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The Natural Bio-Insecticide Spinosad and its Toxicity to Combat Some Mosquito Species in Ismailia Governorate, Egypt

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Abstract: Two formulae of spinosad (Liquid 12% and dust 0.125) were tested in Laboratory and field against some mosquito species in Ismailia Governorate. In the laboratory two types of water were used de-chlorinated and drained (sewage) water. After 24 h, the LC50 value of the liquid formula for *Culex pipiens molesttus* (Forsk) was 0.002 ppm when de-chlorinated water was used. However, the same dose resulted in 100% mortality when field water was used. On the other hand, after 24 h, the LC50 values of dust formula were 0.022 and 0.007 ppm when field and de-chlorinated water were used, respectively. During 10 days follow up in the field, larval mortality percentages of mosquitoes complex ranged from 98.5 to 53.2% at 0.054 ppm when liquid formulation of spinosad was used. However, it ranged from 58.5 to 55.8% at almost the same dose 0.056 ppm when spinosad dust was used. Spinosad liquid formulation performed better initial kill than dust formulation specially at the first two days when almost the same active dose was used. However, Spinosad dust presented a delayed larval mortality, which was absorbed up to pupation indicating better initial activity than the liquid especially in the 1st day.

Key words: Spinosad · *Culex pipiens molesttus* · larval mortality · pupa mortality

INTRODUCTION

The members of *Culex pipiens* complex are the most widely distributed species in the world Hoogstraal *et al.* [1]. In Egypt, the common house mosquito *Cx. pipiens molesttus* (Forsk) has been recorded from all governorates without exception (Wassif [2] and Farghal [4]) causing severe morbidity to man and animals. It is the main vector of *Bancroftian filariasis* [4, 5]. It is also the vector of Rift valley fever in Egypt [6] and diseases caused by other viruses [7].

The prolonged use of synthetic insecticide has been accompanied by harmful effects on human health and the environment [8, 9]. Gain of mosquitoes different types of resistance against chemical insecticides is also a matter of concern [10].

The appearance of such problems has been accompanied by growing interest to switch to use new safe bio-insecticide with a new mode of action specially when dealing with water [11]. Fargahal and Temerak [12] reported that Altosid SR10 exhibited toxic effect on larvae and inhibited adult emergence of *Cx. pipiens molesttus* (Forsk) with no effect on the beneficial aquatic beetle *Dytiscus* spp. Also, Faraghal and Temerak [13] confirmed the efficacy of *Bacillus thuringiensis* subsp *israelensis* (bactimos) to control *Cx. pipiens molestus*.

Spinosad is an insect control product derived from a soil bacterium that combines the advantages of synthetic insecticides with the advantages of traditional biological insecticides on pest species in several orders including Diptera [14]. Temerak [15] indicated that spinosad was not being affected by the existing resistance mechanism to conventional insecticides.

Spinosad has a very low mammalian toxicity and a favorable environmental profile with low persistence and low toxicity to a number of predatory insects [16]. As a result, the United States Environmental Protection Agency has classified spinosad as a reduced risk material [17].

A study was carried out to investigate the susceptibility of larval instars of a laboratory strain as well as a wild strain of *Cx. pipiens* to spinosad (liquid

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formulae) [18]. In order to continue further investigation, the objectives of this study were two items; First it was directed to investigate the susceptibility of larval instars of field and Lab. population of *Cx. pipiens* to two formulae of spinosad (liquid and dust) using two types of water de-chlorinated tap water and field water (sewage water) in the laboratory. Second, to determine the concentration mortality relationship due to spinosad (two formulae) when applied to different mosquito complex species in field breeding site in Ismailia Governorate.

MATERIALS AND METHODS

Chemicals used:

Spinosad: Spinosad formulation used in this study was conserve (12% SC) as well as Tracer (0.125% dust). Both are trademark of Dow Agrosciences Co. It is a naturally occurring mixture of two active components (spinosyn A and D) produced by fermentation of the soil Acctinomycete; *Sacharopolyspora spinosa* Marz and Yao [17].

Methomyl: A conventional insecticide named Lannete 90 SP was selected for purpose of comparison only.

