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Sección Médica

# Control of *Culex quinquefasciatus* and *Cx. saltanensis* (Diptera: Culicidae) with *Bacillus thuringiensis israelensis* in wastewater treatment lagoons

Control de *Culex quinquefasciatus* y *Cx. saltanensis* (Diptera, Culicidae) con *Bacillus thuringiensis israelensis* en lagunas de aguas residuales

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**Abstract:** Industrial or urban wastewater treatment lagoons accumulate nutrients, which allows the proliferation of *Cx. quinquefasciatus*, vector of the etiologic agent of filariasis, and of *Cx. saltanensis,* vector of the protozoan causing malaria in chickens. The goal of this study was to evaluate the effectiveness and persistence of Aquabac® XT 1,200 UTI/mg, Teknar® 3,000 AAU/mg, and Vectobac® AS 1,200 UTI/mg; these products are liquid formulations whose active principles are crystals produced by *Bacillus thuringiensis israelensis*. Products were tested in two wastewater treatment lagoons of a meat cold-storage facility. The lagoons measured 1,419 $m^2$ and 736 $m^2$, and received concentrations of 1 and 2 litres/hectare of each product, with three replicates each. Water pH, conductivity, oxygen and temperature were measured at each collection. *Cx. quinquefasciatus* and *Cx. saltanensis* were found in both lagoons. One litre/hectare concentration controlled 70 to 80% of the larvae in 24 and 48 hours, respectively; two litres/hectare concentration showed the best results, controlling 86 to 99% of the immature mosquitoes, with the highest efficiency observed at 48 hours. None of the products affected water quality according to the abiotic parameters examined. As a result, all products tested efficiently controlled these Culicidae under the local breeding conditions but had low persistence, with seventh-day larval indexes being similar to those recorded before application of the products. Weekly applications are recommended.

**Key words:** Biological control. Bti. Breeding site. Insecticide persistence. Insecticide formulation.

**Resumen:** En lagunas de aguas residuales industriales o domésticas se acumulan nutrientes que conducen a la proliferación de *Cx. quinquefasciatus*, el vector del agente etiológico de la filariasis, y de *Cx. saltanensis*, el vector del protozoo causante de malaria aviar. El objetivo de este estudio fue evaluar la eficacia y persistencia de Aquabac® XT 1.200 IU (s) / mg, Teknar® 3.000AAU (s) / mg, y Vectobac® como 1.200 IU (s) / mg; formulaciones líquidas cuyos principios activos son cristales producidos por *Bacillus thuringiensis israelensis*. Los productos fueron aplicados en dos lagunas de tratamiento de aguas residuales de un frigorífico cuyos espejos de agua miden 1.419 $m^2$ y 736 $m^2$. Las aplicaciones fueron en concentraciones de 1 y 2 litros / hectárea de cada producto, con tres adiciones en cada caso. El pH del agua, la conductividad, el oxígeno y la temperatura se registraron en cada colecta. *Cx. quinquefasciatus* y *Cx. saltanensis* fueron encontrados en ambas lagunas. La concentración de 1 L/ha controló entre el 70 a 80% de las larvas en 24 y 48 horas, respectivamente; por otro lado la concentración de 2 L/ha fue más eficiente al controlar entre 86 y 99% de los mosquitos inmaduros después de 48 horas. Ninguno de los productos afectó la calidad del agua, según los parámetros abióticos examinados. Se concluye que todos los productos analizados controlan de forma eficiente los culícidos en las condiciones de cría locales, con tiempo de permanencia bajo, pues después del séptimo día los índices larvarios fueron similares a los registrados antes de la aplicación de los productos. Se recomiendan aplicaciones semanales.

**Palabras clave:** Control biológico. Bti. Sitio de reproducción. Persistencia insecticida. Formulación insecticida.

## Introduction

The nutrients in decomposing matter found in effluent treatment lagoons promote physical and chemical changes in water. Such changes hinder the preservation of the aerobic aquatic fauna and enhance the breeding of mosquito larvae such as *Cx. quinquefasciatus* Say, 1823 and *Cx. saltanensis* Dyar, 1928 (O´Meara 2010).

*Cx. quinquefasciatus* is an urban and cosmopolitan mosquito, considered the main vector of the etiologic agent of filariasis in Brazil. The pathogen is transmitted in cities such as Manaus, Belém, Recife, Maceió, and Salvador, and other locations in the country (Deane 1951; Rachou 1956). Currently, only the metropolitan area of Recife, Pernambuco is considered an endemic area (Medeiros *et al*. 2003). According to the World Health Organization (WHO), 81 countries were endemic for the disease by the end of the year 2007, where 750 million people underwent treatment, and the aim has been to eradicate the disease (WHO 2008). The control of the arthropod vector is one of the strategies undertaken for such purpose.

