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Biological response of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* berliner var. *kurstaki* at sublethal concentrations

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ABSTRACT

The soybean caterpillar, Anticarsia gemmatalis Hubner (Lepidoptera: Noctuidae), damages this plant. This pest is controlled, mainly, with chemical products which makes it necessary to to develop management strategies for its management in the soybean crop. Feeding and development of A. gemmatalis larvae fed on soybean leaves treated with Bacillus thuringiensis Berliner var. kurstaki at different concentrations (0,5, 1, 2, 4, 6, 8 µg.mL^{-1}) for 24 h were studied in the laboratory. The LC_{50} and LC_{90} after 24 h from hatching and at the last instar, development period, adult emergence, weight gain by larva, pupa weight, and dry food weight ingested besides of A. gemmatalis were evaluated. The LC_{90} value decrease with the development of this insect. Mortality in the third instar of this pest was proportional to the B. thuringiensis concentrations. Emergence of A. gemmatalis adults was higher with 0,5 µg.mL^{-1} concentration of this bacterium and in the control.

Keywords: *biological control; biology; entomopathogen; soybean caterpillar*

Introduction

Soybean [*Glycine max* (L.) Merr.] has great social and economic value as one of the largest vegetable oil sources with low cost and high protein content for human and animal consumption [3]. *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidae), a key pest of the soybean crops in Brazil and other American countries, causes extreme defoliation with a caterpillar consuming about 110 cm^2 of soybean leaves, This makes necessary applications of chemical insecticides in areas with *A. gemmatalis* occurrence, but alternative methods are necessary to manage this pest. The biology of *A. gemmatalis* was studied in the field [7] and this this pest has, normally, five to six instars, with some individuals reaching eight instars [5].

Synthetic insecticides have ecological and toxicological risks, besides high costs, while alternative methods of pest control can be cheaper [14]. Microorganisms used in biological control programs of insect pests, especially from the eighties, reduced conventional insecticide use, lowered environmental contamination, and could increase cultivation revenues [10]. *Bacillus thuringiensis* (Bt), a soil bacteria that produces crystals (cry), has high toxicity to insect pests and is globally considered an effective biopesticide. The toxicity of Bt to insects is due to its proteins of molecular mass ranging from 25 kDa to 140 kDa [4].

The objectives are to study mortality, and development of immature and biological parameters of the soybean caterpillar with *B. thuringiensis* var. *kurstak* (Dipel WP®) at sub-lethal concentrations.

Material and Methods

Soybean caterpillars were obtained from mass rearing of the Laboratory of Biological Control of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais state, Brazil and maintained under laboratory conditions at 25,0 ± 4°C, relative humidity of 70 ± 4%, and photoperiod of 12 hours. Soybean plants were grown in plastic pots in a greenhouse of the UFV without pesticide use.

Fresh leaves of soybean were collected and transferred to laboratory. Disks (10,75 cm^2) from leaves of these plants were prepared one hour before the experiment.

The experiment was carried out in the Laboratory of Biological Control of the Institute of Applied Biotechnology (BIOAGRO) of the UFV. The *Bacillus thuringiensis* var. *kurstaki* (Dipel WP® 16,000 ITU.mg^{-1}) activity was studied with newly emerged *A. gemmatalis* caterpillars in plastic containers (500 mL) with artificial diet until the third instar, when they were individualized in Petri dish (9 cm diameter) for 24 hours without food. Five concentrations (0,5; 1; 2; 4; 6; 8 µg mL) of *B. thuringiensis* were tested, and the control had distilled water. Twenty disks (10,75 cm^2) of soybean leaves per *B. thuringiensis* concentration were dipped for ten seconds and left for thirty minutes to dry under laboratory conditions. After this period, the leaves were transferred to Petri dishes (9,0 x 1,5 cm), each with one *A. gemmatalis* larva. Four replications were used with one larva of this pest each one with soybean leaf disks treated with *B. thuringiensis* according to the treatment or in the control. After this, larvae were transferred to an artificial diet to complete their life cycle.