Laboratory bioassay: The susceptibility of mosquito species (*Culex pipiens*) to spinosad was tested in the laboratory using a methodology adapted from the Elliot larval test [19]. Groups of 20 larvae of the third instar were placed in 300 ml plastic cups containing a solution of spinosad at one of the following concentrations of liquid form 12% (0.03125, 0.015625, 0.007812, 0.003906, 0.001953, 0.001464 and 0.0009765 ppm) and dust form 0.125% (0.03906, 0.0195, 0.00975, 0.00487 and 0.00243 ppm). Population of field strain of *Cx. pipiens* (3rd instar) was used in this study. It's collected from natural breeding site in Farm of Faculty of Agriculture, Sues Canal University. Three groups of larvae were assigned to each treatment (20 larvae each). Tap water left for 24 h was referred as de-chlorinated water and field water (sewage water) were used for the test. in addition to the untreated water as a control. A small quantity of powdered Soya bean and yeast was added to each cup as food. Mortality responses were recorded 24 h later. A larva was classified as dead if it did not move when gently touched with the point of a toothpick. The experiment was preformed three times on different dates. The standard selected product was methomyl 90 SP (Lannete 90% sp) at concentrations of 2.8, 2.7, 2.5, 2.2, 2, 1.9 ppm for field strain and 1.7, 1.5, 1.3, 1 ppm for Lab. strain.

Field application: field trials were done in breading site situates at 2 km in the road between Ismailia and Port Said. This breading site occupies about 10 acres surrounded by houses and cultivated land. Three areas that each equal five square meter of field breading site with depth of 0.175 cm approximately were used for each treatment and a similar untreated area as a control.

The 1st formula of spinosad, was liquid formulation 12.SC% at doses of 1, 1.5 and 2 cm (that equal to 0.027, 0.0405, 0.054 ppm approximately). The 2nd formula was dust formulation 0.125% at doses of 100, 150 and 200 gram (that equal to 0.028, 0.042, 0.056 ppm approximately). Both lab and field experiments were carried out in summer 2005.

Chemical analysis of water: Different types of water were analyzed by using the pipette method as described by Tribouth [20]. Electrical Conductivity (E.C.) of the saturated soil paste extract expressed as (dSm^{-1}) was measured on 5 ml samples of water using conductivity meter according to Richard [21]. The ph of water samples was determined by pH meter according to Page *et al.* [22]. Soluble anions (CO_3^{-2}, HCO_3^{-2}, CL^- and SO_4^{2}) and other cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) were determined according to Richard [21]. Organic matter was determined according to Jackson [23].

Statistical analysis: Data obtained in the present study were statistically analyzed by Duncan's Miltiple Range test [24].

RESULTS AND DISCUSSION

Water analysis: The results of water analysis are shown in Table 1, Organic matter, pH, cation and anions are higher in drained water than other types of water.

Table 1: Chemical analysis of different type of water

Chemical properties	Tap water	De-chlorinated water	Drained water
Organic matter %	0.01	0.01	2.1
pH	8.13	8.13	9.7
EC mmohs cm^{-1}	0.42	0.4	6.5
Cations (mg l^{-1})			
Na^+	2.1	2	15.7
K^+	2.3	2.3	5.3
Ca^{2+}	0.14	0.14	3.2
Mg^{2+}	1	1	1.1
Anions (mg l^{-1})			
HCO_3^{2-}	2.2	2.2	4.1
CO_3^{2-}	0	0	18.6
Cl^-	12	1.8	6
SO_4^{2-}	1.25	1.25	1

Tap water was left for 24 h and then it was considered as de-chlorinated

Table 2: Toxicity of two formula of spinosad (liquid 12% SC and dust 0.125%) under different water resources against *Cx. pipiens* in comparison to the standard methomyl after 24 h

