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Susceptibility of *Aedes aegypti* larvae to temephos and *Bacillus thuringiensis* var *israelensis* in integrated control

Susceptibilidade de larvas de Aedes aegypti ao tratamento integrado com temephos e Bacillus thuringiensis var israelensis

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ANDRADE, C.F.S. & MODOLO, M. Susceptibility of *Aedes aegypti* larvae to temephos and *Bacillus thuringiensis* var *israelensis* in integrated control. *Rev. Saúde públ.*, S. Paulo, **25**: 184-7, 1991. The susceptibility of field collected *Aedes aegypti* larvae was evaluated in terms of median lethal time (LT_{50}) and final mortality, when treated with temephos, *Bacillus thuringiensis* var *israelensis* as well as mixtures of these two agents. Third instar larvae were shown to be more susceptible than early and late fourth instar ones to the entomopathogen. Survival of some individuals when exposed to temephos suggest possible resistance. Temporal synergism in early fourth instar larvae was detected when they were exposed to mixtures of Bti-temephos. The possibility of this integrated treatment is commented on.

Keywords: Aedes. Bacillus thuringiensis, pathogenicity. Cyanamide, toxicity. Mosquito control

Introduction

Increasing interest has been given to the use of microbial insecticides by Brazilian governmental departments in mosquito control programs in urban areas in recent years. However, the current practice in the campaign against *Aedes aegypti* is still the perifocal and focal chemical insecticide treatment based mainly on a granular formulation of temephos against the larval stage[7,8]. Such measures have, in recent years[8], involved the expensive use of many tons of organophosphorous compunds. Furthermore, they have increased the possibility of the development of resistance to such compounds, already detected all over the world, as also to temephos in Caribbe and neighboring countries[9, 11,12].

The high larvicidal activity against mosquitoes of products based on *Bacillus thuringiensis* var. *israelensis* (Bti) has been frequently reported and the feasibility of its use in Brazil has been pointed out[4,10].

In order to achieve efficient mosquito control and avoid the development of resistance, the World Health Organization has recommended the taking of such measures as the mixing, rotation or successive use of insecticides[9]. Our previous research into the use of temephos in association with Bti Against *Culex quinquefasciatus* in sewage showed promising results both under laboratory and field conditions[1].

The present study was undertaken to assess the level of susceptibility of a natural *Ae. aegypti* larval population under laboratory conditions, both to temephos and to a commercial Bti formulation, as well as to mixtures of these two larvicides.

Material and Method

Locality and age of test insect: *Ae. aegypti* larvae were obtained from natural breeding sites such as tyres, discarded tins, cans and flower-pots. Collections were carried out in an urban area (Manoel da Nobrega district) of Campinas, State of S. Paulo, Brazil, during April 1987.

In the laboratory larvae were transferred to petri dishes and separated into third, early fourth and late fourth instars, according to head/body size relation. Young larvae were discarded owing to the difficulty of confirming their identification.

Susceptibility tests: Larvae were treated in

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small polypropylene containers each holding two liters of commercial spring water (Fontagua^R) in such a way as to present a surface area of about 500 cm^2 and 4.5 cm in depth. No additional food was offered and the larvae were kept for ca. 1 h before treatment to permit acclimatization. After this period, sporadic abnormal larvae were replaced by normal ones.

Two replicates of 29 larvae each were used for Bti, temephos and mixture treatments. Another two replicates of 25 untreated larvae were maintained as controls. The calculated amount of larvicide was added to each box in 5 ml of stock solution and homogenated by gently stirring with a glass rod. Vectobac 12 AS^R, an aqueous suspension formulation of Bti containing 1,200 *Ae. aegypti* International Toxic Units (ITU)/mg and Abate 500-E^R emulsifiable concentrate containing 50% w/v of temephos were utilized.

The number of dead larvae and the water temperature were recorded at progressive time intervals until complete mortality or pupation was reached. Susceptibility was compared in terms of final mortality 18 h post-treatment and median lethal time (LT_{50})[4] calculated by log-probit regression using a PC-microcomputer and a BASIC programme. The differences between two LTs_{50} was considered significant or otherwise depending on overlapping existence of their confidence limits ($p < 0,05$).

Table 1. Median lethal time (LT_{50}), confidence limits (CL) and final mortality for concentrations of *Bacillus thuringiensis* var. *israelensis* used against third (L_3) and fourth (L_4) instar larvae of *Aedes aegypti*.

