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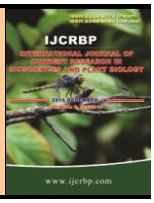

Original Research Article

Mosquitocidal Activity of Spinosad against the Dengue Vector, *Aedes aegypti*

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Abstract

Toxicity of spinosad (2 to 10 ppm) was tested against the larvae and pupae of *Aedes aegypti*. Significant mortality effects were observed in each life stage. Percentage of larval mortality was 95% in 10ppm spinosad against I and II instar larvae; 90, 88 and 82% against III instar, IV instar larvae and pupa respectively. Fitted Probit-mortality curves for larvae indicated the median and 90% lethal concentrations (LC_{50} ppm = LC_{90} ppm) of spinosad for instars I, II, III and IV to be 6.18=9.93, 6.43=9.97, 7.25=10.99 and 7.97=11.73 respectively. There was a significant mortality observed in field trials with spinosad (100 ppm) against III and IV instar larvae and the per cent reduction was 91.08 and it was 75.62% for pupae. The results indicate that spinosad exhibits significant biological activity against different life stages of *Ae. aegypti*. The present study revealed that spinosad is highly potential driving force for the control of *Ae. aegypti* larvae and pupae.

Keywords

Aedes aegypti

Biological activity

Mortality

Spinosad

Introduction

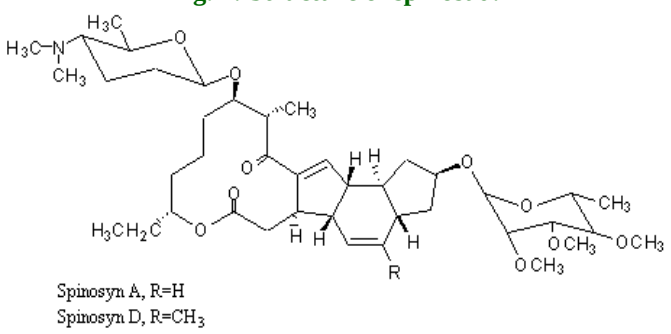
Mosquitoes are the single most group of insects in terms of public health significant. Mosquitoes are the major vectors for many diseases such as the malaria, filariasis and dengue vector, Japanese encephalitis and other fevers. Mosquito-borne diseases have been a major problem in almost all tropical and subtropical countries, and currently there are no successful vaccines against most such diseases. For years, mankind has been exploring various methods to combat threats from mosquito-borne diseases. Many synthetic insecticides are widely used for controlling adult and larval mosquito populations. However, the harmful effects of chemicals on non-target populations and the development of resistance to these chemicals in mosquitoes along with the recent resurgence of different mosquito-borne diseases (Milam et al., 2000) have prompted us to explore alternative, simple, sustainable methods of mosquito control.

Spinosad (Fig. 1) is a naturally-derived insecticide produced by fermentation of *Saccharopolyspora spinosa*, It is a neurotoxin comprising a mixture of spinosyns A and D (hence spinosAD), which are tetracyclic-macrolide compounds that act upon the post-synaptic nicotinic acetylcholine receptor and the GABA receptors. In reality, *S. spinosa* produces a great many spinosyn compounds, but so far, only spinosyns A and D have been developed as the basis for commercial insecticide products. Spinosad is a macrocyclic lactone that causes involuntary muscle contractions, tremors, and eventually paralysis of treated insects (Galvan et al., 2005). The application of spinosad has some advantages

over other measures for control of CPB. For instance, spinosad is naturally derived compound and has low level of toxicity to mammals (Thompson et al., 2000). Spinosad has very fast biodegradation rate with no or low influence beneficial fauna and very low impact on the resistance development. These characteristics are important issues in human health and pest control measures; render spinosad as an appropriate candidate in insect control program. Michaud and Grant (2003) reported that, spinosad fit very well into IPM potato programs. The results of current study indicate that spinosad is relatively fast-acting against *Ae. aegypti* and are consistent with results of later authors. Intoxication began to appear 4-5 h after treatment, whereas several reports is in this instance: spinosad is slower acting than many conventional chemical insecticides (Williams et al., 2003) and ignored the 1 h observations suggested in the WHO protocol specifically to account for the speed of action of spinosad (Bond et al., 2004).

