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Effect of *Bacillus sphaericus* Neide on *Anopheles* (Diptera: Culicidae) and associated insect fauna in fish ponds in the Amazon

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ABSTRACT

We analyzed the effects of *Bacillus sphaericus* on *Anopheles* larvae and on the associated insect fauna in fish farming ponds. Five breeding sites in the peri-urban area of the city of Manaus, AM, Brazil, were studied. Seven samples were collected from each breeding site and *B. sphaericus* was applied and reapplied after 15 days. The samples were made at 24 h before application, 24 h post-application and 5 and 15 days post-application. We determined abundance, larval reduction and larval density for *Anopheles*, and abundance, richness, Shannon diversity index and classified according to the functional trophic groups for associated insect fauna. A total of 904 *Anopheles* larvae were collected and distributed into five species. Density data and larval reduction demonstrated the rapid effect of the biolarvicide 24 h after application. A total of 4874 associated aquatic insects belonging to six orders and 23 families were collected. Regression analysis of diversity and richness indicated that the application of the biolarvicide had no influence on these indices and thus no effect on the associated insect fauna for a period of 30 days. *B. sphaericus* was found to be highly effective against the larvae of *Anopheles*, eliminating the larvae in the first days after application, with no effect on the associated insect fauna present in the fish ponds analyzed.

Introduction

The Amazon environment is rich in water resources, very common mostly due to the existing mesh of rivers, thus enabling the formation of numerous breeding sites for many groups of aquatic organisms (Sioli, 1984). These organisms particularly include insects, where some of them are vectors of pathogens that cause human tropical diseases. Accordingly, mosquitoes occupy a central role because of their plasticity in colonizing different aquatic environments, high density in this environment and food preference for human blood (Tadei and Thatcher, 2000; Forattini, 2002).

Control of the disease, according to the National Program for Prevention and Control of Malaria Brazil, has among other measures, early diagnosis and treatment of patients, major steps for lifting the movement of parasite. They are also recommended vector control measures: indoor residual insecticide application, the treatment of breeding sites with biolarvicides, the use of impregnated mosquito nets and long term, in special situations, spatial fogging of insecticides (Ministério da Saúde/SVS, 2003; Oliveira-Ferreira et al., 2010).

Breeding sites play an essential role in the maintenance of the disease, since adults emerge ready for their daily blood meal (Rodrigues et al., 2008). The control of immature forms can be performed using chemical larvicides, but this technique is not used due to the risk of development of resistance and environmental contamination, and therefore, the use of biological larvicides is increasing. The main representatives of these larvicides are toxic crystal-producing bacteria of the genus *Bacillus* (Galardo et al., 2013). The use of the species *Bacillus sphaericus* Neide, 1904 for mosquito control was advocated in 1985 by the World Health Organization. Since then, larvicides have been produced with this bacterium due to its recognized toxicity to the genera *Anopheles* and *Culex* (Habib, 1989; De Barjac, 1990).

The application of biological larvicides to control immature *Anopheles* sp. is carried out directly at the breeding sites (De Barjac, 1990). However, these environments also have an associated insect fauna, which is formed by several other groups of aquatic insects that share the same habitat as these mosquitoes (Lang and Reymond, 1994). About 13 orders of insects have an aquatic phase, and in some biotopes, they may comprise around

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95% of the macroinvertebrate community. These invertebrates play a key role in the health of the water body, by participating in the cycling of nutrients and the transformation of organic matter, contributing to the flow of energy (Brasil et al., 2014).

The effect of *B. sphaericus* on the associated insect fauna under laboratory and field conditions has been investigated over the past decades, and most of these organisms have not shown any susceptibility to the bacteria (Mulla et al., 1984; Aly and Mulla, 1987; Karch et al., 1991; Rodcharoen et al., 1991; Brown et al., 2004; Merritt et al., 2005).

Rodrigues et al. (2008) conducted field tests in Manaus, Brazil, applying *B. sphaericus* in fish ponds and standing water in pottery, and observed the elimination of larvae at breeding sites 48 h after application. Rodrigues et al. (2013) investigated the effects of *B. sphaericus* in applications on the Negro and Solimões Rivers, where it was found more effective in the black water river than the white water, which has a higher amount of suspended material.

Studies of the effect of *B. sphaericus* against *Anopheles* larvae have been conducted in Brazil, but there is a gap in our knowledge of its action on the associated insect fauna, particularly in the Amazon environment. This study aimed to analyze the effects of *B. sphaericus* on *Anopheles* and the associated insect fauna in fish farming ponds in the peri-urban area of the city of Manaus, Amazonas.

