Some Environmental and Biological Factors Influencing the Activity of Entomopathogenic Bacillus on Mosquito Larvae in Brazil

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The influence of environmental and biological factors on the efficacy of Bacillus thuringiensis serovar israelensis and B. sphaericus as mosquito larvicides are reviewed. The importance of strain dependence, cultivating media/methods, mosquito species/specificity, formulations and their relation to mosquito feeding habits, as well as temperature, solar exposure, larval density and concomitant presence of other aquatic organisms are addressed with reference to the present status of knowledge in Brazil.

Key words: mosquito control - Bacillus thuringiensis s. israelensis - Bacillus sphaericus - environment - biological factors

Mosquitoes (Diptera: Culicidae) are considered the most important group of vectors of diseases, both in number of disease agents and in magnitude of health problems caused worldwide (Merritt et al. 1992). Many populations of mosquito vectors of diseases have developed resistance to synthetic organic insecticides, used mostly during the last half of this century. Thus interest in alternative strategies as well as in integrated control grew (WHO 1982, 1986). Among many methods and processes developed in recent years, bacterial insecticides have been the most successful and also the most widely researched. They are becoming increasingly important in mosquito control. Their specificity and harmlessness to the majority of organisms makes them compatible with current environmental concerns.

Environmental factors such as variability in climatic conditions (light, temperature), physical and chemical conditions present in the breeding sites, concomitant presence of predating and competing organisms may affect the availability and toxicity of bioinsecticides. Specific and strain/population features of agents and target, their formulations or physiological status are also important. Very few data on these topics are presently available in Brazil or in other Latin American countries.

The toxicity of bioinsecticides derived from Bacillus thuringiensis serovar israelensis (BtI) and B. sphaericus is directly dependent on the strain, cultivating media/methods (WHO 1987a, De Barjac 1989). Usually strain identification is done by biochemical, physiological and electrophoretic methods (Zahner et al. 1989, De Barjac & Frachon 1990). Siegel et al. (1993), using cellular fatty acid analysis, identified four commercial brands (Acrobe, Bacitinos, Teknar and Vectobac) as well as IPS 82 as being the same strain of BtI and identified Skeetal as a different one. New isolates and processes are continuously tested. In Brazil, during the last five years, at CPRR/FIOCRUZ a total of 229 experiments involving 193,000 Aedes larvae and 118 tests using 196,200 Culex larvae were performed. Zahner et al. (1990) described the isolation of BtI samples from soils in the Brazilian northeast and their toxicity against Cx. quinquefasciatus, Ae. aegypti and Ae. fluviatilis. Silva et al. (1991) and Rabinovitch et al. (1993) reported the development of new cultivating processes for BtI and BS respectively, presenting both good toxicity and economic viability.

scribed various ultrastructural events in the mid-gut of *An. Cx. quinquefasciatus* and *Ae. aegypti* following ingestion of BS spore/crystal complex. Additionally, Singh and Gill (1988) reported anomalies produced by BS in the CNS of *Cx. quinquefasciatus*. Mulla and Singh (1991) pointed out morphogenetic alterations in the same mosquito species produced by BTI. Most studies aimed to assess the efficacy of BTI and BS against different mosquito species were done with 3rd and 4th instar larvae, but susceptibility can be increased when younger larvae are employed (Mulla et al. 1984, WHO 1984, Lacey & Undeen 1986, Lago et al. 1991). Oliveira et al. (1992) testing three experimental of BTI and three commercial formulation (Vectorbac, Tekanr and Skeetal) on all four larval instars of *Cx. quinquefasciatus* and *Ae. flaviatilis*, observed, in most instances, an inverse proportional relation of toxicity to larval age. When BS (strain 2362) was employed, a similar effect seemed to occur (Oliveira, personal communication).


