

**POTENCY AND PERSISTENCE OF THE BACTERIAL MOSQUITOCIDE
BACILLUS SPHAERICUS AGAINST CULICINE MOSQUITOES UNDER
FIELD CONDITIONS**

By

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Abstract

The biopesticide *Bacillus sphaericus* represents one of the important tools used in the control of mosquito larvae after chemical insecticides. The present study was conducted to investigate the efficacy *B. sphaericus* 2362 (VectoLex) in two different technical powders, ABG-6232 and ABG-6491 against larvae of two mosquitoes, *Culex pipiens* and *Aedes caspius* in El-Arish city, North Sinai, Egypt.

The mosquito larvae were collected from polluted and fresh water (*Cx. pipiens*) and saline water (*Ae. caspius*). The physicochemical characteristics of larval breeding water sites were measured as salinity, conductivity, pH values and temperature.

Susceptibility bioassays showed that *Cx. pipiens* larvae from polluted water sites have high susceptibility to *B. sphaericus* 2362 formulation ABG-6232 (LC₅₀ 0.15-0.33 ppm, LC₉₀: 0.73-9.68 ppm). In case of ABG-6491 formulation, the values were LC₅₀: 0.15-0.33 ppm and LC₉₀: 1.73-9.83 ppm. *Cx. pipiens* larvae collected from fresh water habitat in El-Qusiema, outside El-Arish city, susceptibility to *B. sphaericus* 2362 ABG-6232 had LC₅₀ values ranging between 0.12 and 0.28 ppm, while LC₉₀ ranged between 1.59 and 4.13 ppm. In case of ABG-6491, the LC₅₀ values ranged between 0.13 and 0.28 ppm, while LC₉₀ values ranged between 1.46 and 8.93 ppm.

For *Ae. caspius* larvae from saline water and treated with *B. sphaericus* 2362 ABG-6232, LC₅₀ values ranged between 0.31 & 1.36 ppm, while LC₉₀ ranged between 1.92 & 9.75 ppm. In case of ABG- 6491, the LC₅₀ values ranged between 0.34 & 0.59 ppm, while LC₉₀ values ranged between 1.79 and 11.56 ppm.

Keywords: Egypt, Culicine mosquitoes, Biopesticides, Field efficacy,

Introduction

Anti-mosquito bio-pesticides based on the pathogenic bacterial *B. thuringiensis* var. *israelensis* (*Bti*) and *B. spha-*

ericus (*Bs*) are important means of the mosquito vector control, especially in an integrated vector management (IVM) programme (Dritz *et al.*, 2011).

Many bacterial strains and commercial formulations that have proven successful against various vector species (Medieros *et al*, 2005, Melo *et al*, 2009). Despite their relative efficacy, there was a number of disadvantages that render these bio-pesticides less effective, labour-intensive and expensive. These include low persistence and degradation due to exposure to sunlight UV. Many laboratory or simulated field or field trials improved the efficacy of the present formulations or produce new ones (Morsy and Mazyad, 2000; Lingenfelser *et al*, 2010). Many studies evaluated *Bti* or *Bs* formulation separately or in a mixture of both against many mosquito species of genera *Aedes*, *Anopheles*, *Culex* and *Ochlerotatus* (Brown *et al*, 2004, Majambere *et al*, 2007, Geraldo-Calderon *et al*, 2008, Melo *et al*, 2009, Singh and Prakash, 2009). Toxins and vegetative proteins of these bacteria were tested (Wirth *et al*, 2007, Jones *et al*, 2007). The publication of the complete genome of *B. sphaericus* C3-41 (Hu *et al*, 2008), will help improve the efficacy of the identified proteins or toxins or the identification of novel candidates with strong mosquitocidal activities.

This study aimed to assess the efficacy of two technical powders of *B. sphaericus* 2362, ABG-6232 and ABG-6491 against larvae of two mosquitoes, *Cx. pipiens* (polluted and fresh water) and *Ae. caspius* (saline water) from North Sinai, North-East of Egypt.

