Studies on the *Bacillus sphaericus* Larvicidal Activity against Malarial Vector Species in Amazonia

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In this work, bioassays were carried out in laboratory conditions (average temperature 26 ± 2ºC) to test ten strains of Bacillus sphaericus, *isolated from Brazilian soils against third instar larvae from anopheline species recorded as malaria vectors in Amazonian* - Anopheles nuneztovari *and* An. darlingi. *With the former mosquito, three strains - S_2, S_{20} and S_{46} showed relative activity, in 24 and 48 hr exposure to the* B. spahericus *strains. With the latter only the S_2 and S_{20} were effective in the 48 hr reading. The studied strains that showed the most adequate response in the Amazonian region were S_2 and S_{20} showing broader and more efficient results. Therefore, S_2 was the most effective when the 24 and 48 hr readings were considered, because it showed the greatest relative activity values.*

Key words: malaria - biological control - anopheline - *Bacillus sphaericus*

In the Amazonian region there are great malaria transmission areas mainly those with recent disturbance caused by settling, mining camps and some native villages, where a large number of vectors exists. The methodology used for the control of these mosquitoes, in this region, has been based on intradomicilium chemical control, which has not accomplished the same results as observed in other regions in the southern and southeastern parts of the country.

The importance of new technologies in the seeking of intervention strategies, such as for planning and application of preventive measures and vector control in a selective way is being pointed out. The biological control is an important strategy when prioritizing the enviromental question. For malaria in Amazonian endemic areas, the application of this means of attack on immature forms is one of the most relevant methods for winged density control. Therefore, in order to be implemented, bioassays are needed to evaluate the *Anopheles* species response to the biological control agents.

This work presents the first data obtained on the susceptibility of anopheline larvae, which are pointed out as malaria vectors in Amazonia - *Anopheles nuneztovari* and *An. darlingi*, to ten strains of *Bacillus sphaericus* isolated from Brazilian soils.

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MATERIALS AND METHODS

Anopheline females collected in the suburbs of Manaus, State of Amazonas and in the municipal district of Jaciparaná, State of Rondônia, Brazil, were used in bioassays. Mosquitoes were laid to oviposit one by one in the insectary at a temperature of 26 ± 2ºC, with relative humidity of 80-90%, and were maintained in the laboratory according to Santos et al. (1981).

Ten strains of *B. sphaericus* were utilized in this work. They were isolated from soils colected in the following localities: Brasília, Federal District - S_2, S_{46}; Corumbá, State of Mato Grosso - S_7, S_{11}, S_{15} , S_{17}, S_{20}, S_{21} and S_{24}; Vitória, State of Espírito Santo - S_{32}. These were taken from the Microbian Germoplasm Bank of Centro Nacional de Pesquisas de Recursos Genéticos e Biotecnológicos (CENARGEN). All the strains belong to serotype H5a5b (Barjac 1990, Schenkel et al. 1992, Dias 1992). For comparing Brazilian strains, the *B. sphaericus* strain 2362, the World Health Organization (WHO) standard strain was utilized.

The bioassays were conducted with third instar larvae, with six or seven concentrations of lyophilized bacteria ranging from 0.5 ppb to 50 ppm. The lyophilized of each strain was prepared from cultures in a rotating incubator (30±0.5ºC, 200 rpm) for 48 hr, centrifuged and rinsed with destilled water, frozen and lyophilized from 12 to 15 hr. Same as Brazilian isolates, strain 2362 has been grown and prepared for assay in the same way.

Twenty larvae per concentration tested from each *Anopheles* species were used in one single bioassay. The method used in the observation of mortality and survival of the biossays was the one of Dulmage et al. (1990), with some changes.

The target population average for lethal concentration (LC_{50}) in each period of observation (24 and 48 hr) was estimated through probits analysis (Finney 1981) using the POLO-PC program. The relative activity (RA) was calculated using *B. sphaericus* 2362 as a standard, acording to the following formula:

