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Susceptibility of *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) nymphs to *Bacillus* spp. strains

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Abstract. Soybean crops are subject to various phytophagous insects, among which the Neotropical brown stink bug *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) stands out. The biological control of this pest is one of the viable alternatives to reduce the use of chemical insecticides. This study aimed to evaluate the pathogenic potential of different *Bacillus* spp. strains in the mortality of nymphs of *E. heros* under laboratory conditions. Bioassays were done using the artificial feeding system methodology with Falcon tubes, and seventeen *Bacillus* spp. strains were utilized. As a result, one strain of *Bacillus thuringiensis* caused 100% mortality, and the lethal concentration (LC_{50}) was estimated: 8.42×10^4 spores/mL. In addition, two other strains of *B. thuringiensis* and one strain of *Bacillus cereus* caused mortality above 70%, and a *Bacillus velezensis* caused 55%.

Keywords: *Bacillus cereus*, *Bacillus thuringiensis*, bioassays, biological control.

Euschistus heros (Fabricius, 1798) (Hemiptera: Pentatomidae) is considered one of the main pests in the soybean crop. The damage is caused by the third instar nymphs to adults (Panizzi et al. 2012). These insects feed directly on the grains, harming seed yield and quality. Furthermore, constant attacks by these insects reduce the number of seeds and, to a lesser extent, interfere with the number of pods per plant and the number of seeds per soybean pod (Depieri & Panizzi 2011).

The chemical products available to combat *E. heros* in Brazil, just for the soy crop, include 69 registered products divided into different chemical groups (AGROFIT 2025), and the demand for products to control *E. heros* in the crop continues to increase. Thus, more studies are needed to find different and effective ways to control *E. heros*, contributing to the development of new commercial products that are based on biological agents, like fungi and bacteria.

The genus *Bacillus* is composed of Gram-positive, aerobic or facultatively anaerobic bacteria, with several species of agricultural and biotechnological interest, which can be used to promote plant growth and in the biological control of pests and phytopathogens (Monnerat et al. 2020). One of the main species in the genus is *Bacillus thuringiensis*, an entomopathogenic bacterium that has a high genetic variability and great potential to control insects. Due to its wide distribution capacity, *B. thuringiensis* is found in different environments and is responsible for the production of crystals formed by distinct proteins with insecticidal activity, providing specific treatment for crops without harming the health of animals, humans, and plants (Souza et al. 1999; González-Cabrera et al. 2011). Besides that, *B. thuringiensis* can be included as a suitable biopesticide for farmers to manage the control of some insect pests (Senthil-Nathan 2015).

The amount of research on the mortality activity of *B. thuringiensis* against insects of the order Lepidoptera, which are responsible for major losses in the soybean crop, is more extensive than the few studies available on the entomopathogenic action of this bacterium to control insects of the order Hemiptera (Schünemann et al. 2014). Although the mode of action of *B. thuringiensis* proteins against insects of the Hemiptera order is still unknown, the discovery of *B. thuringiensis* strains that are toxic to these insects is essential (Hayashida et al. 2018). To achieve sustainable management of these pests, studies are needed to improve the application processes of *B. thuringiensis* to control Hemiptera (Li et al. 2011; Schünemann et al. 2014). Furthermore, to control Hemiptera using *B. thuringiensis* strains, improvements in its processes are necessary, since the various studies available that assess the mechanism of action of *B. thuringiensis* toxins on Lepidoptera and insects of different orders have found peculiarities ranging from ingestion of the toxin to activation in the gut (Cristofoletti et al. 2003; Chougule & Bonning 2012; Li et al. 2011).

Besides that, few studies have evaluated the toxic activity of *Bacillus* spp. strains in relation to the mortality of insects of the order Hemiptera. The higher limitation to select strains that can cause mortality in these insects is the non-existence of a precise and trustful methodology. Thus, the objective of this study was to select *Bacillus* spp. strains that cause mortality in *E. heros* nymphs.

