Effectiveness of entomopathogenic fungi for the control of *Euschistus heros*

Vanessa da Silva Caixeta[1], Kaylaine Aparecida Gomes de Souza [1], Gustavo da Silva Leite [1], Flávio Gonçalves de Jesus [1] and André Cirilo de Sousa Almeida [1*]

[1] *Instituto Federal Goiano – Campus Urutaí. Rod. Geraldo Silva Nascimento, km 2,5, 75780-000, Urutaí, GO, Brazil*
* *Corresponding author. E-mail: andre.almeida@ifgoiano.edu.br*

ABSTRACT. The Neotropical brown stink bug, *Euschistus heros*, is considered one of the most important pests of soybean crops. Entomopathogenic fungi are a group of organisms that have attracted attention due to their potential in pest biological control. The aim of this study was to evaluate the control of *E. heros* using different species of entomopathogenic fungi and varying doses of the mycoinsecticide. Two experiments were conducted under laboratory conditions. In the first experiment, the fungi *Metarhizium anisopliae, Cordyceps javanica*, and *Beauveria bassiana* were used to evaluate total and confirmed mortality of *E. heros* in both nymphal and adult stages. A second experiment was conducted to assess adult *E. heros* mortality at different doses of *M. anisopliae* (6.15×10^6, 6.15×10^7, 6.15×10^8, and 6.15×10^9 conidia mL^{-1} + control treatment). Total and confirmed mortality data were analyzed using generalized linear models (GLMs), while concentration data were fitted to a GAMLSS model (Generalized Additive Model for Location, Scale, and Shape). Among the fungi studied, *M. anisopliae* caused the highest total mortality in nymphs. In adults, no significant differences were observed among the species. At different concentrations of *M. anisopliae*, the 6.15×10^8 and 6.15×10^9 conidia mL^{-1} treatments resulted in high total mortality and a shorter interval between mycoinsecticide application and insect death. The results indicate that *M. anisopliae* is a viable alternative for integrated pest management, showing promising potential for controlling *E. heros* under laboratory conditions. Further studies under field conditions are needed for practical validation.

Key words: biological control; bioinputs; *Glycine max*

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INTRODUCTION

Among the pests with the greatest economic importance in soybean [*Glycine max* (L.) Merr.] cultivation in Brazil, the Neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), stands out as the most significant species (Panizzi et al., 2012). *E. heros* is widely distributed in Argentina, Paraguay, and Uruguay. This species begins colonizing soybean plants during the vegetative stage and becomes particularly harmful in the reproductive stage, feeding on the pods. Its feeding can therefore directly affect yield and other grain quality parameters during pod development and grain filling. In addition to the direct damage caused by feeding, the insect's mouthparts can inject toxins that directly impact soybean seed germination (Panizzi et al., 2015; Chiaradia et al., 2016). *E. heros* can cause up to a 30% reduction in soybean yield and account for as much as 60% of all insecticides used in soybean cultivation (Panizzi et al., 2026).

Among the control methods for the Neotropical brown stink bug, chemical control is the most widely used (Ecco et al., 2020). The insecticides employed to manage *E. heros* in soybean primarily belong to three chemical groups (neonicotinoids, organophosphates, and pyrethroids) and can be used individually or in formulated mixtures (AGROFIT, 2026). The limited number of available insecticides, combined with frequent use of the same mode of action, can lead to the selection of resistant insects (Tibola et al., 2021). Furthermore, indiscriminate insecticide use may cause undesirable effects on the environment, human health, and non-target organisms (Torres & Bueno, 2018; Sosa-Gómez et al., 2020). Reducing the use of synthetic insecticides in agriculture has become a global goal. Therefore, the incorporation of multiple, efficient, and environmentally friendly control strategies is necessary. Biological control has emerged as a key strategy for insect management, representing a practical and environmentally safe approach (van Lenteren et al., 2018).

Entomopathogenic fungi are a diverse group of organisms that have attracted attention due to their potential in biological pest control. For stink bug management, entomopathogenic fungi have proven to be effective control agents, as they infect the host directly through contact with the insect cuticle. Furthermore, most entomopathogenic fungi can be easily produced on solid and liquid formulation and can cause epizootics (a large number of insects infected simultaneously), reducing insect populations (Boaventura & Quintela, 2025). Most biopesticides currently registered in Brazil use fungi as the active ingredient (Feria et al., 2022).

