BioAssay

BIOLOGICAL CONTROL

Evaluation of Different Larvicides for the Control of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) under Simulated Field Conditions

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BioAssay: 7:3 (2012)

Avaliação de diferentes larvicidas para o controle de *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) em condições simuladas de campo

RESUMO - A Dengue é uma arbovirose, transmitida pelo *Aedes aegypti*. No Brasil o controle larvário deste mosquito têm sido realizado com o uso do organofosforado temefós, que tem selecionado populações de mosquitos resistentes. Devido a este problema, o Ministério da Saúde tem buscado novos produtos incluindo formulações à base de *Bacillus thuringiensis israelensis* (Bti). O objetivo desse trabalho foi comparar a eficácia e persistência de dois bioinseticidas à base de Bti, um regulador de crescimento - piriproxifen e um produto químico – temefós, em populações susceptíveis e resistentes ao temefós. Os resultados demonstraram que os produtos biológicos à base de Bti controlaram 100% da população por 20 dias e que não houve diferença na suscetibilidade entre as duas populações testadas. Os produtos à base de temefós e piriproxifen causaram 100% de mortalidade em ambas populações por até 60 dias após o tratamento.

PALAVRAS-CHAVE - bioinseticidas, mosquito, inseticidas quimicos.

ABSTRACT - Dengue is an arbovirosis, transmitted by *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). In Brazil the larval control of this mosquito has utilized the organophosphate temephos, which has selected resistant mosquito populations. Faced with this problem, the Ministry of Health is searching for new products including those based on *Bacillus thuringiensis israelensis* (Bti). The aim of this work was to compare the efficacy and persistence of two bioinsecticides based on Bti (VectoBac WDG and VectoBac DT), one growth regulator – pyriproxyfen (Sumilarv 0.5 G), and a chemical product – temephos (Fersol 1G) in susceptible and temephos resistant populations of *A. aegypti*. The results showed that the Bti products gave 100% population control for 20 days and there was no difference in susceptibility among the two mosquito populations. The products based on temephos and pyriproxyfen caused 100% larval mortality in both populations for 60 days after treatment..

KEY WORDS - bioinsecticides, mosquito, chemical insecticides.

Dengue is an arbovirosis, which affects more than 100 countries, reaching around 80 million infections, with 550 thousand hospitalizations and more than 20 thousand deaths per year. It is transmitted mainly by the mosquito *A. aegypti* (Linnaeus) (Diptera: Culicidae), a mosquito originally from the African continent, which has adapted in urban tropical and subtropical environments. Besides representing a threat in disease transmission, large populations of mosquitoes are a nuisance, resulting in losses for tourism and limitations to work and leisure activities. Because of the problems and threats which they represent for society, the mosquito populations should be monitored, and very often the use of control measures is necessary in the urban and rural environments.

The fight against mosquito larvae in their breeding sites, when the physical elimination of these sites is not viable, may be carried out with the periodic application of larvicides (PAHO 1994). The larvicides are divided into three groups: chemicals – organophosphates and pyrethroids, bioregulators – synthetic analogs of insect hormones and biologicals – entomopathogenic bacteria.

In Brazil, the main larvicide used in the control of *A. aegypti* is temephos. However, the continuous use of this organophosphate has selected resistant mosquito populations (Marcoris *et al.* 1999). Thus, the Ministry of Health has sought other alternatives, including products based on *Bacillus thuringiensis israelensis* (Bti) (Vilarinhos 2002).

This work compared the efficacy and persistence of two biological larvicides based on Bti (Vectobac WDG and Vectobac DT, Valent BioSciences Corp.), one growth regulator based on pyriproxyfen (Sumilarv 0.5G, Sumitomo Chemical Corp.) and one chemical product based on temephos (Fersol 1G, Fersol Industria e Comercio S.A.), in populations of *A. aegypti* resistant and susceptible to temephos.

Materials and Methods

The tests were performed under simulated field conditions, in fiberglass water reservoirs with 250 liters capacity. The boxes were kept in a screened greenhouse, partially shaded, allowing exposure to sunlight, at room temperature (25 ± 4°C).