Spinosad was shown to be highly toxic to *Ae. aegypti* and *Anopheles albimanus* Weidemann in the laboratory, and it completely suppressed the development of *Ae. aegypti*, *Culex* spp. and chironomid larvae in semi natural field conditions for periods of 8 to 22 wk, depending on concentration (Bond et al., 2004). Additional studies have reported the larvicidal properties of spinosad in this and other mosquito species (Liu et al. 2004a, 2004b; Cetin et al., 2005; Darriet et al., 2005; Darriet and Corbel 2006; Romi et al., 2006), or as an adulticide in a sugar bait formulation (Muller and Schlein 2006). The suitability of larvicidal compounds for use in insect vector control programs depends on a variety of properties and characteristics, including their persistence in the environment and the behavioral responses of insect vectors that are exposed to the compound. The aim of our study was to evaluate the efficacy of spinosad as a larvicide of *Ae. aegypti* in an important urban habitat.

Fig. 1: Structure of spinosad.



Materials and methods

Rearing and maintenance of *Ae. aegypti*

Initially, eggs were collected in and around Coimbatore (11°N, 77°E) from stagnant water bodies. These were transported to the laboratory on tap water in plastic containers and allowed to hatch at 29°C/28°C in glass trays (18 cm long × 9 cm wide × 3 cm deep) containing 2 L of tap water. After 24 h, first instar larvae were transferred to clean plastic trays (dimensions as above) with 2L of tap water and provided 5 g of ground Pedigree® dog food daily until pupation. Aliquots of 100 pupae were separated from the rearing medium and placed into net cages (42 cm long × 33cm wide × 39cm high) for emergence. Adults were allowed access to 10% sucrose solution *ad libitum via* cotton wick. Female mosquitoes were fed stored chicken blood using the methods described by Meola and Readio (1987). A glass beaker (10cm long × 6 cm diameter) containing tap water for oviposition was placed inside each net cage. Eggs rafts were removed daily, and the eggs allowed hatching and the larvae reared using the methods described above. Mosquitoes were reared and maintained in all cases at 29°C, 50% RH, and a 12:12 (L: D) photoperiod.

Mosquito bioassays

Eggs (n=100 per replicate), first to fourth instars (n=100 per life stage, per replicate), and pupae (n=100 per replicate) of *Ae. aegypti* were exposed to five concentrations of spinosad ranging from 2 to 10ppm. In each case, we used a 1000mL glass beaker (10 cm long × 15cm diameter) containing 2 to 10mg of spinosad and 950-990mL distilled water, respectively, to which 50mg dog biscuit was added. Tests of each concentration of spinosad were replicated three times for each life stage/ instar. Each replicate test for each life stage included single control comprising distilled water and the appropriate quantity (2-10mg) of spinosad final volume. Mortality in all tests was recorded after 24 h. Mortality responses for each life stage/instar were analyzed using analysis of variance procedures with means separation using Tukey's Honestly Significant Difference test (SAS 2003). We used Probit analysis (Finney, 1971) to evaluate the spinosad concentration–response (toxicity) relationship for each life stage/ instar of *Ae. aegypti* and corrected for control mortality before data analysis using a modified Abbott's formula (Abbott, 1925). The level of significance used in all statistical tests was p=0.05.

Field trail

For the field trial the quantity of bacterial insecticide, Spinosad required (Based on laboratory LC_{90} values) for each treatment was determined by calculating the total surface area of the water in each habitat. 100ppm/liter of spinosad mixed with 500L volume of water. The required quantities of spinosad were mixed thoroughly with water in a bucket with constant agitation. Teepol was used as emulsifying agent (0.05%). Field applications of Spinosad were done with the help of a knapsack sprayer. Dipper sampling and counting of larvae monitored the larval density before 72 h after the treatment. A separate sample was taken to determine the species composition of each larval habitat. The percentage of reduction was calculated by the following formula:

$$\text{Reduction (\%)} = \frac{C-T}{C} \times 100$$

Where,
C – is the total number of Mosquitoes in control
T – is the total number of Mosquitoes in treatment

Statistical analysis

The data got from the bioassays was subjected to statistical analysis; the analytical data, together with tables, are presented in appropriate places in the report. SPSS software package was used in computing all the data including Probit analysis, correlation equation, SE of the Mean of the sample.

Results

Table 1 provides the effect of spinosad on various stages, I, II, III, IV and pupae) of *Ae. aegypti*. Considerable mortality was evident after the treatment of Spinosad for all larvae and pupae. The mortality was increased as increasing concentrations. The percentage of mortality of I and II instar larvae was 95% at 10ppm concentration, whereas III and IV instar larvae showed 90 and 88% mortality at 10ppm concentration respectively. However, the pupal mortality was 82% at 10ppm. The effect of larval and pupal mortality was found to be dose dependent. The LC_{50} (LC_{90}) values were 6.18 (9.93) and 6.43 (9.97) in II and I instars; whereas, 7.25 (10.99), 7.97 (11.73) in III and IV instar, and similarly in pupa 8.92 (12.56). The LC_{50} and LC_{90} values were significantly increased with increasing concentration.

