EFFICACY OF A FLOWABLE CONCENTRATE FORMULATION OF *BACILLUS THURINGIENSIS* (H-14) AGAINST LARVAL MOSQUITOES IN SOUTHERN IRAN

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ABSTRACT. A flowable concentrate formulation of *Bacillus thuringiensis* (H-14) [Bactimos FC® (1000 ITU/mg)] was evaluated for the control of mosquito larvae in simulated ponds and natural breeding sites in Kazeroun (Fars Province), southern Iran. A comparison was made with Abate® emulsifiable concentrate. Bactimos FC caused 93–96% anopheline and 97% culicine larval mortality 24 h posttreatment in simulated ponds and natural breeding sites, when used at the rate of 0.2 cc/m². Abate (0.015 cc/m²) resulted in significantly higher anopheline (98.1%) and culicine (100%) mortality at 24 h posttreatment. There was a relatively sharp decline in larval mortality 48 h posttreatment when Bactimos FC was applied. Five-day applications were suggested to prevent pupal production.

INTRODUCTION

Bacillus thuringiensis (H-14) (*B.t.i.*) has been successfully utilized as a microbial insecticide for the control of mosquito larvae (Lacey and Undeen 1986). The protein crystal produced by the bacterium is toxic to Culicidae, Simuliidae and certain Chironomidae and Dixidae (Margalit and Dean 1985) and has no activity against other invertebrate or vertebrate nontarget organisms (Lacey and Mulla 1990). Details of the mode of action and pathogenesis in mosquitoes are reviewed by Lacey and Undeen (1986).

The efficacy of *B.t.i.* is greatly influenced by species, age and feeding behavior of the target mosquito species as well as the vegetative cover, sunlight, water quality, formulation and method of application of the larvicide (Lacey and Lacey 1990). A variety of commercial formulations of *B.t.i.* are produced worldwide. These include wettable powders, granules and flowable concentrates (FC). The latter formulation (FC) has permitted a great number of options for application and, hence, have resulted in more efficacious control (McLaughlin and Vidrine 1984a, 1984b; Yates 1984, Sandoski et al. 1985).

Larviciding by the use of oil and chemical insecticides as a component of integrated mosquito control programs is still used in Iran, especially in anti-malaria campaigns. More than 16 million liters of fuel oil and 45,000 liters of temephos (Abate®) have been used in anti-malaria programs during the past 3 years. However, the need to minimize the environmental impact of these larvicides as well as slowing down the rate of insecticide resistance development has stimulated interest in evaluation of a flowable concentrate formulation of *B.t.i.* in southern Iran, where malaria is still the most important health problem. The present study determined the proper dosage of the larvicide for effective mosquito control, as well as compared its efficacy with Abate.

MATERIALS AND METHODS

The *B.t.i.* formulation used in this study was Bactimos® flowable concentrate (FC) (1,000 ITU/mg), provided by Duphar, Weesp, Holland. Tests were conducted in simulated ponds and natural mosquito breeding sites in Kazeroun, Fars Province (latitude 29° 37′ N and longitude 51° 38′ E), during September to October 1989.

In order to find the proper dosages of Bactimos FC for mosquito larval control, the efficacy of 4 dosages of 0.1, 0.2, 0.3 and 0.5 cc/m² were tested in simulated ponds. Eight ponds, each 1 m², 40–60 cm deep, exposed to sunlight with no vegetation (pH = 7.4), were allocated randomly to each dosage of the larvicide and 8 were left out as controls. The premeasured amounts of larvicide, carried to the field in separate vials, were well mixed with 1 liter of tap water and carefully sprayed during late afternoon to the surface of the pond, with a hand-held plastic sprayer.

For comparing the efficacy of the effective dosage tested of Bactimos FC (0.2 cc/m²) with Abate, 15 simulated ponds, 1 m², 40–60 cm deep, were allocated randomly to each larvicide and 7 ponds were left as untreated controls. Abate emulsifiable concentrate (44% AI) was applied at the rate of 0.015 cc/m². The method of application of the 2 larvicides was the same as described in the previous experiment.

