ENVIRONMENTAL HEALTH PROJECT

Activity Report 112

Eritrea Field Studies on Efficacy of Bacterial Larvicides for Use in Malaria Control

by

Josephat Shililu

December 2001

Prepared for the USAID Mission to Eritrea
under EHP Project 26568/E.X.ER2.LARVCTRL

Environmental Health Project
Contract HRN-I-00-99-00011-00
is sponsored by the
Office of Health, Infectious Diseases and Nutrition
Bureau for Global Health
U.S. Agency for International Development
Washington, DC 20523

Contents

About the Author

Dr. Josephat Shililu is EHP's Resident Advisor for Vector Ecology and Control working with the National Malaria Control Program in Asmara, Eritrea. Dr. Shililu received his M.S. in Zoology in 1992 and his Ph.D. in Entomology /Parasitology in 1997, both from Kenyatta University, Nairobi, Kenya. He has served as a Lecturer in Zoology at Kenyatta University since 1992. He also has held appointments at the International Centre for Insect Physiology and Ecology, Nairobi, as a Consulting Ecologist (1998–2000) and Research Scientist (2001–present). Dr. Shililu has a strong interest in field entomological studies and vector surveillance designed to support and evaluate vector control programs.

Acknowledgments

The study described in this report was first proposed and designed by Dr. Robert Novak of the Illinois Natural History Survey and the University of Illinois in collaboration with the author and officials of the Eritrea National Malaria Control Program. Dr. John Beier of Tulane University and Dr. Robert Fusco of Valent Biosciences made critical contributions to the design and implementation of the study.

We wish to acknowledge the enthusiastic support and assistance of the NMCP Manager, Dr. Tewolde Ghebremeskel, Mr. Mehari Zerom, logistics chief, and Dr. Fesehaye Seulu, entomologist, each of whom had an important role in the success of this study and the subsequent pilot larval control program, now underway.

EHP and the author also acknowledge the contributions of Dr. Andom Ogbamariam, Director for Communicable Diseases Control, Ministry of Health, the Honorable Mr. Saleh Meky, Minister of Health, and Ms. Linda Lou Kelley and Mr. Semere Ghebregiorgis of the U.S. Agency for International Development Mission to Eritrea, for their consistent support of the malaria program and its collaboration with the USAID-sponsored Environmental Health Project.

J. Shililu
EHP Resident Advisor for Vector Ecology and Control
Asmara, Eritrea

G. Brantly
EHP Program Coordinator for Environmental Change and Health Outcomes
Arlington, Virginia, USA

Abbreviations

a.i.	Active ingredient
Bs	Bacillus sphaericus
Bti	Bacillus thuringiensis var. israelensis
ITU	International toxic units
NMCP	National Malaria Control Program
PCR	Polymerase chain reaction
RBM	Roll Back Malaria
WHO	World Health Organization

Executive Summary

Malaria parasite transmission is driven by the temporal and spatial patterns of vector species of anopheline mosquitoes. The distribution patterns of the vector species are dependent on the availability of aquatic habitats, and elimination and treatment of such larval habitats could have dramatic effects on vector populations and the level of malaria transmission. In redefining its long-term vector control strategies in the context of the country's Roll Back Malaria Program, the NMCP has renewed interest in examining larval control as a potentially critical component of the program's integrated vector management program. The semi-arid climatic conditions, the seasonal incidence of malaria and the isolation of towns and villages in the country make larval control an ideal option for reducing the burden of malaria in Eritrea.

If chemical larvicides were used intensively, resistance to these compounds might develop in the mosquito vector. Chemical larvicides also may create environmental problems if they are lethal to non-target species. To meet the challenges of vector resistance to chemical larvicides and environmental safety, the NMCP undertook an evaluation of two alternative bacterial larvicides. The larvicidal activity of the granular formulation of *Bacillus thuringiensis* and *Bacillus sphaericus* was evaluated and compared to that of temephos as used against *Anopheles arabiensis* and other mosquitoes. The primary objective was to determine the optimal application rates and duration of activity for the two biological larvicides. Both larvicides produced significant mortality of immature *Anopheles* and *Culex* species in the test plots. The results have further indicated that application of the two biological larvicides bi-monthly to stream-bed pools, rain pools and similar habitats would maintain control of the anopheline mosquito populations.