*Cx. saltanensis* is an ornithophilic, neotropical mosquito (Lourenço-de-Oliveira & Heyden 1986) that can be infected with *Plasmodium cathemerium*, a sparrow homospory (Gabaldon *et al.* 1988). It has also been considered the primary vector of *Plasmodium juxtanucleare* (Lourenço-de-Oliveira & Castro 1991), etiologic agent of malaria in gallinaceous birds. Sibajev *et al*. (1993) described *Crithidia ricardoi,* a new species of Trypanosomatidae, where *Cx. saltanensis* is considered the primary host. *Cx. saltanensis* colonizes efflu-

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ent treatment lagoons and has been the only mosquito species found in sanitary landfill effluent lagoons in Londrina, Paraná State (Zequi and Lopes 2007).

Both mosquito species are urban vectors, engage in disturbing behaviour during female blood meals, and cause allergies. They are also potential vectors for West Nile virus (WNV), which has been detected in horses in Mato Grosso do Sul (Pauvolid-Correa *et al.* 2011). Because they worsen the quality of life of people living near artificial breeding sites, integrated vector management or control programs are needed. One of the ecologically safest and most efficient biological control methods available is the bioinsecticide *Bacillus thuringiensis* subsp. *israelensis* (Bti) in effluent treatment lagoons or other aquatic environments. The efficiency of Bti has been reported for *Culex* spp. (Amalraj *et al.* 2000; Hallmon *et al*. 2000; Gunasekaran *et al*. 2002; Zequi and Lopes 2007; Bravo *et al.* 2011), although with a persistence of less than 30 days (Amalraj *et al.* 2000; Hallmon *et al*. 2000; Morais *et al*. 2007; Zequi and Lopes 2007). Under some conditions, persistent control of *Aedes aegypti* (Linnaeus, 1762) by Bti is more than 100 days (Mulla *et al.* 2004) or more than five months (Benjamin *et al*. 2005; Melo-Santos *et al.* 2009; Ritchie *et al*. 2010). The efficiency of Bti is inversely proportional to the increase in organic matter (Tetreau *et al*. 2012).

Appropriate biological formulations for effluent treatment lagoons still need to be tested to ensure greater success in controlling these mosquitoes in urban areas. The efficacy of entomopathogenic products depends on several environmental factors, including water quality at the breeding sites, nutrient availability, local climate conditions, number of larvae in the lagoon and solar radiation (Mulla *et al.* 1984; Consoli *et al*. 1995; Lacey 2007).

This study aimed to evaluate the efficiency and persistence of three aqueous suspensions containing *B. thuringiensis israelensis* and to simultaneously monitor water quality (pH, conductivity, and dissolved and saturated oxygen levels) in two wastewater treatment lagoons of a meat cold-storage facility in Jataizinho and Arapongas (Paraná, Brazil).

## Material and methods

**Procedures and tests.** Aqueous suspensions of Aquabac® XT 1,200 UTI/mg (lot F295), Teknar® 3,000 AAU/mg (lot A206673) and Vectobac® AS 1,200 UTI/mg (lot 69-149-N9) were used for the field trials. One urban effluent treatment lagoon was selected in each of the two municipalities studied, Jataizinho and Arapongas, both in the state of Paraná. Jataizinho lagoon measured 33 x 43 m (1,419 $m^2$) and received waste from a swine slaughterhouse. Arapongas lagoon was 33 x 23 m (736 $m^2$), with effluents from a cattle slaughterhouse. Product concentration was determined according to the length and width of each lagoon. Larval colonization site or the site to be treated was defined as the quadrant obtained one meter away from the water's edge and one meter deep, typically the area where larvae occur and feed.

The starting concentration patterns for product application at both sites were one and two liters per hectare following the manufacturers' recommendations for polluted water or high concentration of larvae. To simulate the actual field conditions, the time intervals for each repetition of the applications were defined according to the persistence of the product and initial larval recovery rates in each lagoon. Three applications of each concentration of bio-insecticides were applied every seven days in different ponds. Arapongas lagoon was used to test Vectobac 1 L/ha (March 10-31, 2004), Teknar 1 L/ha (March 31 to April 21, 2004), and Teknar 2 L/ha (April 28 to May 19, 2004). The following products were applied to the Jataizinho lagoon: Aquabac 1 L/ha (March 31 to April 21, 2004), Aquabac 2 L/ha (April 28 to May 19, 2004), and Vectobac 2 L/ha (September 22 to October 13, 2004). The bioinsecticides were applied with a multi-spray atomizing pump in each repetition when the levels of initial larval infestation in ponds were checked.

