LABORATORY BIOASSAYS OF FOUR FORMULATIONS OF *BACILLUS THURINGIENSIS ISRAELENSIS* AGAINST *AEDES POLYNESIENSIS, AE. PSEUDOSCUTELLARIS* AND *AE. AEGYPTI*

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ABSTRACT. Laboratory bioassays using 3 formulations of *Bacillus thuringiensis israelensis*, SAN 402 I, IPS-78, and R153–78 were conducted with 3 species of mosquito: *Aedes polynesiensis, Ae. pseudoscutellaris* and *Ae. aegypti*. Another formulation, Bactimos® was tested with only *Ae. aegypti*. Additional bioassays were performed with *Ae. polynesiensis* in 0.5% salt water. The presence of salt did not deter the action of the bacterium. The 3 mosquito species proved to be susceptible to the various formulations, Bactimos being the most active formulation followed by R153–78, IPS-78 and SAN 402 I.

INTRODUCTION

In 1977, Goldberg and Margalit reported the isolation of a new strain of *Bacillus thuringiensis* which demonstrated rapid larvicidal activity against all five of the mosquito species tested. This strain was later named *Bacillus thuringiensis* var. *israelensis* (*Bti*) by de Barjac (1978a) and has since received much attention for its potential use as a biocontrol agent against mosquitoes and black flies (Anonymous 1979).

Preliminary bioassays have been conducted with mosquitoes (de Barjac 1978b, de Barjac and Coz 1979, Garcia and Desrochers 1979, Goldberg and Margalit 1977, Van Essen and Hembree 1980) and black flies (Undeen and Nagel 1978, Undeen and Berl 1979). The studies demonstrated that susceptibility to *Bti* varies considerably according to the species of mosquito tested. Therefore, bioassays should be performed with each target species before any *Bti* formulations can be put into operational use for that species.

This paper reports on laboratory bioassays performed with 4 formulations of *Bti* against the 3 major dengue vectors in Fiji.

MATERIALS AND METHODS

MOSQUITO CULTURES. Colonies of *Aedes polynesiensis* Marks, *Ae. pseudoscutellaris* (Theobald) and *Ae. aegypti* (Linnaeus) were routinely maintained in an outdoor insectary. They were provided with a mouse blood meal 3 times per week and supplemented with field collected mosquitoes at least once every month. Eggs were collected on paper towelling which were then dried and stored for later use.

Larvae were obtained by soaking the paper towelling and eggs in approximately 2 liters of rain water in plastic trays (32 × 26 × 12 cm) containing liver powder and active dried baking yeast.

BIOASSAYS. The 4 formulations of *Bti* used were SAN 402 I water dispersable concentrate—Sandoz Laboratories, USA and the primary powders IPS-78—Pasteur Institute, France, R153–78 and Bactimos–Roger Bellon Laboratories, France.

Initial suspensions with the IPS-78 were prepared by adding 200 mg of the powder into 5 mls of deionized water in a test tube. The solution was then homogenized by means of a vortex agitator for 3 minutes at high speed. The same procedure was used in preparing

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the initial suspensions of the R153-78 and Bactimos® primary powders except that a 1% solution of Tween 20 in deionized water was used to facilitate putting the powder into a uniform aqueous suspension. Because SAN 402 I is already in solution, the preparation of an initial suspension was not required.

Subsequent serial dilutions were then made from the initial suspensions or from the SAN 402 I by adding 1 ml into 9 mls of deionized water and agitating in a vortex agitator. Aliquots were then placed in a 2 liter conical flask with a measured amount of deionized water, so that the required concentrations of *Bti* were obtained. For the trials with *Ae. polynesiensis* in salt water, the same dilutions were prepared using a 0.5% solution of NaCl.

One hundred fifty mls of the various dilutions were poured into transparent plastic cups (5.5 × 9.5 cm) and 25 late third or early fourth instar larvae were added. A pinch of liver powder was sprinkled onto the surface of each cup for food. The experimental cups were kept in an outdoor insectary and mortality counts were taken at 24 and 48 hours after initial exposure to the *Bti*. Larvae that pupated were not included in the mortality readings.

Each trial consisted of 4 replicates at each of 5 different concentrations and a control. At least 4 trials were conducted on separate days for each species and *Bti* formulation tested. Only one species, *Ae. aegypti*, was tested with the Bactimos preparation.

