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 ricadoi* 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. LC_{50} for *C. saltanensis* immatures fourth instar, was 0.154 ppm and the LC_{95} 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 reation to LC_{50}, 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 polluants 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 reproduz abundantemente em lagoas de tratamento de efluentes. Esse mosquito é vetor de *Plasmodium* spp. e *Crithidia ricadoi* 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 CL_{50} para imaturos de quarto ínstar de *C. saltanensis* foi 0,154 ppm e a $C19_5$ 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 suscetibilidade da larva diminui em 1,50 vezes em relação a CL_{50}, 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.

Culex saltanensis Dyar, 1928 can become a problem in urban centers because they reproduce abundantly in ponds orgnically enriched. They also colonize other natural and artificial breeding sites. *Culex* (*Culex*) *saltanensis* Dyar, 1928 was first collected in the state of Rio de Janeiro by Lourenço de Oliveira (1984). In northern Paraná, Brazil, this mosquito was found by Lopes, (1997a, b), although it was identified as *Culex bahamenis* Dyar & Knab, 1906.

It is vector of the *Plasmodium cathemerium* Hartman, 1927 sparrow hemosporidian (Gabaldon *et al.* 1988), and was considered by Lourenço-de-Oliveira & Castro (1991) as a primary vector of *Plasmodium juxtanucleare* Versiani & Gomes, 1941, a protozoan which causes malaria in birds. A new tripanossomatyd species – *Crithidia ricardoi* Sibajev *et al.* 1993, original host of *C. saltanensis*, was described by Sibajev *et al.* (1993).

The control methods for Culicidae can be cultural, by cleaning the breeding place, taking the vegetation away and doing the appropriate handling, according to the species; or chemical, by using organo-phosporated products, which have restricted use to certain places due to its non-specificity and the high resistance rate presented by insects. There is also the possibility of fighting the insect through a biological and integrated control.

The number of vector insects resistant to chemical pesticides, the long duration of their effects, their non-specificity in the target organisms, and their pollutant action in the atmosphere have encouraged researches on alternative biological pesticides, as the use of entomopathogenic *Bacillus* spp. (Consoli *et al.* 1997). *Bacillus thuringiensis israelensis* (Bti) is specific for larvae of mosquitoes and black fly species, but different 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 somewhat 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 frequent 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 approximately 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 outsskirts of Londrina (51º19'11"W, 23º55'46"S) Paraná, Brazil. The pond measured 130 x 60 m (7,800 m^2) 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 colonized 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 single throw of the entomological net at about 30 cm from the bank. As a reference point, a sample collection was taken before the beginning 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 LC_{50} and LC_{95} were calculated.

RESULTS AND DISCUSSION

We determined from laboratory bioassays that the LC_{50} for *C. saltanensis* immatures, fourth instar, was 0.154 (0.135-0.232) ppm and the LC_{95} was 0.248 (0.189-0.701). The LC_{95} was 1.61 times higher than the respective LC_{50} for larvae of the 4th when assays were conducted at 25.7ºC (Tab. I).

Using the same product, NAYAR *et al.* (1999) reported values of 0.131 and 0.207 ppm respectively for LC_{50} and LC_{90} 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 I. Lethal concentrations (LC_{50} and LC_{95}) of *B. thuringiensis israelensis* (1.200 ITU/mg) on immatures of 4th instars of *C. saltanensis*, kept at an average temperature of 25.7ºC in the bioassay and environmental photoperiod (14L: 10D) with (n = 25 larvae/pot).

Repetitions	LC_{50} (ppm)	Lower Limit (Confidence interval)	Upper Limit (Confidence interval)	LC_{95} (ppm)	Lower Limit (Confidence interval)	Upper Limit (Confidence interval)
1	0.152463	0.135557	0.222450	0.241824	0.184515	0.694718
2	0.145205	0.132875	0.173557	0.210780	0.175525	0.339905
3	0.143506	0.126648	0.189723	0.261490	0.195301	0.573263
4	0.143918	0.128226	0.185389	0.246682	0.189633	0.498011
5	0.182574	0.153139	0.388214	0.280374	0.201531	1.398270
Average	0.154000	0.135000	0.232000	0.248000	0.189000	0.701000

Limatus durhamii Theobald, 1901 and *Limatus flavisetosus* De Oliveira Castro, 1935; and 63.33% for *Trichoprosopon digitatum* (Rondani, 1848); using *B. thuringiensis israelensis* 1000 UTI/mg in the laboratory. The same authors verified with the same preparation a LC_{50} and LC_{95} of 0.042 and 0.33 ppm respectively for late 3rd – early 4 th instar of *C. quinquefasciatus*.

