

Use of a *Bacillus sphaericus* (Strain 2362) Formulations in Control of Malaria Vectors in Brazil

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Abstract

Malaria is an acute febrile infectious disease that is important in public health. In Brazil, there is a high incidence of the disease in the Amazon region, where 99% of the country's cases occur, wherein the disease might be severe. In this study, the use of *Bacillus sphaericus* strain 2362, entomopathogenic bacteria, for the control of malaria vectors was reviewed by surveying scientific articles in the Scielo, Medline and PubMed databases. The selected articles revealed that there is a shortage of studies on the effectiveness of *B. sphaericus* in the control of *Anopheles* in Brazil.

Keywords: *Anopheles*; Biological control of vectors; Entomogenous *Bacillus*; Malaria; Public health

Introduction

Malaria is an acute febrile infectious disease that is important in public health. It is caused by protozoa transmitted by mosquito vectors. In Brazil, there is a high incidence of the disease in the Amazon region, where 99% of the country's cases occur, wherein the disease might be severe. Malaria is a cause of considerable social and economic losses in populations at a risk of infection, especially those living in poor housing and sanitation conditions [1-9].

Malaria vectors belong to the order Diptera, infraorder Culicomorpha, family Culicidae, and genus *Anopheles*. This genus comprises 517 species, of which 70 are medically important, with 54 of these species occurring in Brazil [10-12]. The main vectors of malaria transmission in Brazil are *Anopheles (Nyssorhynchus) darlingi* Root; *Anopheles (Nyssorhynchus) aquasalis* and *Anopheles (Nyssorhynchus) albiparvus* s.l.. Each of these vectors is anthropophilic and commonly feed on blood in households and outdoors [11,13-18].

There are various control methods for malaria vectors such as chemical control by fogging, indoor residual spraying (IRS) screening of doors and windows, repellent lotions and electric repellents, timing of human activities in periods of less vector activity, and long-lasting insecticidal nets (LLINs) or common mosquito nets. Adulticide activity, environmental management, and biological larvicides in breeding sites are less efficient methods of vector control [3,15,19].

Currently, in Brazil, the breeding of *Anopheles* spp. larvae is seldom controlled. Virtually all vector control activities are focused on the adult insect, even though the Ministry of Health of Brazil [3,15,19] and more recently the vector control manual of the World Health Organization [20] have advised the use of entomopathogenic bacteria.

Studies on the effectiveness of *Bacillus sphaericus* strain 2362 against *Anopheles* spp. larvae under field conditions are rare because it is a fairly new vector control method. However, this bacterium is specific in its action, easy to use, and does not interfere with the aquatic habitat in which it is employed. The WHO [20] showed the effectiveness of *B. sphaericus* in anopheline control in laboratory tests, and some researchers of Kenya, Greece, The Gambia, India, the Philippines, Mali, Tanzania, and, Sri Lanka have reported the efficacy of *B. sphaericus* in controlling anophelines; these authors have also suggested the need for further studies on this topic.

Literature Review

A systematic literature review was conducted to identify the historical events of biological control for immature forms of *Anopheles* mosquitoes. The literature review was conducted using a defined search strategy, using the descriptors 'malaria,' 'biological control,' '*Bacillus sphaericus*' and 'National Program for Malaria Control in Brazil.'

Articles were searched in the SciELO database (Scientific Electronic Library Online), MEDLINE - International Database for Medical Literature and PubMed (US National Library of Medicine). In addition to articles, textbooks and the Ministry of Health of Brazil manuals were also included in the literature review. In total, 35 sources were selected, using emphasis on the biological control of malaria vectors as the criterion.

This review article aims to present an overview of the use of the entomopathogenic biolarvicide, *B. sphaericus* strain 2362, in malaria vector control and to report its applicability and effectiveness in Brazil.

Results and Discussion

Of the studies analyzed, we observed that several entomopathogenic bacteria have been used in the control of insects of public health importance. Specifically, *B. sphaericus*, which has a biolarvicide effect due to two toxins of 51 and 42 kDa that act synergistically and that are toxic to mosquito larvae, has been used. Upon ingestion, these toxins destroy the stomach epithelium (midgut) of the larvae, leading to septicemia. This process is favored by the alkaline pH of the larval digestive tract associated with the presence of lipid acids [19,21].

B. sphaericus is easy to handle, does not require special conditions for culture, and shows selectivity in its effects [22]. This biolarvicide does not affect the other fauna and flora in breeding sites in the Amazon

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[23,24]. In addition, their spores are recycled in the cadavers of larvae, which can enlarge its persistence in the breeding period contributing to the persistence of these bacilli in environments rich in organic matter [19,23-27,28].

In Brazil, few field studies have been conducted to determine the effectiveness of biological control by *B. sphaericus* strain 2362. There are several articles detailing laboratory bioassays and controlled field bioassays, but the effective use of this method as an alternative to chemical control of mosquitoes is scarce, even though the Ministry of Health manuals have advocated this approach since 1999 [18]. Experiments conducted with *Aedes* and *Culex* mosquitoes have demonstrated the effectiveness of *B. sphaericus* both in laboratory and field conditions [29,30]. In general, laboratory-controlled bioassays showed satisfactory results; however, state and municipal health managers must implement this method for the health of the environment and staff working in malaria endemic areas. Therefore, we conducted an intervention with *B. sphaericus* strain 2362 in the state of Amapá, in a mining area in the municipality of Calçoene in the Amazon Basin between 2008 and 2009, in breeding sites that originated due to gold mining [31]. These breeding sites were sunny or semi-shaded and, within 48 hours after treatment with a granular formulation of *B. sphaericus*, larvae were reduced by 80% in the breeding sites. Similar results were shown by Majambere et al. [32] in Gambia, Kandyata et al. [29] in Zambia, and Berti Mozer et al. [33] in Venezuela.

The malaria vector control management manual recently published by the WHO recommends the biological control larval like a complementary methods of immature mosquito, especially in areas where first-line interventions (LLINs and/or IRS) have achieved satisfactory entomological and epidemiological results, and some malaria-endemic [20].

Although the use of *B. sphaericus* in Brazil to date has been limited, many countries have adopted this strategy for the control of anophelines. This biocontrol measure may reduce the effects of chemical insecticides on the environment; these measures also show the insecticidal potential, depending on local characteristics such as the type of breeding area, temperature, humidity, solar radiation, and other parameters in real field conditions, as shown by Barjac [34] and Glare and Callaghan [35] with *Bacillus thuringiensis israelenses* and *B. sphaericus*.

The biological control of anophelines with *B. sphaericus* has the advantage of vector specificity. It does not affect fishes, flora, or other insects present in the breeding area, and is innocuous to the handler. Its main disadvantage is the need for training of human resources, because field staff will need to analyze and interpret the effects in the treated breeding areas and determine the long-term results.

B. sphaericus strain 2362 as a biological control method for malaria vectors offers an alternative when resistance of adult anophelines to chemical insecticides is known, as is seen in populations of *Aedes aegypti* in several Brazilian states. In addition, this product is safer to handle and safer for the environment than traditional, chemical control methods.

The implementation of this control method by health managers will require significant planning and intensive management, with investments of time, patience, training, and qualified technical personnel.

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