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ARTICLE

Effectiveness of *Bacillus sphaericus* on *Anopheles nuneztovari* (Diptera: Culicidae) in Amazonia

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ABSTRACT: (Effectiveness of *Bacillus sphaericus* on *Anopheles nuneztovari* (Diptera: Culicidae) in Amazonia). Under laboratory conditions, bioassays were carried out in order to test the effectiveness of eight *Bacillus sphaericus* Neide strains isolated from Brazilian soils, against larvae of *Anopheles nuneztovari*, a potential malaria vector in Amazonia. The findings from this study showed a greater effectiveness of the S14 (CL50 0.07 ppm), S58 (LC50 0.10 ppm), S42 (0.11ppm) and S12 (LC50 0.24 ppm) strains, after being in contact with the larvae for 48h. Compared with the 2362 *B. sphaericus* standard strain (LC50 0.47 ppm), the four strains showed relative potency (around 2-6 times higher), and are therefore a promising control for *Anopheles* species in Amazonia.
Key words: malaria control, biolarvicides, *Anophelines*.

RESUMO: (Efetividade de *Bacillus sphaericus* para *Anopheles nuneztovari* (Diptera: Culicidae) na Amazônia). Foram realizados bioensaios em condições de laboratório, para testar a efetividade de oito estirpes de *Bacillus sphaericus* Neide 1904 isoladas de solo brasileiro, contra larvas de *Anopheles nuneztovari* Gabaldón 1940, espécie com importância na transmissão da malária na região amazônica. Os resultados apontaram maior efetividade para estirpes denominadas S14, S58, S42 e S12, com CL_{50} de 0,07 ppm, 0,10 ppm, 0,11 ppm e 0,24 ppm, respectivamente, em 48 horas de contato com as larvas. Ao serem comparadas com a estirpe padrão 2362, essas quatro estirpes citadas mostraram potência relativa cerca de 2-6 vezes superior, sendo, portanto, promissoras para controle de *An. nuneztovari* na região amazônica.
Palavras-chave: Anofelinos, Biolarvicidas, controle da malária.

INTRODUCTION

According the World Health Organization (WHO 2008), malaria is the widest spread disease, with 3 billion people at risk of infection in 109 countries and territories, and there are around 250 million cases annually that lead to approximately 1 million deaths. "The fundamental action is vector control, which, if effective, will reduce the number of cases requiring treatment. The integration of preventive services (e.g., vector control) into health services primarily oriented towards treatment is a formidable challenge that calls into question the nature and level of planning and implementation of essential vector control functions" (WHO 2006).

In Brazil, nearly all recorded cases of malaria have been from the Amazonian region, representing a serious public health care problem (Rodrigues 2006, Tadei 2008), and, according to data from FUNASA/SISMAL/DIVEP-AM, 99.7% of the cases in this country are from this region. *Anopheles darlingi* Root (1926) is the principal species that transmits malaria along the Amazon. In addition, *A. nuneztovari* Gabaldón (1940), a species frequently found in the periphery areas of Manaus, is considered a vector of human malaria in several localities in Venezuela and Colombia. However, its status as a vector of human malaria is still unknown in the Brazilian Amazon, in spite of the fact that it is sometimes infected with *Plasmodium* sp. (Arruda *et al*. 1986, Scarpassa *et al*. 1999, Tadei *et al*. 1993, Tadei *et al.* 1998).

Presently, new disease fighting alternatives are being sought for minimizing the problems caused by the abusive use of chemical insecticides. Biological control has arisen as an efficient but immature form of control. By integrating biological control with other strategies such as environmental management, insecticide impregnated mosquito nets, indoor spraying and thermal-fogging have been successful in reducing malaria cases. However, the effective deployment of this is a pre-condition for successful and sustained control of malaria (Yousten 1984, Becker 1997, Rodrigues 2006, Litaiff *et al*. 2008).

Bacillus sphaericus entomopathogenics has turned into a major tool used for the development of biolarvicides. These are important because they can selectively target insects, in relation to environmental conditions of a region, and, for this reason, *B. sphaericus* has become a promising vector control for malaria in Amazonia (Dias 1992, Litaiff 2002).

This method, together with other routine controls, has shown positive results for anopheline control in the outskirts of Manaus near aquaculture ponds and puddles in brickyard (Rodrigues *et al*. 2008). The integrated control strategy provides conditions that reduce the quantity of chemical insecticides used, which helps to minimize

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the environmental impacts of the control.

This study aimed to test the toxicity of eight *B. sphaericus* strains, isolated from Brazilian soils, against *A. nuneztovari*, with the purpose of selecting those that were the most promising to be used in a biological control for malaria vectors in the Amazonian region.