The efficacy of Bactimos was also studied in natural mosquito breeding sites by applying the larvicide (0.2 cc/m²) to 7 stream bed pools (pH = 7.5) with no vegetation, totaling 224 m², at 7-day intervals for 1 month. Two pools totaling 50 m² were allocated as untreated controls. A Hudson backpack sprayer equipped with a TX3 nozzle was used for applying the larvicide to the natural breeding sites.

The effectiveness of the larvicides were assessed, using the percent reduction of the 1 + 2 and 3 + 4 instar larvae in posttreatment counts

Table 1. Efficacy of Bactimos FC against mosquito larvae in simulated ponds, Kazeroun, Fars Province, September–October 1989.

Concentration cc/m²	Mosquito larvae[1]	Mean no. of larvae/dip pretreatment	Mean % reduction/larval instars (h posttreatment) 24 h 1+2	24 h 3+4	24 h Total	48 h 1+2	48 h 3+4	48 h Total	72 h 1+2	72 h 3+4	72 h Total
0.1	*Anopheles*	45.4	94.6	100	95.7	77.5	100	81.6	66.6	100	72.7
	Culex	35.6	94.5	100	94.4	64.9	100	78.4	53.7	100	62.1
0.2	*Anopheles*	47.1	95.2	100	96.1	79.4	100	82.5	54.5	100	59.3
	Culex	41.1	97.6	100	97.2	79.0	100	84.9	50.3	100	60.8
0.3	*Anopheles*	60.4	97.6	100	98.1	90.0	100	92.5	72.5	100	76.9
	Culex	26.6	97.4	100	98.5	76.9	100	87.6	57.5	100	66.3
0.5	*Anopheles*	50.7	97.4	100	98.0	86.2	100	88.7	74.1	100	79.4
	Culex	41.5	98.5	100	98.7	85.9	100	88.7	70.0	100	73.9
Control	*Anopheles*	29.8	8.2	3.4	5.4	12.5	9.1	10.5	27.1	10.7	22.7
	Culex	23.2	2.0	100	1.0	12.8	100	7.8	21.2	100	18.1

[1] *Anopheles stephensi, An. superpictus, An. fluviatilis, Cx. theileri, Cx. perexiguus* and *Cx. tritaeniorhynchus.*

Table 2. Comparing the efficacy of Bactimos FC to Abate EC in reducing mosquito larval population in simulated ponds, Kazeroun, Fars Province, September–October 1989.

Larvicide and concentration	Mosquito larvae[1]	Mean no. of larvae/dip pretreatment	Mean % reduction/larval instars (h posttreatment) 24 h 1+2	24 h 3+4	24 h Total	48 h 1+2	48 h 3+4	48 h Total
Bactimos FC, 0.2 cc/m²	*Anopheles*	40.4	91.0	100	93.8	78.8	100	85.5
	Culex	47.0	96.4	100	97.3	79.0	100	84.0
Abate EC, 0.015 cc/m²	*Anopheles*	44.2	97.2	100	98.1	82.5	100	87.0
	Culex	37.7	99.9	100	100	98.7	100	99.1
Control	*Anopheles*	37.2	2.4	0	2.4	0.6	0	0.6
	Culex	20.0	6.0	3.1	7.1	1.1	8.9	10.0

[1] *Anopheles stephensi, An. superpictus, An. fluviatilis, Cx. theileri, Cx. perexiguus* and *Cx. tritaeniorhynchus.*

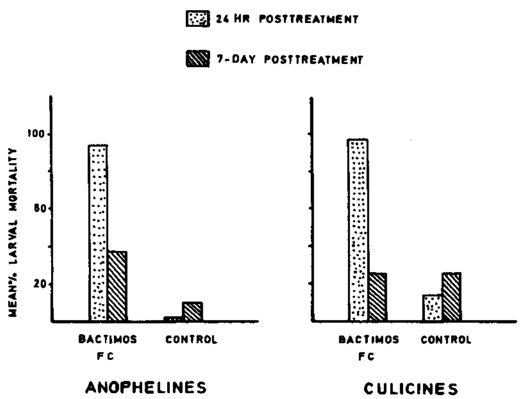

Fig. 1. Larvicidal activity of Bactimos FC in natural mosquito breeding sites in Kazeroun, Fars Province, October 1989.

vs. pretreatment in the treated ponds. The larval population at each pond was determined by taking at least 10 dips per pond. Data were subjected to ANOVA for testing the hypothesis that mean mortality values among treatments were equal.