RESULTS AND DISCUSSION

The results are summarized in Table 1 and Fig. 1. Since control mortalities never rose over 5%, the mortality rates shown are uncorrected for control mortalities. All 3 mosquito species were susceptible to the first 3 formulations of *Bti*, as well as *Ae. aegypti* to the Bactimos preparation. There was a difference in the relative activity of the different *Bti* formulations. The Bactimos was found to be the most active formulation followed by R153–78, IPS-78 and SAN 402 I.

There was little variation in the susceptibility among the 3 species compared to the large variation in susceptibility that de Barjac and Coz (1979) found among different *Aedes* species and even among different *Ae. aegypti* strains. A possible explanation for this discrepancy could be that de Barjac used number of spores/ml to express the concentration of *Bti* whereas we used weight or volume of the formulation per unit volume. This indicates that the weight or volume of formulation per unit volume method may be more accurate for use in the evaluation of different formulations of *Bti*. Steps are now being taken to standardize a bioassay method for *Bti* using the IPS-78 as a reference (Anonymous 1979) and therefore, comparisons with other studies will be facilitated.

The action of the *Bti* on *Ae. polynesiensis* was not affected by the presence of 0.5% NaCl in the water (Figs. 1A and 1B). There was a slight difference when IPS-78 was used but due to the high variability in the 0.5% salt results as indicated by the confidence limits (Table 1), this difference is probably insignificant. Garcia and Desrochers (1979) working with *Aedes dorsalis* (Meigen) and *Culiseta inornata* (Williston) also showed that brackish water does not affect the pathogenicity of *Bti*.

These bioassay results indicate that *Bti* has much potential for the control of *Aedes* mosquitoes in Fiji. Field trials will now have to be performed to further evaluate the bacterium under Fiji field conditions.

ACKNOWLEDGMENTS

The authors thank all those in the Fiji Ministry of Health who made this study possible. Special thanks are due to Messrs. P. Prasad, G. Prakash and R. Ram of the Vector Research Unit, and to Drs. J. U. Mataika and J. A. R. Miles of the Wellcome Virus Laboratory.

Table 1. Susceptibility of 3 species of *Aedes* mosquitoes to 4 formulations of *Bacillus thuringiensis israelensis.*[1]

	Ae. polynesiensis (0.5% salt)		*Ae. polynesiensis*		*Ae. pseudoscutellaris*		*Ae. aegypti*	
	Mean[2]	(C.L.)[3]	Mean[2]	(C.L.)[3]	Mean[2]	(C.L.)[3]	Mean[2]	(C.L.)[3]
SAN 402 I								
PPM(w/v)								
LC50	0.10	(.08–.12)	0.12	(.09–.15)	0.15	(.07–.23)	0.17	(.12–.22)
LC95	0.31	(.26–.36)	0.34	(.21–.47)	0.35	(.21–.49)	0.48	(.43–.53)
Activity[4]								
(ITU/ml)	600		250		267		353	
IPS 78								
PPM(w/v)								
LC50	0.06	(.03–.09)	0.03	(.01–.05)	0.04	(.02–.06)	0.06	(.057–.063)
LC95	0.31	(.12–.50)	0.14	(.01–.17)	0.13	(.08–.18)	0.21	(.19–.23)
Activity[4]								
(ITU/mg)	1000		1000		1000		1000	
R153-78								
PPM(w/v)								
LC50	0.02	(.004–.04)	0.02	(.01–.03)	0.02	(.004–.04)	0.02	(.01–.03)
LC95	0.09	(.01–.17)	0.09	(.07–.11)	0.07	(0–.15)	0.04	(.02–.06)
Activity[4]								
(ITU/mg)	3000		1500		2000		3000	
Bactimos								
PPM(w/v)								
LC50	Not tested		Not tested		Not tested		0.006	(.004–.008)
LC95							0.02	(.01–.03)
Activity[4]								
(ITU/mg)							10,000	

[1] Mortalities were taken after 48 hours of continuous exposure of late third and early fourth instar larvae.

[2] Mean of 4 different trials. Each trial consisted of 4 replicates of 25 larvae at each of 5 different concentrations.

[3] 95% confidence limits.

[4] Activity for formulations determined by comparison of observed LC50 with LC50 of IPS 78, which has an arbitrary value of 1000 International Toxic Units per ml (ITU/mg).