Materials and Methods

B. sphaericus preparations: Two granulated powder preparations of *B.*

sphaericus strain 2362 (ABG-6232 & ABG-6491) from Abbott Laboratories (North Chicago, IL, U.S.A.) were used in laboratory bioassays and field evaluation. The preparations were preserved at a temperature of 5°C. A stock suspension of *B. sphaericus* was prepared by suspending 1 gm of the granular formulation into 100 ml distilled water. Serial dilutions of the stock suspension done in tap water to give five to six concentrations in the range of 0.1 to 10.0 ppm and used in each bioassay. All suspensions were prepared fresh for experimental purposes.

The laboratory susceptibility bioassay tests carried out to determine the sub-lethal concentrations of the two bacterial formulations against the two mosquito species, *Cx. pipiens* and *Ae. caspius* as described (Rodcharoen and Mulla, 1995). Twenty young fourth instar larvae were placed in waxed paper cup containing 100 ml distilled water and treated with different concentrations from 0.001 to 0.75 ppm of bacterial suspensions were tested in each bioassay to yield 10-95% mortality. Larvae were provided fish food (tetra-min) the 48-hr observation period. Larval mortality was assessed 48-hr post-treatment and moribund larvae were also considered dead. All the bioassays were replicated three times.

Evaluation of the efficacy and persistence of *B. sphaericus* 2362 under field conditions: *B. sphaericus* 2362 technical formulation ABG-6232 applied for efficacy and persistence field trials. Because environmental factors such as ultraviolet light would degrade the larvicide, approximately 10X observed

laboratory LC₅₀ for each breeding site used in field experiments. The required amounts of the primary powder suspended in tap water. The aqueous suspension applied with a pressurized hand sprayer (size one liter) at the rate of 0.5-2 gm/m², around the periphery and center of the target larval water habitat.

Field bacterial treatment was tested in El-Arish City, North Sinai Governorate. ABG-6232 preparations were applied to three types of larval breeding sites: polluted water, saline water and fresh water. Samples taken from the periphery and center of each studied larval site were used to estimate larval density prior treatment.

The larval densities of each site at first, second, seventh and fourteenth day post-bacterial treatment recorded. Reduction percent in larval densities post- versus pre-treatment counts were calculated.

Results

Laboratory screening of the susceptibility of two mosquito species showed that the two *Cx. pipiens* populations from polluted and fresh water have comparable susceptibility, and both are more susceptible than the saline water *Ae. caspius* to the two bacterial formulations used based on the LC₅₀ values (ABG-6232 and ABG-6491) and the LC₉₀ values (ABG-6491). Regarding LC₉₀ values to the formulation ABG-6232, the fresh water *Cx. pipiens* population from more susceptible than that from polluted water (~1.8 fold) and the saline water *Ae. caspius* (~2 fold) (Tab. 2).

Efficacy and persistence of *B. sphaericus* 2362 (ABG-6232) under field conditions: For *Cx. pipiens* in the polluted water-breeding site, the recorded mean number of larvae pre-treatment was 9.25larvae/dip. Bacterial treatment caused a reduction of 78.4% in larval number after 24 hrs. Complete reduction of larvae occurred after 48 hrs and remained so to the end of the experiment. This means that the residual activity of this formulation in polluted water habitat continued for 14 days (Tab. 3, Fig. 1).

For the fresh water *Cx. pipiens*, the mean number of pre-treatment larvae was 9larvae/dip. The reduction in larval number is 69.4% at 24 hrs post-treatment, which increased to 80.6% after 48 hrs. The complete reduction of mosquito larvae occurred on the 7th day post-treatment (Tab. 3). However, on the 14th day, the number of larvae started to increase, which indicates a decrease in the residual activity of the bacterial formulation, by 25% of that at day 7 (Tab. 3; Fig. 1).

Saline water habitat: larva-breeding sites was treated with 2gm/m² of *B. sphaericus* 2362 (ABG-6232). Mean number of *Ae. caspius* larvae collected from saline water pre-treatment was 18.75 larvae/dip. Reduction in larvae densities was 54%, 73% & 88% at 1, 2 and 7 days post-treatment, respectively. But, at the 14th day post-treatment, the larval densities started to increase, an indication of decrease in residual activity of this formulation in saline by 12% of that at 7th day (Tab. 3, Fig. 1). These results showed that the tested mosquito species are highly susceptible to the

two technical formulations of the *B. sphaericus* 2362 used. *Cx. pipiens* (from polluted and fresh water habitats) was more susceptible than *Ae. caspius* (saline water habitat) based on laboratory and field assays. The polluted water habitat was the most efficient in retaining the efficacy of the applied bacterial biopesticide (ABG-6232),

followed by fresh and saline water habitats. In all habitats, the biopesticide remained highly effective for 14 days. Many factors contribute to these variations in biopesticide efficacy: the mosquito species, physicochemical properties of water habitats and the biopesticide formulations.