The treatments were made in triplicate and the doses were as recommended by the National Dengue Program for VectoBac WDG and Fersol 1G . For Sumilarv 0.5 G and VectoBac DT, the doses employed were suggested by the producers (Table 1). The 0.01 ppm concentration of pyriproxifen used to be the maximum accepted limit for potable water by the World Health Organization, later modified to 0.3 ppm (WHO 2004). Three untreated boxes were kept as control. The efficacy and persistence of the products were evaluated using larvae from lab colonies of field collected *A. aegypti* populations susceptible and resistant to temephos from the Federal District. The temephos resistant mosquitoes were obtained in the city of Planaltina, as a result of continuous use of temephos for routine dengue control activities (Carvalho *et al* 2004).

Every ten days, the boxes were colonized with 20 second instar larvae of *A aegypti,* from lab rearing colonies maintained at Embrapa Genetic Resources and Biotechnology. Three times a week, 20% of the water volume was removed and refilled, to simulate the field conditions. The boxes were inspected daily and pupae were counted and removed to the lab rearing facility to observe adult emergence. The adult emergence or mortality was scored every ten days. The larvicidal activity and persistence of formulations, expressed as percent mortality, were determined by the difference between the initial number of larvae and the number of pupae produced at the treatments with Bti and temephos. For pyriproxyfen, percent mortality was expressed as the difference between the initial number of larvae and the number of adults produced in comparison with the control data (Mulla *et al* 2004, Vilarinhos & Monnerat 2004, Braga *et al* 2005). The mortality rates were compared by factorial analysis of variance (product x evaluation) and means were compared by Tukey´s test ($P<0.05$). The effect of each product on susceptible and resistant larvae was compared with t-test, using the software Sigmastat v. 3.1 statistical package (Kuo *et al* 1992).

Results and Discussion

The results demonstrated that the Bti based products VectoBac WDG and VectoBac DT maintained 100% mortality up to 20 days after treatment (Fig 1). At one month after treatment there was a reduction in the mortality caused by VectoBac DT in the resistant population. In the 40 days after treatment evaluation only VectoBac WDG caused more than 80% mortality to temephos susceptible population. At 50 days after treatment none of the Bti products achieved more than 80% mortality.

The analysis of variance comparing the effects of the biological products over susceptible larvae over seven evaluations showed significant differences among products (ANOVA $F = 87.7$; 2, 50 d.f..; $p<0.001$) and among evaluations (ANOVA $F = 4.37$; 6, 50 d.f.; $p=0.002$), although there was no significant interaction between products efficacy and evaluations (ANOVA $F = 1.80$; 12, 50 d.f.; $p<0.081$).

Only the formulation WDG showed mortality greater than 80% up to the fourth week in susceptible larvae (Fig 1A). No differences in efficiency were detected in the formulations

Table 1. Products and doses used for the larvicidal persistence tests at Embrapa Genetic Resources and Technology (2004-2005).

Product (potency)	Formulation	Dose
VectoBac® DT (2,200 ITU/mg)	*Bacillus thuringiensis israelensis* Tablet	2 Tablets/100 l
VectoBac® WDG (3,000 ITU/mg)	*Bacillus thuringiensis israelensis* Water Dispersible Granules	1g/500 l
Sumilarv 0.5G®	Pyriproxyfen Sand Granules 0.5%	0.2g/100 l 0.1g/100 l
Fersol 1G	Temephos Sand Granules 1%	1g/10 l

WDG and DT in the average larval mortality of temephos susceptible or resistant populations, in seven evaluations (t test; P > 0.05).