Individuals of *E. heros* were collected from soybean crops in Bahia, Brazil, and reared on a natural diet. Adults were placed in plastic pots (1 L) with the lids cut in the center and with organza-type fabrics fixed to the opening to allow ventilation. To maintain stink bugs breeding, a natural diet consisting of bean pods (*Phaseolus vulgaris*) and cotton moistened with distilled water was offered to serve as a source of water and humidity. Second instar nymphs were used in the present study.

Seventeen strains of *Bacillus* spp. were used in the selective mortality bioassays: six *B. thuringiensis*, three *Bacillus cereus*, three *Bacillus velezensis*, two *Bacillus safensis*, one *Bacillus tropicus*, one *Bacillus amyloliquefaciens*, and one *Bacillus licheniformis*. All strains were characterized and identified based on morphology and molecular phylogeny in previous unpublished studies and are deposited in the Microorganism Collection 01 of SoluBio Tecnologias Agrícolas (SisGen C288D50).

Bioassays were done using the artificial feeding system methodology described by Costa et al. (2022), with adaptations. A sterile Falcon tube (50 mL) containing seven nymphs was utilized as an experimental unit, with five repetitions for each treatment (Fig. 1). About 300 µL of autoclaved artificial diet (200 g of crushed dry pods,

60 g of sucrose, 40 g of crushed peanuts, 10 mL of vitamin solution, and 2 g of nipagin for 1 L of water) were placed over Parafilm® and then 100 µL of the treatments with the bacterium (concentration of 1.0 × 10^8 spores/mL). The control was only the artificial diet, without the bacterium. During seven days, the mortality of insects was observed and counted.

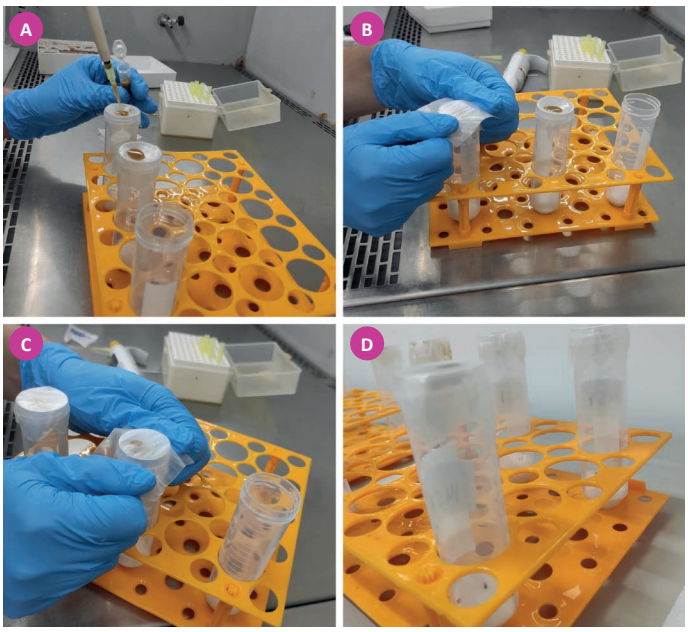

Figure 1. Methodology of mortality bioassays against *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) nymphs using *Bacillus* spp. strains in Falcon tubes. A) Bioassay setup using Falcon tubes and plastic wrap, with the addition of the solution containing diet and bacteria on the plastic; B) Procedure in which a second layer of plastic wrap is placed, forming a bacterial pouch where the nymphs will feed; C) Details of the procedure; D) Overview of the bioassay with Falcon tubes containing *E. heros* nymphs sealed with a double layer of plastic wrap and diet plus bacteria between the layers.