The fungal species *Metarhizium anisopliae* (Metsch.) Sorok., *Beauveria bassiana* (Bals.) Vuill., and *Cordyceps javanica* (Frieder. & Bally) are the most widely used entomopathogenic fungi for insect control, representing 69% of the Brazilian microbial insecticide market (Yamakawa, 2026). Recent studies have shown that entomopathogenic fungi can disrupt *E. heros* feeding, thereby reducing pod damage until the insect's death. According to Almeida et al. (2025), adult *E. heros* treated with *M. anisopliae* reduced feeding by 86%, with feeding completely ceasing by the fifth day compared to untreated insects. Oliveira et al. (2026), comparing the feeding behavior of *E. heros* treated with *M. anisopliae* and *C. javanica*, demonstrated that these fungi are capable of reducing both the frequency and duration of the insect's feeding events.

In addition to causing sublethal effects, entomopathogenic fungi have shown high efficiency in controlling *E. heros*. *M. anisopliae* (BRM 2335) *and B. bassiana* (BRM 14527) caused higher mortality of second-instar *E. heros* nymphs compared to *C. javanica* (BRM 27666), with the highest confirmed mortality observed in *M. anisopliae* (70.6%) (Sousa et al., 2023).

According to Catafesta et al. (2026), different isolates of *M. anisopliae* and *B. bassiana* may exhibit varying levels of *E. heros* mortality, and control efficiency can depend on both the insect's life stage and the fungal isolate. Their results showed that applying entomopathogenic fungi to immature insect stages optimizes the performance of the bioinsecticide in the field. Therefore, we conducted this study to evaluate the efficacy of different entomopathogenic fungal species and various mycoinsecticide doses for the control of *E. heros.*

MATERIAL AND METHODS

The experiment was conducted at the Integrated Pest Management Laboratory (LabMip) of the Instituto Federal Goiano - Campus Urutaí, between May and July 2024. For the experiment, commercial entomopathogenic fungi were obtained: *Cordyceps javanica* (Lalguard C99 WP®, isolate BRM 27666, Lallemand Soluções Biológicas*), Metarhizium anisopliae* (Opala WP®, isolate IBCB 425, Lallemand Soluções Biológicas), and *Beauveria bassiana* (Granada WP®, isolate IBCB 66, Lallemand Soluções Biológicas).

Euschistus heros rearing

Adult *E. heros* were obtained from the colony at Embrapa Rice and Beans, Santo Antônio de Goiás, GO, Brazil. The insects were kept in plastic boxes (25 × 20 × 20 cm) lined with filter paper. Fresh green bean pods (*Phaseolus vulgaris* L.), soybean seeds (*Glycine max* L.), and raw peanut seeds (*Arachis hypogaea* L.) were provided as food. The boxes were cleaned and food was replaced every two days. *Voile* fabric was used as an oviposition substrate. Eggs were collected and transferred to Gerbox boxes (11.0 × 11.0 × 3.0 cm) containing green bean pods and moistened filter paper. For first and secund instar nymphs, only green bean pods were provided as food. From the third instar onward, the diet provided to adults was supplied. Upon reaching the fifth instar, nymphs were transferred to plastic boxes to continue rearing. Insects were reared throughout their development and maintained under controlled conditions (25 ± 2 ºC, 60 ± 10% RH, 14 h photophase).

Evaluation of entomopathogenic fungi

Two experiments were conducted to evaluate the efficacy of entomopathogenic fungi in the control of *E. heros.* The first experiment was carried out on nymphs (third or foirth instar), and the second on adults (approximately 10 days post-emergence). Four treatments were used, corresponding to commercial doses: *Cordyceps javanica* (5×10^6 conidia mL^{-1}), *Metarhizium anisopliae* (6.15×10^8 conidia mL^{-1}), *Beauveria bassiana* (1×10^{10} conidia mL^{-1}), and a control (distilled water with 0.01% v/v Tween 80). Fungal suspension concentrations were determined using a hemocytometer (Brightline Improved Neubauer, New OptikR, São Paulo, SP, Brazil) at 400× magnification. Conidial germination was verified (>98%) on potato dextrose agar (PDA) after 18 h at 26 °C. The fungi were diluted in distilled water with 0.01% v/v Tween 80.