The same analysis applied to temephos resistant larvae showed significant differences among products (ANOVA F = 54.53; 2, 50 d.f.; P<0.001), among evaluations (ANOVA F = 7.99; 6, 50 d.f.; P<0.001) and that there was a significant effect of the period of evaluations on product efficiency, with significant interaction among factors (ANOVA F = 4.41; 12, 50 d.f.; P<0.001). The WDG formulation caused an average mortality (70.0 ± 21.70%) higher than the DT formulation (52.6 ± 23.68%) in resistant larvae considering seven evaluations, with both products causing mortality higher than that observed in untreated controls (4.0 ± 6.37%). Average mortality at the third evaluation for the DT formulation and fourth evaluation for the WDG formulation was inferior to 80%, although up to the fifth evaluation this difference was not significant (Tukey P<0.05) (Fig 1B).

The formulation seems to be a determinant factor for the persistence and efficacy of Bti based larvicides. Vilarinhos & Monnerat (2004) obtained 100% control over three weeks in uncovered and nine weeks in covered fiberglass water boxes treated with VectoBac WDG. Zequi *et al.* (2005) obtained the same level of control in semi covered buckets for 15 days with the same formulation. In the same studies, the formulations VectoBac CG and DT showed inferior persistence.

It was also observed that temephos susceptible and resistant populations are equally susceptible to the Bti based products, not showing cross resistance to temephos. This is not surprising, because it is known that in the majority of cases, insect resistance to *B. thuringiensis* is associated with conformational changes of the toxin receptor sites present in insect midguts (Van Rie *et al.* 1990, Bravo *et al.* 1992a,b) and that the resistance to temephos is associated with high esterase production (Bisset *et al.* 2001).

The products temephos Fersol and Sumilarv 0.5G (pyriproxyfen) in all doses caused 100% mortality in both temephos susceptible and resistant larvae over 60 days (Fig 2). The factorial analysis of variance comparing the effect of the products applied to susceptible larvae over 11 evaluations showed significant differences between the products (ANOVA F = 339.82; 3, 87 d.f.; P<0.001), among evaluations (ANOVA F = 5.84; 10, 87 d.f.; P<0.001) and there was a significant effect of the evaluation period on the efficiency of the products (ANOVA F = 1.70; 30, 87 d.f.; P=0.029). The comparison of the averages in the period of 11 evaluations through the Tukey test (P<0.05) showed that the temephos product (98.9 ± 0.81%; average mortality ± standard error) did not differ from pyriproxyfen in the dose of 0.02 ppm (91.4 ± 2.89%) but was more efficient than the pyriproxyfen in the dose of 0.01 ppm (83.9 ± 5.13%). All treatments caused mortality higher than control (2.9 ± 0.99%). Temephos

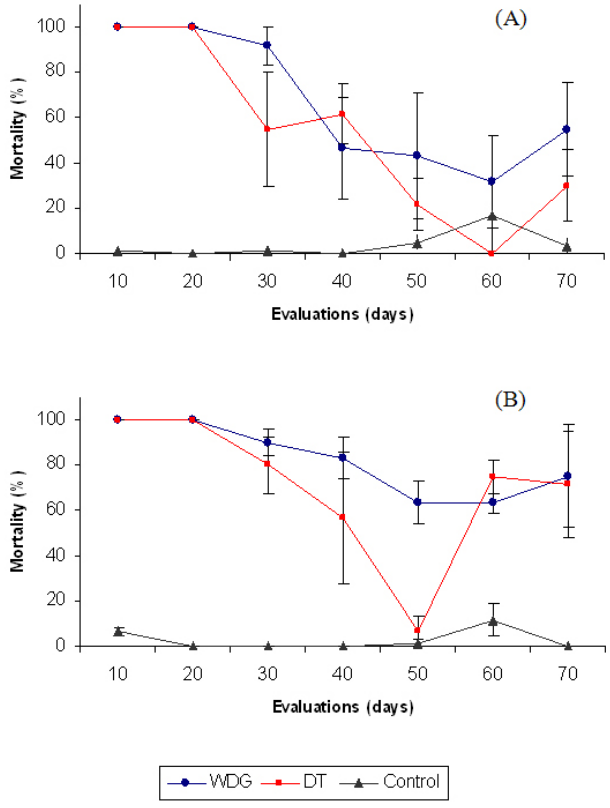

Figure 1. Mortality caused by VectoBac WDG and DT in *Aedes aegypti* larvae (A) resistant and (B) susceptible to temephos.