MATERIALS AND METHODS

The eight *B. sphaericus* strains tested were provided by CENARGEN/EMBRAPA (Brasília), which originated from Corumbá-MS (S4, S6, S12, S14 and S27), Vitória–ES (S3 and S4), and Alta Pará–SP (S58). Bioassays were also performed with the *B. sphaericus* 2362 standard stain. *Anopheles nuneztovari* larvae used in bioassays were kept in containers at 26±2ºC, with a relative humidity of 85%, and a photoperiod of 12L:12D (Scarpassa & Tadei 1990), until reaching the third instar.

Third and fourth instars were equally less susceptible than earlier instars. However, the third instar larvae were used because their rate of food intake is higher than fourth instar larvae, which could influence the results.

For the bioassays, five-cup replicates with three repetitions were set up, which consisted of the following: 20 larvae, the doses of *Bacillus*, and the volume of distillated water needed to fill the cup to 100mL. A control group was set up for each test. The doses tested were obtained through serial dilutions from a 50 parts per million (ppm) standard solution to seven doses: 1.00, 0.50, 0.25, 0.12, 0.06, 0.02 and 0.01 ppm (Dulmage *et al.* 1990). Readings were made every 24, 48 and 72h, after the *Bacillus* application, to record larvae mortality.

For the bioassays, percentiles were corrected using the Abbott's formula when the control group mortality exceeded 5%. LC50 was calculated by the Probit analysis (Finney 1981) with the aid of the program POLO-PC. The relative potency (RP) was calculated based on the relation between the LC50 standard 2362 strain and the LC50 sample strains (Rodrigues *et al.* 1998).

RESULTS

Strains S58, S42 and S14, in 1.0 ppm doses, caused nearly 90% mortality of the *A. nuneztovari* larvae within 24h. The percent mortality of the S12 strain (when also using 1.0 ppm doses) followed, reaching as high as 60% within 24h. The same was observed after 48h of exposure to *Bacillus*. However, after 48h the bioassays with the S4 strain showed a mortality of around 60% using a 1.0 ppm dose. The mortality values of the other four strains fluctuated and only presented higher values at 72h, using the 1.0 ppm dose, which were around 50% (Fig. 1).

The S14, S42, S58 and S12 strains showed the lowest values: 0.07 ppm, 0.10 ppm, 0.11 ppm and 0.24 ppm, respectively. The other strains tested showed a higher LC50 value relative to the 2362 standard strain (LC50 0.47 ppm) Strain effectiveness was also analyzed by considering relative potency (RP). Strains S14, S58, S42 and S12 showed higher larvicide activity compared to the standard strain, with RP values that were 6.71, 4.27, 4.70 and 1.96, respectively. This higher activity was observed during subsequent readings. The remaining strains showed reduced RP values, which were observed for all readings (Tab. 1); the lowest values found were for the S3, S27 and S6 strains (RP 0.02, 0.15 and 0.17, respectively).

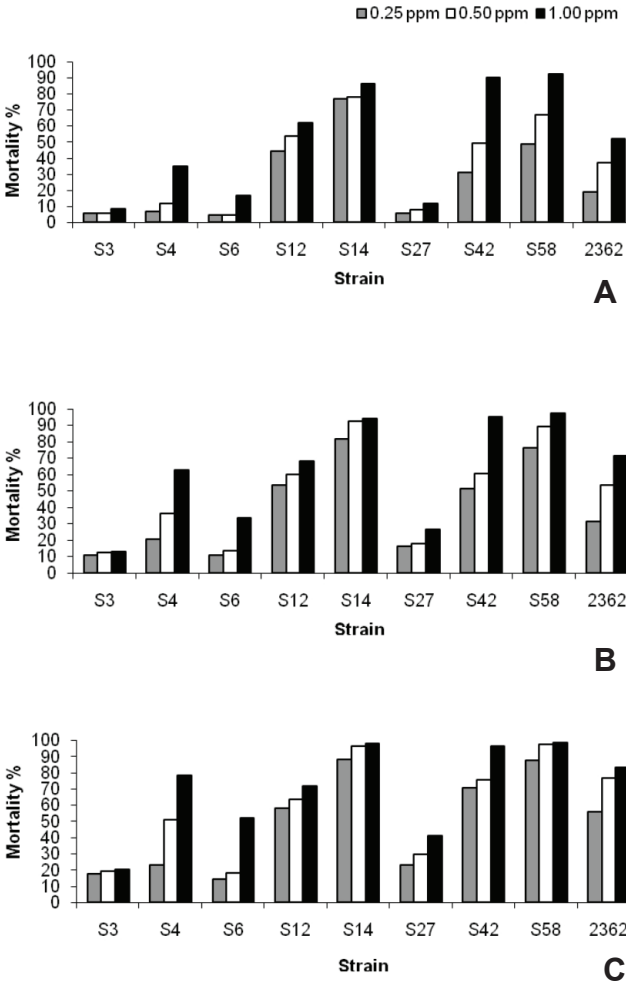

Figure 1. Mortality of *Anopheles nuneztovari* larvae obtained in the bioassays treated with 0.05, 0.50 and 1.00 ppm doses of *Bacillus sphaericus* after 24h (A), 48h (B) and 72h (C) applications.