The data referring to the 17 treatments and the control, each with three replications in different times, were organized and analyzed using R software version 4.4.1 (R Core Team 2024). The data were transformed using the square root and the normality of the residuals from the linear model was assessed using the Shapiro–Wilk test (package "base stats"). The mortality data were subjected to one-way ANOVA followed by Tukey's to identify differences among the treatments, and *p-values* < 0.05 were considered statistically significant (package "agricolae").

The strain that showed 100% mortality in the selective bioassay was used to carry out bioassays and determine the lethal concentration (LC_{50}). To this, the bacteria were multiplied in SM medium until total sporulation and the number of spores was quantified. Then, serial dilutions of the spores were prepared and a bioassay with those dilutions was tested (10^{-1} to 10^{-10}). The method was described in Monnerat et al. (2020). The follow-up, exchange, and readings of these assays were done as described for the selective tests. LC_{50} bioassays were repeated twice at different times.

The LC_{50} value and the confidence interval were estimated with probit regression analysis, according to the method proposed by Finney, 1971. The analyses were conducted using the "drc" statistical package in the R software version 4.4.1 (R Core Team 2024).

As a result, four strains caused 70% or more of mortality in *E. heros* nymphs after seven days and differed statistically from the control group without bacteria (Shapiro-Wilk's normality test: *p-value* = 0.05628; ANOVA: F-value = 65.49; *p-value* < 2e-16; Coefficient of Variation = 14.7 %): three strains of *B. thuringiensis*, one of which caused 100% mortality in all three replicates, and one *B. cereus* (88.34%). *B. velezensis* SBB80 caused 55% and the strains of *B. amyloliquefaciens*, *B. licheniformis, B. tropicus,* and *B. safensis* used in this study did not cause significant mortality (below 50%).

Strain SBB 83 (*B. thuringiensis*) was selected for LC_{50} (lethal concentration 50%) calculation because it caused 100% mortality in *E. heros* nymphs in the selective bioassays. The spore concentration used in the assay was 4.0 × 10^7 spores/mL, and the LC_{50} obtained was 8.42 × 10^4 spores/mL (Confidence Interval: 2.21 × 10^4 – 1.463 × 10^5) (Fig. 2). Costa et al. (2022) demonstrated that *E. heros* is susceptible to different Cry toxins produced by *B. thuringiensis*, corroborating the data presented in this study.

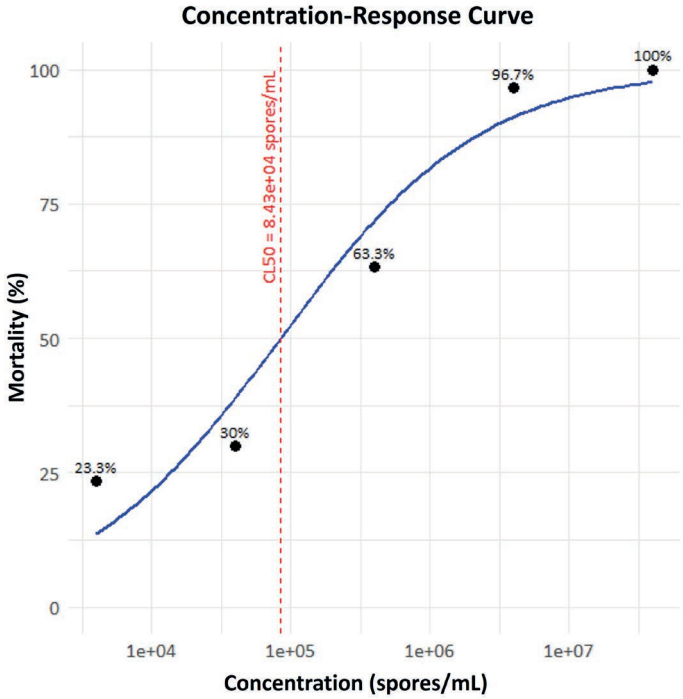

Figure 2. Concentration–response curve of *Bacillus thuringiensis* SBB 83 in mortality of *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) nymphs after seven days.