1 Introduction

The National Malaria Control Program (NMCP) in Eritrea currently uses Temephos for larval control, in conjunction with routine vector control operations that include treated bed nets and DDT spraying for control of adult mosquitoes. One eminent concern is that the malaria vector mosquitoes will develop resistance to Temephos in the future, as the NMCP intensifies operations to meet expectations of the World Health Organization's (WHO) Roll Back Malaria (RBM) Program. Drawbacks such as vector resistance to these compounds, costs and environmental pollution provide a basis for redefining long-term larval control strategies for the country. The NMCP has considered use of the biological insecticides *Bacillus thuringiensis var. israelensis* (Bti) and *Bacillus sphaericus* (Bs). Both biological agents have proved to be useful for control of mosquito species in a variety of breeding habitats (Mulla et al. 1984; Karch et al. 1992), and show very high environmental safety. However, experimental evaluation of both agents specifically as control agents against malaria vectors is still limited. The performance of the microbial agents may be affected by water quality parameters such as organic content, salinity, pH, and water temperature, all which vary by ecology and type of breeding habitat. These variables provide the basis for evaluating the efficacy of these compounds in a variety of ecological and epidemiological settings. One of NMCP's goals is to identify and characterize settings in the country in which vector control, and more specifically larval control, can make a cost-effective contribution to malaria control.

The semiarid climatic conditions, the seasonal incidence of malaria and the isolation of towns and villages in Eritrea make it an ideal country to implement larval control as one of the principal interventions to reduce malaria. In most regions of the country, there is a very short malaria transmission season that coincides with the short rainy season from August to November. However, in some areas, mosquitoes and associated malaria transmission persist throughout the year, even during the long dry season. At key locations throughout Eritrea, larval control using Temephos has proven to be a feasible strategy under these dry and semiarid climatic conditions since mosquito larval habitats sites are discrete and easily targeted by field teams.

2 Objectives

- *Objective #1.* Estimate the optimal application rates and duration of effect for Bti and Bs in representative larval habitats and compare the efficacy with that of Temephos.

- *Objective #2.* Determine the effects of Bti and Bs applications on non-target invertebrate species as compared to Temephos.

3 Materials and Methods

3.1. Study Sites

The efficacy studies were conducted in Tesseney, Elabered and Korbaria, in Gash-Barka, Anseba and Debub zones, respectively. All three sites present different ecologies and lie at different altitudes. The selection of villages for the study was aimed at capturing most of the altitudinal and ecological variations in Eritrea.

The larval habitat types selected included: 1) river and river bed habitats at Elabered in Anseba Zone, 2) temporary ponds and associated seepage areas at Korbaria in Debub Zone and 3) roadside ditches and rain pools at Tessenei in Gash-Barka Zone. These sites represented areas of relatively high adult mosquito abundance and larval productivity as determined by the recent countrywide surveys.

3.2. Experimental Design

3.2.1. Designation of Test Plots

The number of test plots per habitat type and replicates depended on the amount of available larval habitat. Prior to the start of efficacy trials, the test plots were mapped, numbers were assigned to them and they were marked using numbered flags. Each plot was further marked with survey tape to secure the site against human and animal interference.

Each replicate involved six plots so that the three test products (Bti, Bs, and Temephos) relative to an untreated control plot could be tested simultaneously. Six replicate experiments were conducted in Elabered, Anseba zone. In Tesseney and Korbaria the number of replicates were four and five, respectively. Each plot was separated by at least 5 m to prevent any cross contamination.

