EVALUATION OF SPINOSAD ALONE AND IN COMBINATION WITH PLANT OIL EXTRACTS TO *CULEX PIPIENS* L. LARVAE (DIPTERA: CULICIDAE)

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Received on: 9/6/2008 ***Accepted: 4/8/2008***

ABSTRACT

Toxicity of the bioinsecticide spinosad and five plant oil extracts: neem oil, eucalyptus oil, rosemary oil, jojoba oil and garlic oil, against the 4th instar larvae of Culex pipiens were evaluated. Also the joint toxic effect of spinosad with each of these plant oil extracts against the 4th instar larvae of C. pipiens was investigated. While the IC_{50} values of these five plant oil extracts were 54, 287.8, 150.7, 60.5 and 250.6 ppm for neem oil, eucalyptus oil, rosemary oil, jojoba oil and garlic oil, respectively, after 72 hrs of exposure, the IC_{50} of spinosad (0.061ppm). Spinosad was 950, 5029, 2732, 1155, 4190 times more toxic than these extracts, respectively, after 72 the same exposure time. Results indicated that the neem oil was 5.3, 2.7, 1.1, 4.6 times effective than each of the eucalyptus oil, rosemary oil, jojoba oil or garlic oil, respectively, after 72 hrs of exposure. The toxicity of the five plant oil extracts increased with the time elapsed from exposure. Spinosad / oil plant extracts mixtures resulted in a different levels of potentiation expressed by the inhibition of adult formation. The potentiation activity occurs when the mixtures of spinosad (at IC_{25}) with neem oil, rosemary oil, jojoba oil and garlic oil (at IC_{20}, IC_{35}, IC_{50}) after 72 hrs of exposure. Additive effects were resulted from the mixtures of spinosad and eucalyptus.

***Key words**: Spinosad, plant oil extracts, joint action, Culex pipiens.*

INTRODUCTION

Mosquitoes are the most important vectors of certain human diseases including malaria, encephalitis, filariasis and yellow fever. *Culex pipiens pipiens* is a common mosquito found in North Africa and is the primary vector of Bancroftian filariasis in Egypt.

Drawbacks associated with widespread use of synthetic insecticides to control mosquitoes have not only resulted in the development of new mosquito strains resistant to these compounds, but have also caused the death of non-target organisms that feed on mosquito larvae . Accordingly, it is important to find out other alternatives that can effectively control mosquitoes with minimal damage to the environment. Among these alternatives spinosad, a naturally occurring product from the fermentation of the bacterium *Saccharopolyspora spinosa*, which proved to be highly effective as bioinsecticide against many insect pests, and this agent has an excellent friendly environmental and mammalian toxicological profile (Romi *et al* 2006). The toxicity of spinosad against different mosquito species has been evaluated by several investigators such as Romi *et al* 2006; Darriet *et al*., 2005; Bond *et al*.,2004; Cetin *et al*., 2005.

Alternatives from plant origin have received special consideration for vector control pests. Studies on natural plant extractions for their efficacy as mosquito larvicides during the last decade have pronounced their possibilities as alternatives for a certain extent to synthetic chemical insecticides (Rahuman *et al*., 2000; Sun *et al*., 2001; Choi *et al*., 2002; Traboulsi *et al*.,2002; Vatandoost and Vaziri,2004; Wandscheer *et al*,2004; EL-banoby,2005; Traboulsi *et al*.,2005; Nathan 2007; Pitasawat *et al*., 2007).

Certain pesticides being used in pest control are hazardous. In order to reduce these hazards and the development of resistant populations, insect control should be accomplished with fewer applications at far lower doses. This aim might be realized, for example, by combining acute toxicants with other chemicals, such as insect growth regulators or plant oil extracts (El-Guindy *et al*., 1983).

The present study aimed to evaluate the effectiveness of spinosad, five plant oil extracts and their mixtures with spinosad against the 4th instar larvae of *C. pipiens*.

MATERIAL AND METHODS

Mosquito culture: A field strain of *C. pipiens*. larvae was collected from a water pond in Abees area, Alexandria Governorate, Egypt, in August 2001. The obtained larvae were reared under laboratory temperature of 27±1°C and 70±5% R.H., with 14:10 (L:D) photoperiod. The 4th instar larvae were used in this study.

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The tested compounds:

- **Microbial pesticides:**

Spinosad (Tracer® 24% SC).