Insects were individualized in Petri dishes (60 mm), and 1 µL of treatment was applied to the dorsal surface of each nymph and 2 µL to each adult using a pipette (maintaining the same concentration for both). After application, insects were grouped in Gerbox containers, with 10 insects per container, containing green bean pods as a food source. The Gerbox containers were placed in a BOD incubator at 25 °C, with a 12-hour photophase and 70% RH. Live and dead insects were assessed daily for 12 days. To confirm mortality caused from fungal infection, insect cadavers were transferred to 60 mm Petri dishes containing a moistened cotton pad and maintained at room temperature. Insects were considered infected when mycelial or conidial growth was observed on the cadaver (mycosis). This assessment continued until the fifteenth day. The experiments were conducted using a completely randomized design with 30 replicates per treatment, considering each insect as an experimental unit.

Effects of different M. anisopliae concentrations on the control of E. heros

As *M. anisopliae* demonstrated greater pathogenicity and virulence in controlling *E. heros*, it was used in this second stage. Adult *E. heros* (approximately 10 days post-emergence) were employed, following the same procedures for fungal dilution, treatment application, and mortality assessments described above. Four concentrations of *M. anisopliae* were tested: 6.15×10^6, 6.15×10^7, 6.15×10^8, and 6.15×10^9 conidia mL^{-1}, and control treatment (distilled water with 0.01% v/v Tween 80). In total, five treatments were tested, with 30 replicates per treatment, arranged in a completely randomized design.

Statistical analysis

Total and confirmed mortality data were analyzed using generalized linear models (GLMs) with a binomial distribution, and pairwise comparisons of means were performed using Tukey's test at 5% significance. Concentration data were tabulated and fitted to a GAMLSS model (Generalized Additive Model for Location, Scale, and Shape; Rigby & Stasinopoulos, 2005) with a beta-binomial response for the counts of dead insects. A likelihood ratio test was used to evaluate the effect of the concentration factor at 5% significance. 95% confidence intervals were constructed and presented graphically to study the dose–response relationship and the effect of time. All analyses were performed using R software (R Core Team, 2022).

RESULTS AND DISCUSSION

Total mortality of *Euschistus heros* nymphs differed significantly among treatments (Figure 1). The Metarhizium anisopliae treatment was the most effective, causing 96.6% total and confirmed mortality. In contrast, treatments with *Cordyceps javanica* and *Beauveria bassiana* resulted in the lowest total and confirmed mortality (around 60%). All entomopathogenic fungi differed significantly from the control (0% total and confirmed mortality), with no mycosis observed in the control treatment (Figures 1 and 2).

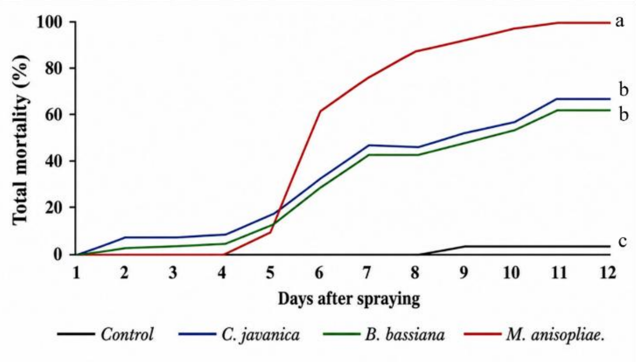

Figure 1. Total mortality of *Euschistus heros* nymphs treated with different entomopathogenic fungi. Means followed by the same letter do not differ significantly according to Tukey's test at 5% probability.

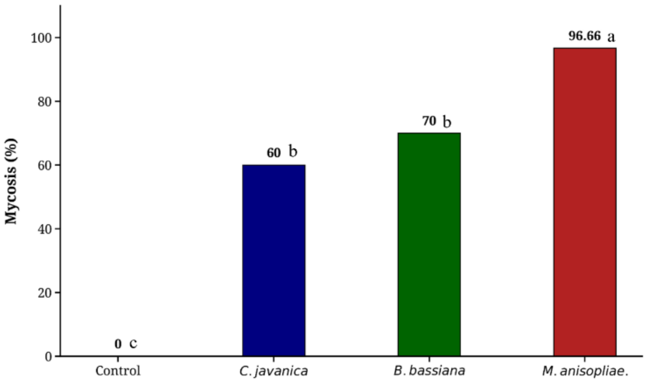

Figure 2. Confirmed mortality of *Euschistus heros* nymphs treated with different entomopathogenic fungi. Means followed by the same letter do not differ significantly according to Tukey's test at 5% probability.

Adult mortality did not differ significantly among treatments (Figure 3). Additionally, confirmed mortality was significantly higher in adult insects treated with fungi compared to the control (Figure 4).