3.2.2. Larvicidal Application Rates

Granular formulation of Bti, serotype H-14 (VectoBac G – 200 International toxic units per mg [ITU/mg]) and Bs, serotype H5a5b, strain 2362 (VectoLex CG – 50 ITU/mg) were tested at the maximum application rate and also at 50% of the maximum application rate. Valent BioSciences Corporation supplied both larvicides. A single plot with Temephos (Abate 47.4% w/w a.i., 500g a.i./l) and an untreated control plot were also run. The larvicides were used at the following application rates:

VectoBac G – (100%)	- 11.2 kg/ha
VectoBac G – (50%)	- 5.6 kg/ha
VectoLex CG – (100%)	- 22.4 kg/ha
VectoLex CG – (50%)	- 11.2 kg/ha
Abate	- 100.0 ml/ha

Calibration of the Maruyama granular spreader was made for 5/8 mesh VectoBac granules and the 10/14 mesh VectoLex CG granules at high and low rates. Temephos application was done using Hudson liquid sprayers.

3.2.3. Larval Sampling

Prior to treatment, mosquito larvae in each plot were sampled by standard dipping methods. A minimum and maximum of five and 10 dips, respectively, were taken at various places in the plot. The number of immature developmental stages (L1, L2, L3, L4 and Pupae) per dip was recorded. Estimates of larval density were then made by calculating the number of larvae per dip. Larval samples were preserved in 70% alcohol and identified later by standard larval taxonomy, with a subset identified to species by PCR. During the course of the trial each plot was sampled for larvae at 24 hours and 48 hours and then once per week for the duration of the study. The trials were terminated when high densities of larvae were detected in all of the treated test plots. Though the size of the larval plots was variable depending on the type of habitat, plots of almost equal dimensions were selected for the study.

3.2.4. Characterization of Larval Habitats

At 24 hours prior to treatment and during the post treatment sampling, the following habitat characteristics were recorded:

a) relative level of pollution, visually determined and ranked 1 to 5 with the clear being 1 and the most polluted being 5

b) pH

c) average depth and volume

d) vegetation (border, emergent, floating)

e) water temperature

f) amount of shade (ranked as 1 for complete sun and 5 for total shade).

3.2.5. Treatment Impacts on Nontarget Invertebrates

Sampling of nontarget invertebrates was done concurrently with mosquito sampling before treatment and post treatment, using standard dipping techniques. The relative densities of the nontarget invertebrates was determined and ranked 1 to 3 with low densities being 1 and high densities being 3. Invertebrate samples were preserved in 75% alcohol for identification to family level using standard taxonomic keys.

3.2.6. Data Forms and Analysis

Standard data forms currently used by Valent BioSciences for trials of this type were used, with only slight modification to account for specific environmental data. Abbott's formula (*Appendix 1*) was used to determine efficacy of all formulations and dosage levels relative to untreated control plots. Graphs showing variation in percent control have been derived from the data.

4 Results

All three larvicides caused significant mortality of immature stages of *An. arabiensis* and other anopheline species. Reduction in larval populations was pronounced in the first 14 days post treatment.

At 24 and 48 hours post treatment, Temephos, VectoBac G and VectoLex CG at 100% and 50% of label rates provided over 90% larval control for *An. arabiensis* in the three different types of breeding habitats tested (i.e., roadside ditches, rivers and river bed pools and ponds). At seven days post treatment onwards the activity of both Bs and Bti tended to vary with breeding habitat. In Anseba zone, about 84% and 98% control was recorded with Bs at 100% and 50%, respectively. Temephos and Bti at both rates, on the other hand, produced at least 75% control at seven days post treatment (Table 1). This shows that both the half and maximum label rates are effective against the anopheline species tested. In Korbaria (altitude 1800 m), the performance of both Bs and Bti at both rates was similar (>85% control). Temephos produced 100% control in this site at seven days post application. In the roadside pools in Gash-Barka, 100% control was recorded for Bti, Bs and Temephos. Data beyond seven days post treatment could not be generated because of drying of the test plots in this site. Overall, the data shows that all three larvicides provide effective control against *Anopheles* and *Culex* larvae up to seven days post treatment.