Considering the mortality rates of *An. nuneztovari*, the results of the different strains tested showed that all four strains were more effective than the standard 2362 strain, but the values found for each strain were very close to each other (Fig. 2).

DISCUSSION

Knowing the larvicidal activity of *B. sphaericus*, when used against target species, is an important step towards applying it as a control. Studying anophelines involves understanding the susceptibility of the instar development of each species (Rodrigues *et al.* 1998). *Anopheles nuneztovari*, which was used in this study,

Table 1. Median Lethal Concentration (LC50) and Relative Potency of strain 2362, obtained from bioassays with *Bacillus sphaericus* on larvae of *Anopheles nuneztovari*.

Strain	LC50 ppm (Fiducial Limit)	Probit equation	Relative Potency*
S3	28.59 (16.01-32.47)	y = 3.60 + 0.96x	0.02
S4	0.67 (0.44-0.99)	y = 5.25 + 1.97x	0.70
S6	2.69 (1.98-2.97)	y = 4.38 + 1.45x	0.17
S12	0.24 (0.13-0.36)	y = 5.65 + 1.43x	1.96
S14	0.07 (0.06-0.10)	y = 7.03 + 2.26x	6.71
S27	3.18 (2.12-4.96)	y = 4.23 + 0.76x	0.15
S42	0.10 (0.83-0.25)	y = 6.11 + 1.55x	4.70
S58	0.11 (0.10-0.34)	y = 5.80 + 0.88x	4.27
2362	0.47 (0.40-0.54)	y = 5.56 + 1.71x	-

*Relative to 2362 *B. sphaericus* strain.

x = log dose.

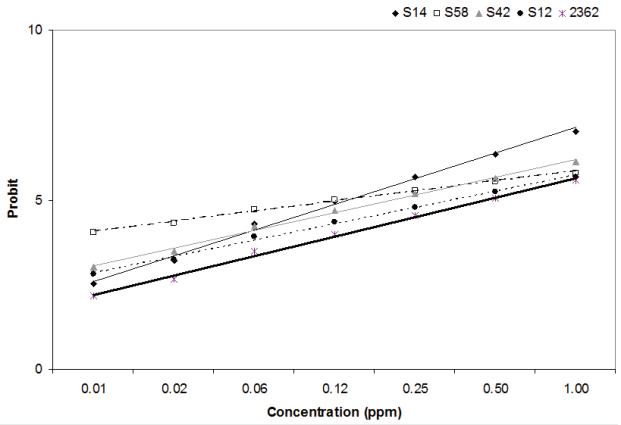

Figure 2. Comparison among *Bacillus sphaericus* strains linear regression lines in bioassays with *Anopheles nuneztovari* larvae after 48h of exposure to S14, S58, S42 and S12 strains.

shows a lower susceptibility than *An. darlingi* and *An. braziliensis* Chagas (1907) to *B. sphaericus* 2362. In addition, for *An. darlingi* and *An. braziliensis*, the third instar larvae are the most resistant to the *B. sphaericus* larvicide (Rodrigues *et al* 1999).

The LC50 values obtained in this study indicated higher toxicity for the S14, S58, S42 and S12 strains, which had a higher relative potency compared to the 2362 standard strain. Relative to the standard strain, when comparing the larvae for the first 48 hours of contact, the activity was nearly seven times higher for the S14 strain, whereas the S42 and S58 strains were about three times more effective. This higher activity was also observed for the subsequent readings.

Other studies using strains isolated from Brazilian soils, with higher larvicide activity than the 2362 standard strain, have been reported for the S1, S2, S5 and L2 strains against *C. quinquefasciatus* and *An. stephensi* (Schenkel *et al*. 1992). High larvicide potency was also observed by Vilarinhos *et al.* (1996) using the S2 strain against *An. albinamus* (LC50 = 5.95µg/L), *An. quadrimaculatus* (LC50 = 12.28µg/L), and *C. quinquefasciatus* (LC50 = 0.25µg/L). The activity of strain S2 using bioassays was analyzed against *An. darlingi* (LC50 = 0.09 ppm) and *An. nuneztovari* (0.12 ppm) (Rodrigues *et al* 1998). Litaiff *et al* (2008) described higher toxicity than the 2362 strain, for bioassays using *B. sphaericus* S15 (LC50 0.04 ppm) and S1116 (LC50 0.05 ppm) strains, to control *An. darlingi.* Because of its high toxicity against several mosquito species, using the S15 strain studied by these authors deserves further investigation, as well as the acquisition of a larger number of spores, for its production as a Brazilian larvicide at a pre-commercial scale (Litaiff 2006).

The findings obtained in this study show that the continuous isolation and evaluation of wild strains against vectors reveals their diversity and larvicide capacity, and points out their great potential for being used in biological insecticides. These findings also indicate that there are useful Brazilian strains, suggesting that further evaluation studies should be conducted, which include strains from Amazonia, to find alternative vector controls.

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