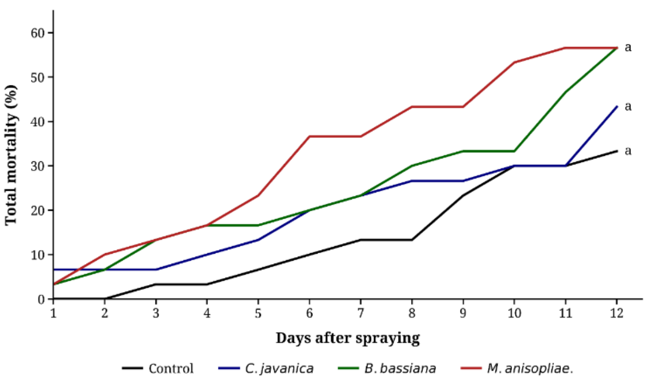

Figure 3. Mortality of adult *Euschistus heros* treated with different entomopathogenic fungi. Means followed by the same letter do not differ significantly according to Tukey's test at 5% probability.

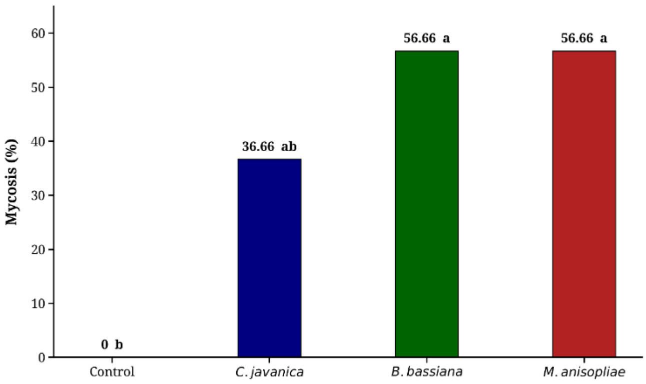

Figure 4. Confirmed mortality of adult *Euschistus heros* treated with different entomopathogenic fungi. Means followed by the same letter do not differ significantly according to Tukey's test at 5% probability.

Significant differences in mortality were observed among the different concentrations of *M. anisopliae* (Figure 5). The dose–response curve showed that the 6.15×10^6 conidia mL⁻¹ dose caused approximately 50% insect mortality, with mortality increasing as the dose increased, reaching over 90% at 6.15×10^9 conidia mL⁻¹.

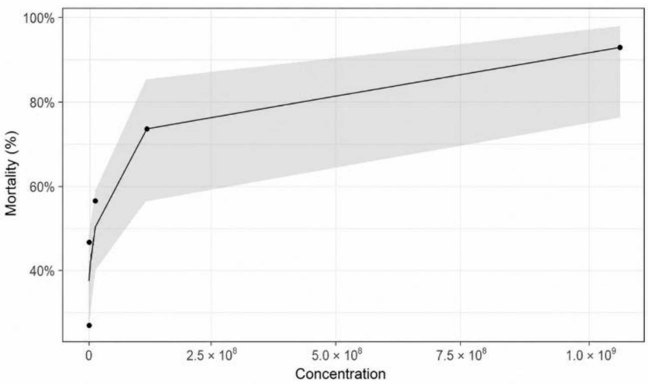

Figure 5. Mortality of adult *Euschistus heros* treated with different concentrations of *Metarhizium anisopliae*.

Significant differences in mortality over time were observed among the evaluated doses (Figure 6). From the seventh day after application, the 6.15×10^8 and 6.15×10^9 conidia mL⁻¹ doses reached 50% mortality, increasing to over 80% and 90%, respectively, by the twelfth day.

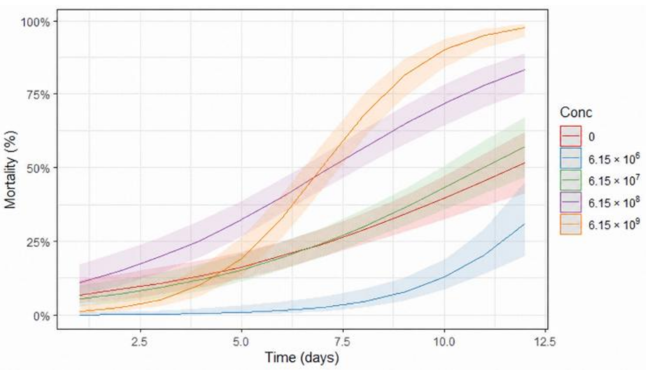

Figure 6. Percentage mortality of adult *Euschistus heros* over time when treated with different concentrations of *Metarhizium anisopliae*.