- **Plant oil extracts used:**

Scientific name	Family	Plant oil extracts tested	Purity (%)
Azadirachta indica	Meliaceae	Neem oil	98
Simmondsia chinensis	Simmondsiaceae	Jojoba oil	97
Eucalyptus sp.	Myrtaceae	Eucalyptus oil	98
Allium sativa	Liliaceae	Garlic oil	98
Rosmarinus officinnalis	Labiatae	Rosemary oil	97

Toxicity of spinosad and the plant oil extracts against *C. pipiens*:

The dosage-mortality tests were conducted with the biocide spinosad. Larval treatments were carried out by exposing early 4th larval instar to various concentrations of the tested compounds in 400 ml glass beakers, containing 100 ml tap water (containing 0.1% triton X-100 as an emulsifier). Each concentration was replicated five times (20 larvae / replicate). The larvae were exposed to the test concentration for 72 hours (Hseish and Steelman, 1974). The control contains the larvae in untreated water. Larvae were given the usual larval food during the exposure time followed. Mortality percentage of larvae and pupae were recorded after 24, 48 and 72 hrs. Live pupae were transferred to untreated water in new glass beakers for further observations, i.e. for records of normal adult emergence, presence of morphological abnormalities or death by the test end (72hrs) cumulative mean mortality percentages were recrded. Partially emerged adults or these found completely emerged but unable to leave the water surface were considered as mortal ones. Mortality percentages were corrected according to Abbott's formula (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The IC_{20}, IC_{35} and IC_{50} were calculated.

Joint toxic action effect of spinosad with plant oil extracts against *C. pipiens*: The joint toxic action of spinosad with each of plant oil extracts tested against 4th larval *C. pipiens* instar was investigated. The IC_{20} of spinosad was mixed with the IC_{25}, IC_{35} or IC_{50} of each oil extract. Toxicity of spinosad plus each plant oil extract tested was evaluated by the following equation (Mansour *et al.*, 1966).

Co-toxicity factor =

$$\frac{\text{observed\% mortality} - \text{expected\% mortality}}{\text{Expected\% mortality}} \times 100$$

This factor was used to categorize the results into three categories as follow:

- +20 or more = potentiation effect .
- Between +20 and -20 = additive effect.
- -20 or less = antagonism.

RESULTS

Toxicity of spinosad and the plant oil extracts against *C. pipiens*:

The toxicity of bioinsecticide spinosad and the plant oil extracts to 4th instar larvae of *C.pipiens* are shown in Table (1). The statistical parameters were calculated according to the method of Lichfield and Wilcoxon (1949). The determination of cumulative mortalities during larval development to pupae and adults have been taken as a criterion for evaluation of the tested bioinsecticide and plant oil extracts against *C.pipiens* mosquitoes.

The effective range concentrations of spinosad, neem oil, eucalyptus oil, rosemary oil, jojoba oil, garlic oil were in respect 0.01-0.2, 50-200, 100-500, 100-500, 50-200,100-500 ppm (Tables 1,2). The corresponding range percentages of adult emergence inhibition were 11-80, 25-89, 28-86, 22-88, 29-84, 20-79%, respectively. The concentrations which inhibit the emergence of 50% of adults (IC_{50} values) after 72hrs of exposure were 0.061, 54, 287.8, 150.7, 60.5, 250.6 ppm, respectively, (Table 3).

The spinosad (IC_{50}=0.061) was 950, 5029, 2732, 1155, 4190 times more toxic than the neem oil, eucalyptus oil, rosemary oil, jojoba oil and garlic oil, respectively, after 72 hrs of exposure (Table 3). Also the obtained data indicated that, neem oil proved to be the most effective among the tested plant oil extracts against *C. pipiens*, followed by jojoba oil, rosemary oil, garlic oil, eucalyptus oil. At the same time, results indicated that the neem oil was 5.3, 2.7, 1.1, 4.6 times more effective than the above plant oils, respectively.

Joint toxic action effect of spinosad with plant oil extracts against *C. pipiens*: Table (4) shows the effect of combinations of the bioinsecticide spinosad with each plant oil extracts tested (neem oil, eucalyptus oil, rosemary oil, jojoba oil and garlic oil) on *C.pipiens* mosquito larvae . The combionations were applied at the IC_{25} value of spinosad (0.025 ppm) and each of IC_{20}, IC_{35} and IC_{50} levels of neem oil (21, 32 and 59 ppm), eucalyptus oil (114, 173 and 309 ppm), rosemary oil (63, 97 and 168 ppm), jojoba oil (25, 41 and 72 ppm) and garlic oil (95, 143 and 257 ppm). Therefore the expected mortality for the mixture of the spinosad and the various concentrations of the tested

plant oil extracts was the sum of the mortalities of each of the concentrations used in the mixture.

