Larvicidal efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* on *Anopheles arabiensis* in Ethiopia

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The second instar larvae of the malaria vector mosquito, *Anopheles arabiensis*, were more susceptible to *Bacillus thuringiensis* var. *israelensis* (IPS-82) and *B. sphaericus* (SPH-88) than the third instar larvae. The LC₅₀ values were 1.0 µg l⁻¹ and 1.8 µg l⁻¹ for IPS-82 against second and third instar larvae respectively, after 48 h of exposure. The LC₅₀ values for SPH-88 were 3.6 µg l⁻¹ against the second instar larvae and 7.6 µg l⁻¹ against the third instar larvae of *An. arabiensis*. The larvicidal efficacy of SPH-88 was significantly less than IPS-82. The potential of IPS-82 for the control of *An. arabiensis* in malaria endemic areas is promising.

Key words: *Anopheles arabiensis*, *Anopheles gambiae* complex, *Bacillus thuringiensis* var. *israelensis*, larvicidal activity.

Mosquitoes are vectors of a variety of widespread human diseases, and the most important disease vectors are members of the subfamilies *Anophelinae* and *Culicininae*. In the subfamily *Anophelinae* there are 400 species of *Anopheles* mosquitoes throughout the world, but only about 60 species are vectors of malaria under natural conditions (Bruce-Chwatt 1985). In Ethiopia, *Anopheles gambiae* complex is the main vector of malaria with the *Anopheles funestus* group, *Anopheles pharoensis* and *Anopheles nili* playing secondary roles (Geberemariam 1988). Two sibling species of the *An. gambiae* complex (*Anopheles arabiensis* and *Anopheles quadriannulatus*) have been identified from Ethiopia (Zahar 1985). *An. arabiensis* is also the main vector of malaria in southern Arabia and most of the African continent including Madagascar and the islands to its north and south (Bruce-Chwatt 1985).

Dichloro-diphenyl-trichloroethane (DDT) and other chemical insecticides with high potency against mosquitoes had encouraged many health workers to press for malaria eradication in the past. Unfortunately, nearly all members of the *An. gambiae* complex became resistant in various degrees to DDT and other organochlorine insecticides over most of tropical Africa (Bruce-Chwatt 1985). In addition, the long-term harmful effects of powerful chemicals to non-target organisms and environment has highlighted the need to develop an alternative. A promising alternative at present is biological control of mosquito vectors.

Among the biological control agents of mosquitoes, *Bacillus thuringiensis* var. *israelensis* (Goldberg & Margalit 1977) and *Bacillus sphaericus* (Kellen et al. 1965) are known to be potent larvicides of mosquito species. However, the efficacy of both microbial control agents is influenced by environmental factors (Darwazeh et al. 1990). Moreover, their efficacy on different mosquito species (Ramoska et al. 1977) and larval stages (Wraight et al. 1981) varies greatly.

The mode of action of both larvicidal bacilli is similar. *B. thuringiensis* produces parasporal inclusions during sporulation. Upon ingestion by susceptible insects larvae, the crystalline inclusions are solubilized in the mid-gut, releasing proteins called delta endotoxins. These proteins (protoxins) are activated by mid-gut proteases, and the activated toxins interact with the larval mid-gut epithelium causing a disruption in membrane integrity, ultimately leading to insect death (Gill et al. 1992). The larvicidal activity of the highly toxic strains of *B. sphaericus* is also due to a large extent to the presence of protein toxins produced during sporulation (Kalfon et al. 1984).

This paper reports the potential of *B. thuringiensis* var. *israelensis* (IPS-82) and *B. sphaericus* (SPH-88) for the control of the main vector of malaria in Ethiopia. Moreover, the...
susceptibility of the different larval instars of *An. arabiensis* towards the two microbial larvicides is described.

### Materials and Methods

**Bacterial Strains and Mosquito Species**

International standard strains of *B. thuringiensis* var. *israelensis* (IPS-82) and *B. sphaericus* (SPH-88) were obtained from the Institute of Pasteur, Paris, France.

Larvae of *An. arabiensis* were obtained from colonies maintained at the insectary of the National Organization for the Control of Malaria and Other Vector-borne Diseases, Nazareth, Ethiopia. The rearing procedures of the colonies maintained at the insectary are described in Mekuria et al. (1991).

**Bioassays**

*B. thuringiensis* var. *israelensis* and *B. sphaericus* were tested against second and third instar larvae of *An. arabiensis* at the Nazareth Insectary, Ethiopia.

