SCIENTIFIC NOTE

Toxicity of *Bacillus* Species to Larvae of *Lutzomyia longipalpis* (L. & N.) (Diptera: Psychodidae: Phlebotominae)

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Toxicidade de Espécies de *Bacillus* a Larvas de *Lutzomyia longipalpis* (L. & N.) (Diptera: Psychodidae: Phlebotominae)

RESUMO - Foi feito um estudo comparativo da susceptibilidade de larvas de terceiro estádio de *Lutzomyia longipalpis* (L. & N.) (Diptera: Psychodidae: Phlebotominae), vetor da leishmaniose visceral americana, a duas estirpes de *Bacillus thuringiensis* sorovar *israelensis* e uma de *Bacillus sphaericus*, todas patogênicas a dípteros Culicidae, além de uma de *B. thuringiensis* sorovar *morrisoni*, patogênica a larvas de *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). Larvas de *L. longipalpis* mostraram-se susceptíveis às duas amostras de *B. thuringiensis* sorovar *israelensis*, mas com baixa e nenhuma susceptibilidade às estirpes de *B. sphaericus* e de *B. thuringiensis* sorovar *morrisoni*, respectivamente.

PALAVRAS-CHAVE: Insecta, controle microbiológico, leishmaniose.

ABSTRACT - A study was conducted to compare the susceptibility of third instar larvae of *Lutzomyia longipalpis* (L. & N.) (Diptera: Psychodidae: Phlebotominae), the vector of the American visceral leishmaniasis to two strains of *Bacillus thuringiensis* serovar *israelensis*, one strain of *Bacillus sphaericus* (all pathogenic to Diptera Culicidae) and a strain of *B. thuringiensis* ser. *morrisoni* (pathogenic to larvae of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae)). Larvae of *L. longipalpis* showed similar susceptibility to the two strains of *B. thruringiensis* ser. *israelensis*, while *B. sphaericus* and *B. thuringiensis* ser. *morrisoni* showed low and no larvicidal effect to this vector, respectively.

KEY WORDS: Insecta, microbial control, leishmaniasis.

Control programs against phlebotomine sandfly vectors of leishmaniasis depend largely on household application of conventional insecticides (Gratz & Jany 1994, Perich *et al*. 1995, Service 1996). These applications target only adult populations since no practical means to control the immature stages of this pest are available, due to limited knowledge of their breeding sites in nature.

Diptera Nematocera larvae, including phlebotomine sandflies have a highly alkaline midgut, live in humid habitats and feed on decaying matter or associated fungi (Panizzi & Parra 1991). Although information regarding their breeding sites is scarce, it is likely that phlebotomine larvae are detritivores, similar to the majority of soil inhabiting invertebrates which consum dead organic matter in various decomposition stages. For this reason they can live in many microhabitats, such as fallen tree trunks, rock holes and cracks, humus, and so on. Such conditions are suitable for the development and long-term persistence of many entomopathogens, which can be used to control phlebotomine larvae (Warburg *et al*. 1991). This presents an opportunity, alone or in combination with other methods to control vectors of the leishmaniasis.

The spore forming bacteria of the genus *Bacillus* is efficient against important Diptera vectors of diseases, particularly Culicidae and Simulidae (Barjac & Sutherland 1990, Priest 1992, Rozendaal 1997, Vilarinhos *et al*. 1998). The susceptibility of sandfly larvae to *Bacillus thuringiensis* serovar *israelensis* was previously demonstrated by Barjac *et al*. (1981) against *Lutzomyia longipalpis* and *Phlebotomus papatasi*. Pener & Wilamowski (1996) showed that *P. papatasi* is susceptible to *B. sphaericus*. Such studies do not clarify the pathogenicity of *Bacillus* to sandfly larvae and no study has compared toxicity of entomopathogenic *Bacillus* species to sandfly larvae.