Table 1. Mortality of *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) nymphs with the use of *Bacillus* spp. strains after seven days.

Strain (SBB)	Species	Mortality (%)	
12	*Bacillus amyloliquefaciens*	13.33 ± 3.33	e
22	*Bacillus licheniformis*	21.67 ± 5.00	de
54	*Bacillus thuringiensis*	25.00 ± 1.66	de
80	*Bacillus velezensis*	55.00 ± 5.00	c
83	*Bacillus thuringiensis*	100.00 ± 0.00	a
84	*Bacillus velezensis*	31.63 ± 4.97	d
108	*Bacillus cereus*	88.34 ± 11.67	ab
109	*Bacillus thuringiensis*	70.00 ± 6.67	c
119	*Bacillus velezensis*	30.00 ± 6.67	de
133	*Bacillus cereus*	31.66 ± 5.00	d
134	*Bacillus tropicus*	25.00 ± 8.33	de
135	*Bacillus safensis*	21.67 ± 5.00	de
136	*Bacillus cereus*	23.33 ± 3.33	de
137	*Bacillus safensis*	18.33 ± 8.33	de
139	*Bacillus thuringiensis*	15.00 ± 5.00	de
142	*Bacillus thuringiensis*	71.67 ± 8.34	bc
143	*Bacillus thuringiensis*	63.33 ± 0.00	c
Control	----	13.33 ± 3.33	e
CV		**14.7%**	

Percentage of mortality values (means ± SE) followed by the same letter in the column do not differ statistically (Tukey's test $p < 0.05$).

Bacillus thuringiensis is a bacterial species widely recognized for its biocontrol potential against immature stages of several insect orders, particularly Lepidoptera, Coleoptera, and Diptera (Praça et al. 2004; Verma et al. 2024). Recent studies show that strains of this species also have the ability to control several other insect orders, including

sucking species from the order Hemiptera (Dorta et al. 2020; Costa et al. 2022). Strain SBB 83 was recently characterized, and its toxic effect was confirmed against immature stages of four Lepidoptera species (de Castro et al. 2025). The genes encoding the proteins Cry1Ab24, Cry1Bd2, Cry1Ea7, Cry1Ia37, Cry2Aa9, Cry2Ab41, and Spp1Aa1 were identified in the bacterial genome (Castro et al. 2025), and these may be involved, either individually or synergistically, in the mortality of *E. heros* nymphs. Based on the results of the present study, this strain shows strong potential for use in the control of various insect pest species from both Lepidoptera and, now, Hemiptera.

In addition to *B. thuringiensis*, this study reports, for the first time, the mortality caused by *B. cereus* and *B. velezensis* against insects of the order Hemiptera. *Bacillus cereus*, which is phylogenetically related to *B. thuringiensis* and *Bacillus tropicus* (Ehling-Schulz et al. 2018). *Bacillus velezensis* is a species that has gained increasing recognition for its strong antagonistic effect against phytopathogenic fungi and nematodes (Ye et al. 2018; Rabbee et al. 2019). However, some studies have shown that strains of *B. velezensis* can cause mortality against insects (Alahmed et al. 2024). Among the likely enzymes produced by this bacterium that may act against insects are proteases, chitinases, lipases, amylases, and cellulases (Ajuna et al. 2023). Therefore, although the species is primarily used for the control of phytopathogens, *B. velezensis* may also play a complementary or secondary role in insect control.

In conclusion, this study confirms the existence of *Bacillus* strains, especially *B. thuringiensis*, with the potential to control *E. heros* nymphs and highlights the need for further studies to validate their toxic effects under laboratory, greenhouse and field conditions, aiming at the development of a potential new biological product for commercial use.

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Authors' Contributions

MTC: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing; GCG: Investigation; BBRC: Investigation; GTR: Investigation; ADCFL: Investigation; SCLM: Formal analysis, Investigation; RGM: Project administration, Supervision.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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