Biological control of *Culex* (*Culex*) *saltanensis* Dyar, (Diptera, Culicidae) through *Bacillus thuringiensis israelensis* in laboratory and field conditions

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**ABSTRACT.** *Culex* (*Culex*) *saltanensis* Dyar, 1928 can become a problem in urban centers because they reproduce abundantly in ponds organically enriched. It is vector of the *Plasmodium* spp. and *Crithidia ricardoi* Sibajev et al. 1993. This research verifies the efficacy of *Bacillus thuringiensis israelensis* on *C. saltanensis* in two temperature situations, both in laboratory and field conditions. LC₅₀ for *C. saltanensis* immatures fourth instar, was 0.154 ppm and the LC₉₅ was 0.248 ppm an average temperature of 25.7°C. When exposed at a constant temperature of 12±1°C and a photoperiod of 14L:10D, had its susceptibility decreased in 1.50 times in relation to LC₉₅ when compared to room temperature. *B. thuringiensis israelensis* is highly efficient in the control of this mosquito in natural environment with a high level of pollutants using the concentration of 2 liters/hectare, with applications every 15 days.

**KEY WORDS.** Bioassay; mosquito control; immature Culicidae.

**RESUMO.** Controle biológico de *Culex* (*Culex*) *saltanensis* Dyar, (Diptera, Culicidae) através de *Bacillus thuringiensis israelensis* em condições de laboratório e campo. *Culex* (*Culex*) *saltanensis* Dyar, 1928 pode se tornar um problema nos centros urbanos, porque reproduzem abundantemente em lagos de tratamento de esfuentes. Esse mosquito é vetor de *Plasmodium* spp. e *Crithidia ricardoi* Sibajev et al. 1993. O objetivo dessa pesquisa foi verificar a eficácia de *Bacillus thuringiensis israelensis* sobre *C. saltanensis* em condições de temperatura no laboratório e campo. A CL₅₀ para imaturos de quarto instar de *C. saltanensis* foi 0,154 ppm e a CL₉₅ foi 0,248 ppm em temperatura ambiente média de 25,7°C. Quando o bioensaio foi conduzido à temperatura constante de 12±1°C e fotoperíodo de 14L:10E, a susceptibilidade da larva diminui em 1,50 vezes em relação a CL₉₅ quando comparado a temperatura ambiente. *B. thuringiensis israelensis* é eficiente no controle desse mosquito em seu ambiente natural com altos índices de matéria orgânica, usando 2 litros/hectare com aplicações a cada 15 dias.

**PALAVRAS-CHAVE.** Bioensaio; controle de mosquito; imaturo.
ent levels of susceptibility are reported. In general, Culex Linnaeus, 1758, species are rather susceptible, followed by Aedes spp. Meigen, 1818, while Anopheles spp. Meigen, 1818 some-
what less susceptible to products based on B. thuringiensis israelensis (MULLA, 1990). The efficiency of B. thuringiensis israelensis on several species of Culicidae has been verified by LACEY & LACEY (1981), BECKER et al. (1992), BROWN et al. (1998a, b), RODRIGUES et al. (1998), Su & MULLA (1999), BROWN et al. (1999), NAYAR et al. (1999), BROWN et al. (2000) and AMALRAJ et al. (2000) among several other researches.

This research verifies the efficacy of B. thuringiensis israelensis on C. saltanensis in two temperature situations, both in laboratory and field conditions, taking into account its fre-
cquent presence in large organically enriched sites, its potential as a vector of pathogenic agents, and the fact that there is no history of biological control of this species.

**MATERIAL AND METHODS**

About 30 rafts of C. saltanensis eggs were collected on a weekly basis from an effluent treatment pond of a soft drink factory in Londrina, Paraná.

The eggs were conditioned in a 40 x 28 cm tray, 3.5 cm deep, with 1.800 mL of well water. They were placed inside an acclimatized incubator (BOD) at 27 ± 1ºC and a photoperiod of 14L:10D. The immatures were fed daily with 30 mg of “Dog Show” (food for puppies), mill-triturated in particles of approxi-
mately 1 mm. After the sixth day of development the larvae, which were already in the 4th instar, were used in bioassays, and no food was added to the trays 24 hours before the test.

Bacillus thuringiensis israelensis Vectobacâ – AS, 1.200 ITU/mg Lot nº 53-040-N9 was used both for laboratory and field tests.

The bioassays were based on LACEY (1997) and the World Health Organization for bacterial larvicides for public health use (DRAFT-WHO 1999). One was done with repetitions in different days when the average temperature was approximately 25.7ºC, and a natural photoperiod (14L:10D), and the other with a constant temperature of 12 ± 1ºC, and a photoperiod of 14L:10D for conditioning in incubator.