Bioassays were carried out according to the test procedure obtained from the World Health Organization collaborating centre for entomopathogenic *Bacillus* (Institute of Pasteur, Paris, France). 50 mg of the standard powders (IPS-82 and SPH-88) were placed in 20 ml penicillin flasks to which were added 10 ml of deionized water and 15 glass beads (6 mm diameter). The suspension was thoroughly homogenized by shaking on a vortex mixer.

Stock solutions were prepared in test tubes by serial dilution of the homogenates in deionized water, and appropriate dilutions were used in the test against the different larval stages. The bioassay was carried out by adding known volumes from the appropriate dilution in plastic cups containing deionized water. Five different concentrations of the standard powders were prepared in duplicate for each assay. In each assay, 20 mosquito larvae were added to a final test volume of 150 ml. Each test was repeated three times on different dates. Duplicate cups which contained 20 mosquito larvae in 150 ml deionized water without the test material served as the control.

**Efficacy of Strains**

Mortality was determined at 24 and 48 h, based on the counting of live larvae. Mortality in 48 h were subjected to probit analysis on a computer program and the LC₅₀ values of live larvae. Mortality in 48 h were subjected to probit analysis on a computer program and the LC₅₀ values were calculated as in Finney (1971). The p values were calculated for comparisons of the differences in the susceptibility of the larval instars to each strain and also for the comparisons of the efficacy of the two standard strains against the two instar levels. When control mortality exceeded 5%, the mortalities of treated groups were corrected according to Abbott's formula (Swaroop 1966). Test results with control mortality greater than 10% were discarded.

### Results and Discussion

The use of microbial agents for the control of mosquito vectors of disease has been recommended by various authorities during the last two decades. Evaluation of the efficacy of microbial larvicides on important vector mosquitoes provides the basic information required for the potential utilization of the strain for the control of mosquito-borne diseases.

The susceptibility of the most important vector mosquito of malaria in our region, *An. arabiensis* was investigated. The activity of *B. thuringiensis* var. *israelensis* (IPS-82) and *B. sphaericus* against larvae of *An. arabiensis* is summarized in Table 1. The LC₅₀ values of IPS-82 were found to be 1.0 and 1.8 µg ml⁻¹ against the second and third instar larvae respectively showing the high susceptibility of the mosquito larvae.

A recent review by Porter et al. (1993), concluded that in general species of *Aedes* and *Culex* larvae are more sensitive than species of *Anopheles* to *B. thuringiensis* var. *israelensis*. It is reported by Foo & Yap (1982) that the susceptibility of mosquito larvae to *B. thuringiensis* var *israelensis* depends on the mosquito species within a genus. Our result on *An. arabiensis* shows that there could also be other species of *Anopheles* which are more susceptible than species belonging to the genera *Culex* and *Aedes*. A recent comparative study on the efficacy *B. thuringiensis* var. *israelensis* against *An. arabiensis* and *Cx. quinquefasciatus* showed that *An. arabiensis* was more susceptible than *Cx. quinquefasciatus* (Seyoum 1995).

Abdel-Hammed et al. (1990) determined the efficacy of *B. thuringiensis* var. *israelensis* (IPS-82) against larvae of *Aedes aegypti*. A comparison of our result on larvae of *An. arabiensis* with the finding of Abdel-Hammed et al. (1990) on larvae of *Ae. aegypti*, shows that *An. arabiensis* is more susceptible than *Ae. aegypti*.

Nugud & White (1982) have evaluated the efficacy of IPS-78 (the first international standard of *B. thuringiensis* var. *israelensis*) and Abbott and Sandoz formulations of *B. thuringiensis* var. *israelensis* on second instar larvae of *An. arabiensis*. In our study, the efficacy of IPS-82 preparation on the same mosquito larvae was found to be appreciably higher than reported for those preparations. Therefore, *B. thuringiensis* var. *israelensis* (IPS-82) has great potential for the control of *An. arabiensis* which is the most important vector mosquito in the region.

In contrast to IPS-82, SPH-88 showed a significantly lower efficacy against both second and third instar larvae of *An. arabiensis* (Table 1). Statistical analysis also showed that all the data are comparable as indicated by the chi-squared test.

Strains of *B. sphaericus* are known to have high activity towards larvae of *Culex*, variable toxicity to *Anopheles* depending on the species, and are inactive against *Aedes* larvae (Ramoska et al. 1977). Seyoum (1995) has also recently shown that the larvicidal efficacy of SPH-88 against larvae of *Culex quinquefasciatus* is higher than that of the same larval stages of *An. arabiensis*. *Cx. quinquefasciatus* is