Material and methods

Bioassays in field

The sampling sites are located in the peri-urban region of Manaus, Central Amazonia in northern Brazil. Five artificial breeding sites of *Anopheles*, namely fish culture ponds, were selected: C1 (S 03°04′32.2″, W 59°53′07.4″); C2 (S 03°02′07.6″, W 59°53′30.2″); C3 (S 03°03′45.8″, W 59°51′11.3″); C4 (S 03°02′44.4″, W 59°53′09.0″) and C5 (S 03°02′43.9″, W 59°53′09.0″) (Fig. 1). The larvicide used was VECTOLEX CG®, formulated granules (Valent BioSciences Corporation), concentrated dried serotype H5a5b at 7.5% with a power of approximately 670 Bs international toxin units (ITU), containing corn oil and corn cob granules. We used the dose recommended by the manufacturer, i.e., 11 kg/ha.

The applications were done manually by reaching across the edge and over a perimeter of 3 m of the breeding center. Field bioassays lasted 30 days at each pond and two applications (application and reapplication) of biolarvicide were performed with an interval of 15 days between them. Samples of *Anopheles* larvae and associated insect fauna were obtained at the following times: pre-application – sampling performed before application; and after application of biolarvicide – three post-application samples and three post-reapplication samples at the following times: 24 h, 5 days and 15 days. The period of larvicide application began in December 2011 and lasted until April 2012, where there were 9 field trips at each breeding site, totaling 45 for the whole bioassay.

Sampled of insects

Anopheles larvae were collected for 20 min at three randomly selected points on the edge of each breeding site using an entomological scoop with volumetric capacity of about 350 mL, 11 cm aperture and capable of handling a meter. During sampling, the 4th instar larvae of *Anopheles* were separated and taken to the Malaria and Dengue Laboratory of the National Institute of Amazonian Research, maintained under laboratory conditions and grown to adulthood to facilitate species identification. The identification of *Anopheles* was performed using the dichotomous key proposed by Consoli and Oliveira (1994).

Aquatic insects were collected using an aquatic insect net, at four random points for 30 s at each point (Merritt et al., 2005). Subsequently, the material was fixed in 70% alcohol and brought to the laboratory where it was screened with the aid of a stereomicroscope. The insects found were identified to the lowest possible taxonomic level using dichotomous keys (Merritt and Cummins, 1996; Pes et al., 2005; Pereira et al., 2007). Subsequently, the individuals were classified according to the functional trophic groups separated into shredders, scrapers, collectors, filter feeders and predators, following recommendations by Merritt and Cummins (1996) and Cummins et al. (2005).

Data analysis

To characterize the insect fauna, the relative abundance (%) of aquatic insects and species of *Anopheles* (Magurran, 1988) was calculated. To evaluate the effect of *B. sphaericus* on *Anopheles*, larval reduction (%) was obtained by using the three post-application and post-reapplication data. The index uses the number of larvae before and after application of biolarvicide, obtaining the percent reduction of *Anopheles* larvae during the experiment (Mulla et al., 1986). Larval density (LNMH) was determined before and after application of biolarvicide at breeding sites LNMH was obtained according to the formula described by Tadei et al. (2007) and was superimposed on abundance data over time insect fauna associated to check the behavior of these populations during the biolarvicide action period.

To evaluate the effect of *B. sphaericus* on insect fauna associated vectors, richness and diversity data were analyzed by linear regression analysis, with the aid of the Statistica Statsoft 10.0 program. The independent variable was the application of *B. sphaericus* and dependent was the richness and diversity of associated insect fauna. The relationship between richness/diversity and the use of biolarvicide was assessed by regression models that best fit the data distribution. The values obtained after two biolarvicide application cycles at the five artificial breeding sites were taken into account.

Results

A total of 905 larvae of *Anopheles* were collected identified in five species: *Anopheles darlingi*, *Anopheles albitarsis*, *Anopheles braziliensis*, *Anopheles triannulatus* and *Anopheles nuneztovari*. The relative abundance showed that *A. darlingi* (54%) was the most abundant species and *A. albitarsis* (1%) the least. Considering the associated insect fauna, 4874 specimens belonging to 6 orders and 23 families were collected. Chironomidae was the most abundant family with 51%, followed by Ceratopogonidae (14%) and Coenagrionidae (11%), and the least abundant was Gerridae (0.02%) (Table 1). Among the functional trophic groups, the collectors were the most abundant in three of the five breeding sites analyzed (C2: 85%, C3: 45.5% and C4: 60%); in the other two, the predators were the most abundant (C1: 73.5% and C5: 68%). The group of shredders was the least abundant (C4: 0.2%) in all breeding sites analyzed.

Effects on Anopheles

At three of the five treated breeding sites (C1, C2 and C5), the larvae were eliminated at 24 h (100%) and up to 5 days after the application of biolarvicide, and at C4, the reduction was 98%. After reapplication, the larval reduction rate was high in the initial readings but decreased 15 days after re-application. At breeding site C3, 24 h after *B. sphaericus* application, larval reduction was 56%, and at five days, negative values were obtained for larval reduction, because there was an increase in larvae. After reapplication, 100% reduction in larvae was observed after 24 h and also after 15 days (Table 2).