INSTAR	L_3	L_3	L_4 early	L_4 early	L_4 late
Concentration (ITU/l)	2,500	5,000	2,500	5,000	5,000
LT_{50} (min)	261.2	235.0	391.4	298.0	362.1
Lower CL	251.7	220.1	379.2	291.5	355.4
Upper CL	271.1	250.9	404.1	304.7	369.0
Mortality (%)	100	100	100	100	96.8

Results

The mean water temperature recorded during the experiment was of 17.8 ± 1.2 °C. No mortality was observed among the control group, thus eliminating the need for the application of any corrective formula.

Table 1 presents the results in terms of LT_{50} and final mortalities of *Ae. aegypti* larvae treated with Bti. Third instar (L_3) larvae were shown to be more susceptible to Bti than fourth instar ones (L_4). A significantly shorter time, to cause 50% mortality of L_3 larvae was obtained for both concentrations, though the same total mortality was achieved in both L_3 and early L_4 larvae. The relatively high LT_{50} obtained among late L_4 larvae to 5,000 ITU/l as well as the partial final mortality indicate their low susceptibility to Bti. The regression lines and the angular coefficients for Bti treatment against L_3 and L_4 larvae can be found in Figure 1.

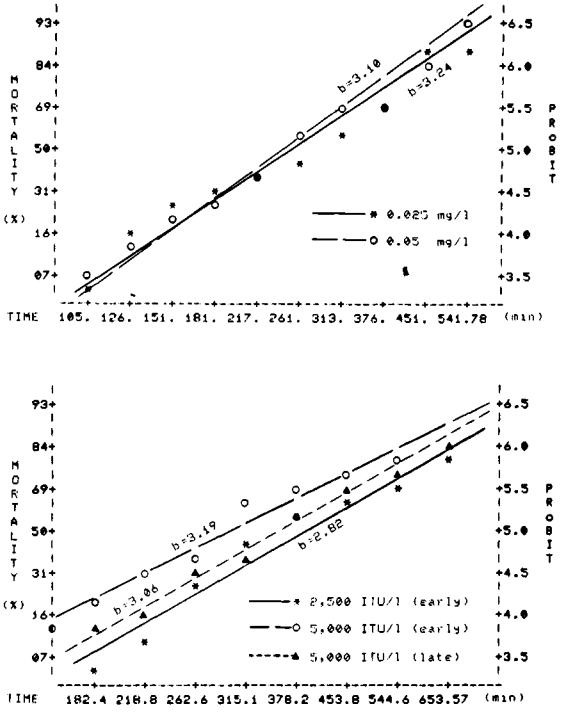

Figure 1. Time-mortality curves for 2 concentrations of *Bacillus thuringiensis* var. *israelensis* used against third (A) as well as early and late forth instar (B) *Aedes aegypti* larvae (b = angular coefficient).

Early L_4 larvae presented LTs_{50} to temephos near to 4 h with a significant variation between the two concentrations. Although temephos LTs_{50} were shown to be lesser then those of Bti against early L_4 larvae, a greater survival rate was observed among the former treatments, resulting in 96.4% and 89.8% as final mortalities, respectively, after 18 h (Table 2). Figure 2 presents the regression lines and the angular coefficients for early L_4 larvae treated with the chemical insecticide.

Mixtures of temephos and Bti were evaluated against early L_4 larvae in two different concentrations. The higher mixture was equivalent to the sum of the lowest concentrations of the two larvicides when evaluated separately. The other was equal to 1/2.5 of this mixture. The

Table 2. Median lethal time (LT_{50}), confidence limits (CL) and final mortality for 2 concentrations of temephos used against early fourth instar *Aedes aegypti* larvae.

Concentration (mg/l)	0.025	0.05
LT_{50} (min)	256.4	244.6
Lower CL	249.3	240.7
Upper CL	263.8	248.7
Mortality (%)	96.4	89.8

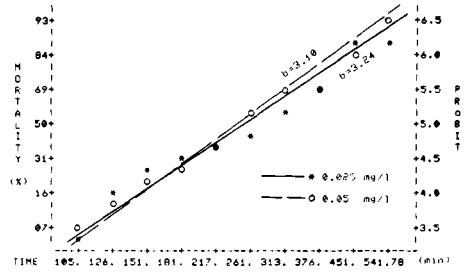

Figure 2. Time-mortality curves for 2 concentrations of temephos used against early L_4 *Aedes aegypti* larvae (b = angular coefficient).

Ae. aegypti larvae treated showed low susceptibility to the lower mixture resulting in a TL_{50} close to 6.2 h and a final mortality of 91.2%. For the higher mixture the TL_{50} was close to 3.2 h with a final mortality of 98.4% (Table 3). Regression lines and angular coefficients for Bti and temephos mixtures used against early L_4 larvae are shown in Figure 3.