The knowledge of the particle size that are optimally ingested by mosquito larvae in their natural habitats and the areas where they forage could greatly enhance the success of BTI and BS as particulate larvicides (Merrit et al. 1992). The same authors, considered most *Anopheles* as lentile planktonic swimmers, surface feeders with a collecting-filtering feeding mode that use microorganisms and detritus as the main food resource. *Culex* spp. and *Ae. aegypti* were regarded mainly as planktonic swimmers and divers: the former is filter-feeding on microorganisms and detritus in the water column, the latter is mostly shredding on organic surfaces and sediments, ingesting microorganisms, detritus and parts of dead invertebrates. Additionally, Dahl et al. (1993) pointed out that *Cx. quinquefasciatus* larvae were able to select the size of the particles ingested and that *Ae. aegypti* usually ingested larger particles than the former species. Avissar et al. (1994) observed that shredding larvae ingest filamentous particles more easily than sphaerical ones, the reverse occurring with filter feeders. Starved larvae and larvae exposed to phagostimulants can also ingest much larger amounts of particles than otherwise (Dadd et al. 1982, Rashed & Mulla 1989). Very little is known of the feeding mode, feeding micro-habitat, food resources and preferred particle size of vectors and potential vectors in Brazil, especially of malaria vectors. Research of these topics should be very important in regard to ingestable biolarvicides.

Temperature is another important factor influencing the efficacy of BTI and BS insecticides. Substantial decreases in temperature are usually related to a reduced larvicidal activity. Wright et al. (1981), Mulla et al. (1985, 1988), Becker et al. (1992), Toussignant et al. (1992) and Chui et al. (1993) reported a reduction of BTI toxicity proportional to the number of freezing and thawing cycles. Carvalho-Pinto and Consoli (1994a) tested BS strain 2362 against *Cx. quinquefasciatus* at temperatures of 15, 20 and 25°C and observed a reduction of LC 50 with increasing temperature. Since most research on this topic was done in temperate climates, data in tropical climates concerning local vectors should be desirable.

Solar or direct ultra-violet exposure can sometimes impair BTI and BS activity. De Barjac (1989), Ignoffo et al. (1981), Lacey (1984), Priest (1992), Becker et al. (1992) observed that BTI had its activity reduced 2.5 to 4.0 fold after 7 hr of solar exposition in Germany, when tested against *Cx. pippens* and *Ae. aegypti*. When tested in Brazil, BS (strain 2362) also showed a progressively reduced activity against *Cx. quinquefasciatus* when exposed to 1, 2, 4, 6 and 8 hr of direct sunlight (Carvalho-Pinto & Consoli 1994a). In this case, as well, additional data obtained at tropical conditions should be useful.

Becker et al. (1992) and Chui et al. (1993) reported an inverted relation between larval density and efficacy of BTI against *Cx. pippens* and *Ae. vexans*. Carvalho-Pinto and Consoli (1994a), using 10, 25, 50 or 100 *Cx. quinquefasciatus* fourth instar larvae in 150 ml samples, failed to observe significant differences (α = 0.05) in the activity of BS (strain 2362) both after 24 or 48 hr.

The influence of the concomitant presence of other aquatic organisms, predators or others, in mosquito breeding places in relation to the efficacy of BTI or BS has been addressed in several publications. Becker et al. (1992) showed that the presence of the filter-feeder *Daphnia curvisrostris* can reduce the quantity of BTI toxins in the water and consequently its efficacy. Marten et al. (1993), on the other hand, reported a synergic action of BTI with three genera of cyclopoid copepods (*Macrocylops, Mesocylops* and *Acanthocylops*). Orduz and Axtell (1991) observed the compatibility of BTI and BS with the fungal pathogen *Lagenidium giganteum* in control of mosquito larvae. Reyna et al. 1993, in Clark and Suarez (1993) also observed the synergic action of *Buenoa* sp. (Hemiptera: Notonectidae) and BTI against *Cx. quinquefasciatus*. The possibility of survival and reproduction of entomopathogenic bacteria inside other organisms has been considered. Smański (1990) detected a few BTI spores in the faeces of the fish.
Pimephales promelas after two weeks. Belostoma micanultum (Hemiptera: Belostomatidae) is a common predator in Brazilian water habitats, that may include mosquito breeding places (Consoli et al. 1989). While BS could be detected in the gut of this predator up to 10 days after its ingestion of infected larvae, no evidence of BS replication could be observed there (Carvalho-Pinto et al. 1993). The development of B. micanultum, its reproductive ability and number predated larvae was not substantially influenced by the ingestion of BS infected Cx. quinquefasciatus larvae (Carvalho-Pinto & Consoli 1994b).

The complex environmental and biological factors influencing both bioinsecticides and their targets should be considered together in each instance to optimize mosquito control. New data, specially from tropical regions, where mosquitoes and mosquito-borne diseases are major problems, will be very necessary.

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