**Measurement of abiotic factors**. Water pH, conductivity and dissolved and saturated oxygen were checked before each application of products and collection of immatures, using Gehaka CG 220, Gehaka PG 1400 and Oakton DO 300 instruments. The environmental temperature and relative humidity of the site were monitored using a thermo-hygrometer (Gehaka) at 10 m from the lagoon edge and three meters above the soil, in a shady area.

**Specimen collection and procedures.** Larvae were collected before (pre-treatment sample) and one, two, five and seven days after bioinsecticide application (20 samples per product and concentration); physical and chemical water and environmental parameters were simultaneously measured. Larvae were collected at each corner of the pond using a nylon net (20 cm in diameter and 0.1 mm mesh) at a distance of one meter from the edge. Larvae collected were counted in the laboratory; 5% of the larvae collected at $4^{th}$ instar of each site were mounted on a microscope slide with Hoyer's solution for species identification.

**Statistical analysis.** The treatments for the control of Culicidae were considered: three products in two different concentrations and seven days of assessment. For data analysis by ANOVA was found not to homogeneity. To achieve this significance, data were transformed into square root (x +0.5). For the averages of variables (pH, conductivity and oxygen) of the treatments were compared by Tukey test at 5%. ANOVA and the Tukey test at 5% significance were performed, using the SPSS program (SPSS Inc. 2005).

## Results and discussion

Larvae found in both lagoons belonged to the species *Cx. quinquefasciatus* and *Cx. saltanensis*. Similar larval density was found for the two species in the Jataizinho lagoon. Infestation rate of *Cx. quinquefasciatus* in the Arapongas lagoon was higher. Teknar (1 L/ha) controlled 70.1% of the larval population 24 h after application; less control was observed thereafter, declining to 45.9% 48 h after application. The same product applied at a concentration of 2 L/ha was more efficient 48 h after application, reaching 85.4% of control as compared to the initial level (Table 1). At the 2 L/ha concentration, control was efficient until the fifth day (Table 1). The different concentrations had similar initial impacts, whereas the higher product concentrations increased the residual activity of the product.

Gunasekaran *et al.* (2004) controlled more than 80% of *Cx. quinquefasciatus* in sewage using Teknar HP-D (1,200 IU/mg) at the 2 L/ha concentration twice a week, at three-day

**Table 1.** Efficacy of Teknar (1 L/ha concentration) against *Cx. quinquefasciatus* and *Cx. saltanensis* applied to the effluent treatment lagoon of a cattle slaughterhouse (Arapongas, Paraná), with three replicates performed (March 31 to April 21, 2004; April 28 to May 19, 2004), and measurements of abiotic factors.

| Larvae/ Parameter | Number of days larvae exposed to the product | | | | | | |
|---|---|---|---|---|---|---|---|
| | Concentration | Application | 1 day | 2 days | 5 days | 7 days | CV% |
| Total larvae | 1L/ha | 29,697 | 8,891 | 16,060 | 14,863 | 37,999 | - |
| | 2L/ha | 29,695 | 6519 | 4350 | 7451 | 17308 | - |
| Mean larval count | 1L/ha | 2,474.75* ab+ | 740.92 b | 1,338.33 ab | 1,238.58 ab | 3,166.58 a | 63.2# |
| | 2L/ha | 2,474.58 a | 543.25 b | 362.50 b | 620.92 b | 1,442.33 ab | 79.3# |
| Mean pH | 1L/ha | 7.10 a | 7.07 a | 7.14 a | 7.10 a | 7.1 a | P > 0.05 |
| | 2L/ha | 6.81 ab | 6.87 ab | 6.94 ab | 7.06 a | 6.77 b | 1.2 |
| Conductivity (µS) | 1L/ha | 165.87 a | 168.60 a | 166.17 a | 174.44 a | 171.34 a | P > 0.05 |
| | 2L/ha | 183.11 ab | 186.21 ab | 183.73 ab | 178.40 b | 187.47 a | 1.6 |
| Dissolved oxygen (mg/L) | 1L/ha | 0.97 a | 1.05 a | 1.33 a | 2.33 a | 0.32 a | P > 0.05 |
| | 2L/ha | 0.68 b | 0.88 ab | 0.91 ab | 2.40 a | 0.84 ab | 31.9 |
| Saturated oxygen (%) | 1L/ha | 12.20 a | 12.86 a | 16.91 a | 32.14 a | 3.9 a | P > 0.05 |
| | 2L/ha | 6.13 b | 9.62 b | 10.02 b | 47.91 a | 7.53 b | 58.7 |

* Original data; results were square root (x + 0.5) transformed for determination of larval mortality rate.
\+ Same letters on same row indicate no difference according to the Tukey test at 5% significance level.
\# Coefficient of variation.

intervals. Mulla *et al.* (2003), using high doses of Bti, failed to extend control of *Culex*. These results show that there are many biotic and abiotic factors that affect the action of Bti. According to Lacey (2007), the efficiency of Bti can be affected by temperature, solar radiation, turbidity, presence of vegetation, mosquito species, strategies and rates of food ingestion by the larvae, among other factors.