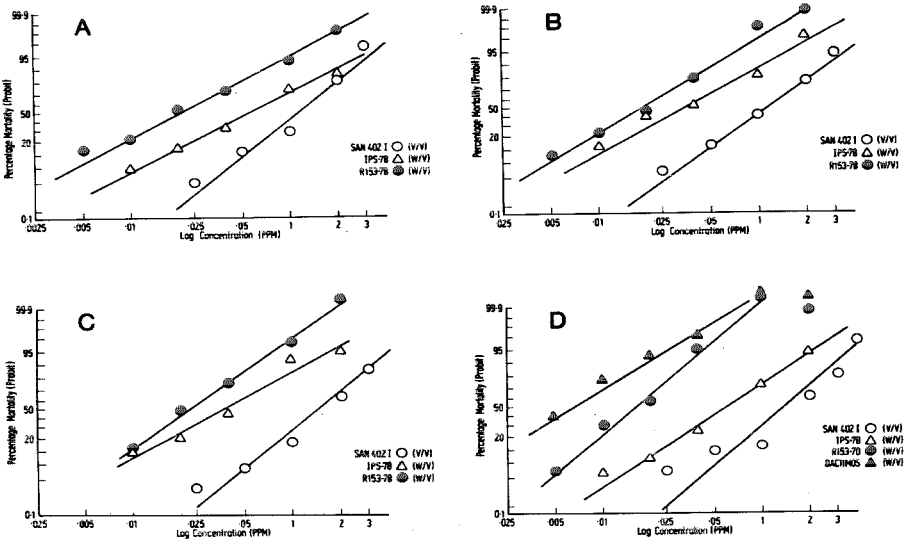

Fig. 1A-D. Dose-mortality curves obtained with 3 species of *Aedes* mosquitoes exposed to 4 formulations of *Bacillus thuringiensis israelensis*.* (A) *Aedes polynesiensis* in .5% salt (B) *Ae. polynesiensis* (C) *Ae. pseudoscutellaris* (D) *Ae. aegypti.*

* Mortalities were taken after 48 hours of continuous exposure of late third and early fourth instar larvae. The values are a mean of 4 different trials. Each trial consisted of 4 replicates of 25 larvae at 5 concentrations.

MSG and MKT were initially supported by a Canadian International Development Agency Award and later by the United Nations Volunteers/World University Service of Canada joint sponsorship programme. Research funds were provided by grants to JSP from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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EVALUATION OF PYRETHRIN AND TWO SYNTHETIC PYRETHROIDS ALONE AND IN MIXTURES WITH MALATHION AS ULV GROUND AEROSOLS AGAINST RICELAND MOSQUITOES

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ABSTRACT. Permethrin, pyrethrin, resmethrin and malathion were tested as ULV ground aerosols against caged, field collected *Anopheles quadrimaculatus* and *Culex quinquefasciatus*. Pyrethrin-malathion, resmethrin-malathion (91%) mixtures were also tested. The synthetic pyrethroids alone were more effective at 1 hr posttreatment than any mixture or malathion alone.

In recent years ultralow volume (ULV) cold aerosol generators have become important for control of adult mosquito populations. For adequate control, mosquito re-infestation following treatment often requires nightly applications of insecticides. Many commonly used insecticides such as malathion require several hours to kill adult mosquitoes. Although excellent control may be obtained for those mosquitoes present at the time of spraying, subsequent influx of mosquitoes from outside the treatment zone may completely replace those killed. There is thus a need for compounds that possess quick knockdown, such as synthetic pyrethroids. These are expensive, however, and generally show short residual. Mixing pyrethroids with malathion may produce an economical compound with quick knockdown and effective residual. There are no prior data on potentiation available. This study was conducted at the University of Arkansas Rice Research and Experiment Center at Stuttgart, in 1977–78, to evaluate pyrethrin pyrethroid/malathion mixtures.

Effective control of the riceland mosquitoes *Psorophora columbiae* (Dyar and Knab) and *Anopheles quadrimaculatus* Say by ULV ground aerosols of resmethrin and pyrethrin was reported by Coombes and Meisch (1976). Thompson and Meisch (1977) found ULV ground aerosols of permethrin very effective against *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

Two synthetic pyrethroids and pyrethrin were mixed with malathion, and evaluated in 1977–78 as ULV ground aerosols against caged, field collected mosquitoes.

In tests conducted in 1977, pyrethrin-malathion (5%/91%, PY/MA) mixtures along with standard formulations of pyrethrin (5%) permethrin (25%), and malathion (91% were evaluated against adult *An. quadrimaculatus*. The

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