Research Note

Bacillus sphaericus interferes with the development of *Brugia malayi* in *Aedes aegypti*

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Abstract

Aedes aegypti (black-eyed Liverpool strain) were exposed to a sublethal dose (LD_{25}) of *Bacillus sphaericus* and were fed to *Mastomys coucha* infected with *Brugia malayi*. The development of the filarial parasite was found to be arrested mostly at the second larval stage. The infection ($P< 0.05$), infectivity rates ($P< 0.001$) and L_3 load ($P< 0.001$) were found to be reduced significantly in the treated group.

Bacillus sphaericus is considered as an alternative agent for microbial control of mosquitoes, following the isolation of highly larvicidal strains of this bacterium (2362) from the blackfly (Vilarinhos *et al.*, 1996). The lethal factor is a toxin which is produced during sporulation. The spores, when engulfed by mosquito larvae, are disintegrated by the gut enzyme, causing lysis of the gut, which in turn results in the death of the larvae (Young *et al.*, 1990). Although there is no visible effect on adults emerged from larvae treated with sublethal dosages of this biocide, there might be residual effects upon mosquitoes which could result in a reduction in their capacity as vectors (Young *et al.*, 1990). *Bacillus sphaericus* has also been shown to reduce the susceptibility of *Anopheles stephensi* to *Plasmodium yoelii* (Noireau & Karch, 1983) and *A. quadrimaculatus* to *P. berghei* (Young *et al*., 1990). Though several workers have studied the development of filarial nematodes in mosquitoes (Ewert, 1959; Franz & Zielke, 1980; Oda & Wada, 1980), the effect of the biocide on the development of filarial parasite in mosquitoes has not previously been reported. Therefore, the present study considers the effect of *Bacillus sphaericus* on the development of *Brugia malayi* in *Aedes aegypti* (black-eyed Liverpool strain).

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Third instar larvae of *A. aegypti* were treated with a sublethal dose (LD_{25}) of *Bacillus sphaericus* (2362). The emerging female mosquitoes were kept in separate cages and fed to *Mastomys coucha* infected with *Brugia malayi*. The microfilarial (mf) density in the blood of the animal was determined by microscopic examination of the peripheral smears before feeding and the mf count was recorded as the number of mf per 20 μl of blood. The mosquitoes were maintained at 27°C, with a relative humidity of about 80–90% and were provided with 5% glucose solution. After 14 days incubation, the mosquitoes were dissected and examined under the microscope for evidence of infection as shown by the presence of different larval stages (1st, 2nd and 3rd stage) of *Brugia malayi*. The infection rate (percentage of mosquitoes positive for any developing larvae), infectivity rate (percentage of mosquitoes positive for infective larvae) and L_3 load (number of infective third stage larvae per infective mosquito) were calculated for both treated and control groups. The mean mf density was 100 per 20 μl of blood.

The infections in the control and test mosquitoes were compared (table 1). The mosquitoes treated with *Bacillus sphaericus* were significantly less susceptible to infection than the controls. The infection rate, infectivity rate and L_3 load were also found to be reduced significantly in the treated groups ($P< 0.05$, $P< 0.001$ and $P< 0.001$ respectively) (table 1), whereas the growth of the

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Table 1. Effects of *Bacillus sphaericus* on the development of *Brugia malayi* in *Aedes aegypti*.

	Number of mosquitoes dissected	Mosquitoes positive for			Total number of L_3	Infection rate %	Infectivity rate %	L_3 load
		L_1	L_2	L_3				
Exposed	132	11	55	22	60	66.6	16.6	2.7
Control	148	8	10	96	519	77.0	64.8	5.4

L_1, first stage larvae; L_2, second stage larvae; L_3, third stage larvae.

Table 2. The prevalence (%) of larval *Brugia malayi in Aedes aegypti* exposed to *Bacillus sphaericus*.

	No of mosquitoes + ve for L_1, L_2, L_3	Mosquitoes positive for L_1		L_2		L_3	
		Number	%	Number	%	Number	%
Exposed	88	11	12.5	55	62.5	22	25
Control	114	8	7.0	10	8.8	96	84.2

L_1, first stage larvae; L_2, second stage larvae; L_3, third stage larvae.

parasite was mostly arrested at the second larval stage (L_2) (table 2).

Young *et al.* (1990) found no significant differences in the oocyst load when *Bacillus sphaericus* treated *Anopheles quadrimaculatus* were infected with the malaria parasite *Plasmodium berghei*. However, Mohapatra *et al*. (1996) obtained similar results to the present study in terms of infectivity rates but also found the L_3 load of the parasite in the mosquitoes to be significantly lower ($P < 0.001$) in the chitin synthesis inhibitor (triflumuron and hexaflumuron) treated mosquitoes than in controls.

Therefore, in field trials larval mortality may not represent the entire effect on the reduction of filariasis transmission. A significant reduction in the proportion of filarial larvae surviving to adults in mosquito larvae exposed to *Bacillus sphaericus* may also play a major role in vector control.

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