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Effects of a formulation of *Bacillus thuringiensis* on the parasitoid *Trichogramma pretiosum* Riley,1879 (Hymenoptera: Trichogrammatidae) under laboratory conditions

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Introduction

Assessing the potential environmental effects of biopesticides is an important part of its development and registration process. In Brazil little attention has been given to this approach, but now with the new approved rules for biopesticides registration that issue will be increasingly considered (COSAVE, 1997).

Among the potential safety issues related to the use of microorganisms for pest control pathogenicity and toxicity effects on non-target beneficial organisms are the main concern (OECD, 1995; USEPA, 1989; Cook et al, 1996). In addition to that potential hazards there are those posed by microbial contaminants and by substances used in the formulation of biopesticides (e.g. stickers, carriers, spreaders, UV protectors) (Burges, 1981).

Bacillus thuringiensis var. *kurstaki* is a biopesticide widely applied to control lepidopteran pests (Navon, 1993). In Brazil it is specially applied in reforestation areas (Zanuncio, 1993), and there are little published information about its safety, and when it exists it is usually of restrict access .Lambert & Peferoen 1992 discussed about the use and mode of action of Bt in pest control, although almost nothing is known about its ecology and its role in nature.They showed the necessity of evaluating the relationship between the pathogens utilized in microbial pest control and their natural enemies; that will be another stage of the research in environmental impact assessment.

Trichogramma pretiosum, a beneficial hymenopteran used in large scale for controlling lepidopteran pests in Brazil, is easy to rear in laboratory (Parra, 1996 and 1997) and occurs naturally under field conditions in different crops. Therefore it is a good representative of the agriculturally important wasps family Trichogrammatidae that could be used as a biological indicator of potential hazards of pesticides.

This study refers to the evaluation of the effect of a formulation of *Bacillus thuringiensis* var. *kurstaki* (Btk) on the parasitism capacity and adult emergence of the nontarget Hymenoptera *T. pretiosum.* The main goal was to develop and validate protocols for evaluating adverse effects of biopesticides on nontarget egg parasite insects.

Material and Methods

The bioassays about parasitism capacity and adult emergence were developed at the „Costa Lima" Quarantine Laboratory - EMBRAPA/CNPMA, Jaguariúna, State of Sao Paulo. The assays were maintained in incubators at 25 ± 1°C, 60 ±10 % RH , and 14 h photophase.

Biopesticide: The commercial product used, Dipel® from Abbott Laboratórios do Brasil ıda, is based on *Bacillus thuringiensis* var. *kurstaki*, strain HD-1. It has 17600 IU/mg, and ,5 % w/w of inert content. About 2.6 x 10^9 viable spores/mL were detected at the mmercial product by pour plate counting in nutrient agar (Thompson & Stevenson, 1984). e recommended dose of Dipel® for field application is 500 mL/ha diluted in approx. 50 L. erefore the dosages selected for the bioassays were: the recommended dose (RD) rresponding to 2,6 x 10^7 CFU/mL and the inactivated recommended dose (IRD) rresponding to the RD autoclaved at 120°C for 20 minutes. Distilled water was used as the ntrol treatment (C).

Parasitoid: The strain of *T. pretiosum* used was T-Pr from the collection of *ichogramma* strains of the Insect Biology Laboratory at the Department of Entomology in ALQ/USP, Piracicaba, State of São Paulo. The maintenance and multiplication of *T. etiosum* in laboratory were accomplished in glass tubes (10,0 cm of height x 1,20 cm of ımeter) covered with hidrofilic cotton, using eggs of the alternative host *Anagasta ehniella* for *T. pretiosum* parasitization, according to methodology of Parra (1997). The ıerged adults were fed on pupe honey. All the rearing steps were developed into controlled ubators as mentioned above.

