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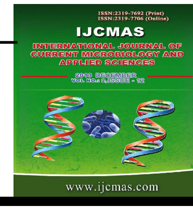

Original Research Article

Effect of microbial metabolite (Spinosad) against larval stages of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi*

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A B S T R A C T

Keywords

Spinosad; *Saccharopolyspora spinosa;* larvicidal activity; Mosquito sps

The naturally-derived insecticide Spinosad (TRACER) was evaluated against larval *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* under laboratory conditions. Laboratory bioassays showed that the 24 hLC_{50} andLC_{90}values against late 3rd and early 4th instars were estimated, at three different (0.01,0.1,1ppm) concentrations. Microbial metabolite Spinosad which showed significant effect against all the three dangerous mosquito species LC_{50} value of *Culex quinquefasciatus,*was 0.43, LC_{90} was 1.04,LC_{50} of *Aedes aegypti*was0.46,LC_{90}was1.27 and $LC_{50}$of*Anopheles stephensii*0.29, $LC_{90}$was 0.81. From this we concluded that spinosad have larvicidal effect on mosquitoes.

Introduction

Many species of mosquitoes are not blood eaters, and many of those that do create a "high to low pressure" in the blood to obtain and it do not transmit disease. In the bloodsucking species, only the females suck blood. Furthermore, even among mosquitoes that do carry important diseases. Among those the dangerous species such as *Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi* were focused in this study which is causing serious damaging to public.

Mosquitoes are members of the family nematocerid flies: the Culicidae (from the Latin *culex*, genitive *culicis* meaning "midge" or " gnat") (Jaeger, Edmund C. 1959). The word "mosquito" (formed by *mosca* and diminutive *ito*) is from the Spanish or Portuguese for "little fly". (Brown, Lesley 1993). Superficially, mosquitoes resemble crane flies (family Tipulidae) and chironomid flies (family Chironomidae); as a result, casual observers seldom realize the important differences between the members of the respective families.

In particular, the females of many species of mosquitoes are blood-eating pests and dangerous vectors of diseases, whereas members of the similar-looking Chironomidae and Tipulidae are not.

Culex quinquefasciatus (earlier known as *Culex fatigans*), the southern house mosquito, is the vector of lymphatic filariasis caused by the nematode *Wuchereria bancrofti* in the tropics and subtropics. It is also a vector of avian malaria in birds. This species is the primary vector of several arboviral diseases in the Southern United States, most particularly West Nile virus, and filariasis in India. It is a strong-winged domestic species seen all over India in and around human dwellings. Rapid urbanization and industrialization without adequate drainage facilities are responsible for its increased dispersal.

Anopheles is a genus of mosquito first described and named by J. W. Meigen in 1818. About 460 species are recognized; while over 100 can transmit human malaria, only 30–40 commonly transmit parasites of the genus Plasmodium, which cause malaria in humans in endemic areas. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malarial parasite species (to humans) – *Plasmodium falciparum*. *A.stephensi* is an important vector for both human and rodent malaria species such as: *Plasmodium falciparum* and *Plasmodium berghei*. In rural areas, the larvae of *A. stephensi* may exist in many aquatic habitats, such as: ponds, streams, swamps, marshes, and other sources of standing water (Rueda, 2008).

They may also occupy smaller environments, such as: tree holes, leaf axils, and man-made containers. (Harbach, 2007).Though larvae of *A. stephensi mysorensis* exclusively prefer to occupy stone pots and earthenware containers. (Sinka*etal*., 2011)This species is also able to endure high amounts of salinity, and have been found to breed readily in water where the salinity is equal to or even surpassing that of sea water (Maouchehri *et al.*, 1976) *A. stephensi* is considered to be endophilic and endophagic, Most larvae feed on microorganisms and particle matter that is suspended in water (Harbach, 2007)However, later in development, adult males will feed on the nectar of flowers, whereas females will take blood meals, which help produce viable eggs(Malhotra *etal*.,2000).

