Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Larvicidal and pupicidal activity of spinosad against the malarial vector *Anopheles stephensi*

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ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 11 April 2011
Accepted 15 June 2011
Available online 20 August 2011

Keywords:
Spinosad
Saccharopolyspora spinosa
Anopheles stephensi
Larvicides

ABSTRACT

Objective: To investigate the larvicidal and pupicidal activity of spinosad against *Anopheles stephensi* Listen. **Methods:** Spinosad from the actinomycete, *Saccharopolyspora spinosa* was tested against *Anopheles stephensi* at different concentrations (0.01, 0.02, 0.04, 0.06 and 0.08 ppm.), and against first to fourth instar larvae and pupae. **Results:** The larval mortality ranged from 36.1 ±1.7 in (0.01 ppm) to 79.3±1.8 (0.08 ppm) the first instar larva. The LC_{50} and LC_{90} values of first, second, third and fourth instar larva were 0.001, 0.031, 0.034, 0.036 and 0.0113, 0.102, 0.111, 0.113, respectively. The pupal mortality ranged from 33.0±2.0 (0.01 ppm) to 80.0±0.9 (0.08 ppm). The LC_{50} and LC_{90} values were 0.028 and 0.1020, respectively. The reduction percentage of *Anopheles* larvae was 82.7%, 91.4% and 96.0% after 24, 48, 72 hours, respectively, while more than 80% reduction was observed after 3 weeks. **Conclusions:** In the present study spinosad effectively caused mortality of mosquito larvae in both the laboratory and field trial. It is predicted that spinosad is likely to be an effective larvicide for treatment of mosquito breeding sites.

1. Introduction

The success of insecticide-based control programmes in reducing the prevalence of insect vector-borne diseases[1,2] has been accompanied by growing interest regarding the harmful effects of wide scale and prolonged use of synthetic insecticides on human health and the environment[3]. Mosquito resistance to a number of conventional chemical insecticides is also a matter of current concern[4].

Spinosad is a mixture of tetracyclic macrolide neurotoxins, spinosyn A and D, produced during the fermentation of the soil actinomycete *Saccharopolyspora spinosa*. As such, it may be considered as a bioinsecticide[5]. Spinosad is highly toxic to Lepidoptera, Diptera and some Coleoptera has a unique mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptors[6]. Spinosad was shown to be highly toxic to *Aedes aegypti* (*Ae. aegypti*) and *Anopheles albimanus* in the laboratory, and it completely suppressed the development of *Ae. aegypti*, *Culex* spp., and chironomid larvae in seminatural field conditions for periods of 8 to >22 wk, depending on concentration[7]. Additional studies have reported the larvicidal properties of spinosad in this and other mosquito species[8] or as an adulticide in a sugar bait formulation[9].

Spinosad has a very low mammalian toxicity and a favorable environmental profile with low persistence and low toxicity to a number of predatory insects[10]. As a result, the United States Environmental Protection Agency has classified spinosad as a reduced risk material[11].

In this study, we aimed to determine the susceptibility of *Anopheles stephensi* (*An. stephensi*) to spinosad. These species were selected because of their importance as vectors of malarial, *Plasmodium vivax*. Until recently, control of *An. stephensi* was based on the use of DDT, which has been recently phased out in favour of household applications of organophosphates and pyrethroids[12].

The objectives of this study are two-fold. Firstly, we aimed to determine the concentration-mortality relationship for *An. stephensi* was exposed to spinosad in the laboratory.

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Secondly, we tested the duration of protection offered by spinosad when applied to urban breeding sites to inhibit the reproduction of *An. stephensi*.

2. Materials and methods

2.1. Test mosquitoes

The present study was conducted at Entomology Lab, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India. Larvae of *An. stephensi* were obtained from a laboratory colony maintained in the vector Research Unit. Mosquitoes used in the experiments described below were reared using filtered dechlorinated tap water. All laboratory procedures involving mosquitoes were performed at (26±1)℃, LD 12:12 h light cycles and 75%-85% relative humidity. The larvae were fed on a powdered mixture of dog biscuits and dried yeast powder at a ratio of 3:1.

2.2. Collection of eggs

The eggs of *An. stephensi* have been collected from local (in and around Coimbatore districts) drinking water bodies, water stored container and stagnant ditches with the help of 'O' type brush, for the laboratory bioassay. These eggs have been brought to the laboratory and have transferred to 18 cm × 13 cm × 4 cm size enamel trays containing 500 mL of water and keep for larval hatching. First to fourth instar larvae and pupae of *An. stephensi* were used to screen the larvicidal and pupicidal activity of commercial insecticide spinosad.