Parasitism capacity assays: Three bioassays were accomplished for the evaluation of the asitism capacity. Sterilized eggs of *A. kuehniella* were prepared dipping them for 30 onds in each of the Btk doses (RD, IRD) or in the control (C) separately. Sixty prepared gs were offered to each of the 30 *T. pretiosum* females, daily, until the females death. The asitized eggs were stored in glass tubes (6,5 cm length x 0,7 cm diameter) covered with 'C film in incubators already regulated, during 10 days. The number of blackened gs/female was registered daily as confirmation of parasitism. The average percentages of asitism in a cumulative period of 10 days were compared by the Tukey test at 5% of obability.

T. pretiosum adults emergence: For the parameter „emergence percentage", two bioassays re accomplished. Three hundred *T. pretiosum* eggs already parasitized (Parra, 1997) were ı in the Btk doses (RD and IRD), or in the control C separately. The averages of the daily ergence percentage were observed during a period of 10 days after the treatment, and ılyzed by the test t of Student.

sults and Discussion

rasitism capacity: under the conditions of this study, it was observed that the average of asitism /female was larger for the control (C) than for the RD and IRD treatments. There s not significant difference in the average of parasitism among the doses RD and IRD gures 1 and 2). It was observed in the three bioassays a high variability in the parasitism acity among the females (from 0 to 30 eggs parasitized / day). In a general way the number parasitized eggs decreased along the period (Figure 1). The higher number of parasitized gs/female could be observed 24-48 hours after the treatment, even in the control (C). In the and IRD there was an accentuated decline of the number of parasitized eggs/female after third day (almost zero) when compared to the control (C) (Figure 1). *Adults emergence:* the adult emergence percentage in the total observation period it was verified that the ntrol (C) differed statistically from RD and IRD treatments in the two bioassays and there s no significant differences between RD and IRD (Figure 3). For the control (C) there was a % average of emergence, while it was inferior to 20% for the RD and IRD treatments.

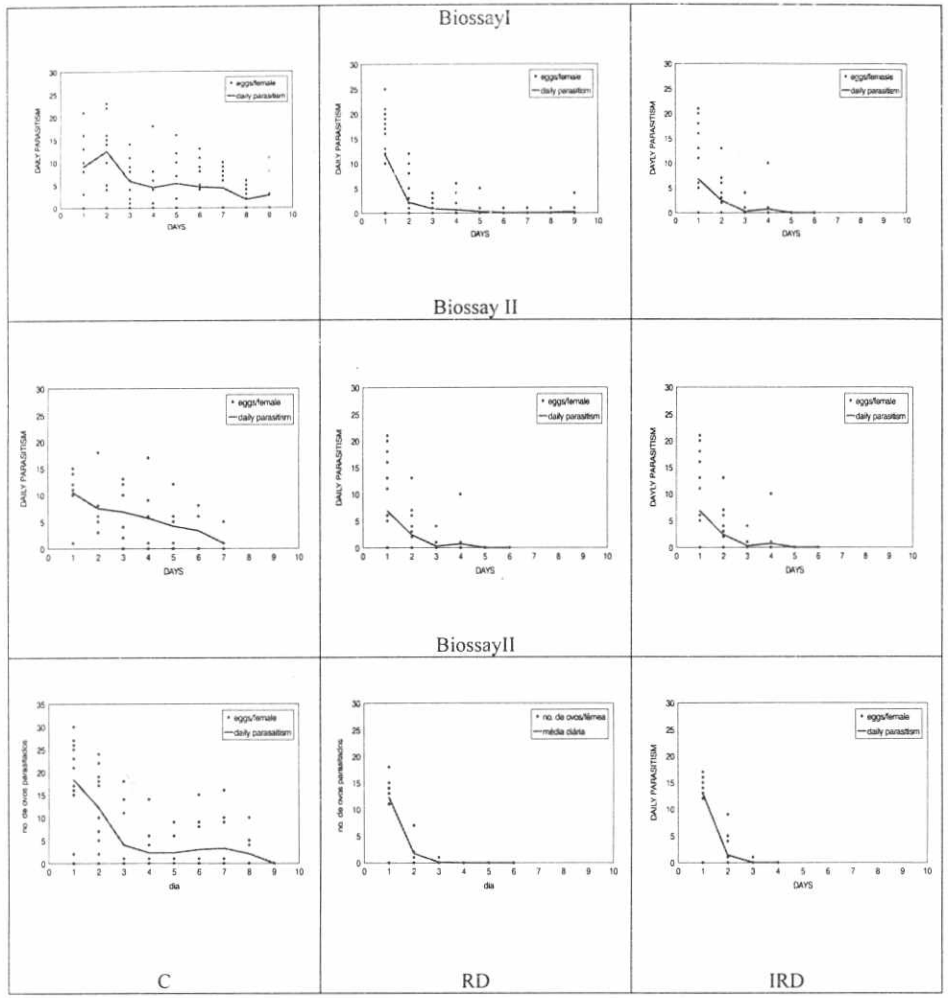
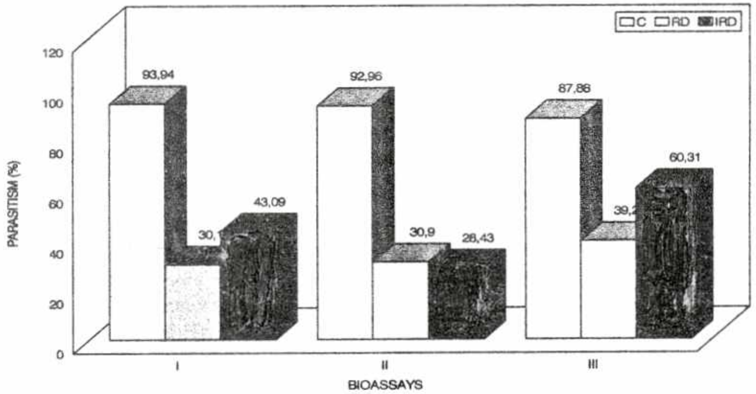


