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BACILLUS SPHAERICUS INHIBITS HATCHING OF PHLEBOTOMINE SAND FLY EGGS[1]

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ABSTRACT. The effect of *Bacillus sphaericus*, at various concentrations, on hatching of phlebotomine sand fly eggs was examined using laboratory bioassays. Aqueous suspensions of *B. sphaericus*, strain 2362, inhibited hatching of eggs of *Phlebotomus duboscqi* and *Sergentomyia schwetzi* by 95% at concentrations as low as 0.05 and 0.11 mg/cm^2, respectively. In contrast, *B. sphaericus* did not affect the ability of pupae to emerge as adults.

KEY WORDS *Bacillus sphaericus*, ovicide, sand fly, *Phlebotomus dubascqi, Sergentomyia schwetzi*, control

Bacillus sphaericus Neide has been extensively investigated as a mosquito larvicide and has shown significant biological activity against several genera of mosquitoes, particularly *Culex* (Lacey and Singer 1982, Lacey et al. 1984, Mulla 1986) and has recently shown promise against phlebotomine sand flies (Robert et al. 1997). In contrast, *B. sphaericus* is not effective against many species of *Aedes* mosquitoes or blackflies (Hougard and Back 1992). In addition to toxic effects, sublethal concentrations of *B. sphaericus* cause delayed mortality and a wide range of external morphogenetic aberrations in larval, pupal, and adult *Culex quinquefasciatus* Say mosquitoes (Lacey et at. 1987, Mulla et al. 1991).

In response to a recent study (Robert et al. 1997) demonstrating *B. sphaericus* larvicidal effects against phlebotomine sand flies, we initiated a study to establish whether this bacterial agent has any effect on hatching of sand fly eggs. This article is the first report of ovicidal effects of *B. sphaericus* against phlebotomine sand fly eggs.

Two different aqueous formulations of *B. sphaericus* strain 2362 (Spherimos®) were used in this study. The two formulations were provided by the World Health Organization (WHO), Geneva, Switzerland, and Novo Nordisk Bioindustrials, Inc., Danbury, CT, respectively. Bioassays were conducted using *Phlebotomus duboscqi* Neveu-Lemaire, a vector of cutaneous leishmaniasis in Kenya (Beach et al. 1984) and *Sergentomyia schwetzi* Alder, Theodor, and Parrot, a nonvector nuisance species. The sand fly colonies were originally established from wild-caught specimens collected from Baringo District, Kenya.

Three separate tests were conducted on 3 separate days. Each test consisted of 4 replicates at each concentration. For each replicate 10 3 to 4-day-old eggs were placed on the bottoms of plaster-covered (1 cm deep), plastic petri dishes (4.8-cm diam). Serial dilutions of *B. sphaericus*/water suspensions were added to the individual dishes at concentrations of 0 (control), 0.01, 0.02, 0.04, 0.08, and 0.16 mg/cm^2. A total volume of 2.0 ml of liquid was applied to each dish because this volume evenly covered the plaster surface. The suspension was quickly absorbed by the plaster. Data from the hatching tests were analyzed using a probit plane procedure based on a modified Gauss–Newton algorithm using a computer program developed at Letterman Army Institute of Research, Presidio of San Francisco, CA. Calculation of the confidence limits was based on the method of Goldstein (1964). The analysis for the 3 tests, consisting of 12 replicates of each concentration, yielded effective dosage to inhibit hatching of 50% of the eggs (ED_{50}) and 95% effective dosage values (ED_{95}) and the corresponding 95% confidence intervals (CIs). Statistical significance between formulations was determined by nonoverlapping 95% CIs.

Both *B. sphaericus* formulations were effective in inhibiting hatching of eggs of *P. duboscqi* and *S. schwetzi* (Table 1). A significantly lower ($P < 0.05$) concentration of the WHO formulation (0.05 mg/cm^2) inhibited 95% of *P. duboscqi* eggs from hatching compared to the Novo Nordisk formulation (0.56 mg/cm^2). However, the 2 formulations were equally effective in inhibiting hatching of eggs of *S. schwetzi.*

In addition to preventing egg hatching, some larvae did not fully eclose from the egg and died within 24 h. Wraight et al. (1981) demonstrated that 1st-instar mosquito larvae are extremely susceptible to *B. sphaericus*, compared with later instars. Whether the mortality of sand fly 1st-instar larvae was caused by *B. sphaericus* toxins crossing the egg barrier or was due to the larvae eating the toxin

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Table 1. Hatching response of eggs of *Phlebotomus duboscqi* and *Sergentomyia schwetzi* treated with various concentrations of *Bacillus sphaericus*.

Formulation	P. duboscqi		S. schwetzi	
	ED_{50}[1] (95% CI)[3]	ED_{95}[2] (95% CI)	ED_{50} (95% CI)	ED_{95} (95% CI)
Novo Nordisk	0.05 (0.02–0.20)	0.56[4] (0.10–0.91)	0.03 (0.01–0.05)	0.12 (0.07–0.43)
World Health Organization	0.03 (0.02–0.04)	0.05[4] (0.04–0.06)	0.03 (0.03–0.04)	0.11 (0.10–0.22)

[1] Effective dosage to inhibit 50% of eggs from hatching; expressed as mg/cm^2.
[2] Effective dosage to inhibit 95% of eggs from hatching; expressed as mg/cm^2.
[3] 95% confidence interval.
[4] Values are significantly different between formulations, based on nonoverlapping 95% CIs, $P < 0.05$.

after emergence was unclear. Pupae, which do not feed, were placed on plaster as described above and treated with topical applications of *B. sphaericus* (0.05 mg/cm^2). This treatment did not significantly affect adult emergence from the pupae. This would suggest that *B. sphaericus* does not have a lethal topical effect on sand flies and must be ingested to cause mortality, similar to it's action in mosquitoes.

These data indicate that *B. sphaericus* may have potential as an ovicidal agent for phlebotomine sand fly control. This and previous studies (Robert et. al. 1997) indicate that bacterial insecticides may have important potential for sand fly control.

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