

SCIENTIFIC NOTE

Effect of Deltamethrin on Germination and Virulence of *Beauveria bassiana* (Bals.) Vuill. on *Triatoma infestans* (Klug)

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Efeito de Deltametrina na Germinação e Virulência de *Beauveria bassiana*
(Bals.) Vuill. em *Triatoma infestans* (Klug)

RESUMO - Foi avaliada a ação do inseticida deltametrina sobre a capacidade de germinação e virulência do fungo entomopatogênico *Beauveria bassiana* (Bals.) Vuill. contra ninfas de 3º estágio de *Triatoma infestans* (Klug) (Hemiptera: Reduviidae). Após 12 horas em cultura submersa, observou-se uma diminuição significativa da germinação de conídios de *B. bassiana* em proporção ao aumento da concentração do inseticida. Na mais alta dose aplicada, 550 µg/ml, foi observado retardamento da germinação com 24 e 36 horas e interrupção do desenvolvimento do fungo após esse tempo. A exposição de ninfas de 3º estágio a concentrações subletais de deltametrina não alterou de forma significativa a virulência de *B. bassiana*.

PALAVRAS-CHAVE: Insecta, fungo entomopatogênico, piretróide, manejo integrado de pragas.

Chagas Disease, a chronic parasitic disease caused by the protozoan *Trypanosoma cruzi* (Chagas), is a serious health problem in Latin America (WHO 1991). It is normally transmitted to humans by blood-sucking triatomines (Hemiptera: Reduviidae). In Argentina and Brazil, *Triatoma infestans* (Klug) is the main vector of Chagas Disease (Schofield 1994), and its control is extensively made by pyrethroids, particularly deltamethrin (Zerba 1989). As an alternative to chemical control, the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. was shown to be a

potential candidate for biological control of triatomines (Luz & Fargues 1997). The simultaneous application of entomopathogenic fungi and reduced doses of chemical insecticides was first suggested by Telenga in 1964, based on the observation of epizootics which occurred after chemical treatments against insect pests (Ferron 1985). Several other studies showed that advantageous interactions, synergism and potentiation, can be observed when fungus and insecticides are simultaneously applied (Pristavko 1966, Ferron 1985, Barjan *et al.* 1995). Synergism is a toxico-

logical interaction between two or more toxic agents, where the effect of the joint action is significantly higher than the sum of the individual effects. Potentiation is a particular form of synergism, where one of the involved chemicals is applied at a non-toxically dose (Eaton & Klaassen 1996). This study was carried out to verify the effect of flowable deltamethrin on *in vitro* *B. bassiana* conidial germination and to determine if the virulence of this fungus against *T. infestans* could be potentiated by its combination with sublethal concentrations of deltamethrin.

The tests were realized with a strain of *B. bassiana* (CG306 from Embrapa Culture Collection, Brasília, DF), originally isolated in Brazil from *Thyanta perditor* (Fabr.) (Hemiptera: Pentatomidae). This strain was selected based on its high virulence against *T. infestans* in low humidity conditions (Luz *et al.*, un-

published). A commercial formulation of deltamethrin (flowable, 2.5% AI; AgrEvo, San Isidro, Argentina) was used for the tests. This formulation was specifically developed for control of Chagas Disease vectors. Germination tests were carried out with five concentrations of deltamethrin, 5.5, 55.0, 181.5, 363.0 and 550.0 µg/ml, in 50 ml aliquots of liquid complete media (1 mg FeSO₄, 0.5 g KCL, 1.5 g KH₂PO₄, 0.5 g MgSO₄ x 7 H₂O, 6 g NaNO₃, 1 mg ZnSO₄, 1.5 g hydrolysed caseine, 0.5 g yeast extract, 10 g glucose, 2 g peptone, and 1 liter of distilled water). Each aliquot was inoculated with *B. bassiana* conidia to a final concentration of 10⁶ conidia/ml. The percent germination was determined on the basis of the number of conidia producing germ tubes, that were at least twice as long as the length of the conidial diameter. One hundred conidia/replicate were counted. The

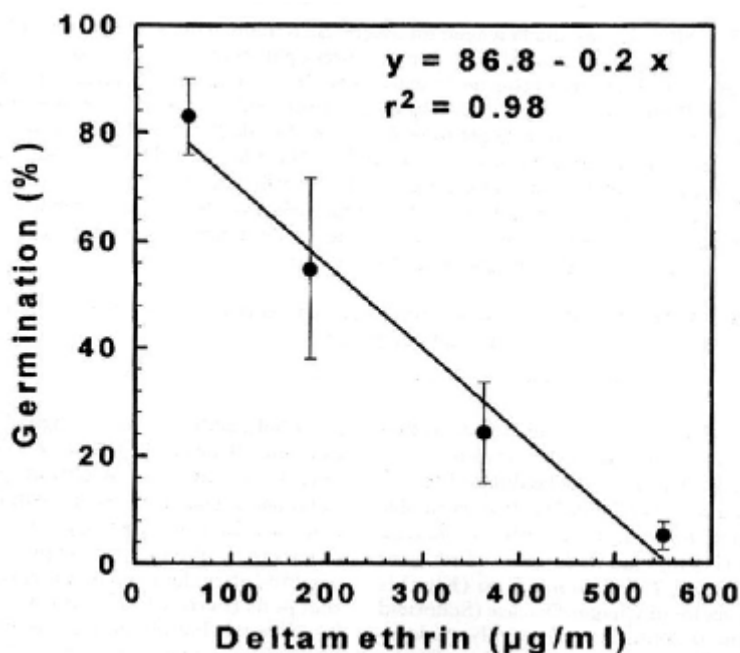


Figure 1. Effect of flowable deltamethrin on germination of *Beauveria bassiana* (CG306) conidia after 24 h of incubation in complete liquid media at 25°C. Each value is the mean of 4 replicates \pm SD, counting 100 conidia/replicate.

