Evaluation of the naturally-derived insecticide spinosad against *Culex pipiens* L. (Diptera: Culicidae) larvae in septic tank water in Antalya, Turkey

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ABSTRACT: The naturally-derived insecticide spinosad (Conserve® SC) was evaluated against larval *Culex pipiens* L. (Diptera: Culicidae) under laboratory and field conditions in Antalya, Turkey. Laboratory bioassays showed that the 24 h LC_{50} and LC_{90} against late 3rd and early 4th instars were estimated at 0.027 and 0.111 parts per million, 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 ai/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 *Cx. 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 *Cx. pipiens*. ***Journal of Vector Ecology*** **30 (1): 151-154. 2005.**

Keyword Index: Culex pipiens, biological control, spinosad, septic tank.

INTRODUCTION

Spinosad is a natural fermentation product produced by an actinomycete, *Saccharopolyspora spinosa* Mertz and Yao. 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-derived insecticide has been reported to have no adverse effects on predatory insects such as ladybirds, lacewings, big-eyed bugs, or minute pirate bugs (Kirst et al. 1992, DeAmicis et al. 1997, Copping 2001, Williams et al. 2003). Spinosad acts as a stomach and contact poison and degrades rapidly in the environment (Cisneros et al. 2002). An immediate effect of ingestion is the cessation of feeding, followed 24 h later, by paralysis and death. This compound is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and GABA receptors (Watson 2001). Spinosad has little toxicity to birds and mammals (Bret et al. 1997, Breslin et al. 2000). There is no cross-resistance to synthetic and traditional biological insecticides (Salgado 1997, 1998). Moreover, spinosad is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Saunders and Bret 1997).

We report here the results of studies to determine the relative effectiveness of spinosad against *Culex pipiens* L. (Diptera: Culicidae) larvae in septic tank water under laboratory and field conditions in Antalya, Turkey.

MATERIALS AND METHODS

To assess the susceptibility of larval *Cx. pipiens* to spinosad, field-collected late 3rd and early 4th instars were exposed to septic tank water, treated with five concentrations (0.01-0.12 parts per million ai spinosad) using the methods of WHO (1996). Ten ml of Conserve® SC (containing Spinosyn A and Spinosyn D 120 g/l ai; NAF 313) was dissolved in unchlorinated water that served as the stock solution for the above concentrations. Groups of 25 larvae, collected from a septic tank in Varsak, Antalya, Turkey during August 2004, were transferred into plastic containers each containing 1 L of untreated septic tank water using a Pasteur pipette. Each concentration was then applied onto the water surface of each container with an automatic pipette. Four replicates for each concentration and control (septic tank water only) were used. Experiments were repeated three times. Larval mortality was recorded 24 h after treatment. Larval mortality was determined by dividing the total number of dead larvae by the initial number of late 3rd and early 4th instars. During the study no food was offered to the larvae. A 12L:12D photoperiod, 60±10% relative humidity, and temperature of 27±2 °C were maintained in the laboratory during all bioassays.

Field studies were conducted in Varsak, Antalya during August 2004. Spinosad (Dow AgroSciences, Estuary Road King's Lynn Norfolk PE30 2JD, United Kingdom) was evaluated at field application rates of 25, 50, 100, and 200 g ai/ha. These rates were based on preliminary studies that resulted in between 10 and 95% larval reduction under field-conditions at 24 h. Test material was mixed with tap water in a hand compression sprayer and applied using the procedures of Yapabandara and Curtis (2002). Application volumes for each septic tank were determined by measuring the surface area of each septic tank, while including a pump emission rate of about 600 ml/min. A total of 20 septic tanks were used in this study. Four septic tanks were maintained untreated as controls.