The LC_{50} and LC_{90} after 24 h and at last instar, development period (third instar to end of pupa period), larva weight gain, pupa weight, and dry weight of food ingested and feces were evaluated to obtain the *B. thuringiensis* effect on *A. gemmatalis* development and biology. The weight was obtained with an analytical precision scale (± 0,001 g) in milligrams.

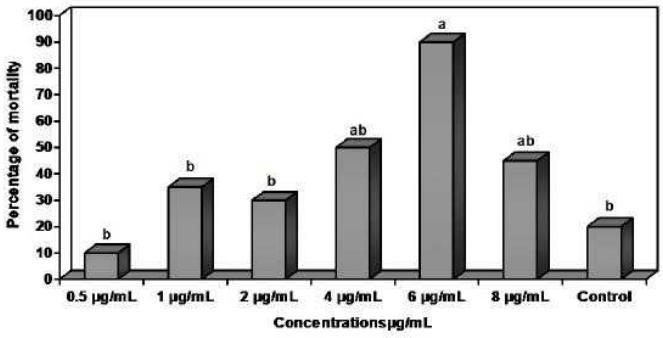
Mortality (%) of *A. gemmatalis* was corrected with Abbott's formula [1]. The effective *B. thuringiensis* concentration was analyzed using probit. Data from the biology experiment was subjected to variance analysis (ANOVA).

Differences between treatments were determined by Tukey's significant difference test (P ≤ 0,05).

Results and Discussion

The *B. thuringiensis* effects on third instar *A. gemmatalis* larvae showed that this insect is more susceptible at early than at later instars. The mortality of this pest was higher after 4 µg.mL^{-1} than with other concentrations lower (Figure 1). The a toxicity de LC_{90} of *B. thuringiensis* in after larva period was folding higher than the LC_{90} for the 24 h after treatment (Table 1).

Figure 1 Percentage of mortallity



Fonte: autor

Figure 1 Concentration–mortality responses of *Anticarsia gemmatalis* after 24 hours feeding on soybean leaf treated with *Bacillus thuringiensis* sub-lethal concentrations. Means followed by the same letter (s) do not differ at 5% according to Tukey test.

Table 1 Toxicity (LC50 and LC90) of Bacillus thuringiensis to 3rd larvae of Anticarsia gemmatalis fed on soybean leaf

	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Slope (±SE)	χ^2
After 24h of treatment	4.65	32.70	1.513 ± 0.19	99.486
After larva period	4.49	12.46	2.893 ± 0.25	157.112

Fonte: autores

The development of caterpillars neonates at end of pupa period (days), weight gain, pupa weight, and dry weight of food ingested and faeces on larva of *A. gemmatalis* was not modified after treatment with *B. thuringiensis* at all concentrations (Table 2).

Table 2 Development period (DP), last instar larvae weight (LW), pupa weight (PWeight), food dry weight ingested (DWeight) and faeces weight (DWFaeces) of *Anticarsia gemmatalis* with of sub-lethal concentrations of *Bacillus thuringiensis kurstaki* (Dipel WP®) in the laboratory

Parameters	*Bacillus thuringiensis* concentrations				
	0.5µg/mL	1µg/mL	2µg/mL	4µg/mL	Control
DP* (days)	28.94±0.70a	28.13±0.75a	25.5±0.00a	28.88±0.25a	25.88±0.25a
LWeight	275.7±12.39a	240.8±11.23a	226.7±6.03a	239.4±45.27a	258.8±29.73a
P weight	242.60± 0.60a	206.2±13.53a	204.0±18.23a	246.7±21.53a	203.8± 5.76a
DWeight	1.793±0.40a	1.837±0.11a	1.789±0.24a	1.476±0.10a	1.838±0.30a
DW Faeces (g)	0.235±0.002a	0.191±0.122a	0.194±0.131a	0.234±0.058a	0.155±0.001a

Means followed by the same letter (s), per row, do not differ at 5% probability according to Tukey test. Statistical analyzes was not made with 6 and 8 µg/mL concentrations due to their number of replications lower than three of larvae completing this stage. * From 3rd instar larvae at end of pupal period.