Table 1: Physicochemical characteristics of the studied mosquito larval collection water sites in Al-Arish City.

| Water site | Mosquito spp. | Salinity (gm CL/L) | Conductivity (μmohs/cm) | pH | Temp. (°C) | Areas in Al-Arish district |
|------------|--------------------|--------------------|-------------------------|----------|------------|----------------------------|
| Polluted | <i>Cx. pipiens</i> | 0.06-0.23 | 402-579 | 6.8-8.8 | 16-38 | Dahiat El-Salam |
| Fresh | <i>Cx. pipiens</i> | 0.05-0.23 | 150-254 | 6.9-9.0 | 16-37 | El-Qusiema, |
| Saline | <i>Ae. caspius</i> | 1.8-2.5 | 1177-1993 | 6.5-10.5 | 14-38 | El-Masaeed |

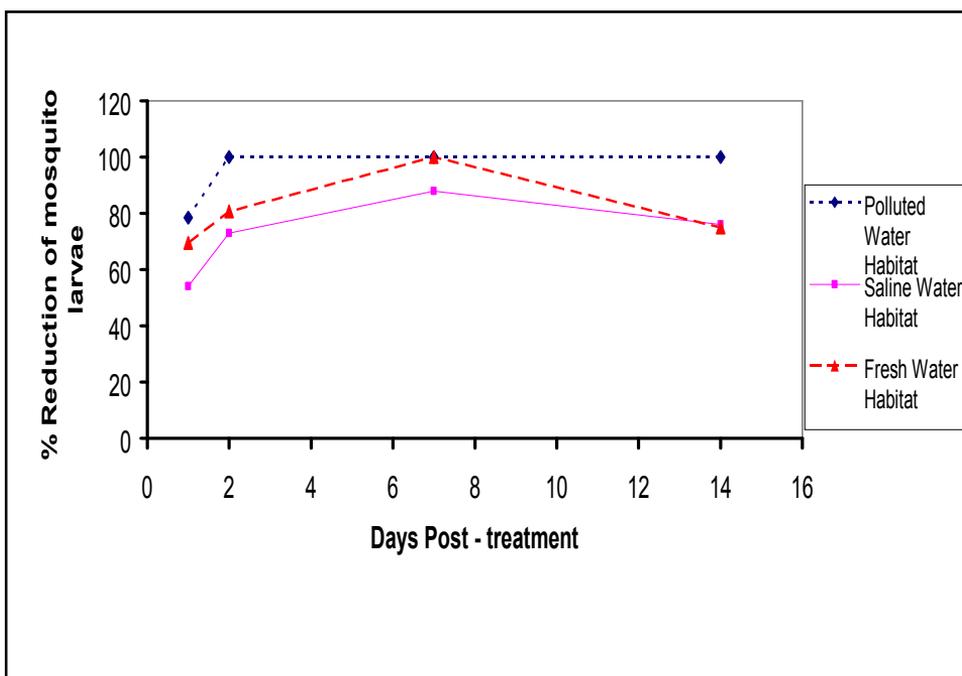


Fig. 1: Efficacy of *B. sphaericus* 2362 (ABG-6232) against *Cx. pipiens* and *Ae. caspius* larvae in three different breeding habitats.