The obtained results for Co-toxicity in Table 4 showed that all combinations for spinosad with the five tested plant oil extracts produced different levels of potentiation expressed by the inhibition of adult formation. It is quiet evident that, the mixtures of spinosad (at IC_{25}) with the neem oil, rosemary oil or jojoba oil (at IC20) after 72 hrs of exposure resulted in potentiating effect and the Co-toxicity factors (CTFs) were +28.8, +20.0, +26.6, respectively, (Table 4). When spinosad at (IC_{25}) were mixed with the IC_{35} of the test plant oil, potentiation effect were also obtained (Table 4). The CTFs were +15.09 when spinosad was mixed with neem oil after 24 hrs of exposure. This value was +25.0 and +23.3 when spinosad were mixed with neem oil, jojoba oil, respectively, after 48 hrs of exposure. The values were +31.66, +28.33 and 21.66 when spinosad were mixed with neem oil, jojoba oil and garlic oil, respectively, after 72 hrs of exposure (Table 4).

Table (4) shows the CTFs when the IC_{25} of spinosad was mixed with the IC_{50} of the plant oil extracts. The CTFs were +20.0 and 21.3 when spinosad were mixed with neem oil and jojoba oil, respectively, after 24 hrs of exposure. The values were +28.0 and 22.66 when spinosad was mixed with neem oil and jojoba oil, respectively, after 48 hrs of exposure. The values were +32.0, +21.33, +29.33 and +24.0 when spinosad was mixed with neem oil, rosemary oil, jojoba oil and garlic oil, respectively, after 72 hrs of exposure.

Results revealed that, the potentiation activity occurs with the mixture of spinosad with neem oil or rosemary oil or jojoba oil or garlic after 72 hrs of exposure. Additive effects were only resulted from the mixtures of spinosad and eucalyptus.

DISCUSION

Bioinsecticides are claimed to be safer for beneficial organisms than conventional compounds, and they have attracted considerable attention for their inclusion in IPM programs (Darvas and Polgar, 1998; Schneider *et al.*, 2003; Schoonover and Larson, 1995). As spinosyns are produced by fermentation of an actinomycete, spinosad has been classified as a biopesticide (Copping and Menn, 2000).

Secondary metabolites obtained from the indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control (Nathan, 2007). In the present study, neem oil was more toxic than the other oil plant extracts tested against the 4th instar larvae of *C. pipiens*, after 72 hrs of exposure. Generally, it can be concluded that the response of the 4th instar larvae of the present strain depends entirely on the type of the essential oil used and its concentration. The fluctuations in the percentage inhibition of adult survivors obtained from the different concentrations of the tested oil plant extracts against this strain support this conclusion. The results are in agreement with those obtained by Nathan (2007). He stated that the oil plant extracts from the forest redgum, *Eucalyptus tereticornis* Sm. (Myrtaceae) showed strong larvicidal, pupicidal and adulticidal activity against mosquito vector *Anopheles stevensi*. Also, Pitasawat *et al.*, (2007) found that all of the volatile oils, extracted from the five aromatic plants, *Carum carvi* (caraway), *Apium graveolens* (celery), *Foeniculum vulgare* (fennel), *Zanthoxylum limonella* (mullilam) and *Curcuma zedoaria* (zedoary), exerted significant larvicidal activity against *Anopheles dirus* and *Aedes aegypti*. The delayed lethal effect of the tested plant oil extracts, however, is more likely to be caused by a disturbance of the endocrine mechanisms that regulate moulting and metamorphosis. This mechanism of action has been postulated previously for neem seed kernel extracts (Rembold 1984; Zebitz 1986) and *Melia volkensii* fruit extracts (Mwangi and Mukiama, 1988).

The use of insecticides can be reduced by combining them with other chemicals, such as insect growth regulators or plant oil extracts. The strong synergistic effect observed by Darriet and Corbel (2006) between pyriproxyfen and spinosad allows a reduction in both pyriproxyfen and spinosad amounts by 5 and 9 fold to kill almost 100% mosquitoes. The joint toxic action of spinosad with each of the five plant oil extracts against the 4th instar larvae of *C. pipiens* was investigated. Results indicated that all combinations between the bioinsecticide spinosad with the tested oil plant extracts produced different levels of potentiation expressed by the inhibition of adult formation. These results were compatible with the results obtained by Guirguis *et al.* (1991). They reported that the binary mixture of the wild plant *Suaeda fruticosa* extract with methamidophos, chlorpyrifos, methomyl, fenvalerate and fenpropathrin against *Spodoptera littoralis* larvae produced potentiation.