The regression equation and Chi-square values were X = 0.3613, Y = -2.3245 in II instar larvae which were significant. The Chi-square value showed increase of 1.668 in III instar larvae of *Ae. aegypti*. In this spinosad treatment, II and III instars were found to be more susceptible than the other stages. Overall, spinosad treatment at 10 ppm concentration was greatly reduced the larval and pupal states at 24 h mortality observation.

Table 1. Results of Probit analyses of mortality responses of *Ae. aegypti* larvae and pupae to spinosad application.

Stages	% mortality (Mean ±SE)	50% Confidential		95% Confidential				Regression Coefficient		$\chi 2$
				LCL		UCL		(X)	(Y)	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}			
I Instar	95 (24.2±0.25) [a]	6.18	9.93	5.63	9.10	6.72	11.13	0.3412	-2.1089	0.683
II Instar	95 (23.0±0.26) [a]	6.43	9.97	5.91	9.18	6.96	11.12	0.3613	-2.3245	0.727
III Instar	90 (19.4±0.28) [b]	7.25	10.99	6.71	10.07	7.84	12.36	0.3425	-2.4846	1.668
IV Instar	88 (16.2±0.30) [bc]	7.97	11.73	7.41	10.71	8.63	13.31	0.3403	-2.7132	0.387
Pupa	82 (12.2±0.37) [d]	8.92	12.56	8.32	11.40	9.72	14.46	0.3528	-3.1501	0.741

LCL - Lower Confidential Limit, UCL – Upper Confidential Limit; Within column mean (±SE) followed by the same letter (s) are not significantly different at 5% level; Chi-Square value significant at $p \leq 0.05$ level.

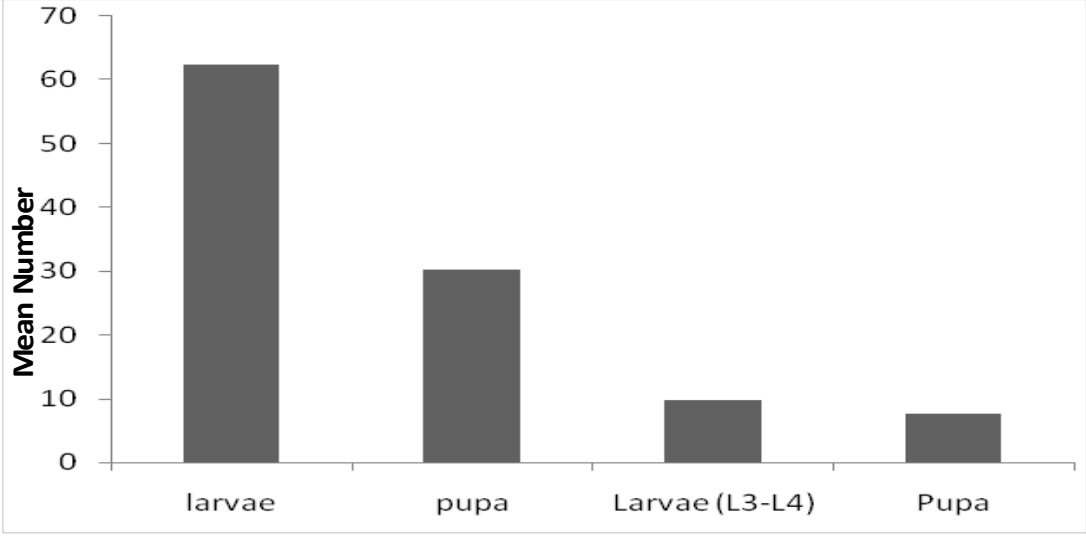
Table 2 provides the field evaluation data on the population reduction of larval and pupal stages of *Ae. aegyptii* at Vadavalli Village, Coimbatore, Tamil Nadu, India. The concentration at 100ppm/liter of spinosad added 500L of water in the tank. The sample from the mosquito habitat was taken up to 5 dipping/sample and also for five replicates were set to observe the mosquito larval and pupal percentage mortality at 72 h duration of post treatment. In each replicate, the number of live larvae and pupae were counted per dipping. In first replicate, the per cent reduction was 85.89 and 67.54 for larvae and pupae respectively. Similar trend was observed for 2, 3, 4 and 5th replicates. The overall per cent reduction was 91.08 for larvae and 75.62 for pupae of *Ae. aegypti*. Fig. 1 shows the field trail post treatment of spinosad-mean survival of larvae, pupae in control and in experiment. In control, the mean number of larvae survived was 62.4 and of pupa 30.2; whereas, in

post treatment, the mean percentage of reduction was 5.56 for larvae and 7.6 for pupae of *Ae. aegypti*. There was a significant reduction of larval and pupal mortality observed in pre and post treatment of spinosad.