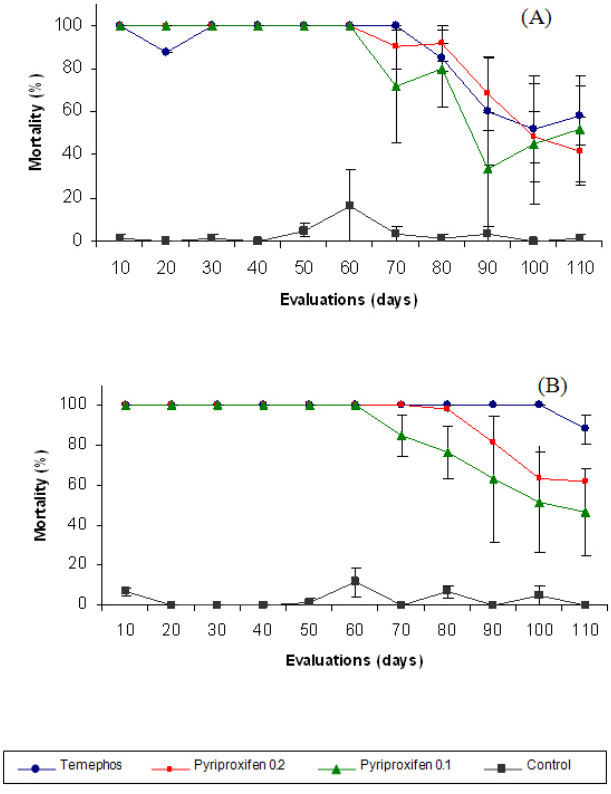

Figure 2. Percent mortality caused by Sumilarv 0.5G (pyriproxyfen) and Fersol 1G (temephos) in *Aedes aegypti* larvae (A) resistant and (B) susceptible to temephos.

maintained an efficiency of 100% mortality to susceptible larvae up to 100 days, while pyriproxyfen efficacy was reduced to below 80% at 90 days after treatment for the 0.02 ppm dose and 80 days for the 0.01 ppm dose.

The same analysis applied to temephos resistant larvae showed significant difference between treatments (ANOVA $F=141.53$; 3, 87 d.f.; $P<0.001$) and among evaluations (ANOVA $F=8.82$; 10, 87 d.f.; $P<0.001$) and there was no interaction between evaluations and treatments. The average mortality in the experiment did not differ among the products temephos (87.6 ± 4.43%; average ± standard error) pyriproxyfen 0.02 ppm (85.5 ± 4.35%) and pyriproxyfen 0.01 ppm (80.2 ± 6.02%). All treatments produced mortalities higher than those seen for untreated control resistant larvae (3.2 ± 1.57%). In the same way, it was observed that with temephos susceptible larvae pyriproxyfen had its efficiency reduced after the eighth evaluation, while temephos had its efficiency reduced to below 80% mortality at the ninth evaluation (Fig 2B).

The mortality of temephos resistant larvae dropped significantly at the 90 day evaluation showing that the efficacy result of temephos in this population differs from that obtained in the susceptible population. The treatments with pyriproxyfen in both doses controlled both populations at 100% for 60 days. After this period the efficacy fell up to the 80 day evaluation to below 80% adult emergence mortality. In some evaluations differences in the pyriproxyfen larvicidal effect between populations were observed. It seems that the population resistant to temephos is also less susceptible to pyriproxyfen. This is a surprising finding because there are no literature data detailing cross resistance between temephos and pyriproxyfen. The *A. aegypti* population from Planaltina used in this study, although characterized as resistant to temephos, did not have its resistance ratio determined; but presented average mortality of 50% with the WHO diagnostic dose of 0.012 ppm, used to determine the resistance ratio. This study suggests that it is important to investigate the behavior of *A. aegypti* populations with higher levels of resistance to temephos, when exposed to pyriproxyfen.

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