Larvae in septic tanks were sampled using a white plastic dipper with a capacity of 500 ml at 1, 7, and 14 days after treatment. A minimum of five dips was taken at various points along the surface of each septic tank and the number of larvae per dip recorded. Water, pH, and temperature were also recorded in each tank during sampling. Percent larval reduction of *C. pipiens* in each treated septic tank was calculated using the formula of Mulla et al. (1971):

$$\% \text{ reduction} = 100 - [(C_1/T_1) \times (T_2/C_2) \times 100]$$

Where C_1=pretreatment population density in control habitat; C_2=postreatment population density in control habitat; T_1=pretreatment population density in treatment habitat; T_2= postreatment population density in treatment habitat.

Simultaneous laboratory bioassays of water from spinosad-treated and untreated septic tanks were conducted to assess the residual activity of the product under field conditions of daily dilution by septic tank pumps. Bioassay procedures used the methods of Ali et al. (1999). One day before, and at intervals of 1, 7, and 14 days after treatment, 1-liter water samples (at surface) were collected from treated and control septic tanks and taken to the laboratory. Larval bioassays were conducted immediately after collection of water samples using 25 field collected 3rd and 4th *C. pipiens* instars. Testing continued until all larvae died or developed to the adult stage.

Statistical analysis

LC_{50} and LC_{90} values were calculated using the probit analysis program of Schoofs and Willhite (1984). All data were subjected to ANOVA (SPSS 1999). Percentage mortalities were compared by a chi-square test (P>0.05). Mean larval mortality and abundance data were compared among concentrations within time and between time within concentrations using Duncan's Multiple Range Test ($P < 0.05$) (SPSS 1999).

RESULTS AND DISCUSSION

Spinosad 24 h LC_{50} and LC_{90} values for late 3rd and early 4th instar *C. pipiens* were calculated at 0.027 (0.002-0.057) and 0.111 (0.054-5.383) ppm, respectively. Adult emergence was eliminated at concentrations above 0.06 ppm in laboratory studies. In controls, mortality ranged from 1-4% for 3rd and 4th instars while all other larvae pupated and emerged successfully. In field studies, application rates of 100 and 200 g ai/ha were significantly more effective than those of 25 and 50 g ai/ha (Table 1). At 1 and 7 days after treatment there was complete larval reduction in septic tanks treated at 100 g ai/ha and 200 g ai/ha. At 14 days, reduction was significant between 79 and 83%, respectively, at these two application rates. The highest application rates were also equally effective during the 14-day persistence study. In the larval bioassays with treated septic tank water, spinosad generally showed little (<6%) larval toxicity 14 days after treatment at any of the application rates (Table 2). This indicated that the addition of waste water and/or high organic materials may quickly degrade and/or dilute spinosad residues. During the evaluation periods, septic tank water temperatures ranged between 27^o and 30^{o}C and pH ranged between 6.8 and 7.6.

Activity of spinosad against mosquitoes has previously been reported by Bond et al. (2004). They found that spinosad applied at a concentration of 10 ppm inhibited adult production of *Aedes aegypti* (L.) for a 22-week period in unpolluted water. It is well-known that the organic content of water affects the longevity of compounds when applied as mosquito larvicides (Mulla and Darwazeh 1988, Mulla 1995). Indeed, our study showed that the minimum effective dosage for spinosad that caused 100% larval reduction of *C. pipiens* for 1-7 days was considerably greater (i.e. 100 to 200 g ai/ha) for septic tanks with a moderate residual effect for an additional 7–14 days after treatment. This product may provide an alternative when one compares the reduced risk to humans and its excellent environmental profile compared with synthetic insecticides of similar use (Saunders and Bret 1997). Stark and Vargas (2003) showed that spinosad was at least five times less toxic than diazinon (OP) compared to *Daphnia pulex* at a similar rate of application. Liu et al. (2004) reported that three strains of *Culex quinquefaciatus* Say showed high levels of susceptibility to spinosad in the southeastern United States. Also, Hyun et al. (2003) reported that 74 to 6-fold pyrethroid-resistant *Anopheles sinensis* (L.) larvae were found to be as nearly susceptible to spinosad as chlorfenapyr in laboratory tests.