Table 2. Field evaluation of spinosad (72 h post treatment) for the control of III and IV instar larvae (L3-4) and pupae of *Ae. aegypti*.

Live larvae (L3–4) and pupae (P)/sample (72h post treatment)															
Conc.	Sample	Control		Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Grand total	
		L3-4	P	L3-4	P	L3-4	P	L3-4	P	L3-4	P	L3-4	P	L3-4	P
100 ppm/L	1	73	21	10	13	8	12	6	7	4	4	4	6	6.4	8.4
	2	70	33	8	11	6	6	6	7	3	5	0	3	4.6	6.4
	3	58	30	9	8	8	8	7	6	6	6	4	8	6.8	7.2
	4	52	30	10	11	7	6	8	9	5	3	1	5	6.2	6.8
	5	59	37	7	6	6	10	4	11	2	8	0	11	3.8	9.2
	Total	312	151	44	49	35	42	31	40	20	26	9	33	27.8	38
	Mean reduction	62.4	30.2	8.8	9.8	7	8.4	6.2	8	4	5.2	1.8	6.6	5.56	7.6
	Reduction (%)	0	0	85.89	67.54	88.78	72.18	90.06	73.50	93.58	86.75	97.11	78.14	91.08	75.62

Fig. 2: Field trail post treatment of spinosad-mean live larvae, pupae in control and experiment.



Discussion

Spinosad has also been shown to have a high level of activity against larvae of various mosquito species, such as *Ae. aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, *Cx. pipiens*, *Anopheles albimanus*, *An. stephensi* and *An. quadrimaculatus* in recent studies (Bond et al., 2004; Cetin et al., 2005; Dariet et al., 2005; Dariet and Corbel, 2006; Paul et al., 2006; Romi et al., 2006; Pridgeon et al., 2008). Spinosadbased product (Laser 4.8% emulsifiable concentrate) was evaluated in laboratory bioassays against laboratory-reared mosquito strains of 3 species of medical importance: *Ae. aegypti*, *An. stephensi*, and *Cx. pipiens*. Spinosad was particularly effective against larvae of *Aedes* and *Culex*, with a less marked activity against anophelines (24 h median lethal concentration = 0.0096, 0.0064, and 0.039 mg/liter, respectively), showing a persistence of the insecticide action of about 6 wk in laboratory containers (Romi et al., 2006). In the present study 24 h lethal concentration of the suspension, containing concentrate formulation of spinosad (LC_{50}) against III and IV instar larvae of *Ae. aegypti* was estimated.

The field and the laboratory strains of *Rhizopertha dominica* Fabr. (Coleoptera: Borstrychidae) were highly susceptible to spinosad, and one of the field strains was less susceptible to spinosad than the laboratory strain (Huang et al., 2004). Spinosad was very effective in suppressing *R. dominica* and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) populations in stored wheat. Spinosad also effectively prevented breeding of *Culex* (Diptera: Culicidae) mosquitoes and chironomids (Diptera: Chironomidae) (Bond et al., 2004). The results of the present study showed that spinosad was very effective in the control of *Ae .aegypti*.

Therefore, in order to maximise the negative effects of the chemicals on the environment and natural enemies in the management of pests, the natural insecticide could be integrated into IPM programmes. The present experiment showed the strong efficacy of the spinosad on *Ae. aegypti* when applied at high rates, resulting in complete control. Further research is needed to understand the reduced susceptibility to spinosad.

The results of the present study showed that spinosad is highly toxic to larval and pural stages of *Ae. aegypti* in the laboratory and field condition. Spinosad is a naturally derived biorational insecticide with an environmentally favorable toxicity profile, so we investigated its potency against mosquito larvae (Diptera:Culicidae). By laboratory bioassays of a suspension concentrate formulation of spinosad (Tracer®), the 24 h lethal concentration (LC_{50}) against *Ae. aegypti* third and fourth instars was estimated at 2 to 10 ppm following logit regression. The concentration – mortality responses of III and IV-instar larvae of *Ae. aegypti* sites for 72h at 100 ppm, spinosad prevented the breeding. In contrast, the bacterial insecticide *Bacillus thuringiensis* var. *israelensis* (*Bti*, Vectobac® AS) performed poorly with just 2 weeks of complete inhibition of *Ae. aegypti* breeding. Spinosad also effectively prevented breeding of *Culex* mosquitoes and chironomids in both trials to a degree similar to that of temephos (Bond et al., 2004). It is concluded from the observations recorded in the present study that spinosad could be used as an effective larvicidal agent for application in mosquito breeding sites.

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