Table 1. Comparison of Percent Control Using Bs, Bti, and Temephos for Larval Control

Site/Zone	Days Post-Treatment	Larvicide						
		BS 100	BS 50	BTI 100	BTI 50	TEM	BTI - WDG 50	BTI- WDG 100
Elabered,	1	90.2	93.8	96.2	90.6	97.0		
Anseba	2	86.9	98.3	100.0	98.5	97.0		
	7	84.4	97.8	77.5	75.7	74.9		
	14	83.8	73.8	54.0	57.0	73.7		
	21			23.6	19.7			
Korbaria,	1	95.5	94.3	99.5	96.8	92.7		
Debub	2	86.9	87.3	89.1	97.7	97.3		
	7	91.9	97.3	94.0	94.6	100.0		
	14	75.8	84.2	73.4	78.7	84.7		
	21	82.5	93.2	83.1	91.7	100.0		
	28	67.8	23.9	78.8	100	53.2		
Tesseney,	1	100	100	100	100	100	100	92.7
G/Barka	2	99.9	100	100	99.2	100	100	99.4
	3	100	100	100	100	100	100	87.8
	4	100	100	100	100	100		
	5	100	100		73.9	100		
	6		100		47.6	100		
	7		100			100		

In Korbaria, Bti at the 100 and 50% rate produced appreciable control over 21 days post treatment (Figure 1). A mean percent control of 89% was recorded at 28 days post treatment. Bs, on the other hand, was effective at the maximum rate at 28 days. Control with Bs at 50% broke down after 21 days post treatment. Temephos gave a 100% control at 21 days post treatment, but only 50% control at 28 days post treatment. The data show that the three larvicides produced appreciable control at least up to three weeks post treatment in this site.

In Elabered (river bed breeding sites) only a 14-day control by the three larvicides was achieved (Figure 2). The difference between the two sites in the activity of the larvicides arose mainly from the type of breeding habitat and associated water qualities. In Elabered, the riverbed breeding sites were prone to water seepage through the sandy substratum, thereby leading to dilution effect. Furthermore, given that there was water outflow, though at a very slow pace, there was evidence of drifting of the light granular material to one end of the plot or downriver altogether, meaning that the full dose could not have been achieved in all instances. A third factor that could have contributed to the relatively low activity in this site was the presence of algal growth that could have compromised feeding activity of larvae on the larvicidal agent. Anopheline larvae are highly associated with algal growth. Formulations that can penetrate such algal masses would be an option for further testing for specific situations. Previous entomology studies show that over 90% of anopheline breeding activity in the country takes place on river edges and riverbed pools. This becomes the critical target for larval control intervention by the biological agents.