procedure was repeated 4 times. Bioassays were conducted with 5-10 day old third instar nymphs of *T. infestans*, that were not fed after the last molt. Insects were obtained from a laboratory colony, where they were fed on chicken and maintained at 25°C. Two sublethal concentrations of flowable deltamethrin were selected for the tests: 0.5 and 5.0 ng a.i./cm², the latter being the concentration affecting 10% of the treated individuals (EC₁₀) 72h after application (data not shown). Filter paper disks (Whatman #1.7 cm diameter) were impregnated with a dilution of the flowable formulation in sterile distilled water (SDW). The treated filter papers were dried for 24 h at room temperature and placed at the bottom

Nymphal mortality was recorded daily for 15 days after the fungal application. Conidia concentration to kill 50% of the treated insects (LC₅₀) was calculated by probit method (SAS Institute 1985). Survival time distribution was analyzed by Wilcoxon test (SAS Institute 1985).

The percentage of conidial germination decreased significantly as a function of deltamethrin concentration (Fig. 1). At the highest deltamethrin concentration (550 µg/ml), a delay in the formation of the germ tube was observed and the germination rate at 24 h was 5.3 ± 2.6 %, compared to 78.8 ± 4.3 % in the control. Fungal development was totally interrupted after 36 h incubation. Lower

Table 1. Lethal concentrations (LC₅₀) calculated for *Triatoma infestans* third instar nymphs treated with *Beauveria bassiana* (CG 306) and sublethal concentrations of deltamethrin.

Time of exposure (days)	LC ₅₀ (conidia x 10 ⁵ /ml) ¹ (95% C.I.)		
	<i>B. bassiana</i> alone	<i>B. bassiana</i> plus deltamethrin (0.5 ng/cm ²)	<i>B. bassiana</i> plus deltamethrin (5.0 ng/cm ²)
10	2.1a (0.9-3.7)	2.5a (1.8-3.2)	3.3a (2.5-5.1)

¹Each value was calculated from data obtained in 4 replicates (N = 560). Values followed by the same letter in a same row are not significantly different, based on overlap of 95% confidential intervals (C.I.).

of plastic containers (7 cm diameter and 4 cm high). Groups of 20 nymphs were continuously exposed to each film. The containers were covered with gauze tied by rubbers, and kept at 26°C. At 24 h of exposure, nymphs were submersed in fungal suspensions of 10⁵, 3x10⁵, 10⁶, 3x10⁶, 10⁷, 3x10⁷, and 10⁸ conidia/ml. After conidial application, nymphs were replaced on the insecticide-treated filter papers, and maintained at 26°C and 50% relative humidity. Control insects were submersed only in SDW. The same conidial concentrations were applied to nymphs exposed to filter papers treated with SDW. The procedure was replicated 4 times for each treatment.

deltamethrin concentrations did not affect fungal germination (≥ 98%) after 24 h. Regarding fungal virulence at 10 days after conidial application, the LC₅₀ values ranged from 2.1 to 3.3 x 10⁵ conidia/ml (Table 1). No significant differences (P > 0.05; based on overlap of 95% C.I.) were observed between LC₅₀ values for nymphs exposed to the combinations of 0.5 or 5.0 ng/cm² of deltamethrin with *B. bassiana*, and *B. bassiana* alone. Survival time 50% varied from 5 to 7 days for the highest and lowest conidial concentration, respectively (Table 2). In all concentrations tested, no significant differences were observed between the distribution values for deltamethrin

Table 2. Estimate survival time 50% (days, C.I. 95%) of *Triatoma infestans* third instar nymphs exposed to sublethal concentrations of deltamethrin and treated with *Beauveria bassiana* conidia.

Deltamethrin (ng/cm ²)	Concentration of conidia/ml ¹ (95% C.I.)					
	3x10 ⁵	10 ⁶	3x10 ⁶	10 ⁷	3x10 ⁷	10 ⁸
0	7 (6-14)	6 (5-7)	5 (5-6)	5 (5-6)	5 (5-6)	5 (5-6)
0.5	7 (6-11)	6 (5-7)	5 (5-6)	5 (5-6)	5 (4-5)	5 (4-6)
5	7 (6-10)	6 (5-8)	5 (5-6)	5 (5-6)	5 (4-5)	5 (5-6)

¹Each value is the mean of 4 replicates (N = 560). There was no significant difference between values in a same column.

treated and untreated insects (Wilcoxon test, $P > 0.05$). Despite the lack of potentiation by sublethal concentrations of deltamethrin on the virulence of *B. bassiana* against *T. infestans*, the evaluation of the association with other chemical insecticide should be pursued. Otherwise, other isolates of *B. bassiana* should also be tested in association with deltamethrin.

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