The yellow fever mosquito, *Aedes aegypti* is a mosquito that can spread the dengue fever, chikungunya and yellow fever viruses, and other diseases. The mosquito can be recognized by white markings on legs and a marking in the form of a lyre on the thorax. The mosquito originated in Africa(Laurence Mousson *etal*, 2005), but is now, found in tropical and subtropical regions throughout the world(Womack, 1993). *Aedes aegypti* is a vector for transmitting several tropical fevers. Only the female bites for blood which she needs to mature her eggs. To find a host, *Aedes aegypti* are attracted to chemical compounds that are emitted by mammals. These compounds include ammonia, carbon dioxide, lactic acid, and octenol (Dennis O'Brien, 2010). *Aedes aegypti* is a day biting mosquito which means the mosquito is most active during daylight, for approximately two hours after sunrise and several hours before sunset. The mosquito rests in indoors, in closets and other dark places. Outside, they rest where it is cool and shaded. The males of all species of mosquitoes do not bite humans or animals of any species, they live on fruit. The female of *Aedes aegypti* feed not only on fruit, but also on blood.

As of 2010 dengue fever is believed to infect 50 to 100 million people worldwide a year with 1/2 million life threatening infections (Whitehorn and Farrar, 2010). It has dramatically increased in frequency between 1960 and 2010 by 30 fold. This increase is believed to be due to a combination of urbanization, population growth, increased international travel and global warming. The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific (WHO 2009).

Spinosad is a natural fermentation product produced by an actinomycete, *Saccharopolyspora spinosa*. This compound is a mixture of spinosyns A and D.Spinosad (spinosyn A and spinosyn D) are a new chemical class of insecticides that are registered by the United States Environmental Protection Agency(EPA) to control a variety of insects. The active ingredient is derived from a naturally occurring soil dwelling bacterium called *Saccharopolyspora spinosa*, a rare actinomycete reportedly collected from soil in an abandoned rum distillery on a Caribbean Island in 1982 by a scientist on vacation (Mertz and Yao, 1990). It has not been found in nature since that time, and was subsequently described as a new species. The bacteria produce compounds (metabolites) while in a fermentation broth. The first fermentation-derived compound was formulated in 1988. Spinosad has since been formulated into insecticides that combine the efficacy of a synthetic insecticide with the benefits of a biological pest control organism.

Spinosad is a mixture of two tetracyclic macrolide neurotoxins, spinosyns A and D, those target the nicotinic acetyl-choline and GABA receptors of the insect's nervous system, leading to paralysis and death. Spinosad is currently used in agriculture to control dipteran, lepidopteron, thysanopteran and some coleopteran pest species in a diversity of crops worldwide.

Materials and Methods

Collection of eggs and maintenance of larvae

Laboratory colonization of mosquitoes

The eggs of *A. stephensi* were collected from road side ponds of Chidambaram, Tamilnadu and also from water stored containers with the help of 'O' type brush. These eggs were brought to the laboratory and transferred to 18 X 13 X 4 cm size enamel trays containing 500 ml of water for larval hatching. The mosquito larval and pupal culture was maintained in the laboratory. The plastic jars will be kept in 90 X 90X 90 cm size mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part was covered with a muslin cloth and bottom of the cage was fitted with 10% sugar solution for a period of three days before they will be provided with animal for blood feeding. The mosquito colony was maintained at 70-85% relative humidity (RH), 28 ± 2^{o}C temperature and 14:10 light and dark photoperiod cycle. The larvae were fed with powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquito and 5% glucose solution and honey were given to the male adult mosquitoes (Aarthi and Murugan, 2010).

Preparation of Spinosad

Naturally derived insecticide SPINOSAD, Trade name - Tracer was purchased from Bharathi Agro Centre at Athur (Salem District), Tamil Nadu, India. Required quantity of Spinosad was thoroughly mixed with distilled water to prepare various concentrations like 0.01 ppm, 0.1 ppm, 1ppm, where 1 ppm is equivalent to 1mg\liter.

Mosquito larvicidal activity

Larvicidal activity of Spinosad against three mosquito species *viz.,Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefacialus* were observed under *in vitro* conditions. The larvicidal activity was carried out at different concentrations by preparing the required stock solution by following the standard procedure (WHO, 1996). The desired concentrations of the test solution were achieved by adding 1.0 ml of an appropriate stock solution to 249 ml of dechlorinatedwater;6 replicates for each concentration were maintained. Twenty five numbers of late third larvae introduced into the beaker were obtained from the laboratory colony. The larval mortality in both treated and control was recorded after 24 hours.