Table 2: Susceptibility of larval populations to *B. sphaericus* 2362 commercial formulations, ABG-6232 & ABG-6491 from water sites in Al-Arish City.

| Water Site | Mosquito* | ABG-6232 | | | |
|------------|--------------------|------------------------|------------------------|------------------------|------------------------|
| | | LC ₅₀ (ppm) | | ABG-6491 | |
| Polluted | <i>Cx. pipiens</i> | 0.24 (0.15-0.33) | LC ₉₀ (ppm) | LC ₅₀ (ppm) | LC ₉₀ (ppm) |
| Fresh | <i>Cx. pipiens</i> | 0.20 (0.12-0.28) | 5.21 (0.73-9.68) | 0.24 (0.15-0.33) | 5.78 (1.73-9.83) |
| Saline | <i>Ae. caspius</i> | 0.84 (0.31-1.36) | 2.86 (1.59-4.13) | 0.25 (0.13-0.28) | 5.2 (1.46-8.93) |

*18 populations tested for each collection site.

Table 3: Efficacy of *B. sphaericus* 2362 (ABG-6232) against *Cx. pipiens* and *Ae. caspius* larvae in three different natural breeding habitats.

| Breeding Site | Mosquito | Bacterial dosage (gm/m ²) | Mean larvae/dip | | | | |
|---------------|--------------------|---------------------------------------|---------------------|------------------------------|----------------|--------------|--------------|
| | | | Pre-treatment (day) | Post-treatment (Reduction %) | | | |
| | | | D0 | D1 | D2 | D7 | D14 |
| Polluted | <i>Cx. pipiens</i> | 1.5 | 9.25 | 2 (78.4) | 0 (100) | 0 (100) | 0 (100) |
| Fresh | <i>Cx. pipiens</i> | 1 | 9 | 2.75 (69.4) | 1.75 (80.6) | 0 (100) | 2.25 (75) |
| Saline | <i>Ae. caspius</i> | 2 | 18.75 | 8.5 (54.7) | 5 (73.3) | 2.25 (88) | 4.5 (76) |

Discussion

In the present study, *B. sphaericus* 2362 (ABG-6232) provided extremely effective control of mosquito larvae in all breeding sites, especially polluted and fresh breeding ones, against *Cx. pipiens* larvae and to a lesser degree against *Ae. caspius* larvae in saline breeding habitat. Field application of *B. sphaericus* resulted in significant reduction of *Cx. pipiens* populations (Wirth *et al*, 2001) and in *An. species* (Wirth *et al*, 2000). The appropriate formulations of *B. sphaericus* showed significant residual activity against *Cx. pipiens* in the highly polluted breeding sites. Extended activity of *B. sphaericus* was explained by Lacey and Udeen (1986) to be due to recycling of spores in larval cadavers or to persistence of sufficient quantities of toxin in the habitat. Also, the extended control can be explained by the fact that larvae ingest spores settled into bottom water and

mud (Matanmi *et al*, 1990). On the other hand, it has been shown that *B. sphaericus* provides a higher level of control in fresh than polluted water (Mulla *et al*, 1988). They concluded that in general, higher rates than that of the fresh water rates are required to achieve satisfactory control of larvae in polluted water breeding sites.

B. sphaericus has the potential to persist and recycle under field conditions, for up to 3 months (Jones *et al*, 1990). In previous studies, a powder preparation of strain 2362 proved to be the most active preparation tested, yielding excellent control of stagnant and flood water mosquitoes such as *Cx. tarsalis*, *Cx. pipiens*, *Ae. nigromaculis* and *Ae. melanimon* (Chabanenko *et al*, 1992). A number of factors can influence the level and duration of larvicidal effect of *B. sphaericus* in mosquito habitats. These include water quality and depth, solar radiation, target spe-

cies and larval density (Yousten *et al.*, 1984). Moreover, non-target organisms can play an important role in recycling *B. sphaericus*, since the bacterium was able to germinate in the larval guts of *Chironomus* species and other filter-feeding arthropods such as *Daphnia* (Karch *et al.*, 1990).

In the present study, high efficacy of *B. sphaericus* in the polluted breeding site was related to the presence of high densities of aquatic invertebrates and arthropods.