The objective of this study was to compare the susceptibility of *L. longipalpis*, the vector of the American visceral leishmaniasis, to four strains of entomopathogenic *Bacillus*. The *Bacillus* strains used were: (1) *B. thuringiensis* ser. *israelensis* LFB/FIOCRUZ 584, from the standard powder IPS-82; (2) *B. thuringiensis* ser. *israelensis* LFB/FIOCRUZ 710, isolated from soil of the Monte Paschoal National Park, Bahia, Brazil, and serotyped by Dr. H. de Barjac; (3) *B. thuringiensis* ser. *morrisoni*, LFB/FIOCRUZ 756, isolated from Brazilian soil of João Pessoa, Paraíba, Brazil, which was also serotyped by Dr. H. de Barjac; and (4) a standard strain of *B. sphaericus* ATCC 2362. All strains were supplied by the Collection Culture of the Genus *Bacillus* (CCGB), of the Department of Bacteriology of the "Instituto Oswaldo Cruz." The IPS 82 was originally supplied by Dr. H. de Barjac from the Pasteur Institute, Paris. *B. thuringiensis* ser. *morrisoni* proved pathogenic to larvae of *Anticarsia gemmatalis* (Hübner) (Lep. Noctuidae), while the other three strains have previously shown larvicidal action against dipterous Culicidae.

L. longipalpis larvae were obtained from a colony maintained through 78 generations in the insectary of the Leishmaniasis and Onchocerciasis Vectors Laboratory of the Entomology Department of the Instituto Oswaldo Cruz (Wermelinger *et al*. 1987).

Two concentrations of each bacteria were tested (10 mg and 20 mg of dry weight) of biomass bearing delta-endotoxin crystals and spores of *Bacillus*, obtained by bacterial growth in a New Brunswick 14 liter capacity fermentator loaded with a soya flour, divalent metals and yeast extract liquid culture medium, for 22 hours at 33°C. This biomass was harvested by continuous centrifugation (3500 rpm). Samples of equivalent biomass to dry weigh material were mixed and homog-

enized with 1 g of autoclaved larval food, and used during seven days in each experimental lot. After this period, all lots received normal food, a commercial aquarium fish food "Vitormonio", first used by Rangel *et al*. (1985).

Each experimental unit was represented by 20 third instar *L. longipalpis* larvae, kept in 150 x 20 mm petri dishes with a 6 mm thick plaster of Paris in its bottom. Each dish was placed in a cylindrical plastic pot (170 mm diameter by 70 mm height), and covered with a filter paper. The experiment was developed at 25°C in a B.O.D. incubator with relative humidity above 90%. Each bacterium concentration had four replications, and the control had at least five replications.

The experiment was carried out in three phases: the first with *B. sphaericus* ATCC 2362 and *B. thuringiensis* ser. *israelensis* LFB 584; the second with *B. thuringiensis* ser. *israelensis* LFB 584 and *B. thruringiensis* ser. *israelensis* LFB 710; and the third with *B. thuringiensis* ser. *israelensis* LFB 710 and *B. thuringiensis* ser. *morrisoni* LFB 756. Readings (three per week) were performed until the total number of surviving pupae in all dishes was recorded. Larval food was changed during each observation in order to avoid excessive fungal growth. Mortality rates of *L. longipalpis* larvae were based on total number of surviving pupae. Average mortality rates in the three phases, including the controls, were compared using Tukey's test (Pimentel Gomes 1990) at 5% probability level.

Data on the results of the tree phases are presented on Table 1. In the first phase, *B. sphaericus* was slightly virulent to *L. longipalpis* larvae with mortalities of 35% and 28,7% while *B. thuringiensis* ser. *israelensis* LFB 584 showed mortality above 70%. Mortalities atributed to *B. sphaericus* were not significantly different from the control while *B. thuringienis* ser. *israelensis* showed higher mortality than both *B. sphaericus* and the control. In the second phase, no significant differences were observed between three of the four mortality attributed to two strains of *B. thuringiensis* ser. *israelensis*. The mortality of 36.2% obtained for the LFB 584 strain in a concentration of 20 mg/g was statistically different from the 60% obtained for the concentration of 10 mg/g of this strain, and from averages of both concentrations (61.2%) of the strain LFB 710, and from mortality of 9.1% in the control. In the third phase, *B. thuringiensis* ser. *morrisoni* seemed to be nonpathogenic to *L. longipalpis* larvae, with mortality rates of 23.7% and 21.1% for the concentration of 10 mg/g and 20 mg/g, respectively. These results were lower than the mortality rate of 25% obtained in the control (Table 3). The LFB 710 strain of *B. thuringiensis* ser. *israelensis* showed 77.5% and 81.2% mortality rates with 10 mg/g and 20 mg/g respectively, which were significantly different from the 25% obtained in the control.