High conductivity levels and low concentrations of dissolved and saturated oxygen were recorded for the lagoon water. However, none of the abiotic parameters evaluated was affected by Teknar at the 1 L/ha concentration. High colonization of breeding sites in alkaline water with pH 7 to 9 for *Cx. quinquefasciatus* (Tables 2 to 3) had been previously reported by Fernández *et al*. (1986). They also suggested that low oxygen indexes at breeding sites with high amounts of organic matter are associated with the presence of protozoans with high reduction potential such as *Metopus* sp., an indicator of polluted water, this being the appropriate breeding site for the mosquito.

Vectobac at the 1 L/ha concentration controlled 82.4 and 85.6% of the lagoon larvae 24 and 48 h after application, respectively, but the larval count returned to initial levels 5 days after application of this product (Table 2). The 2 L/ha concentration controlled 98.1% of the initial larval popula-

**Table 2.** Efficacy of Vectobac AS (1 and 2 L/ha concentrations) against *Cx. quinquefasciatus* and *Cx. saltanensis* applied to the effluent treatment lagoons of a cattle (Arapongas, Paraná) and a swine slaughterhouse (Jataizinho, Paraná), with three replicates performed (March 10-31, 2004; September 22 to October 13, 2004), and measurements of abiotic factors.

| Larvae/ Parameter | Number of days larvae exposed to the product | | | | | | |
|---|---|---|---|---|---|---|---|
| | Concentration | Application | 1 day | 2 days | 5 days | 7 days | CV% |
| Total larvae | 1L/ha | 4,948 | 869 | 713 | 4,846 | 11,168 | - |
| | 2L/ha | 49,207 | 929 | 369 | 22,772 | 49,536 | - |
| Mean larval count | 1L/ha | 412.33* ab+ | 72.42 b | 59.42 b | 403.83 ab | 930.67 a | 87.7# |
| | 2L/ha | 4,100.58 ab | 77.42 c | 30.75 c | 1,897.66 b | 4,128.00 a | 53.7# |
| Mean pH | 1L/ha | 7.13 a | 7.11 a | 7.13 a | 7.06 a | 7.16 a | P > 0.05 |
| | 2L/ha | 7.49 a | 7.57 a | 7.52 a | 7.55 a | 7.52 a | P > 0.05 |
| Conductivity (µS) | 1L/ha | 188.22 a | 189.59 a | 197.31 a | 191.91 a | 174.90 b | 2.2 |
| | 2L/ha | 172.91 a | 173.22 a | 171.36 a | 167.34 a | 178.72 b | P > 0.05 |
| Dissolved oxygen (mg/L) | 1L/ha | 1.21 a | 2.13 a | 1.56 a | 1.86 a | 1.76 a | P > 0.05 |
| | 2L/ha | 6.54 a | 6.39 a | 6.05 a | 6.22 a | 5.42 a | P > 0.05 |
| Saturated oxygen (%) | 1L/ha | 15.44 b | 25.54 b | 18.27 b | 23.21 a | 22.07 b | P > 0.05 |
| | 2L/ha | 93.24 a | 90.66 a | 83.73 a | 86.89 a | 73.34 a | P > 0.05 |

* Primary data; results were square root (x + 0.5) transformed for determination of larval mortality rate.
\+ Same letters on same row indicate no difference by the Tukey test at 5% significance level.
\# Coefficient of variation.