Statistical analysis

The percentage of mortality was calculated using Abbott's formula (Abbott, 1925).

% Mortality = <u>Mortality at treatment-mortality at control X 100</u>

100- Mortality at control.

The statistical evaluation of LC_{50}, LC_{90}, regression equation and 95% confidence limit, LCL and UCL were calculated from the data, which was carried out by probit analysis (Finney, 1971).The experimental data were processed statistically by applying the SPSS software, version 11.5 (Panse and Sukhatine, 1978). The critical difference was worked out at five per cent probability level for significant results (P=0.05).

Result and Discussion

Mosquito larvicidal activity of spinosad against *culex quinquefasciatus*

Larvicidal activity of spinosad was tested against *Culex quinquefasciatus* larvae at different concentrations. The LC_{50} value of spinosad was in the range of 0.43 ppm against *Culex quinquefasciatus* (Table-1). The $LC_{90,}$ LCL, UCL and $\chi 2$ values were also interpreted.

Mosquito larvicidal activity of spinosad against *aedes aegypti*

Larvicidal activity of spinosad was tested against *Aedes aegypti* larvae at different concentrations. The LC_{50} value of spinosad was in the range of 0.46 ppm against *Aedes aegypti* (Table-1). The $LC_{90,}$ LCL, UCL and $\chi 2$ values were also interpreted

Mosquito larvicidal activity of spinosad against *anopheles stephensi*

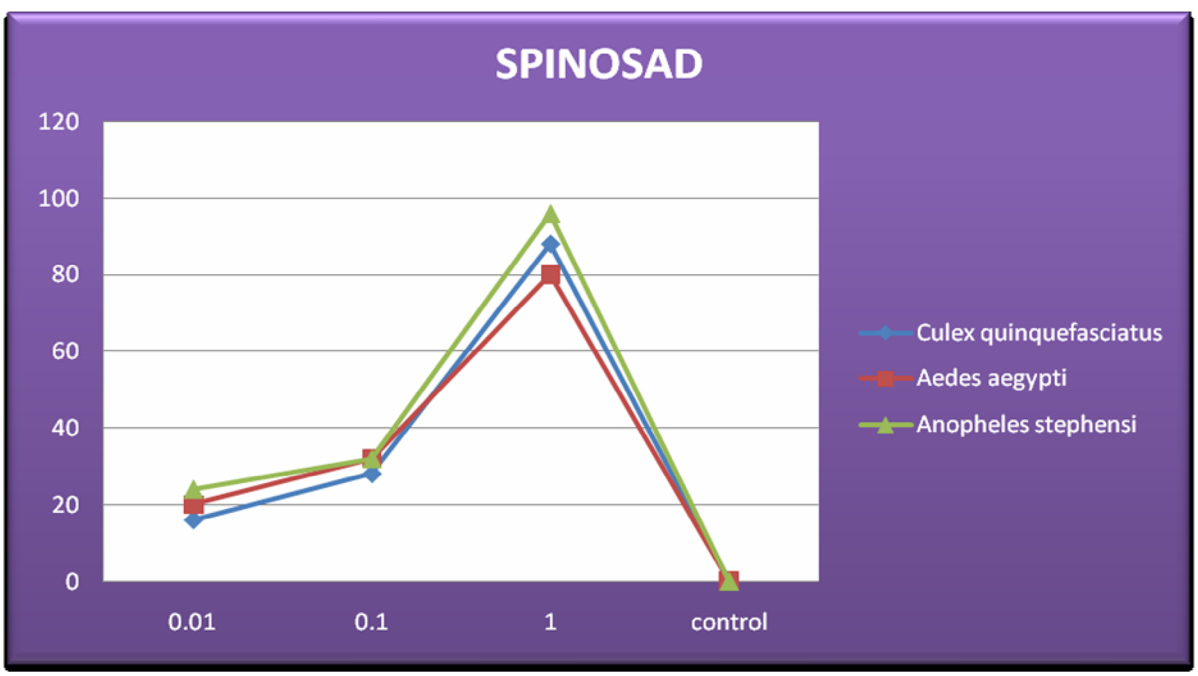
Larvicidal activity of Spinosad was tested against *Anopheles stephensi* larvae at different concentrations. The LC_{50} value of spinosad was in the range of 0.29 ppm against *Anopheles stephensi* (Table-1). The $LC_{90,}$ LCL, UCL and $\chi 2$ values were also interpreted.