Among the stink bug species that damage soybean crops, *E. heros* is considered the most important species across all soybean-producing regions in Brazil, receiving the highest number of chemical insecticide applications. Despite the extensive area treated with chemical insecticides, control failures and low control efficiencies have been reported.

A portfolio of technologies can be employed for the control of stink bugs in soybean crops, including the use of parasitoids such as *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Platygastridae), with approximately ten registered commercial products available for brown stink bug management. The use of entomopathogenic fungi is a viable and compatible alternative that can be combined with other control methods, without affecting non-target organisms such as the parasitoids mentioned above.

This study evaluated the efficacy of different entomopathogenic fungal species in controlling *E. heros*. The results showed that *M. anisopliae* was more effective than *C. javanica* and *B. bassiana* during the nymphal stage. In the adult stage, no significant differences were observed among treatments. Similarly, Catafesta et al. (2026) reported higher control efficiency of *M. anisopliae* and *B. bassiana* in *E. heros* during the early developmental stages compared to adults. This greater efficacy of the mycoinsecticide during the insect's early developmental stages has also been observed in other pentatomid species, such as *Diceraeus melacanthus* (Dallas, 1851), *Nezara viridula* (Linnaeus, 1758), and *Tibraca limbativentris* Stål, 1860 (Silva et al., 2015; Portilla et al., 2022).

Producers frequently report difficulties or inconsistencies in pest control when using commercial or on-farm prepared mycoinsecticides (Faria et al., 2023). Defensive compounds produced by adult pentatomid stink bugs on the insect cuticle have been reported to exert fungistatic or fungicidal effects on conidia (Lopes et al., 2015). Immature and adult insects produce these compounds in different amounts, which may affect fungal propagules present on the insect cuticle, reducing susceptibility to the fungus (Pareja et al., 2007).

In addition to compounds produced on the insect cuticle, pentatomid resistance to entomopathogenic fungi can be explained by additional chemical defenses, in which insects release volatile compounds that act as defense or alarm pheromones (Moraes et al., 2008). These compounds are produced and stored in the dorsal abdominal glands (DAGs) of nymphs and in the metathoracic glands (MTGs) of adults (Aldrich, 1988).

The low susceptibility of adult insects to entomopathogenic fungi, along with the role of defensive compounds in preventing fungal propagules from penetrating the host cuticle, suggests that field applications should not target the adult stage. Entomopathogenic fungi can even have ovicidal effects on *E. heros* eggs. *M. anisopliae* was able to reduce nymph hatching rates to less than 3% by degrading the egg chorion through hyphal growth and penetration. During fungal development, lipids from the insect egg were observed to be directed into the hyphae, suggesting that these lipid reserves are used as a nutrient source (Silva et al., 2025).

This study demonstrated that increasing the conidial concentration of *M. anisopliae* increases mortality and reduces the time interval between application and death in adult *E. heros*. Similarly, Almeida et al. (2025) reported that higher doses of *M. anisopliae* led to increased *E. heros* mortality and a reduced time between application and insect death. Sublethal effects of the fungus were also observed, including reduced feeding activity and decreased damage to soybean seeds.

Euschistus heros treated with *M. anisopliae* showed a reduction in feeding activity of up to 86% by the fourth day after fungal application, and feeding ceased entirely by the fifth day compared to untreated insects. This reduction in feeding indicates that even when insects remain alive in the field, their feeding is impaired, consequently reducing crop damage (Almeida et al., 2025). These behavioral changes suggest that entomopathogenic fungi compromise the ability of *E. heros* to initiate and maintain effective feeding. One hypothesis for these observations is that fungal infection disrupts neuromuscular coordination, possibly through the production of mycotoxins such as destruxins (associated with *Metarhizium* spp.) or cordycepin (produced by *Cordyceps* spp.), which have been shown to interfere with insect physiology (Zhang et al., 2025; Oliveira et al., 2026).

CONCLUSION

The mycoinsecticide was more effective during the immature stage of the insect, and product dose influenced control efficiency. Variables such as pest developmental stage and product dose impact the field efficacy of entomopathogenic fungi. *Metarhizium anisopliae* represents a viable alternative for integrated pest management, showing promising results for the control of *E. heros* under laboratory conditions. Additional studies under field conditions are required for practical validation.

CONFLICT OF INTEREST

None to declare.

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