**Table 3.** Efficacy of Aquabac XT (1 and 2 L/ha concentrations) against *Cx. quinquefasciatus* and *Cx. saltanensis* applied to the effluent treatment lagoon of a swine slaughterhouse (Jataizinho, Paraná) with three replicates performed (March 31 to April 21, 2004; April 28 to May 19, 2004), and measurements of abiotic parameters.

| Larvae/ Parameter | Number of days larvae exposed to the product | | | | | | |
|---|---|---|---|---|---|---|---|
| | Concentration | Application | 1 day | 2 days | 5 days | 7 days | CV% |
| Total larvae | 1L/ha | 27,527 | 6,480 | 7,836 | 16,403 | 30,804 | - |
| | 2L/ha | 28,255 | 1,288 | 648 | 12,462 | 25,091 | - |
| Mean larval count | 1L/ha | 2,293.92* ab+ | 540 b | 653 ab | 1,366.92 ab | 2,567.00 a | 71.2# |
| | 2L/ha | 2,354.48 a | 107.33 b | 54 b | 1,038.50 ab | 2,090.92 a | 78.3# |
| Mean pH | 1L/ha | 7.44 a | 7.60 a | 7.64 a | 7.42 a | 7.52 a | P > 0.05 |
| | 2L/ha | 7.7 a | 7.38 bc | 7.57 ab | 7.22 c | 7.62 ab | 1.4 |
| Conductivity (μS) | 1L/ha | 159.69 a | 167.49 a | 171.60 a | 164.98 a | 167.63 a | P > 0.05 |
| | 2L/ha | 190 a | 192.32 a | 191.76 a | 188.71 a | 196.93 a | P > 0.05 |
| Dissolved oxygen (mg/L) | 1L/ha | 4.88 a | 3.06 a | 3.87 a | 4.67 a | 4.81 a | P > 0.05 |
| | 2L/ha | 4.44 b | 5.51 ab | 4.84 ab | 6.79 a | 4.15 b | 13.5 |
| Saturated oxygen (%) | 1L/ha | 64.18 a | 37.77 a | 49.84 a | 65.51 a | 62.91 a | P > 0.05 |
| | 2L/ha | 51.70 b | 67.20 b | 56.20 b | 93.21 a | 42.59 b | 31.0 |

* Primary data; results were square root (x + 0.5) transformed for determination of larval mortality rate.
\+ Same letters on same row indicate no difference by the Tukey test at 5% significance level.
\# Coefficient of variation.

tion 24 h after application and 99.3% at 48 h, but there was an increase in the number of larvae on the fifth day (Table 2). Hallmon *et al.* (2000) controlled *Cx. quinquefasciatus* with Vectobac AS, two to three days after application in plastic containers, with repeated applications every ten days. Zequi and Lopes (2007) found that Vectobac at 2 L/ha effectively controlled *Cx. saltanensis* in slurry lagoons for up to 15 days, with 100% larval mortality 24 h after application, and recommended biweekly applications for similar conditions. Amalraj *et al.* (2000) tested Vectobac AS at 1.2 and 2.4 L/ha concentrations at smaller breeding sites, obtaining 80% control of larvae of *Cx. quinquefasciatus* for 1.8 days in septic cesspools. Conductivity was the only chemical parameter to decrease on the seventh day after Vectobac application at the 1 and 2 L/ha concentrations. Such a decrease might not have been related to the bioinsecticide application because higher product concentration did not produce significant changes or the application of 1L/ha was sufficient to maximally alter conductivity (Table 2). A fluctuation in the physical and chemical parameters (pH and conductivity) between the fifth and seventh day was observed with 2 L/ha concentration but without statistical difference (Table 1). Such changes are typical of lagoons with continuous effluent influx and higher or lower needs for organic matter, depending on the intensity of activities of the waste producer.

Aquabac XT controlled 76.5 and 71.5% of the larvae at a concentration of 1 L/ha one and two days after application, respectively (Table 3). At 2 L/ha concentration, 95.4% control was observed 24 h after application and 97.7%, 48 h after application (Table 3); the initial larval counts were reached again on the seventh day. Aquabac XT seems to have caused changes in pH and oxygen (saturated and dissolved) only at the 2L/ha concentration (Table 3), but these parameters returned to almost baseline levels after 7 days.

All field trials were conducted at 19.2 to 29.4 ºC water temperatures, environmental temperatures between 11.1 and 38 ºC, and 25 to 99% relative humidity (Table 4). Such fluctuations are common in northern Paraná State during the summer (Fritzsons *et al*. 2008), when most of the reproductive activity of Culicidae occurs by colonization of the breeding sites, assuring a high egg hatching rate and constantly high quantity of larvae. Egg rafts were collected at every sampling period, indicating the presence of adults near the lagoons, assuring the immediate recolonization of breeding sites (data not shown). Camargo *et al*. (1994) reported 93% eclosion

**Table 4.** Environmental and water temperature and relative humidity in lagoons where formulations containing *Bacillus thuringiensis israelensis* were applied, with three replicates for each concentration.