Although the cost of this biological insecticide is greater than organophosphate insecticides, it is less harmful to humans and the environment. Moreover, spinosad appears to be a viable alternative larvicide, thereby preventing further pollution of ground water, surface water, and other components of the ecosystem.

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REFERENCES CITED

Ali, A., M.A. Chowdhury, M.I. Hossain, M.U. Ameen, D.B. Habiba, and A.F.M. Aslam. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. J. Am. Mosq. Contr. Assoc. 15: 43-47.

Bond, J.G., C.F. Marina, and T. Williams. 2004. The naturally

Table 1. Effect of spinosad treatment (0-200 g ai/ha) on the number of *Culex pipiens* larvae present in septic water tanks Figures in parentheses indicate percentage reduction.

Application rate (g ai/ha)	Mean no. larvae[x]/ dip ± SE			
	Pre-treatment	Days after treatment 1	7	14
25	21 ± 1.9 (0)	16 ± 3.7 (14)a[α], A[β]	19 ± 4.0 (40) a, B	26 ± 4.2 (0) a, C
50	36 ± 5.0 (0)	9 ± 1.5 (74)b, A	16 ± 3.3 (74) b, A	29 ± 6.5 (40) a, B
100	43 ± 6.9 (0)	0 ± 0.0 (100) c, A	0 ± 0.0 (100) c, A	10 ± 2.9 (79) b, B
200	29 ± 4.4 (0)	0 ± 0.0 (100) c, A	0 ± 0.0 (100) c, A	8 ± 2.3 (83) b, B
Control	24	21	36	29

[α]Means within a row followed by the same lower case letter are not significantly different, Duncan's multiple range test ($P<0.05$).
[β]Means within a column followed by the same capital letter are not significantly different, Duncan's multiple range test ($P<0.05$).
[x]All instars.

Table 2. Larvicidal activity of spinosad collected from septic tanks following application of 0-200 g ai/ha. Activity determined by bioassay against late 3rd and early 4th instar *Culex pipiens* in the laboratory (Mean abundance ± SE).

Time after treatment (days)	Untreated Control	Spinosad 25 g ai/ha	50 g ai/ha	100 g ai/ha	200 g ai/ha
Pre-treat	2.0 ± 1.1 a[x],A[y]	0.0 ± 0.0 a,A	1.0 ± 0.7 a,A	1.3 ± 0.7 a,A	0.3 ± 0.3 a,A
1	2.0 ± 1.4 a,A	22.0 ± 6.3 b,B	42.0 ± 0.0 b,C	54.6 ± 7.9 b,C	78.3 ± 7.7 b,D
7	0.6 ± 0.6 a,A	6.8 ± 3.3 a,A	2.0 ± 1.3 a,A	45.0 ± 9.0 b,B	50.0 ± 6.9 c,B
14	0.6 ± 0.6 a,A	1.0 ± 0.7 a,A	1.3 ± 0.8 a,A	3.0 ± 1.6 a,A	5.67 ± 2.5 a,A

[x]Means within a row followed by the same lower case letter are not significantly different, Duncan's multiple range test ($P<0.05$).
[y]Means within a column followed by the same capital letter are not significantly different, Duncan's multiple range test ($P<0.05$).

derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Med. Vet. Entomol. 18: 50-56.

Breslin, W.J., M.S. Marty, U. Vedula, A.B. Liberacki, and B.L. Yano. 2000. Developmental toxicity of spinosad administered by gavage to CD rats and New Zealand white rabbits. Food Chem. Toxicol. 38: 1103-1112.

Bret, B.L., L.L.Larson, J.R. Schoonover, T.C. Sparks, and G.D. Thompson. 1997. Biological properties of Spinosad. Down to Earth 52: 6-13.

Cisneros, J., D. Goulson, L.C. Derwent, D.I. Penagos, O. Hernández, and T. Williams. 2002. Toxic effects of spinosad on predatory insects. Biol. Contr. 23: 156–163.

Copping, L.G. and J.J. Menn. 2001. Biopesticides: a review of their action, applications and efficacy. Pest Manag. Sci. 56: 651–676.