L. longipalpis larvae proved to be more susceptible to *B. thuringiensis* ser. *israelensis* than *B. sphaericus* and *B. thuringiensis* ser. *morrisoni*. *B. thuringiensis* ser. *israelensis* is pathogenic to sandfly larvae (Barjac *et al*. 1981), to mosquitoes of the genus *Culex, Anopheles*, *Aedes* and *Mansonia* (Nicolas 1992) and also to larvae of other Diptera in the Blephariceridae (Boisvert *et al*. 1985), Chironomidae (Ali *et al*. 1981, Boisvert *et al*. 1985, Mulla *et al*. 1990, Molloy 1992), Ceratopogonidae (Lacey & Kline 1983), Muscidae (Temeyer 1984), Sciaridae (Osborne *et al*. 1985, Keil 1991) and Tipulidae (Waalwijk *et al*. 1992, Smiths *et al*. 1993) which indicates that this bacteria is pathogenic to a wide spectrum of Diptera larvae species.

Larvicidal activity of *B. thuringiensis* ser. *israelensis* to *L. longipalpis* or to other species of sandfly vectors is poorly studied and needs to be more carefully evaluated because larval susceptibility to bacterial toxins varies considerably, even between closely related taxa. It may also vary with different conditions such as temperature, nutrition and larval stage. For example, in our results *B. sphaericus* showed slight pathogenicity to *L. longipalpis* larvae, but under different conditions, Pener and Wilamowski (1996) observed high susceptibility of 13 ± 2 and 20 ± 2 days

Table 1. *L. longipalpis* mortality rates (%) with *B. thuringiensis* ser. *israelensis* LFB 584 (Bti 584) LFB 710 (Bti 710), *B. sphaericus* ATCC 2362 (Bs 2362) and *B.thuringiensis* ser. *morrisoni* LFB 756 (Btm 756)

Replication	Percentage of mortality				
	Control	10 mg/g[1]	20mg/g	10 mg/g	20mg/g
1st phase		Bs 2362		Bti 584	
r1	15	50	40	55	90
r2	15	30	20	75	60
r3	20	40	15	85	70
r4	0	20	40	75	75
Mean	12.5±8.66 b	35.0 ±12.90 b	28.7±13.14 b	72.5±12.58 a	73.7±12.50 a
2nd phase		Bti 584		Bti 710	
r1	5	60	30	60	70
r2	15	35	25	45	55
r3	15	70	45	75	65
r4	15	75	45	65	55
r5	5	-	-	-	-
r6	0	-	-	-	-
Mean	9.1±6.64 c	60.0±17.79 a b	36.2±10.30 b	61.2±12.50 a	61.2±7.50 a
3rd phase		Btm 756		Bti 710	
r1	5	30	20	75	85
r2	25	15	5	55	95
r3	30	20	45	90	60
r4	35	30	15	90	85
r5	25	-	-	-	-
r6	30	-	-	-	-
Mean	25.0 ±10.48 a	23.7±7.50 a	21.2±17,02 a	77.5±16.58 b	81.2±14.93 b

[1]mg of bacterial biomass/g of larval diet. (-) no replications were made
Means followed by the same letter are not significantly different at 0.05 probability level (Tukey)

old larvae of *P. papatasi* to *B. sphaericus*.

Mortalities in the control (first and third phases) were higher than 10%, which must be discarded according to Alves *et al.* (1998). However, normal mortality in experimental conditions and insetary, from third instar to pupae ranged from 10% to 30%. For these reason mortalities in the control groups can be accepted, although these rates should be kept as low as possible.

We conclude that *L. longipalpis* larvae are more susceptible to *Bacillus thuringiensis* ser. *israelensis* than *B. sphaericus* and *B. thuringiensis* ser. *morrisoni*. In comparison with *B. t. israelensis*, the other two strains proved to have low or none larvicidal activity.

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