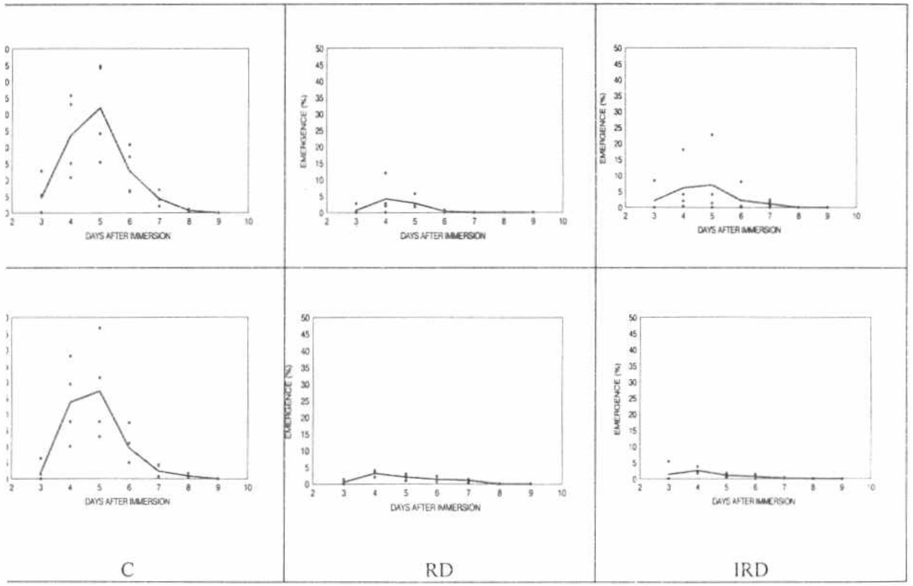
Fig. 1: Daily parasitism capacity of *T. preciosum* females maintained on *A. kuehniella* eggs emmersed in BtK doses (RD and IRD) and distilled water (C). Temperature of 25±1°C, 60% RH, and 14 h photophase.

One of the explanation of the results observed could be attributed to the ingredients of the Btk formulation that could have caused the formation of a physical barrier on the eggs, diminishing the parasitism capacity and the emergence of the parasitoid. Also it could be related to a subproducts generated during the inativation process (120°C) of the Btk suspension or to a toxin produced by Btk.