Anopheline larval populations in the simulated ponds consisted of *Anopheles stephensi* Liston (83%), *An. superpictus* Grassi (10%), *An. fluviatilis* James (7%); and the culicine population comprised *Culex theileri* Theobald (66.1%), *Cx. perexiguus* Theobald (23.7%) and *Cx. tritaeniorhynchus* Giles (10.2%). The anopheline and culicine larval populations in natural breeding sites consisted of *An. dthali* Patton (66.4%), *An. turkhudi* Liston (17.2%), *An. stephensi* (9.4%), *An. superpictus* (7%); *Cx. bitaeniorhynchus* Giles (60%), *Cx. mimeticus* Noe (28%), *Cx. perexiguus* (9%) and *Cx. tritaeniorhynchus* (3%).

RESULTS AND DISCUSSION

The results of the test to determine the optimum dosage for field application of Bactimos FC against anopheline and culicine larval populations, used at the rates of 0.1, 0.2, 0.3 and 0.5 cc/m² in 1 m² simulated ponds, are presented in Table 1. The lowest concentration of this formulation (0.1 cc/m²) caused about 95% total reduction (L1-4) in both anopheline and culicine larval populations at 24 h posttreatment. Although 100% reduction was observed in the L3 + 4 of both anopheline and culicine populations throughout the 72 h posttreatment observation, nevertheless there was a sharp decline in total percent of larval reduction (L1-4) after 48 h posttreatment due to increases in the number of L1-2 larval populations. This may be attributed to the rapid settlement of the *B.t.i.* toxins. An analysis of variance revealed that differences among dosages had no significant effect on 24 h posttreatment anopheline and culicine total larval reductions. Dosage, also, caused no significant difference on culicine larval (L1-4) reductions in 48 and 72 h posttreatments, where on average 85 and 66% reductions were achieved, respectively. However, dosage had a significant effect ($P < 0.05$) on 48 and 72 h posttreatment anopheline total larval reductions. In this study, the 0.2 cc/m^2 concentration of Bactimos FC was chosen as the optimum rate for field application, as 96–97% of the larval populations were reduced by 24 h posttreatment.

When the efficacy of Bactimos FC (0.2 cc/m^2) was compared with Abate in 1 m^2 ponds (Table 2), the former caused 93.8% and 97.3% total (L1-4) anopheline and culicine larval mortality in 24 h posttreatment, respectively. Abate caused 98.1 and 100% total (L1-4) anopheline and culicine mortality, during the same period of time, respectively. The analysis of variance showed a significant difference ($P < 0.05$) between the effectiveness of the 2 larvicides in reducing anopheline and culicine larvae at 24 h posttreatment. There was also a significant difference ($P < 0.05$) in total culicine larval mortality at 48 h posttreatment where Abate and Bactimos FC caused 99 and 84% mortality, respectively. However, the difference between mortality rates of anopheline larvae at 48 h posttreatment was not significant.

The application of Bactimos FC (0.2 cc/m^2) to natural mosquito breeding sites, at 7-day intervals for 1 month, caused on average, 94.7% anopheline and 98.4% culicine larval mortality in 24 h posttreatment (Fig. 1). The weekly larval counts which were conducted right before the next larviciding program revealed on average, 28.5 and 25.8% reduction of anopheline and culicine larval populations, as assessed by comparing the present counts to the last larviciding pretreatment counts. This means that a good number of L3 + 4 and even pupae were observed in the treated ponds within 1 wk after the treatment. Therefore, the 7-day larviciding interval was not considered a proper timing application for Bactimos FC and 5-day intervals were recommended.

This study affirms the potential of the Bactimos FC as an effective larvicide which can well be used for the management of anopheline and culicine populations in southern Iran, especially where slowing down the rate of insecticide resistance development may be of major concern.

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