Toxicity parameter	De-chlorinated water				Field water
	Spinosad liq.	Spinosad dust	Methomyl		Spinosad dust
	Field strain	Field strain	Lab. strain	Field strain	Field strain
LC10	0.001 (0.001-0.001)	0.001 (0.001-0.003)	0.715 (0.576-0.887)	1.508 (1.28-1.78)	0.003 (0.001-0.006)
LC20	0.001 (0.001-0.002)	0.002 (0.001-0.004)	0.906 (0.784-1.050)	1.783 (1.60-2.00)	0.005 (0.003-0.010)
LC50	0.002 (0.002-0.003)	0.007 (0.005-0.011)	1.430 (1.340-1.530)	2.463 (2.33-6.60)	0.022 (0.013-0.037)
LC90	0.006 (0.004-0.010)	0.040 (0.020-0.083)	2.859 (2.230-3.660)	4.023 (3.30-4.91)	0.175 (0.044-0.691)
Slope	2.227	3.802	1.712	1.463	5.088
Alph (0.05) CHI2	1.55	1.72	3.18	1.88	0.84

Table 3: Species composition of mosquito population in the field

Mosquito species	Percentage of larva
Culex pusillus	37%
Cx. pipiens	35%
Aedes caspius	25%
Anopheles multicolor	3%

Laboratory tests: Toxicity of two formulae of spinosad under different water resources against the 3rd larval instar of *Cx. pipiens* in comparison to the standard methomyl after 24 h is shown in Table 2.

All rates of liquid formulation (0.03125, 0.015625, 0.007812, 0.003906, 0.001953, 0.001464 and 0.0009765 ppm) after using field water performed 100% kill. So LC50 was not calculated.

After 24 h, values of LC50s were 0.002 and 0.007 ppm for the liquid and the dust formulations respectively when de-chlorinated water was used. This indicated that liquid form was more toxic than dust in de-chlorinated water after 24 h. Dust formulation performed little better results in de-chlorinated water (LC50=0.007) than in field water (LC50=0.022). However, this trend was opposite with the liquid in field water (all rates used performed 100% mortality) versus de-chlorinated (LC50=0.002). We believe that high level of organic matter in field water facilitate the mosquito larvae to swallow more chemicals, hence the difference effect was considerable very high over lab water. The liquid form (LC50=0.002) as well as the dust of spinosad (LC50=0.007) proved to be more toxic than the standard methomyl (LC50=2.462).

Field tests:

Complex of mosquito species %: Four species of mosquitoes were recorded in the field and their % share are shown in Table 3.

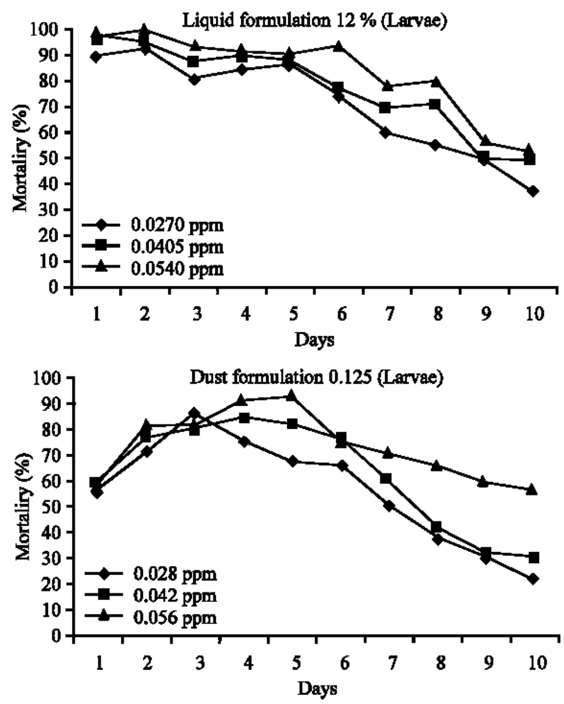

Fig. 1: Mortality % of mosquito larval complex after being treated with liquid and dust formulations of spinosad in the field

Complex of field water indicated that *Culex* spp were representing 72% of the total complex. The lowest was recorded for Anopheles as 3%.