It not be attributed to the pathogenicity of Btk on the parasitoid that provoked the adverse effects, because IRD showed no significant difference in relation to dose RD (Figures 2 and 4). Effect of such components of a Btk formulation has been suggested by Havert (1982) to explain the effect of that biopesticide on *Hippodamia convergens* Quérin-Méneville and ysopa carnea Stephens. Alternatively, the observed adverse effect could be related to :eivable toxin produced by Btk as observed by Dulmage et al. (1981) in louse. Muck et al, 1 observed some effect of Btk on the parasitoids *Apanteles glomeratus* and *Pimpla* *onellae.*



2: Accumulated percentage of parasitism during a period of 10 days of *T. pretiosum* ntained on *A. kuehniella* eggs immersed in BtK doses (RD and IRD) and destilled water Temperature 25±1°C, 60±10% RH, and 14 h photophase.



; 3. Daily percentage of emergence of *T. pretiosum* adults mainned on " *kuehniella* eggs

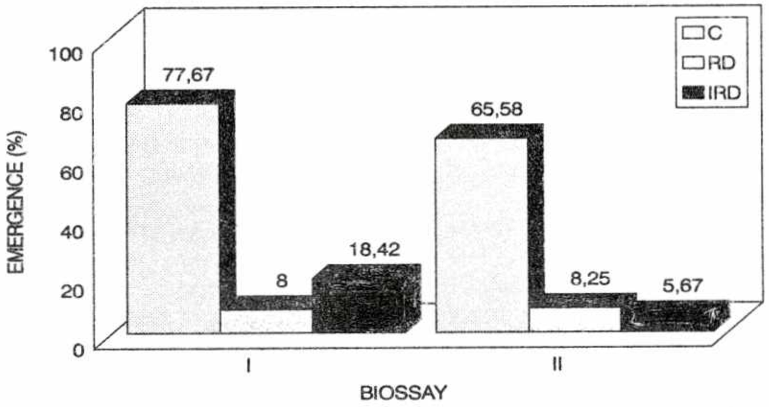


Fig. 4: Accumulated percentage of emergence during a period of 10 days of *T. pretiosum* maintained on *A. kuehniella* eggs immersed in BtK doses (RD and IRD) and destilled water (C). Temperature 25±1°C, 60±10% RH, and 14 h photophase.

In the world literature it is told in a general way that Btk in different formulations, dosagens and exhibition methods has not been causing adverse effects on Trichogramma spp. (Forsberg et al. 1976, Kapustina, 1977; Kostadinov, 1979; Legotai, 1980; Krieg et al.1980 and Rodrigues & Trumble, 1993). Exception were done to the papers of Franz et al. 1980 and Korostel & Kapustina (1975) that showed reduction in the production of eggs per female of T. *cocoeciae,* when adults of the parasitoid were treated by contact with Dipel® 54,7 x 10 9 spores g-1. Also Hassan & Krieg (1975) showed that the product Bactospeine® reduced the ability of the parasitism of T. *cocoeciae,* which was attributed to the presence of a β-toxin.. Those authors also verified that adults of *T. cocociae* were repelled when in contact with Btk in the formulations Bactospeine® and Dipel® in the choice tests .In Brazil, Marques (1993) working under the greenhouse condition, verified that Btk (Dipel formulation) affected the emergency of *T. pretiosum* of treated eggs, but did not influenced the ability of parasitism of the parasitoid, no reason was attributed to this effect.

Other works evaluating the effect of Btk on different beneficial insects didn't show occurrence of adverse effects (Kaya & Dunbar, 1972; Salama & Zaki, 1983; Wallner & Surgedner, 1974 and Campos & Gravena, 1984).

The results obtained in this research have a direct practical application in Brazil, where the appraised product has been used together with *T. pretiosum*, in the Northeast area for the control of *Tuta absoluta*, important pest of the tomato. Complemental studies evaluating only the components of the formulation can elucidate the obtained results better.

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