2.3. Preparation of extract

Spinosad was purchased from Kalpatharu Pesticide Limited, Coimbatore, Tamil Nadu, India. Spinosad 2.5%, copolymer of ethylene oxide and propylene oxide 0.17%, ammonium salt of naphthalene sulphonic acid 0.11%, polyalkyl siloxane 1.00%, prophylene glycol 4.14%, polysaccharide gum 0.15%, hydrated magnesium aluminum silicate 0.92% and water 9.00%, were of 100% w/w, and active specifically against insects. This product is labelled for use as an agricultural insecticide for control of lepidopteron and thrips pests of vegetables. Required quantity of spinosad was thoroughly mixed with distilled water to prepare various concentrations, ranging from 0.01 to 0.08 ppm.

2.4. Larvicidal bioassay

The susceptibility of each species of mosquito to spinosad was tested in the laboratory using a methodology adapted from the Elliot larval test[13]. Groups of 25 larvae of the first to fourth instar were placed in 150 mL plastic cups containing a solution of spinosad at one of the following concentrations: 0.01, 0.02, 0.04, 0.06, and 0.08 ppm active ingredients. Five groups of larvae were assigned to each treatment. Additional cup of water kept as a control, after 1 hour exposure, larvae were transferred to cups containing 100 mL clean dechlorinated water. A small quantity of powdered soya bean and yeast were added to each cup as food. Mortality responses were recorded after 24 hours. A larva was classified as dead if it did not move when gently touched with the point of a toothpick. The experiment was performed three times on different dates. The LC_{50} and LC_{90} were determined by a probit analysis program[14]. Control mortality was accounted by the formula of Abbott[15].

2.5. Pupicidal activity

A laboratory colony of mosquito pupae has been used for pupicidal activity. Groups of 25 larvae of the first to fourth instar were placed in 150mL plastic cups containing a solution of spinosad at one of the following concentrations: 0.01, 0.02, 0.04, 0.06, and 0.08 ppm active ingredients. Each experiment was conducted with three replicates, with a final total number of 100 pupae for each concentration. Mortality responses were recorded 24 h later. The LC_{50} and LC_{90} were determined by a probit analysis program[14]. Control mortality was accounted for by the formula of Abbott[15].

2.6. Field trial bioassay

The field trial study was carried out at mosquito breeding sites in the Bharathiar University campus. The field trials were conducted by using required concentration of bacterial pesticide in different breeding habitat such as overhead tank, cement tank and cement container, respectively. Selection of the localities was decided on the basis of the breeding potential and operational convenience. Field application of the bacterial pesticides was done with the help of a knapsack sprayer (or) hand sprayer. Biopesticide has sprayed uniformly at the surface of the water in each habitat. The mean larval density was calculated on the basis of 5 dips per each habitat. Prior to the experiment the surface area of the breeding habitat was measured along with the pre-spray density of larvae. After the treatment the post-spray density of larvae has been recorded after 24, 48 and 42 hours. Successive observations were made at an interval of three days. The percentage reduction was calculated by the following formula[16, 17].

$$\% \text{ Reduction} = 100 \quad \frac{C_1 \times T_2}{C_2 \times T_1} \quad \times 100$$

Where, C_1 and T_1 are pre-treatment density and T_2 and C_2 are the post-treatment density of larvae per dip in the control and treated habitats, respectively.

2.7. Statistical analysis

The percentage mortality observed (%M) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed using the

computer software SPSS 14 version and MS EXCEL 2003 to find the LC_{50}, regression equations (Y = mortality; X = concentrations) and regression coefficient values.

3. Results

The larval (first to fourth instar) and pupal mortalities after the treatment of spinosad at different concentrations (0.01, 0.02 0.04 0.06, 0.08 ppm) were showed in Table 1. The larval mortality ranged from 36.1 (0.01 ppm) to 79.3 (0.08 ppm) in the first instar larva, and from 30.0 (0.01 ppm) to 73.3±2.0 (0.08 ppm) in fourth instar larvae. Similar trend has been noticed for all larval instar of malarial vector, *An. stephensi* at different concentration of spinosad treatment. The pupal larval mortality ranged from 33.0 (0.01 ppm) to 80.0 (0.08 ppm). The LC_{50} and LC_{90} values increased from the 1st instar larvae to the 4th. The LC_{50} and LC_{90} values increased from the 1st instar larvae to the 4th and the value were 0.028 and 0.102, respectively (Table 2).

The field trail bioassay was carried out in two different breeding sites: Overhead tank and aquaculture tank at Bharathiar University Campus, Coimbatore, India. Larvae has been collected from these breeding sites were identified as *An. stephensi*.