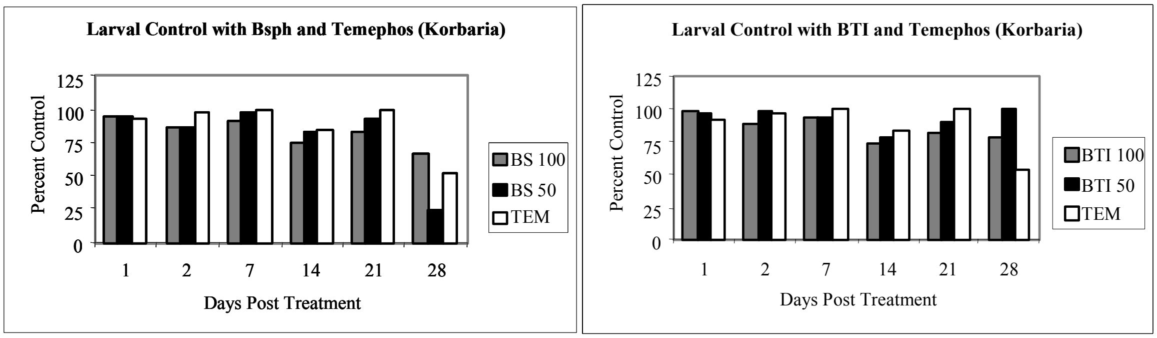

Figure 1. Percent control of Culex and Anopheles larvae in Korbaria, Debub zone

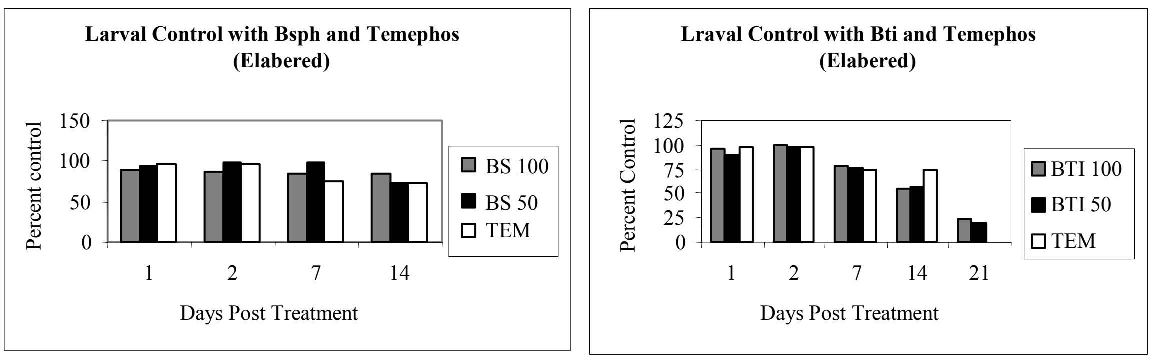

Figure 2. Percent control of Anopheles larvae in Elabered, Anseba zone

5 Conclusions

- Bti and Bs provided control on river edge and riverbed habitats for a two-week period. Application of the two larvicides every two weeks would be necessary to maintain control. A similar application cycle would be appropriate for rain pools and roadside ditches. The maximum and 50% application rates for Bti and Bs produced equivalent control over the two-week period. To reduce costs the two compounds could be used at 50% maximum label rates.

- The larvicidal activity for Bti and Bs was variable depending on breeding habitat, mosquito species and general ecology. A three-week larvicidal activity was recorded for Bs at 100% and only two weeks at 50% rate in ponds at an altitude of 1800 m. Application with Bs at the maximum label rates on a three-week cycle would be an appropriate operational regime for ponds and similar breeding sites within this ecological stratum. Bti applied at a 50% rate every three weeks would also be appropriate only under these specific conditions.

- Considerations should be given for formulations appropriate for specific habitats such those with high algal content, or edges of slow-flowing rivers where anopheline larvae abound. It will be important to test activity of the wettable dispersible granule formulation (VectoBac WDG and VectoLex WDG) for such habitats.

- The data show that Bti, Bs and Temephos are effective for similar periods. It is therefore possible to use the three larvicides on a rotational basis.

References

1. Karch. S; Asidi A; Manzami M. and Salaun J.J. 1992. Efficacy of *Bacillus sphaericus* against malaria vector *Anopheles gambiae* and other mosquitoes in swamps and rice fields in Zaire. *J. Am. Mosq. Control Assoc. 8 (4): 376–380*.

2. Mulla M.S; Darwaseh H.A; Davidson E.W. and Gulmage H.T. 1984. Efficacy and persistence of microbial agent *Bacillus sphaericus* for the control of mosquito larvae in originally enriched habitats. *Mosq. News. 44: 166–173*

<u>Appendix 1. Abbott's formula</u>

$$\text{Adjusted Percent Mortality} = \frac{\text{Percent Test Mortality - Percent Control Mortality}}{\text{100\% Control Mortality}} \times 100$$