| Product / concentration | Water temperature ºC | | | Environmental temperature ºC | | Relative humidity % | |
|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | Min. | Max. | Min. | Max. |
| Teknar, 1 litre | 28.0 | 23.3 | 32 | 16.7 | 38 | 33 | 99 |
| Teknar, 2 litres | 24.6 | 20.7 | 32.4 | 11.1 | 29.6 | 36 | 99 |
| Vectobac, 1 litre | 29.4 | 24 | 34.3 | 13.5 | 37.9 | 39 | 99 |
| Vectobac, 2 litres | 27.6 | 23.9 | 30.8 | 11.3 | 35.7 | 25 | 98 |
| Aquabac, 1 litre | 27.4 | 23.1 | 32.3 | 14.6 | 33.7 | 35 | 99 |
| Aquabac, 2 litres | 22.8 | 19.2 | 26.4 | 18.0 | 32.8 | 39.5 | 99 |

from egg rafts at 27 ºC for *Cx. quinquefasciatus*. Vianna *et al.* (1996) showed that hatching of eggs of *Cx. quinquefasciatus* was not significantly affected by temperature under natural conditions, reaching 90% eclosion during a large part of the year. Zequi and Lopes (2012) found a 97.48% egg hatching rate for *Cx. saltanensis*, with emergence occurring between 12.29 and 13.12 days for males and females, respectively, at 27 ºC and relative humidity of 80 ± 5% in the laboratory. These data on the reproductive ecology of the species and the limits of temperature changes in the environment suggest that temperature does not affect the reproductive behaviour of the species. Therefore, the population changes observed were directly related to the pathogenic action of the bioinsecticide, where high temperature reduces the time between egg hatching and the emergence of adults, thereby facilitating a potential rapid recolonization of the site. High temperature and excess of nutrients significantly increase larval density at breeding sites. Such conditions indirectly affect the impact of Bti because high temperatures and ultraviolet rays affect crystal persistence in the environment (Lacey 2007). According to Tetreau *et al*. (2013), ultraviolet rays increase the sensitivity of mosquitos to Bti, thereby increasing its efficiency for short-term periods. At low temperatures, food intake and screening by the larvae occur at lower rates, requiring higher product concentration to achieve better results (Becker *et al.* 1992).

Larval density was high in both lagoons (Tables 1 to 3). This might have hindered the persistence of Bti action. Becker *et al*. (1992) argue that Bti efficacy decreases linearly as larval density increases. Insecticide efficiency also decreases with higher solar intensity and presence of other competitors for filtration, such as the micro-crustacean *Daphnia.* Nayar *et al*. (1999) also tested the effect of larval densities and light intensity on Bti efficiency, with results similar to those obtained by Becker *et al.* (1992) and to our results in this study. Morais *et al.* (2007) found a high infestation by *Cx. quinquefasciatus* in the Pinheiros River in the city of São Paulo, even after applications of adulticides, organophosphorus larvicides, and *B. sphaericus*. They related such a high infestation to the breeding site's potential for mosquito development, such as its large amount of nutrients. This same phenomenon was observed in lagoons where the products were applied, probably competing directly with Bti crystals during the larval filtering process during feeding. The low levels of dissolved oxygen found in the lagoons can account for the very few aquatic predators found, allowing for the proliferation of Culicidae (Juliano 2009).

## Conclusion

Biological products containing Bti, such as those tested in our trials are an ecological alternative to chemical insecticides for controlling Culicidae in effluent treatment lagoons. None of the products has high persistence rates and must be applied weekly at the 2 L/ha concentration for efficient control and environmental safety. Because of the limited persistence of biological products in this environment and the high-pressure colonization by Culicidae, depending on the context, Bti does not exhibit the same efficiency for different places. Therefore, the control strategy must be adapted to each location to be treated, because effluent treatment lagoons are sites conducive to the breeding of Culicidae and are difficult to control, thereby requiring constant monitoring.

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## Literature cited

AMALRAJ, D. D.; SAHU, S. S.; JAMBULINGAM , P.; BOOPATHI , P. S.; KALYANASUNDARAM, M. P. K. 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Tropica 75: 243-246.

BECKER, N.; ZGOMBA, M.; LUDWIG, M.; PETRIC, D.; RETTICH, F. 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. Journal of the American Mosquito Control Association 8: 285-289.

BENJAMIN, S.; RATH, A.; CHIANG, Y. F.; LEE, H. L. 2005. Efficacy of a *Bacillus thuringiensis israelensis* tablet formulation, VectoBac DT®, for control of dengue mosquito vectors in potable water containers. Southeast Asian Journal of Tropical Medicine and Public Health 36: 879-892.

BRAVO, A.; LIKITVIVATANAVONG, S.; GILL, S. S.; SOBERÓN, M. 2011. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology 41: 423-431.