The field experiment was done in a landfill leachate pond, in the outskirts of Londrina (51º19’11”W, 23º55’46”S) Paraná, Brazil. The pond measured 130 x 60 m (7,800 m²) and is highly polluted both by organic material and chemicals. Vectobacâ was used at two liters per hectare, which is the average dosage recommended by the manufacturer for these conditions, with a motorized bomb for sprinkling.

The pond where the field experiment was done was colon-
ized only b C. saltanensis, and the experiment was replicated twice on 24/X to 24/XI/2000. Before the application, six points of larvae collection were determined, equally distant in the pond and covering all its diameter. An entomological net with 1mm was used for the capture of larvae, as described by LOPES & LOZOVEI (1995). The immatures were collected in a singlethrow of the entomological net at about 30 cm from the bank. As a reference point, a sample collection was taken before the be-
going of the application, and other subsequent collections were systematically taken daily 1-4 after application and again seven and 15 days after application.

In each collection procedure, live larvae were counted. Ten percent of collected larvae were mounted on slides in Hoyer’s medium for subsequent identification.

Temperature and precipitation data were supplied by the Agronomic Institute of Paraná (IAPAR), which is approximately 5 km away from the experiment location.

The laboratory tests were analyzed using Probit analysis, and the respective LC50 and LC95 were calculated.

**RESULTS AND DISCUSSION**

We determined from laboratory bioassays that the LC50 for C. saltanensis immatures, fourth instar, was 0.154 (0.135-
0.232) ppm and the LC95 was 0.248 (0.189-0.701). The LC95 was 1.61 times higher than the respective LC50 for larvae of the 4th when assays were conducted at 25.7ºC (Tab. I).

Using the same product, NAYAR et al. (1999) reported val-
ues of 0.131 and 0.207 ppm respectively for LC50 and LC95 in larvae of Culex nigripalpus Theobald, 1901, in 4th instars. This species belongs to the same sub-genus as C. saltanensis and is also found in ponds with polluted water in urban areas. This helps to explain the similar results we report here.

LACEY & LACEY (1981) reported respective mortality rates of 92% for Culex (Culex) mollis Dyar & Knab, 1906; 92.96% for

<table>
<thead>
<tr>
<th>Repetitions</th>
<th>LC50 (ppm)</th>
<th>Lower Limit (Confidence interval)</th>
<th>Upper Limit (Confidence interval)</th>
<th>LC95 (ppm)</th>
<th>Lower Limit (Confidence interval)</th>
<th>Upper Limit (Confidence interval)</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td>4</td>
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<td>0.128226</td>
<td>0.185389</td>
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</tr>
<tr>
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<td>Average</td>
<td>0.154000</td>
<td>0.135000</td>
<td>0.232000</td>
<td>0.248000</td>
<td>0.189000</td>
<td>0.701000</td>
</tr>
</tbody>
</table>
temperatures.

The mosquito susceptibility increases or decreases according to the species, and also due to the influence of abiotic conditions such as pollution, water depth turbidity, temperature, associated microflora, ionic composition of water, presence of larval food, an canopy and biotic conditions of the environment (Lacey & Undeen 1986).

Culex saltanensis, when exposed to B. thuringiensis israelensis at a constant temperature of 12 ± 1°C and a photoperiod of 14L:10D, had its susceptibility decreased in 1.5 times in relation to LC50, when compared to room temperature (Tab I and II). In these conditions, the LC95 was 1.68 times higher in relation to the LC50 (Tab. II). Both the kinetics of toxin activation and feeding rate (hence the amount of toxin consumed) are governed by ambient temperatures.

Lacey & Oldecro (1983) reported an direct and positive relationship between mortality and temperature with early 4th instar Culex quinquefasciatus exposed to 3.4x10^4 viable spores/mL of B. thuringiensis (H-10) at 17.7, 24.3 and 31°C.

Becker et al. (1992) found a distinct difference in the effectiveness of Bti between 5°C and 8°C in the 2nd and 4th instars of Aedes vexans (Meigen, 1830). In low temperatures there is a reduction of water filtering by the larva, which suggests that the amount of product used with low temperatures should be increased. The LC95 was 1.56 times higher in bioassays with immatures kept at low temperatures than in the environment (Tabs I and II).