Table.1 Larvicidal activity of Spinosad against three different Mosquito species

Mosquito species	Lethal concentrations*				
	IV instar	Dose (ppm)	Mortality (%)	χ2	Microbial metabolite
	LC50 LC90				
Culex quinquefasciatus	0.43 1.04 (0.27-0.63) (0.80-1.53)	0.01 0.1 1.0 Control	16 28 88 0	0.312	**Spinosad**
Aedes aegypti	0.46 1.27 (0.26-0.72) (0.94-2.06)	0.01 0.1 1.0 Control	20 32 80 0	0.358	
Anopheles stephensi	0.29 0.81 (0.15-0.47) (0.59-1.27)	0.01 0.1 1.0 Control	24 32 96 0	0.002	

*All values are in ppm; Figures in 95% confidential limits

Fig.1 Graphical representation of Spinosad concentration against *Culex quinquefasciatus*, *Aedes aegyptiand Anopheles stephensi*



The efficacy of Spinosad was tested against three different mosquito species namely, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi.* The larvicidal activity of mosquitoes varies at different concentrations their mortality percentage also differs. This shows that when the concentration increases the mortality percentage also increases.

Huseyin Cetin*et al.*, 2005 worked on the naturally-derived insecticide spinosad (Conserve® SC) against larval *Culex pipiens*L. (Diptera: Culicidae) under laboratory and field conditions in Antalya, Turkey. The same species of mosquito whichwe used under laboratory condition. Their concentration range differs from us. Laboratory bioassays showed that the 24 hLC50 and LC90 against late 3rd and early 4th instars were estimated at 0.027 and 0.111 parts per million, (ppm) respectively, while adult emergence was eliminated at concentrations above 0.06 ppm. Larval mortality from septic tanks that were treated with spinosad at rates of 25, 50, 100 and 200 g a.i/ha ranged between 22 to 78% 1 day after application. At 7 days post-treatment, larval mortality ranged from 2 to 50% and at 14 days mortality was <10% for all treatments.

Larval bioassays of the water from those septic tanks treated at 100 and 200 g ai/ha resulted in an elimination of *Culex pipiens* larvae 7 days after treatment. After this time, larval reduction declined to 79 and 83%, respectively, 14 days after treatment. Larval reduction in septic tanks treated at the two lowest rates (i.e. 25 and 50 g ai/ha) ranged from 14 to 74% during the 14-day study. These results indicated that spinosad can be considered an effective larvicide for treatment of septic tanks against *Culex pipiens.* They have compared the laboratory larvicidal activity with that of the septic tank field treatment. Laboratory bioassays and the larval species tested were very similar to that of (Bond *et al.*, 2004) Spinosad proved to be highly toxic to larvae of both species of mosquitoes in laboratory assays. The response of *Aedes aegypti* larvae closely followed the logit model, with an estimated LC50 of 0.025 ppm (spinosad). As we tested in our work the same microbial metabolite spinosad was tested against *Aedes aegypti mosquito larvae.* Bond *et al* used different ppm concentrations to test the mosquito sp. such as0.001, 0.003, 0.01, 0.03, 0.1 ppm. Whereas we used the concentrations viz., 0.01, 0.1, 1.0 which is more or less similar to that study.

(Nareshkumar *et al.,* 2011) research reveals the toxicity effect of Spinosad and Neem Seed Kernel Extract (NSKE) against different larval stages of *Anopheles stephensi* and *Chironomus circumdatus*. The similar methodology, the concentrations range which is most probably similar, and the mosquito larvae used in this study is also same among the three species used in our study.

(Romi *et al*, 2006)studied the efficacy of a Spinosad-based product(Laser® 4.8% emulsifiable concentrate) was evaluated in laboratory bioassays against laboratory-reared mosquito strains of 3 species of medical importance: *Aedes aegypti, Anopheles stephensi,* and *Culex pipiens.*Spinosad was particularly effective against larval *Aedes*and *Culex*, with a less marked activity against*Anopheles* (24-h median lethal concentration = 0.0096,0.0064, and 0.039mg/liter, respectively), showing apersistence of the insecticide action of about 6 wk in laboratory containers, which is a very similar work with little differentiation in the range in ppm concentration and larval species.