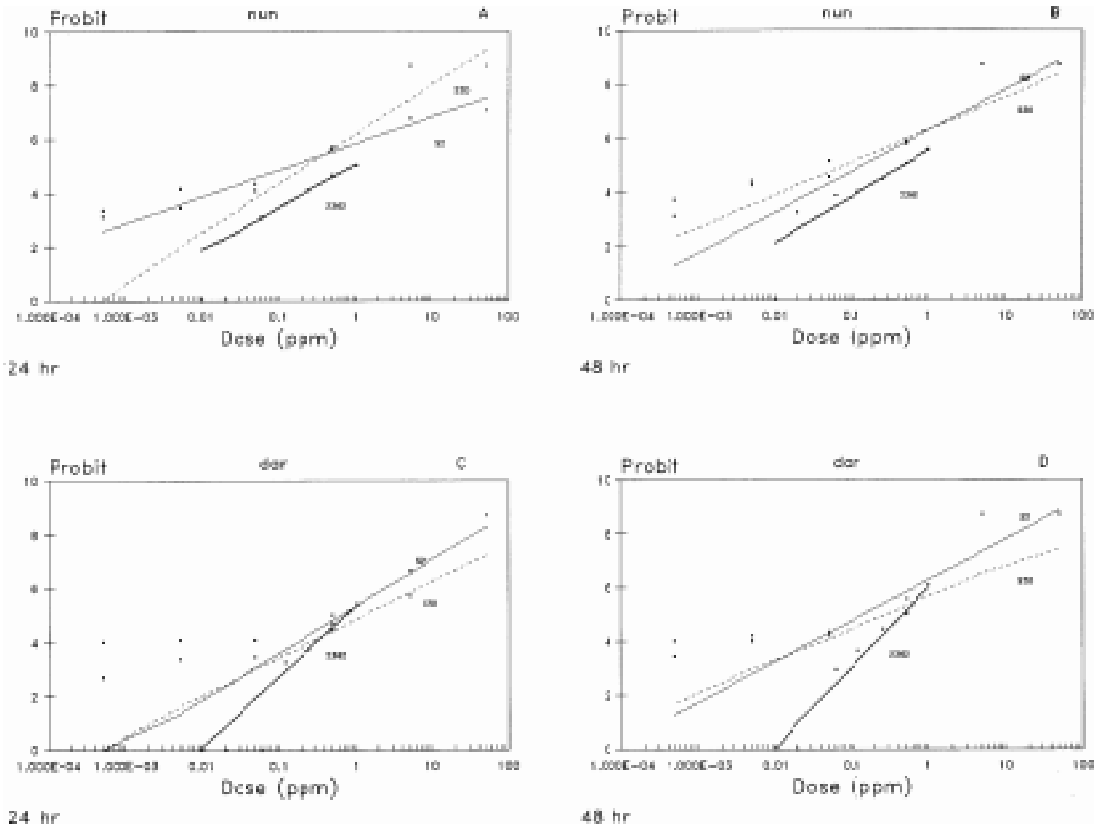
$$RA = - \frac{LC_{50} \text{ standard}}{LC_{50} \text{ sample}}$$

The values of RA were calculated for 48 hr results.

RESULTS AND DISCUSSION

The larvicidal activity of the ten Brazilian strains tested can be evaluated by the association of the LC_{50} values (Table I) with the RA (Table II). Regarding *An. nuneztovari*, it is observed that the S_2, S_{20} and S_{46} are the most effective with LC_{50} values between 0.084-0.127 ppm in 48 hr reading. In this last reading, considering *B. sphaericus* 2362 as a standard, the three cited strains showed RA varying from 3.7 to 5.6 times. The RA shows values above four units, except for S_2 in the 48 hr reading (Table II). Few strains were effective in the control of *An. darlingi*, when we associated LC_{50} to RA. The S_2, S_{20} and S_{46} were again stressed (only in the 48 hr reading) with RA around four and five times.

Figure shows the graphic representation of the dosage response line for the S_2, S_{20} and S_{46} strains to the two species. Figure also shows the values for the 2362 WHO standard strain. It is observed that for *An. nuneztovari* (Figs 1A, 1B) the S_2, S_{20} and S_{46} strains were more effective than the 2362. It has also been shown that the linear regression straight lines of the 2362 strain, in both Figs, presents a relatively parallel pattern to the S_2 and S_{20} straight lines. In *An. darlingi* (Figs 1C, 1D) the LC_{50} values of S_2 and S_{20} are close to the 2362 strain, in the 24 hr reading. The S_2 is more effective being close to the S_{20} and 2362 values, in 48 hr. The linear regression straight lines of S_2, S_{20} and 2362 are divergent in the minor concentration doses (abscissa), for they show zero mortality (24 hr) or next to 10% (48 hr - S_2 and S_{20}). There are no studies about biological activity of *B. sphaericus* against anophelines which are important in the Amazonian region. S_2 and S_{20} straight lines cross with the one from 2362 in values near the LC_{50} from the three strains. The RA data (Table II)



Dose response line of *Bacillus sphaericus* Brazillian strains against third instar larvae *Anopheles* species.