The survival of *A. gemmatalis* third instar caterpillars varied with the concentrations *B. thuringiensis* (0,5; 6 and 8 µg.mL^{-1}). The percentage of adult emergence of this pest decreased as the *B. thuringiensis* concentrations increased (Figure 2).

Figura 2 Percentage of adults emergence

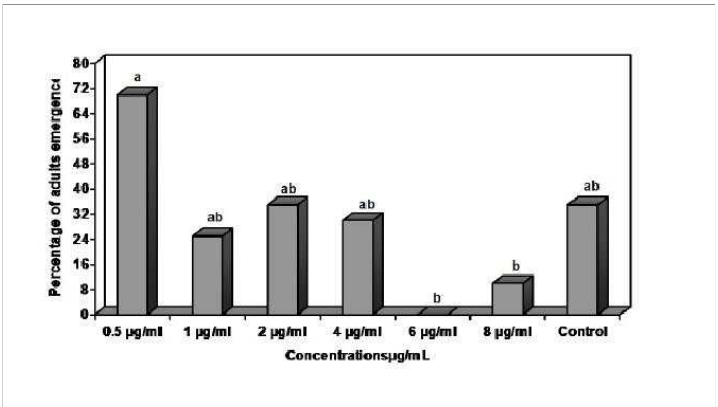


Figure 2 *Anticarsia gemmatalis* adult emergence (%) with different concentrations of *Bacillus thuringiensis*. Means followed by the same letter (s) do not differ at 5% according to Tukey test.

Fonte: autor

The Cry proteins of the bacterium *B. thuringiensis* kill Lepidoptera insect pests [6] with lower environmental impact than most chemical insecticides [8]. Differences in *A. gemmatalis* susceptibility to the *B. thuringiensis* concentrations may be due to the protein Cry 1Ba levels from the gene Cry1B [12].

The increasing mortality (%) of *A. gemmatalis* third instar larvae after 24 h, starting at the concentration of 4 $\mu g.mL^{-1}$ in a dose-dependent manner, will reduce adult emergence at higher *B. thuringiensis*. These results are similar to those of other studies with this bacteria on *Apis mellifera* Linnaeus (Hymenoptera: Apidae), *Ostrinia nubilalis* (Hiibner) (Lepidoptera: Crambidae, *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) and storage pests [7-9]. *Bacillus thuringiensis* crystals are solubilized and activated by proteases in the insect midgut. The δ-endotoxins (Cry toxins) bind to midgut epithelial cells; and form pores, causing cytoplasm vacuolization and increasing cellular volume cell lysis which eventually leads to insect death [10].

Lower values of the LC_{50} and LC_{90} for *A. gemmatalis* at the larva period end compared to those after 24 hours may be due to the action mode of *B. thuringiensis* needing 24 h to express its toxicity. Besides the impact of this bacterium reduces the energy (i.e., total protein, glycogen, and lipids) by lowering food digestion due to its toxic effect reducing digestive enzymes as a-amilase e proteases. Besides, *B. thuringiensis* toxins bind to cell receptors of the epithelial of the medium intestine resulting in its perforation [15].

The lack of impact on development period, last instar larvae, and pupa weight with *B. thuringiensis* concentrations lower than 4 $\mu g.mL^{-1}$ will allow the larva recovering after healing its medium intestine. This is due to low *B. thuringiensis* toxins binding to medium intestin receptors or to insecticide activation by insect proteases [2]. Similar effects were reported on larva and pre-pupa (days), pupa weight (g), sex ratio, survival (%) and larvae-adult period of *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera: Noctuidae) submitted to this protein Cry1Ac, even being specific to Lepidoptera [13]. The mortality of third instar *A. gemmatalis* was higher with *B. thuringiensis* at the concentration of 4 $\mu g.mL^{-1}$ or higher in a dose-dependent manner, without effect in the biological parameters studied.

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