالسيهالوثرين هى 76.2%و71.3% للسلالتين المعملية و الحقلية على الترتيب. وكذلك تم دراسة تأثير تلك الزيوت المختبرة بعد المعاملة مع المبيد المختبر السيهالوثرين على قيم الـI_{50} (تركيز المبيد اللازم لتثبيط50% من النشاط الأنزيمى).وأيضا دراسة ثابت التثبيط (K_i) ولقد أثبتت النتائج حدوث أنخفاض فى تلك القيم وكانت أعلى نسبة للأنخفاض عند معاملة زيت الفوليك وزيت الكيمسول مع السيهالوثرين على التوالى وقد وجد أن هذه المركبات أظهرت تثبيط تنافسى على نشاط أنزيم أدينوسين تراى فوسفاتيز. ومن نتائج هذه الدراسة يتبين أن التأثير التثبيطى لمبيدات البيرثرويد يمكن تشجيعه بأضافة زيت الفوليك وزيت الكيميسول على الترتيب، وبذلك يمكن أن يكون للزيوت المعدنية دور فى زيادة سمية المبيدات خاصة عند خلطها مع مبيدات البيرثرويد، وعلى هذا يمكن الأستعانة بها فى برامج المكافحة.