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Beauveria bassiana* and *Metarhizium anisopliae* in the control of *Euschistus heros

Abstract – The objective of this work was to investigate whether isolates of *Beauveria bassiana* and *Metarhizium anisopliae*, native from the state of Rio Grande do Sul, Brazil, exhibit entomopathogenic activity against nymphs and adults of the brown stink bug (*Euschistus heros*). The evaluated treatments were: distilled water (control), thiamethoxam + lambda-cyhalothrin, *Beauveria bassiana* PL 63, *Metarhizium anisopliae* strain E9, and native isolates of *B. bassiana* (B-2 and Bb-15) and *M. anisopliae* (1M, 7M, 9M, 11M, 12M, and 13M). *Metarhizium anisopliae* isolates (7M and 11M) were shown to be effective, standing out as entomopathogenic agents against nymphs and adults of the brown stink bug. The obtained results provide support for field trials and the incorporation of these isolates into integrated pest management strategies.

Index terms: biological control, brown stink bug, entomopathogenic fungi.

Beauveria bassiana* e *Metarhizium anisopliae* no controle de *Euschistus heros

Resumo – O objetivo deste trabalho foi investigar se isolados de *Beauveria bassiana* e *Metarhizium anisopliae*, nativos do estado do Rio Grande do Sul, Brasil, apresentam atividade entomopatogênica contra ninfas e adultos do percevejo-marrom (*Euschistus heros*). Os tratamentos avaliados foram: água destilada (controle), tiametoxam + lambda-cialotrina, *Beauveria bassiana* PL 63, estirpe E9 de *Metarhizium anisopliae* e isolados nativos de *B. bassiana* (B-2 e Bb-15) e *M. anisopliae* (1M, 7M, 9M, 11M, 12M e 13M). Isolados de *M. anisopliae* (7M e 11M) se mostraram eficazes, tendo-se destacado como agentes entomopatogênicos contra ninfas e adultos do percevejo-marrom. Os resultados obtidos fornecem suporte para ensaios de campo e para a incorporação desses isolados em estratégias de manejo integrado de pragas.

Termos para indexação: controle biológico, percevejo-marrom, fungos entomopatogênicos.

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The soybean [*Glycine max* (L.) Merrill] crop is attacked by numerous insect pests, which are responsible for reducing yield and increasing production costs (Bueno et al., 2026). In Brazil, Pentatomidae stink bugs are the most damaging to the soybean crop (Bueno et al., 2024). The brown stink bug [*Euschistus heros* (Fabricius, 1794)] stands out as the most prevalent species, whose adults and nymphs feed on developing seeds, causing injuries (Singh et al., 2023).

Given that the soybean crop has a substantial economic importance in many Brazilian regions, the effective control of the brown stink bug

is essential to ensure the continuity of agricultural production and economic sustainability in the country. To mitigate the negative effects caused by these pests, producers must adopt strategies such as integrated pest management (Möhring et al., 2025).

In integrated pest management, the use of biological inputs has been gradually expanding (Scott-Brown et al., 2025), mainly due to the negative impacts on agricultural ecosystems caused by many traditional insect control methods, such as the intensive use of chemical insecticides (Barathi et al., 2024). This trend is further attributed to the effective pest control of these inputs and to their lower environmental impact compared with that of chemical products, as well as to their specificity and selectivity, which help preserve the natural enemies present in the ecosystem (Galli et al., 2024). Cai & Dimopoulos (2025) added that, although bioinsecticides target insect pests, they derive from living organisms, such as fungi, bacteria, viruses, and protozoa, being regarded as a safer and more environmentally sustainable than chemical insecticides.

The use of biological products has also emerged as a promising strategy in the control of brown stink bug populations. However, there is still a lack of bioinsecticides formulated with native isolates, such as those from Southern Brazil, which may be adapted to the macroenvironment where they are to be applied. Examples include fungi from the genera *Beauveria bassiana* (Balsamo) Vuillemin, 1912 and *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883. Although the biocontrol of the brown stink bug through *B. bassiana* and *M. anisopliae* has been reported (Porto & Garcia, 2022), studies on the use of these native entomopathogens continue to be scarce. Therefore, to select microorganisms with a higher efficacy, it is important to search for native isolates from the target region that naturally exhibit entomopathogenic activity against the brown stink bug.

The objective of this work was to investigate whether isolates of *B. bassiana* and *M. anisopliae*, native from the state of Rio Grande do Sul, Brazil, exhibit entomopathogenic activity against nymphs and adults of the brown stink bug.