The development of strategies for the rational use of insecticides within the framework of insect pest management requires a great deal of research. There is a tendency with insect pest management research to emphasize the alternative non insecticides. There is at present a great need for independent work to identify reduced dosage levels that provide adequate control. Finally, we can conclude that, the trend of using the insecticides in mixtures with the plant oil extracts, for controlling insect pests, can lead to a reduction of insecticides field doses, enhance the role of beneficial insects and reduce the cost of insect control process.

ACKNOWLEDGMENT

I gratefully acknowledge Dr. M. Radwan, Dept. of Pharmacology. Fac. of Pharmacy for providing plant oils tested and Dow Agroscience Co. for providing the spinosad.

Table (1): The delayed effect of spinosad on the 4th larval instar of *C. pipiens*.

Conc. (ppm)	Larval mortality* (%)			Pupation (%)	Adult emergence			Obs. – Exp.	Contribution to X^2
					Total (%)	Inhibition(%)			
	24hrs	48hrs	72hrs			Obs.**	Exp.		
0.01	2	3	5	95	89	11	9	2	0.005
0.025	8	11	16	84	76	25	25	0.0	0.001
0.05	17	21	25	75	59	41	41	0.0	0.001
0.1	25	28	33	67	48	58	60	-2	0.0017
0.15	47	52	54	46	29	71	71	0.0	0.001
0.2	75	83	89	11	11	80	77	3	0.005
0.0(Cont.)	-	2	3	97	96	4			

*Five replicates, 20 larvae each.
**Corrected by Abbott's formula (Abbott,1925)
X^2 at 5% probability level = 7.81
X^2 from the data = 0.97
The line is good fit and the data are significantly homogeneous.
Slope function (s) = 4.18
IC_{50} = 0.07 ppm
flC_{50} = 1.18
Fiducial limits of IC_{50} = 0.059 – 0.083 ppm
Slope = 14.03

Table (2): Effects of 72 hr. exposing 4th larval instar of *C. pipiens* to the plant oil extract on percentages of larval mortality, pupation and emerged adults

Compound	Concentrations (ppm)	Larval mortality (%)	Pupation (%)	Adult emergence (%)		IC_{50}**
				Total (%)	Inhibition*	
Neem oil	50 – 200	20 – 87	80 – 13	64 – 9	25 – 89	55
Eucalyptus oil	100 – 500	16 – 80	84 – 20	68 – 7	28 – 86	290
Rosemary oil	100 – 500	30 – 81	70 – 19	61 – 10	22 – 88	152
Jojoba oil	50 – 200	16 – 90	84 – 10	66 – 6	29 – 84	62
Garlic oil	100 – 500	23 – 65	77 – 35	63 – 15	20 – 79	252
Water (Cont.)	_	2 – 4	96 – 98	88 – 94	5 – 10	-

*Corrected by Abbott's formula
** Lichfield and Wilcoxon (1949).

Table (3): Toxicity of spinosad and plant oil extracts against the 4th larval instar of *C.pipiens* at different exposure periods:

Insecticides	Time (hrs)	IC_{50}*	IC_{50} confidence limits	Slope
Spinosad	24	0.07	0.059-0.083	14.03
	48	0.066	0.056-0.078	14.02
	72	**0.061**	0.049-0.071	14.02
Neem	24	55	50.72-59.6	4.2
	48	54.4	50.2-58.7	4.2
	72	**54**	49.79-58.1	4.2
Eucalyptus	24	290	260.92-322.08	2.7
	48	289.1	259.1-321.2	2.7
	72	**287.8**	258.78-319.4	2.6
Rosemary	24	152	132.97-173.42	3.0
	48	151.6	133.1-172.5	3.1
	72	**150.7**	132.7-171.5	3.0
Jojoba oil	24	62	52.63-72.72	2.2
	48	61.2	52.2-71.6	2.4
	72	**60.5**	51.1-71.3	2.3
Garlic oil	24	252	214.79-295.42	2.0
	48	251.1	213.2-294.9	2.0
	72	**250.6**	215.1-294.2	2.1

* Lichfield and Wilcoxon (1949).