TABLE I

Values of the mean lethal concentration (LC_{50}, ppm) of *Bacillus sphaericus* Brazillian strains against *Anopheles* species

Strains	Species			
	An. nuneztovari		*An. darlingi*	
	24 hr	48 hr	24 hr	48 hr
S_2	0,169	0,127	1,187	0,092
S_7	1,519	0,783	1,006	0,337
S_{11}	0,613	0,462	4,740	2,592
S_{15}	0,634	0,534	0,383	0,142
S_{17}	4,253	4,229	0,252	0,174
S_{20}	0,205	0,084	0,493	0,097
S_{21}	0,652	0,661	1,753	0,647
S_{24}	0,466	0,239	0,669	0,254
S_{32}	260,908	273,885	2,572	23,012
S_{46}	0,159	0,105	1,119	0,106
2362	0,895	0,470	0,735	0,457

TABLE II

Relative activity[a] values of 48 hr readings of *Bacillus sphaericus* Brazilian strains against *Anopheles* species

Strains	Relative activity	
	An. nuneztovari	*An. darlingi*
S_2	3,709	4,967
S_7	0,601	1,356
S_{11}	1,020	0,176
S_{15}	1,676	3,218
S_{17}	0,111	2,626
S_{20}	5,607	4,711
S_{21}	0,712	0,706
S_{24}	1,971	1,799
S_{32}	0,001	0,019
S_{46}	4,431	4,310

a: standard strain is *B. sphaericus* 2362.

revealed that the S_2 and S_{20} strains were effective for the two species either at 24 or at 48 hr readings. Considering the other tested strains, in a general way, the RA values were lower than when compared to standard *B. sphaericus* 2362 strain. Therefore, it is observed that the Brazilian strains that presented a potential for the control of the anopheline immature forms were S_2, S_{20} and S_{46}, at 24 or 48 hr readings.

The studies on new strains against anopheline immature forms have been carried out in different parts of the world. Mulla (1986) tested larvicidal activity of different *B. sphaericus* 2362 preparations, and of 1593 and 2297 strains against *An. albimanus* and *An. quadrimaculatus*. In relation to the two *Anopheles* species, an activity variation of mortality rate caused by *B. sphaericus* 2362 was observed *An. albimanus* was from 8 to 25 times more sensitive than *An. quadrimaculatus*. Formulations showed different levels of larvicidal activity, according to the 2297 AP and SD strain formulations and to the 1593 Sawdust formulation, which showed high larvicidal activity levels against *An. albimanus* and low activity against *An. quadrimaculatus*. Studies with different *B. sphaericus* strains, distributed into the six serotypes, were made testing *An. sthephensi* third instar larvae. Comparatively, the average LC_{50} on *An. stephensi* larvae were around 10^{-5} FWC dilutions (Thiery & Barjac 1989).

Other strains from different places in Brazil, that were also isolated at CENARGEN, were used by Schenkel et al. (1992) in bioassays with *An. albimanus*. These authors tested S_1, S_2, S_5 and L_2 (all isolated from Brasília) against *An. stephensi* larvae, taking the mortality rate observed with *B. sphaericus* 2362 as a standard. They observed that only L_2 did not show larvicidal activity similar to the 2362 isolated strain, against this mosquito species. The S_2 strain showed a toxicity that also affects other genera and *Anopheles* sub-genera. *B. sphaericus* entomopatogenic strain was first reported in a soil sample from Distrito Federal, pointing to S_2 (Schenkel et al. 1992). Later characterization and biological activity showing that S_2 is equaly or even more toxic than 2362 for *An. albimanus* and *An. quadrimaculatus*. Besides these, S_2 was biochemicaly characterized and differenciated from 2362 through lipid gaseous chromagraphy of the cell wall (Vilarinhos 1991).

The continuos isolation of new strains from native ones has indicated that many of these isolates showed a greater larvicidal activity than the 2362 strain.

Concluding, it should be pointed out that in several experiments in which S_2 was used, against several *Anopheles* species, this strain showed high larvicidal activity often greater than the standard 2362 strain.

Brazilian strains of *B. sphaericus* show a greater larvicidal activity against *Anopheles* species, the malarian vectors in Amazonia, in relation to the standard 2362 strain. *An. nuneztovari* third instar larvae were more susceptible to strains: S_2, S_{20}, and S_{46} in 24 and 48 hr exposure. In *An. darlingi* only S_2 was effective in the two readings. The S_{20} showed greater activity only in the 48 hr reading. These data revealed that S_2, S_{20} and S_{46} strains were potentialy the most adequate for the Amazonian region, against the studied species immature forms. Therefore, S_2 can be pointed out as the most effective one, for it showed activity in both readings with higher RA values.

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