The insects infected with entomopathogens were collected in the municipality of Passo Fundo, in the state of Rio Grande do Sul, Brazil (28°15'41"S, 52°24'45"W). The insect pests, specifically the

soybean looper [*Rachiplusia nu* (Guenée, 1852)] and the brown stink bug, were collected in soybean fields. The fungal spores present on the surface of the insect pests were scraped off and transferred to Petri dishes containing potato dextrose agar medium. After the isolates had grown, they were subcultured. Based on colony morphology (Gebremariam et al., 2024), fungi from the genera *Metarhizium* and *Beauveria* were identified.

The experiment consisted of the following 12 treatments: control, distilled water; thiamethoxam + lambda-cyhalothrin (Engeo Pleno S, Syngenta Proteção de Cultivos Ltda., Paulínia, SP, Brazil), as a chemical control standard; *Beauveria bassiana* PL 63 (Boveril Plus, Koppert Brasil, Piracicaba, SP, Brazil); *Metarhizium anisopliae* strain E9 (Metarril WP E9, Koppert Brasil, Piracicaba, SP, Brazil); *B. bassiana* isolate B-2, collected from the brown stink bug; *B. bassiana* isolate Bb-15 standard, from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); *M. anisopliae* isolate 1M, the *M. anisopliae* Ma 557-1 standard from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); *M. anisopliae* isolate 7M, from the brown stink bug; *M. anisopliae* isolate 9M, from the soybean looper; *Metarhizium* isolate 11M, from the soybean looper; *M. anisopliae* isolate 12M, from the soybean looper; and *M. anisopliae* isolated 13M, also from the soybean looper.

For the bioassays, second-instar nymphs and adults of the brown stink bug were used, all sourced from a colony maintained at the Entomology Laboratory of Universidade de Passo Fundo, located in the state of Rio Grande do Sul, Brazil. The bioassays were conducted in a growth chamber, at 25°C, with a 12 hour photoperiod, humidity control, and a supply of green bean (*Phaseolus vulgaris* L.) as food for nymphs and adults. The treatments were arranged in a completely randomized design, with five replicates, i.e., five adults placed in each of the 60 used Petri dishes (140×15 mm), and five nymphs in each of the 60 plastic containers (120×50 mm).

For treatment applications, a spray suspension containing 1.0×10^6 entomopathogenic fungal spores per millimeter was used (Alfenas et al., 2016). In the evaluations of adults and nymphs, the number of dead insects was recorded on the first, third, fifth, seventh, tenth, and fourteenth day after infestation (DAI). The dead insects were transferred to a humid chamber to

confirm the mortality of the fungus. The obtained data were subjected to the analysis of variance, and the means of the treatments were compared by Tukey's test, at a 5% significance level.

Concerning the control of brown stink bug nymphs, differences among the biocontrol treatments became evident on the tenth and fourteenth DAI. On both days, in addition to the standard treatment with the commercial chemical product based on the active ingredients thiamethoxam + lambda-cyhalothrin, the use of the native *M. anisopliae* isolate 7M led to nymph mortality exceeding 54 and 61% of that observed for the control on the tenth and fourteenth DAI, respectively (Table 1). By the end of the experiment on the fourteenth DAI, the values obtained for *M. anisopliae* isolates 1M (63%), 9M (60%), and 11M (52%) and for *B. bassiana* PL 63 (52%) and B-2 (48%) did not differ significantly from that found for 7M. Similarly, Sousa et al. (2023) evaluated the efficacy of six entomopathogenic fungal isolates against the brown stink bug, concluding that the highest mortality of 70.6% in second instar nymphs was caused by *M. anisopliae*.

In the control of brown stink bug adults, the native isolate of *M. anisopliae* 11M achieved mortality rates of 81% on the fifth DAI and 70% on the seventh DAI,

higher than those of the control. At the tenth DAI, both biological insecticides and fungal isolates did not differ significantly from the chemical insecticide based on the thiamethoxam + lambda-cyhalothrin. The native isolate 11M was the earliest (at fifth DAI) not to differ statistically from the chemical insecticide, showing a faster efficacy than the other isolates that required more time to reach levels comparable to those of the control (Table 2).

According to the literature, fungi of the genus *Metarhizium* act through hydrophobic interactions between their spores and the cuticle of the host insect, enabling adhesion under favorable environmental conditions (Bihal et al., 2023). After germination and cuticle penetration, the fungus rapidly colonizes internal tissues, causing paralysis and insect death (Boaventura & Quintela, 2025). In the present study, mycelial growth and sporulation were registered on the brown stink bug during post-mortem observations, and the *M. anisopliae* 11M isolate produced characteristic green-colored spores on the insect's body.