DeAmicis, C.V., J.E. Dripps, C.J. Hatton, and L.L. Karr. 1997. Physical and biological properties of the spinosyns: Novel macrolide pest-control agents from fermentation. In: P. A. Hedin, R. M. Hollingworth, E. P. Masler, J. Miyamoto, and D. G. Thompson (eds.) *Phytochemicals for Pest Control,* Symposium Series 658, pp. 144–154. American Chemical Society, Washington, D.C.

Hyun, S.E., Y.I. Park, H.I. Lee, W.J. Lee, Y.H. Shin, and J.C. Shim. 2003. Insecticide susceptibilities of *Anopheles sinensis* (Diptera: Culicidae) larvae from Paju-shi, Korea. Korean J. Entomol. 33: 33-37.

Kirst, H.A., K.H. Michel, J.S. Mynderse, E.H. Chio, R.C. Yao, W.M. Nakatsukasa, L.D. Boech, J.L. Occlowitz, J.W. Paschal, J.B. Deeter, and G.D. Thompson. 1992. Discovery, isolation, and structure elucidation of a family of structurally unique fermentation-derived tetracyclic macrolides. In: D.R. Baker, J.G. Fenyes, and J.J. Steffans (eds.) *Synthesis and Chemistry of Agrochemicals III*, pp. 214–225. American Chemical Society, Washington, D.C.

Liu, H.Q., E.W. Cupp, K.M. Micher, A.G. Guo, and N.N. Liu. 2004. Insecticide resistance and cross resistance in Alabama and Florida strains of *Culex quinquefaciatus*. Med. Entomol. 41: 408-413.

Mulla, M.S., R.L. Norland, D.M. Fanara, H.A. Darwazeh, and D.W. Mc-Kean. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64: 300-307.

Mulla, M.S. 1995. The future of insect growth regulators in vector control. J. Am. Mosq. Contr. Assoc. 11: 269-273.

Mulla, M.S. and H.A. Darwazeh. 1988. Activity of insect growth regulators against mosquito larvae in dairly wastewater lagoons. J. Am. Mosq. Contr. Assoc. 4: 322-325.

Salgado, V.L. 1997. The mode of action of spinosad and other insect control products. Down to Earth 52: 35-44.

Salgado, V.L. 1998. Studies on the mode of action of spinosad: Insect symptoms and physiology correlates. Pest. Biochem. Physiol. 60: 91-102.

Saunders, D.G. and B.L. Bret. 1997. Fate of spinosad in the environment. Down to Earth 52: 14–20.

Schoofs, G.M. and C.C. Willhite. 1984. A probit analysis program for the personal computer. J. Appl. Toxicol. 4: 141-144.

SPSS 1999. Base 10.0 Application Guide, SPSS, Inc., 426 pp.

Stark, J.D. and R.I. Vargas. 2003. Demographic changes in *Daphnia pulex* (Leydig) after exposure to the insecticides spinosad and diazinon. Ecotoxicol. Environ. Safety 56: 334-338.

United States Enviromental Protection Agency. 2002. Pesticides: topical and chemical fact sheets: larvicides for mosquito control. URL: www.epa.gov/pesticides/factsheets/pesticides4mosquitos.htm.

Watson, G.B. 2001. Actions of insecticidal spinosyns on c-aminobutyric acid responses from small-diameter cockroach neurons, Pest. Biochem. Physiol. 71: 20-28.

WHO 1996. Report of the WHO Informal Consultation on the evaluation and testing of insecticides, WHO, Geneva, 7-11 October. CTD/WHO PES/IC/96.1.

Williams, T., J. Valle, and E. Viñuela. 2003. Is the naturally derived insecticide spinosad® compatible with insect natural enemies? Biocont. Sci. Technol. 13: 459-475.

Yapabandara, A.M.G.M. and C.F. Curtis. 2002. Laboratory and field comparisons of pyriproxfen, polystyrene beads and other larvicidal methods against malaria vectors in Sri Lanka. Acta Trop. 81: 211-223.