Effect of spinosad on mosquito complex larvae: As shown in Fig. 1, during 10 days follow up in the field, larval mortality percentage ranged from 89.9 to 37.5% with a peak on the second day (92.9%) at 0.027 ppm when liquid formulation of spinosad was used. However, it ranged from 56.6 to 21.6% with a peak in the

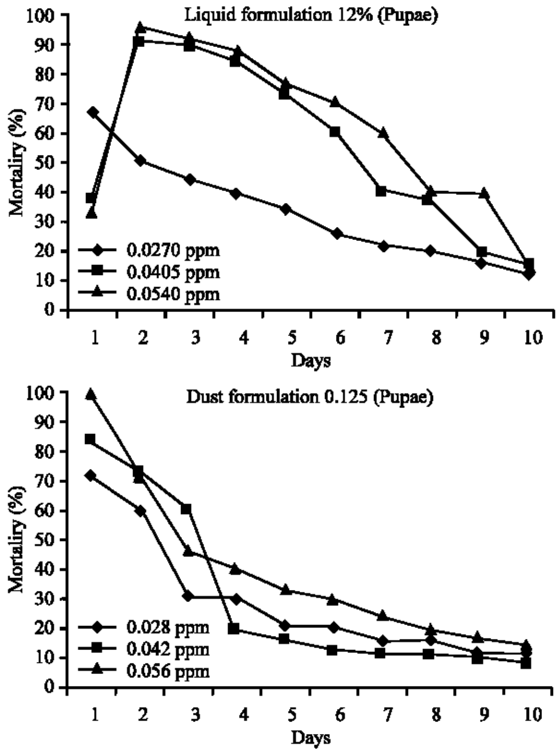

Fig. 2: Mortality % of mosquito pupae complex after being treated with liquid and dust formulations of spinosad in the field

3rd day (85.8%) at 0.028 ppm when spinosad dust was used.

Also, larval morality ranged from 97% to 53.2% at 0.0405 ppm, when liquid formulation was used . However, it ranged from 58.5% to 30.9% with a peak on the fourth day (85.6%) at 0.042 ppm when dust was used.

Moreover, larval mortality ranged from 98.5% to 53.2% with a peak in the second day (100%)at 0.054 ppm when liquid was used while it ranged from 58.5% to 55.8% with a peak in the fifth day (92.4%) at 0.056 ppm., when dust was used.

At all the tested concentrations, the liquid formula resulted in significantly higher levels of mortality compared with the dust formula at the level of 5% (P=0.006, F=7.99). At each of the three tested concentrations the liquid formula resulted in significantly higher levels of mortality compared with the dust formula at the level of 5% (P=0.046, F=3.26). Applying Duncan's Miltiple Range resulted in no significant variation between the three tested concentration of the liquid formula. On the other hand the recorded mortality at 0.056 ppm of the dust formula was significantly higher than that at 0.028 ppm.

The above confirm that liquid form was more toxic than dust with better initial kill. Comparing our results with Bond *et al.* [25] proved that *Cx. pipiens* is more susceptible to spinosad than other species, *Ae. aegypti* (LC50=0.025 ppm) and*An. albimanus* (LC50=0.024 ppm).

Effect of spinosad on mosquito complex pupae: As shown in Fig. 2, during 10 days follow up in the field, pupal mosquito complex mortality percentage ranged from 66.7 to12.5% at 0.027 ppm from liquid formulation. However, it ranged from 71.4 to 11.4% at 0.028 ppm from the dust. Also, it ranged from 37.8 to 15.5% at 0.0405 ppm from the liquid versus from 82.9 to 8.0% at 0.042 from the dust. Furthermore, it ranged from 33.3 to 15.0% at 0.054 ppm from the liquid while it was from 100 to14.3% at 0.056 ppm from the dust. At all the tested concentrations significant differences were recorded between the 6 treatments. Comparable mortality percentages were recorded at the three-dust concentration. On the other hand significantly higher mortality percentages were recorded at 0.054 ppm and 0.0405 ppm compared to 0.027 ppm of liquid formula at the level of 5% (P=0.025, F=2.8).

This can reflect that dust was far better than the liquid on pupae initial kill specially with the lowest dose. The last may confirm the slow acting of dust over liquid. Spinosad dust also presented a delayed larval mortality which was absorbed up to pupation.

CONCLUSIONS

Spinosad liquid formulation performed better initial kill than dust formulation specially at the first two days when almost the same active dose was used. However, Spinosad dust presented a delayed larval mortality which was absorbed up to pupation indicating better initial kill than the liquid.

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