Table 4: The joint action of spinosad at IC_{25} with each plant oil extract at IC_{20},IC_{35} or IC_{50} on the 4th larval instar *C. pipiens*:

Plant Oil	After 24 hrs of exposure			After 48 hrs of exposure			After 72 hrs of exposure		
	Expected % Mortality	Observed % Mortality	Co-toxicity factor	Expected % Mortality	Observed % Mortality	Co-toxicity factor	Expected % Mortality	Observed % Mortality	Co-toxicity factor
	Spinosad plus Plant oils IC20								
Neem	45	53	+15.09	45	55	+22.2	45	58	+28.8
Eucalyptus	45	46	+2.22	45	49	+8.88	45	51	+13.33
Rosemary	45	48	+6.66	45	51	+13.33	45	54	+20.00
Jojoba	45	49	+8.88	45	53	+17.7	45	57	+26.6
Garlic	45	47	+4.44	45	50	+11.11	45	53	+17.77
	Spinosad plus Plant oils IC35								
Neem	60	72	+20.0	60	75	+25.0	60	79	+31.66
Eucalyptus	60	63	+5.0	60	64	+6.6	60	70	+16.66
Rosemary	60	66	+10.0	60	69	+15.0	60	71	+18.33
Jojoba	60	70	+16.66	60	74	+23.3	60	77	+28.33
Garlic	60	65	+8.33	60	68	+13.3	60	73	+21.66
	Spinosad plus Plant oilsIC50								
Neem	75	90	+20.0	75	96	+28.0	75	99	+32.0
Eucalyptus	75	80	+6.66	75	84	+12.0	75	89	+18.6
Rosemary	75	82	+9.33	75	87	+16.0	75	91	+21.33
Jojoba	75	90	+20.0	75	91	+21.3	75	97	+29.33
Garlic	75	81	+8.0	75	86	+14.66	75	90	+20.0

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الملخص العربي

تقييم الإسبينوساد بمفرده ومخلوط مع زيوت نباتية مستخلصه لبرقات الباعوض الكيولكس بيبينز (دبترا: كيوليسيدي).

حسام الدين مجدى زهران

قسم الحشرات الإقتصادية – كلية الزراعة – الشاطبى – جامعة الإسكندرية – مصر

أجريت دراسة على سمية المبيد الحيوى الإسبينوساد وخمسة زيوت نباتية هى زيت النيم ، زيت الكافور ، زيت الحصالبان ، زيت الجوجوبا ، زيت الثوم ضد العمر الرابع ليرقات بعوضة الكيولكس بيبينز . كذلك تمت دراسة الفعل السام المشترك لمبيد الإسبينوساد مع الزيوت النباتية السابق ذكرها ضد العمر الرابع ليرقات بعوضة الكيولكس بيبينز.

أظهرت النتائج أن سمية مركب الإسبينوساد LC_{50} (التركيز المطلوب لتثبيط خروج ٥٠% من الحشرات الكاملة – ٠,٠٦١ جزء فى المليون) كان ٥٠٢٩،٩٥٠، ٢٧٣٢، ١١٥٥، ٤١٩٠ ضعف سمية زيت النيم ، زيت الكافور ، زيت الحصالبان ، زيت الجوجوبا ، زيت الثوم بعد ٧٢ ساعة من المعاملة ، على الترتيب. كما أظهرت النتائج أن زيت النيم كان أكثر سمية بمقدار ٥,٣ ، ٢,٧ ، ١,١ ، ٤,٦ مرة من زيت الكافور ، زيت الحصالبان ، زيت الجوجوبا ، زيت الثوم بعد ٧٢ ساعة من المعاملة ،على الترتيب. وكانت قيم الـ LC_{50} للخمسة زيوت النباتية هى ٥٤، ٢٨٧,٨ ، ١٥٠,٧ ، ٦٠,٥ ، ٢٥٠,٦ جزء فى المليون لزيت النيم ، زيت الكافور ، زيت الحصالبان ، زيت الجوجوبا ، زيت الثوم بعد ٧٢ ساعة من المعاملة ، على الترتيب. كما أن سمية الزيوت الطبيعية تتزايد مع الوقت. نتج عن خلط الإسبينوساد مع الزيوت الطبيعية مستويات مختلفة من التأثير التنشيطى الذى إنعكس على تثبيط خروج الحشرات الكاملة.

تم خلط قيم الـ LC_{50} من الإسبينوساد مع قيم الـ LC_{20} ، LC_{35} ، LC_{50} من الزيوت الخمسة النباتية المستخدمة وأظهرت النتائج حدوث تأثير تنشيطى فى حالة خلط الإسبينوساد مع زيت النيم ، زيت الحصالبان ، زيت الجوجوبا ، زيت الثوم بعد ٧٢ ساعة من المعاملة ولكن لم يظهر هذا التأثير فى حالة خلط الأسبينوساد مع زيت الكافور حيث كان تأثير إضافي فقط.