CAMARGO, M. F.; SILVA, I. G.; ELIAS, C. N. 1994. Ciclo evolutivo do *Cx. quinquefasciatus* (WIEDMANN. 1828) (Diptera: Culicidae) em condições de laboratório. Revista de Patologia Tropical 3: 191-195.

CONSOLI, R. A. G. B.; CARVALHO-PINTO, C. J.; OLIVEIRA, M. A.; SANTOS B. S.; LAMOUNIER, M. A.; ALVES, R. S. A.; SILVA, C. M. B.; RABINOVITCH, L. 1995. Some environmental and biological factors influencing the activity of entomopathogenic *Bacillus* on mosquito larvae in Brazil. Mémórias do Instituto Oswaldo Cruz 90: 121-124.

DEANE, L. M. 1951. Observações sobre alguns hábitos dos adultos de *Cx. fatigans*. O principal transmissor da filariose em Belém. Pará. Revista do Serviço Especial de Saúde Pública 4: 423-464.

FERNÁNDEZ, M. C.; CARMEN, M. D.; LAZCANO, J. A. B.; ORTEGA, J. A. N. 1986. Influencia de algunos factores abióticos sobre las fluctuaciones de la población larval de *Cx. quinquefasciatus*, Say 1823 (Diptera: Culicidae). Revista Cubana de Medicina Tropical 38: 281-288.

FRITZSONS, E.; MANTOVANI, L. E.; AGUIAR, A. V. 2008. Relação entre altitude e temperatura: uma contribuição ao zoneamento climático no Estado do Paraná. Revista de Estudos Ambientais 10: 49-64.

GABALDON, A.; ULLOA, G.; ZERPA, N. 1988. *Plasmodium cathermerium* cepa de Icteridae inoculable a palomas, patos y pavos; sus vectores y utilidad en enseñanza e investigación. Boletín de la Dirección de Malariología y Saneamiento Ambiental 28: 53-68.

GUNASEKARAN, K.; PRABAKARAN, G.; BALARAMAN, K. 2002. Efficacy of a floating, sustained release formulation of *Bacillus thuringhiensis* ssp. *israelensis* in controlling *Cx. quinquefasciatus* larvae in polluted water habitats. Acta Tropica 83: 241-247.

GUNASEKARAN, K.; BOOPATHI DOSS, P. S.; VAIDYANATHAN, K. 2004. Laboratory and field evaluation of Teknar HP-D, a biolarvicidal formulation of *Bacillus thuringiensis* ssp. *israelensis*, against mosquito vectors. Acta Tropica 92: 109-118.

HALLMON, C. F.; SCHREIBER, E. T.; VO, T.; BLOOMQUIST, M. A. 2000. Field trials of three concentrations of laginex as biological larvicide compared to vectobac 12 as a biocontrol agent for *Cx. quinquefasciatus*. Journal of the American Mosquito Control Association 16: 5-8.

JULIANO, S. A. 2009. Species interactions among larval mosquitoes: Context dependence across habitat gradients. Annual Review of Entomology 54: 37-56.

LACEY, L. A. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. Journal of the American Mosquito Control Association 23: 133-163.

LOURENÇO-DE-OLIVEIRA, R.; CASTRO, F. A. 1991. *Cx. saltanensis* Dyar 1928. Natural vector of *Plasmodium juxtanucleare* in Rio de Janeiro. Brasil. Memórias do Instituto Oswaldo Cruz 86: 87-94.

LOURENÇO-DE-OLIVEIRA, R.; HEYDEN, R. 1986. Alguns aspectos da ecologia dos mosquitos (Diptera: Culicidae) de uma área de planície (Granjas Calábria) em Jacarepaguá. Rio de Janeiro. IV. Preferências alimentares quanto ao hospedeiro e freqüência domiciliar. Memórias do Instituto Oswaldo Cruz 81: 15-27.

MEDEIROS, Z.; CESSE, E. P.; MENEZES, J. A.; LESSA, F. 2003. Controle da filariose linfática no Brasil, 1951-2000. Epidemiologia e Serviços de Saúde 12: 77-86.

MELO-SANTOS, M. A. V. D.; ARAÚJO, A. P. D.; RIOS, E. M. M.; REGIS, L. 2009. Long lasting persistence of *Bacillus thuringiensis* serovar. *israelensis* larvicidal activity in *Aedes aegypti* (Diptera: Culicidae) breeding places is associated to bacteria recycling. Biological Control 49: 186-191.