Nayar et al. (1999) report an LC50 of 0.152, 0.139 and 0.140 ppm in bioassays kept at 15°C, 25°C and 35°C respectively, for C. nigripalpus 4th instar exposed to Vectobac® 12 AS - 1.200 ITU/mg, in 24 hours. Brown et al. (2000), using B. thuringiensis israelensis with 1.279x10^5 ITU/mg, verified that a LC95 for Culex anulirostris Skuse, 1889 larvae of the 3rd instar were among 0.013x10^9 (0.01 to 0.02x10^9) ITU/mg in laboratory conditions.

In field conditions Vectobac® AS was efficient in the control of this mosquito (Fig. 1), using the average concentration recommended for the product (two liters / hectare) for highly polluted environments. Before the first application 12,146 C. saltanensis immatures were collected. There was 100% mortality of larvae 24 hours after application of the product. The larva index remained low for up to 15 days. The second application continued to control 100% of the immatures present in this pond (Fig. 1).

The application of Vectobac every 15 days can offer efficient results for the control of this species. These data are important because there no record of control for this mosquito until now.

Brown et al. (1998a) controlled 3rd instar Culex sitiens, Wiedeman, 1828 larvae using 0.0077 liters/hectare of Vectobac 12 AS in laboratory conditions to reach the LC50, and 0.011 liters/ hectare to reach the LC95. In this experiment in Londrina, there was a mortality rate of 100% of larvae, 24 hours after the application of the product in a 2.0 liters / hectare concentration in field.

Table II. Lethal concentrations (LC50 and LC95) of B. thuringiensis israelensis (1.200 ITU/mg) on immatures of 4th instars of C. saltanensis, maintained in the bioassay at a constant temperature of 12 ± 1°C and photoperiod 14L:10D (n = 25 larvae/pot).

<table>
<thead>
<tr>
<th>Repetitions</th>
<th>LC50 (ppm)</th>
<th>Lower Limit (Confidence interval)</th>
<th>Upper Limit (Confidence interval)</th>
<th>LC95 (ppm)</th>
<th>Lower Limit (Confidence interval)</th>
<th>Upper Limit (Confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<tr>
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<td>0.259309</td>
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</tr>
<tr>
<td>Average</td>
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<td>0.206000</td>
<td>0.297000</td>
<td>0.387000</td>
<td>0.300000</td>
<td>0.818000</td>
</tr>
</tbody>
</table>

Fig. 1. Number of C. saltanensis larvae collected before and after the application of Vectobac® AS (1,200 ITU/mg), 2 liters/hectare after two consecutive applications at the landfill leachate pond in Londrina, Paraná, Brazil.
BROWN et al. (1999) compared two organophosphorates, a growth regulator and B. thuringiensis israelensis, on Aedes vigilax (Skuse, 1889), in Queensland, Australia, and verified that the biological product (Bti) was efficient in the control of the mosquito in laboratory and field conditions, and it did not affect the survival of Leander tenuicornis (Decapoda, Palaeonidae) a non target species with the environment. It also did not affect the quality of the water, while the organophosphorates influenced its pH and turbidity.

SKOVENDE & SANOGO (1999), while testing Bacillus sphaericus and B. thuringiensis israelensis in cesspools and rain puddles, in Burkina Faso, Ouagadougou, Africa, reported that B. sphaericus applied at the rate of 3.0 g/m² reduced C. quinquefasciatus 99% for at least 28 days in cesspits, whereas the same dosage of two Bti granules and commercial liquid formulations of Bs and Bti gave 95% control for 8-14 days. The levels of control obtained with the two liquid products were not different. B. thuringiensis had a reported inferiority compared to B. sphaericus in polluted waters. This was due to the low dosage of B. thuringiensis used. Nevertheless, it had a larger action spectrum, killing C. quinquefasciatus, Culex decens Theobald, 1901 and also Culex cinereus Theobald, 1901.

The experiment a polluted habitat in Londrina proved Bti to be efficient, and somewhat different from other results reported in the literature that show lower efficiency of Bti in highly-polluted breeding places, when the average dosage recommended by the manufacturer was used in locations.

CONCLUSIONS

Bacillus thuringiensis israelensis (Vectobac® AS - 1.200 ITU/mg) controls C. saltansensis 4th instar larvae with an LC₅₀ of 0.154 ppm and an LC₉₅ of 0.248 at room temperature. At 12 ± 1°C, this product presented a decrease in efficiency of about 1.50 times in relation to the LC₉₅ at room temperature under controlled laboratory conditions.

Vectobac® AS - 1.200 ITU/mg is highly efficient in the control of this mosquito in its natural environment with a high level of pollutants using the concentration of 2 liters / hectare, with applications every 15 days.

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REFERENCES


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