Gloria E Antonio *et al*., 2008 have worked on paradoxical effects of sub lethal exposure to the naturally derived spinosad in the dengue vector mosquito, *Aedes aegypti* in which they used the method to maintain the mosquito, the Spinosad insecticide (trade name) which is very similar to that of our study.

In Determination and confirmation of the LC50 value also they used the same method .the ppm concentration range which is more or less similar to our study and the results 50% mortality value was calculated using t-test which is also similar.

So, from this we can conclude from the results and discussion that Spinosad, a biopesticide that has high toxic effect against three mosquito species *Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi,* and. The effect was seen at higher concentration only (10 PPM).

References

Aarthi, N., and Murugan K. 2010. Larvicidal and repellent activity of *Vetiveria zizanioides* L, *Ocimum basilicum* Linn and the microbial pesticide Spinosad against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae) J. Biopest. 3(1 Special Issue) 199 – 204.

Bond, J.G., Marina, C.F. and Williams T. 2004. The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Med. Vet. Entomol.18:50–56

Brown Lesley., 1993. The New shorter Oxford English dictionary on historical principles. Oxford [Eng.]: Clarendon. ISBN 0-19-861271-0.

Darriet, F., Duchon S and Hougard, J.M. 2005. Spinosad: a new larvicide against insecticide-resistant mosquito larvae. J Am. Mosq.Contr.Assoc.21:495–496

Dennis O'Brien., 2010. "ARS Study Provides a Better Understanding of How Mosquitoes Find a Host". U.S. Department of Agriculture. Archived from the original on 8 October 2010.Retrieved 2010-08-27.

Harbach, R.E., 2007. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa*. 1668: 591-629.

Huseyin, C., Atila Y, and James E. Cilek. 2005. Evaluation of the naturally-derived insecticide spinosad against *Culex pipiens*L. (Diptera: Culicidae) larvae in septic tank water in Antalya, Turkey. J. Vector. Ecol. 30 (1): 151-154.

Jaeger, Edmund C. 1959. A Source-Book of Biological Names and Terms. Springfield, Ill: Thomas. ISBN 0-398-06179-3.

Laurence, M., Catherine D, Thomas G, Francis S, Marie V and Anna-Bella Failloux. 2005. "Phylogeography of *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations" *Genetics Res.* 86 (1): 1–11.

Liu, H. , Cupp EW, Guo A and Liu, N. 2004. Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. J. Med. Entomol. 41:946–952

Malhotra, P.R., Jatav, CP, and Chauhan, R.S. 2000. Surface morphology of the egg of *Anopheles stephensi stephensi* sensustricto (Diptera, Culicidae). Italian. J. Zool. 62: 147-

151.

Maouchehri, A.V., Javadian E, Eshghy, N, &Motabar, M. 1976. Ecology of *Anopheles stephensi* Liston in southern Iran. Trop. Geographical Med. 28: 228-232.

Mertz, E.P., and Yao, R.C. 1990. *Saccharopolyspora spinosa*. Isolated from soil collected in sugar rum still. Inter. J. Syst. Bacteriol. 40: 34-39.

Romi, R., Proietti S, Di Luca M, and Cristofaro, M. 2006. Laboratory evaluation of the bioinsecticide Spinosad for mosquito control. J. American .Mosquito Control Asso. 22 (1):93-6.

Rueda, L.M. , 2008. Global diversity of mosquitoes (Insecta: Diptera: Culicidae). Hydrobiologia. 595 477-487

Sinka, M.E., Bangs, M.J, Manguin, S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething P W, Elyazar, IRF, Kabaria CW, Harbach RE and Hay, S.I. 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasites. Vectors. 4: 1-46.

Whitehorn, J., and Farrar, J. 2010. "Dengue". Br. Med. Bull. 95: 161–73.

WHO., 2009. pg.3

Womack, M., 1993. "The yellow fever mosquito, *Aedes aegypti*". Wing Beats. 5 (4): 4.