MORAIS, S. A.; MARRELLI, M. T.; NATAL, D. 2007. Observações sobre aplicações de controle em ecótopo com alta infestação de *Cx. quinquefasciatus* Say (Diptera, Culicidae). Revista Brasileira de Entomologia 51: 246-251.

MULLA, M. S.; CHANEY, J. D.; RODCHAROEN, J. 1993. Elevated dosages of *Bacillus thuringiensis* var. *israelensis* fail to extend control of *Culex larvae*. Bulletin of the Society of Vector Ecologists 18: 125-132.

MULLA *et al.* (2003), MULLA, M. S.; DARWAZEH, H. A.; DAVIDSON, E. W.; DULMAGE, H. T.; SINGER, S. 1984. Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and safety to nontarget organisms. Mosquito News 44: 336-42.

MULLA, M. S.; USAVADEE, T.; APIWAT, T.; JAKKRAWAM, C. 2004. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. Journal of the American Mosquito Control Association 20: 64-73.

NAYAR, J. K.; KNIGHT, J. W.; ALI, A.; CARLSON, D. B.; O'BRYAN, P. D. 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar *israelensis* against two Florida mosquito species. Journal of the American Mosquito Control Association 15: 32-42.

O´MEARA, G. F.; CUTWA-FRANCIS, M.; REY, J. R. 2010. Seasonal variation in the abundance of *Cx. nigripalpus* and *Cx. quinquefasciatus* in wastewater ponds at two Florida dairies. Journal of the American Mosquito Control Association 26: 160-166.

PAUVOLID-CORRÊA, A.; MORALES, M. A.; LEVIS, S.; FIGUEIREDO, L. T. M.; COUTO-LIMA, D.; CAMPOS, Z.; NOGUEIRA, M. F.; DA SILVA, E. E; NOGUEIRA, R. M. R.; SCHATZMAYR, H. G. 2011. Neutralizing antibodies for West Nile virus in horses from Brazilian Pantanal. Memorias do Instituto Oswaldo Cruz 106: 467-474.

RACHOU, R. G. 1956. Transmissores da filariose bracroftiana no Brasil. Revista Brasileira de Malariologia e Doenças Tropicais 8: 267-279.

RITCHIE, S. A.; RAPLEY, L. P.; BENJAMIN, S. 2010. *Bacillus thuringiensis* var. *israelensis* (Bti) provides residual control of *Aedes aegypti* in small containers. American Journal Tropical Medicine and Hygiene 82: 1053-1059.

SIBAJEV, A.; PACHECO, R. S.; SOARES, M. J.; CUPOLILLO, E.; SANTOS, A. B.; MOMEN, H. 1993. *Crithidia ricardoi* sp. n. a new species of trypanossomatidae isolated from *Cx. saltanensis* Dyar. 1928 (Diptera. Culicidae). Memórias do Instituto Oswaldo Cruz 88: 541-545.

SPSS INC. 2005. SPSS® for Windows®. Version 14.0 [computer program]. Chicago, SPSS Inc.

TETREAU, G.; CHANDOR-PROUST, A.; FRÉDÉRIC, F.; STALINSKI, R.; AKHOUAYRI, I.; PRUD´HOMME, S. M.; RAVETON, M.; REYNAUD, S. 2013. Contrasting patterns of tolerance between chemical and biological insecticides in mosquitoes exposed to UV-A. Aquatic Toxicology 15: 389-397.

TETREAU, G.; RENAUD, S.; DYLANN, K.; SYLVIE V.; JEAN-PHILIPPE, D.; STÉPHANE, R.; LAURENCE, D. 2012. Decreased toxicity of *Bacillus thuringiensis* subsp. *israelensis* to mosquito larvae after contact with leaf litter. Applied and Environmental Microbiology 78: 5189-5195.

VIANNA, E. E. S.; COSTA, P. R. P.; RIBEIRO, P. B. 1996. Oviposição e longevidade de adultos do *Cx. quinquefasciatus* Say 1823 (Diptera: Culicidae) em condições ambientais em Pelotas. RS. Revista Brasileira de Parasitologia Veterinária 5: 47-52.

WHO. 2008. Global programme to eliminate lymphatic filariasis. World Health Organization 83: 333-348.

ZEQUI, J. A. C.; LOPES, J. 2007. Biological control of *Culex* (*Culex*) *saltanensis* Dyar, (Diptera, Culicidae) through *Bacillus thuringiensis israelensis* in laboratory and field conditions. Revista Brasileira de Zoologia 24: 164-68.

ZEQUI, J. A. C.; LOPES, J. 2012. Development of the immature stages of *Cx. saltanensis* Dyar (Diptera, Culicidae) under laboratory conditions. Revista Brasileira de Entomologia 56: 106-110.

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