In overhead tank, the pre-treatment larval density was 69.0±0.8 and the post treatment larval density were 18.3±1.2, 9.6±0.5, 4.0±0.8 in 24, 48 and 72 hours, respectively. The percent reduction of *Anopheles* larvae were 82.7%, 91.4 % and 96.0% after 24, 48, 72 hours, respectively, while more than 80% reduction was observed after 3 weeks. In aquaculture tanks, the larval density were 13.0±1.6, 6.5±0.5 and 2.5±0.7 after 24, 48 and 72 hours, respectively. The reduction of larval growth was 77.0 % in 24 h, followed by 98% reduction after 72 h. The analysis of one way ANOVA showed significance between aquaculture and overhead tanks ($P<0.01$).

Table 1
Larval and pupal toxicity effect of spinosad on *An. stephensi* (%)(Mean±SD).

Larval &Pupal stage	Mortaliy				
	0.01 ppm	0.02 ppm	0.04 ppm	0.06 ppm	0.08 ppm
I	36.1±1.7	49.2±2.1	60.0±2.5	73.4±2.2	79.3±1.8
II	33.4±1.2	43.0±2.1	58.2±1.2	72.0±1.2	79.0±1.7
III	28.0±2.1	44.0±1.2	55.8±1.2	68.2±2.2	75.1±5.0
IV	30.0±2.0	42.2±2.2	56.1±1.7	70.3±3.0	73.3±2.0
Pupa	33.0±2.0	49.0±0.9	59.0±2.1	72.0±1.4	80.0±0.9

Table 2
LC_{50} and LC_{90} values of larval and pupal toxicity effect of spinosad on *An. stephensi* Listn.

Larval & Pupal stage	LC_{50}	LC_{90}	Regression equation	95%confidence limit		Chi-square value (X^2)
				LCL	UCL	
I	0.001	0.011	Y=1.194 X+0.014	0.037	0.007	1.98
II	0.031	0.102	Y=-0.559 X+0.180	0.023	0.088	0.85
III	0.034	0.111	Y=-0.603 X+17.008	0.028	0.095	2.71
IV	0.036	0.113	Y=-0.574 X+16.431	0.027	0.096	0.80
Pupa	0.028	0.102	Y=-0.488 X+17.345	0.020	0.088	2.03

Significance at 0.05% level at DMRT; LCL: lower confidence limit, UCL: upper confidence limit.

4. Discussion

Spinosad, is a natural product of the fermentation of the bacterium *Saccharopolyspora spinosa*, and is a highly effective bioinsecticide against a broad range of agriculturally important insect pests. This agent has an excellent environmental and mammalian toxicological profile. Romi *et al*[18] has studied the efficacy of a spinosad-based product (Laser® 4.8% emulsifiable concentrate) by evaluating activity of laboratory bioassays against laboratory-reared mosquito strains of 3 species, *Aedes aegypti*, *An. stephensi* and *Culex pipiens*. Spinosad was particularly effective against larval *Aedes* and *Culex*, with a less marked activity against anophelines (24 h median lethal concentration=0.0096, 0.0064, and 0.039 mg/L, respectively), showing a persistence of the insecticide action of about 6 week in laboratory containers.

Bond *et al*[7] have been reported the naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Spinosad is a naturally derived biorational insecticide with an environmentally favorable toxicity profile, so we investigated its potency against mosquito larvae (Diptera: Culicidae).

The spinosad treated larvae and pupae had significant mortality and this toxicity is mainly due to the toxin produced by the bacterium, *Saccharopolyspora spinosa*. Further, Cisneros *et al*[19] reported that spinosad acts as a stomach and contact poison and degrades rapidly in the environment. An immediate effect of ingestion is the cessation of feeding, followed by paralysis and death 24 h later. This compound is a neurotoxin with a novel mode of

action involving the nicotinic acetylcholine receptor and GABA receptors[20]. This compound is a mixture of spinosyns A and D. It has shown activity against *Lepidoptera*, *Thysanoptera*, and other insect orders such as *Diptera*. This naturally-derivedinsecticide has been reported to have no adverse effects on predatory insects such as ladybirds, lacewings, big-eyed bugs, or minute pirate bugs[21].

Spinosad kills insects through activation of the acetylcholine nervous system by nicotinic receptors. The mode of action is unique and incompletely understood. Continuous activation of motor neurons causes insects to die of exhaustion. There may be some effects on the GABA and other nervous systems[11, 22-25]. When spinosad is applied to water, very little hydrolysis occurs, and the substance can be persistent. In the absence of sunlight, half lives of spinosyn A and D are at least 200 days. In water exposed to sunlight, photodegradation occurs[26].

In the present study spinosad also effectively caused mortality of mosquito larvae at the larboratory and field trial. It is also predict that spinosad is likely to be an effective larvicide for treatment of mosquito breeding sites.

Conflict of interest statement

We declare that we have no conflict of interest.

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