Table 3. Median lethal time (LT_{50}), confidence limits (CL) and final mortality for 2 mixtures of *Bacillus thuringiensis* var. *israelensis* with temephos used against early fourth instar *Aedes aegypti* larvae.

Concentrations Temephos (mg/l) Bti (ITU/l)	0.01 + 1,000	0.025 + 2,500
LT_{50} (min)	372.8	194.6
Lower CL	363.3	188.9
Upper CL	382.6	199.3
Mortality (%)	91.2	98.4

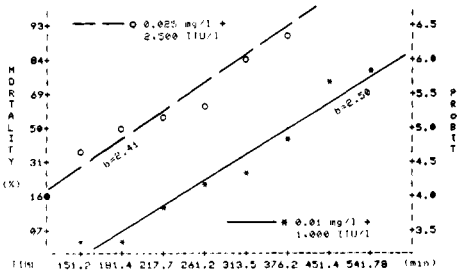

Figure 3. Time-mortality curves for 2 mixtures of temephos and *Bacillus thuringiensis* var. *israelensis* used against early L_4 *Aedes aegypti* larvae (b = angular coefficient).

Discussion

The rapid mode of action of Bti formulations against *Ae. aegypti* larvae has been reported ever since the discovery of this entomopathogen. Very high experimental concentrations such as 900,000 ITU/l against L_3 larvae or 12,000 ITU/l against L_4 *Ae. aegypti* larvae are able to cause LTs_{50} in time intervals as short as 12 or 25 min., respectively[5,6].

In the present work, *Ae. aegypti* larvae present LTs_{50} ranging from ca. 4 to 6.5 h when subjected to field recommended doses of Bti, and except for those in late fourth instar no surviving larvae were recorded. The differences observed in larval susceptibility among different larval ages may be due to a greater filtration rate in early L_4 larvae, as compared with those in third or late fourth instars. Such results permit us to reinforce existing recommendations as to a criterious choice of aged larvae and the use of those in early L_4 when seeking to compare susceptibility to larvicides with "per os" effect.

According to the tentative diagnostic dosages proposed by the World Health Organization (Brown)[3], survival of any *Ae. aegypti* larvae from exposure to 0.02 mg/l of temephos would indicate the possibility of resistance among the population tested. In the present work survival both to 0.025 and 0.05 mg/l was obtained, thus making full multiple-concentration tests imperative to confirm the level of resistance among Brazilian populations of *Ae. aegypti* larvae.

The two mixtures of larvicides evaluated showed a significant antecipation in the LTs_{50} for early L_4 larvae. The higher mixture provoked 50% mortality one hour earlier than the chemical insecticide alone and 3.3 hours earlier than that caused by the bacteria alone. The lower mixture, though 2.5 times less strong, was sufficient to cause 50% mortality in 6.3 h and a final mortality of up to 90%. Such a performance may be considered equivalent in terms of LT_{50} to Bti alone when evaluated at 2,500 ITU/l. This implies a classic temporal synergism, as described by Benz[2].

The integration of biological and chemical insecticides to control mosquito larvae may have as its main advantages the more rapid onset of death, thus controlling L_4 larvae which otherwise would escape control starving for pupation, as well as permitting more economical treatment. As an additional advantage, the integrated control may also reduce the normal pressure towards the resistance selection to a routinely used chemical insecticide. According to pest management precepts, the monitoring of resistance development is in any case

imperative. The use of sub-doses of more than one active ingredient in integrated control must take a double resistance risk into account, and a good monitoring programme must always be undertaken.

ANDRADE, C.F.S. & MODOLO, M. Susceptibilidade de larvas de *Aedes aegypti* ao tratamento integrado com temephos e *Bacillus thuringiensis* var *israelensis*. *Rev. Saúde públ.*, S. Paulo, **25**: 184-7, 1991. A susceptibilidade de larvas de *Aedes aegypti* coletadas no campo foi avaliada em termos do tempo letal mediano (TL_{50}) e da mortalidade final, quando tratadas com temephos, *Bacillus thuringiensis* var *israelensis* ou misturas desses dois agentes. As larvas de terceiro estádio mostraram-se mais suceptíveis ao patógeno do que aquelas no início ou no fim do quarto estádio. A sobrevivência de alguns indivíduos aos tratamentos com temephos permite sugerir a possibilidade de resistência. Foi detectada a existência de sinergismo temporal, quando larvas no início do quarto estádio foram tratadas com as misturas do Bti com o temephos. A possibilidade do tratamento integrado é comentada.

Descritores: Aedes. Bacillus thuringiensis, patogenicidade. Cianamida, toxicidade. Controle de mosquito.

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