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 ± 1ºC and a photoperiod of 14L:10D, had its susceptibility decreased in 1.5 times in relation to LC_{50}, when compared to room temperature (Tab I and II). In these conditions, the LC_{95} was 1.68 times higher in relation to the LC_{50} (Tab. II). Both the kinetics of toxin activation and feeding rate (hence the amount of toxin consumed) are governed by ambient temperatures.

LACEY & OLDACRE (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 31ºC.

BECKER *et al.* (1992) found a distinct difference in the effectiveness of Bti between 5ºC and 8ºC in the 2nd and 4th instars of *Aedes vexans* (Meigen, 1830). In low temperatures there is a reduction of water filtering by the larvae, which suggests that the amount of product used with low temperatures should be increased. The LC_{95} 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 LC_{50} of 0.152, 0.139 and 0.140 ppm in bioassays kept at 15ºC, 25ºC and 35ºC respectively, for *C. nigripalpus* 4th instar exposed to Vectobac® 12 AS – 1.200 UTI/mg, in 24 hours. BROWN *et al.* (2000), using *B. thuringiensis israelensis* with 1.279x10^{9} ITU/mg, verified that a LC_{95} for *Culex annulirostris* 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).

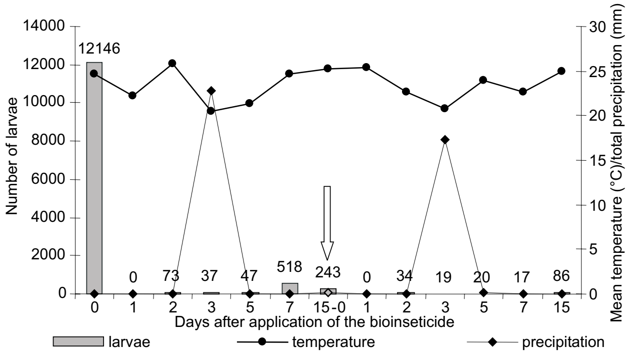

Fig. 1. Number of *C. saltanensis* larvae collected before and after the application of Vectobac® AS (1,200 ITU/mg), 2 liters/hectare after two consecutive applications at the landfill leachate pond in Londrina, Paraná, Brazil.

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 $LC_{50,}$ and 0.011 liters / hectare to reach the LC_{95}. 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 (LC_{50} and LC_{95}) 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 ± 1ºC and photoperiod 14L:10D (n = 25 larvae/pot).

Repetitions	LC_{50} (ppm)	Lower Limit (Confidence interval)	Upper Limit (Confidence interval)	LC_{95} (ppm)	Lower Limit (Confidence interval)	Upper Limit (Confidence interval)
1	0.226313	0.210190	0.252941	0.304320	0.268293	0.380083
2	0.235985	0.206157	0.332931	0.437540	0.317333	1.130193
3	0.243057	0.219600	0.288563	0.353388	0.295566	0.497584
4	0.236977	0.204310	0.348851	0.474337	0.330697	1.402827
5	0.211426	0.191136	0.259309	0.367833	0.287613	0.679261
Average	0.231000	0.206000	0.297000	0.387000	0.300000	0.818000

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, Palaemonidae) 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.

Skovmand & 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/m^2 reduced *C. quinquefasciatus* 99% for at least 28 days in cesspits, whereas the same dosage of two Bti granules and comercial 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. saltanensis* 4th instar larvae with an LC_{50} of 0.154 ppm and an LC_{95} of 0.248 at room temperature. At 12 ± 1ºC, this product presented a decrease in efficiency of about 1.50 times in relation the to LC_{50} 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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