The present results also revealed that *B. sphaericus* provided effective extended mosquito control in all types of breeding sites especially the polluted habitat. This agreed with that of Karch *et al.* (1990) and Mulla *et al.* (2001). Decline in immature populations followed by a substantial decline in adult mosquitoes (Mulla *et al.*, 2001). The granule formulation proved to be highly effective against the polluted stagnant water mosquito (Lacey and Lacey, 1990). The present study agreed with Mulla (1991) who reported that the binary crystalline toxins produced by *B. sphaericus* strain 2362 have high activity against larvae of *Culex* mosquitoes, and with Mulla *et al.* (1997) who reported that *B. sphaericus* 2362 granular formulations gave high mortality of *Cx. pipiens* field conditions especially in highly polluted water. Besides, Lacey *et al.* (1988) reported that *B. sphaericus* activity was positively related to the amount of food available to mosquito larvae in artificial container. The duration of control is also dependent on the product, the transient nature of some sites, and the

target mosquito larvae (Skovmand and Sanogo, 1999).

Spores of *B. sphaericus* persisted for months in larval habitats and cadavers (Berry *et al.*, 1987). Spores grow in larval cadavers, which provide protection for the bacteria from the deleterious effect of the sunlight (Des Rochers and Garcia, 1984). *B. sphaericus* recycled in dead larvae both in the laboratory and in the field, producing an increase of 100 to 1000 fold in spore numbers (Davidson *et al.*, 1984). Nicolas *et al.* (1987b) stated that the final amount of spores recycled in dead larvae as affected by organic matter.

In the present study, *B. sphaericus* 2362 (ABG-6232) proved to be persistent and more active against *Cx. pipiens* larvae in the deep, shaded, polluted mosquito breeding site than in the fresh breeding one which was relatively less in depth and exposed to sunlight. This observation agrees with that of Mulligan *et al.* (1980) who reported several reasons for extended suppression of mosquito breeding sites, namely, water movement, depth, rains, and exposure to sunlight known to reduce *B. sphaericus* activity under field conditions.

The present results revealed less efficacy and persistence of *B. sphaericus* against *Ae. caspius* larvae in the saline breeding site. This may be because the water in this breeding site was shallow, completely exposed to sunlight and contained solid materials. This agreed with Nicolas *et al.* (1987a). But, Davidson (1985) recorded many advantages of *B. sphaericus* such as its

persistence and ability of recycling, the author stated that its main disadvantage was its relatively narrow mosquito host range since it was virtually inactive against *Ae. aegypti* and certain other *Aedes* species.

Additional advantage of this microbial agent is being environmentally safe and without adverse effects on dominant macro-invertebrate fauna, especially predacious insects (Mulla *et al*, 1984). The microbial agent *B. sphaericus* proved to be an excellent alternative to chemical larvicides for mosquitoes control, especially *Cx. pipiens*, the main vector of Bancroftian filariasis in Egypt (Hougard *et al*, 1993). This bacterial agent is environmentally safe due to its host specificity, required in very low dosage, easy to prepare commercially in a large-scale and its less costly (Bhattacharya, 1998).

Undoubtedly, mosquitoes-borne diseases are threat worldwide (Mikhail *et al*, 2009). In Egypt, the re-emerging of *Aedes aegypti* in Aswan Governorate (Essam *et al*, 2006) and in Toshka Project (Shoukry and Morsy, 2011; Shoukry *et al*, 2012), which is the main vector of Yellow fever (CDC, 2010a), Dengue hemorrhagic fever (El-Bahnasawy *et al*, 2011) and Chikungunya viruses (CDC, 2010 b).

Moreover, the recent demonstration of the Yellow fever in the Northern Sudan is another risk health problem (Markoff, 2013).

On the other hand, the threatening of the Rift Valley fever (El-Bahnasawy *et al*, 2013) and West Nile fever (Abbassy *et al*, 1993; Darwish *et al*, 1996; Kro-

man *et al*, 2012) to Egypt are all risk factors paving the way to an urgent need for mosquito control by a feasible ecological friend agent.

Conclusion

The study carried out revealed various levels of efficacies of the tested bacterial agents due to experimental conditions, material used and most important the specific susceptibility and ecology of the tested mosquito.

To obtain comprehensive evaluation of their efficacies, all available formulations of these bio-pesticides, should be used on a range of mosquito vectors in different geographic regions in all possible larval habitats.

No doubt, the public health, the veterinary and the agricultural authorities must take into consideration that the control of the mosquito-vectors of infectious diseases with a safe and environmental agent like bacillus is the first feasible control measure to prevent transmission of such infectious diseases to man. The prevention was and is always better than treatment in a country like Egypt which lies at the cross roads of many countries.

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