The obtained results are an indicative that entomopathogenic fungi can serve as valuable allies for soybean producers, provided all necessary precautions are taken to ensure the effectiveness of these biologicals

Table 1. Mortality of brown stink bug (*Euschistus heros*) nymphs at different days after infestation (DAI) following spraying with entomopathogenic fungi⁽¹⁾.

Treatment ⁽²⁾	Mortality (%)					
	1 DAI	3 DAI	5 DAI	7 DAI	10 DAI	14 DAI
Control	0.0b	4.0b	12.0b	20.0b	24.0b	28.0bc
Standard treatment	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
PL 63	12.0b	20.0b	24.0b	32.0b	40.0b	52.0abc
Strain E9	0.0b	4.0b	4.0b	20.0b	28.0b	40.0bc
B-2	0.0b	4.0b	12.0b	24.0b	28.0b	48.0abc
Bb-15	0.0b	0.0b	9.0b	9.0b	14.0b	18.0c
1M	4.0b	17.0 b	17.0b	29.0b	41.0b	63.0abc
7M	4.0b	20.0 b	20.0b	32.0b	52.0ab	72.0ab
9M	8.0b	12.0 b	16.0b	28.0b	40.0b	60.0abc
11M	4.0b	16.0 b	24.0b	28.0b	44.0b	52.0abc
12M	0.0b	0.0b	8.0b	8.0b	16.0b	20.0bc
13M	0.0b	0.0b	8.0b	12.0b	20.0b	40.0bc
Coefficient of variation (%)	68.43	73.24	72.81	72.05	66.36	50.81

⁽¹⁾Means followed by equal letters, in the same column, do not differ from each other by Tukey's test ($p \leq 0.05$). ⁽²⁾Control, distilled water; Standard treatment, thiamethoxam + lambda-cyhalothrin; B-2, *B. bassiana* isolate B-2 from the brown stink bug; Bb-15, *B. bassiana* isolate Bb-15 standard from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); 1M, *M. anisopliae* isolate Ma 557-1 from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); 7M, *M. anisopliae* isolate 7M from the brown stink bug; 9M, *M. anisopliae* isolate 9M from the soybean looper (*Rachiplusia nu*); 11M, *M. anisopliae* native isolate 11M from the soybean looper; 12M, *M. anisopliae* isolate 12M from the soybean looper; and 13M, *M. anisopliae* isolate 13M from the soybean looper. ⁽²⁾CV, coefficient of variation.

Table 2. Mortality of brown stink bug (*Euschistus heros*) adults at different days after infestation (DAI) following spraying with entomopathogenic fungi⁽¹⁾.

Treatment ⁽²⁾	Mortality (%)				
	1 DAI	3 DAI	5 DAI	7 DAI	10 DAI
Control	0.0b	4.0b	12.0c	24.0b	64.0b
Standard treatment	100.0a	100.0a	100.0a	100.0a	100.0a
<i>Beauveria bassiana</i> PL 63	8.0b	18.0b	26.0bc	71.0ab	96.0ab
<i>Metarhizium anisopliae</i> strain E9	0.0b	12.0b	28.0bc	56.0ab	96.0ab
B-2	0.0b	4.0b	24.0bc	56.0ab	84.0ab
Bb-15	4.0b	8.0b	24.0bc	56.0ab	80.0ab
1M	8.0b	24.0b	48.0bc	64.0ab	84.0ab
7M	0.0b	12.0b	40.0bc	64.0ab	88.0ab
9M	4.0b	16.0b	40.0bc	68.0ab	88.0ab
11M	4.0b	32.0b	64.0ab	80.0a	96.0ab
12M	0.0b	8.0b	24.0bc	56.0ab	96.0ab
13M	0.0b	4.0b	48.0bc	76.0ab	84.0ab
Coefficient of variation (%)	59.29	64.34	54.14	38.82	18.21

⁽¹⁾Means followed by equal letters, in the same column, do not differ from each other by Tukey's test ($p \leq 0.05$). ⁽²⁾Control, distilled water; standard treatment, thiamethoxam + lambda-cyhalothrin; B-2, *B. bassiana* isolate B-2 from the brown stink bug; Bb-15, *B. bassiana* isolate Bb-15 standard from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); 1M, *M. anisopliae* isolate Ma 557-1 from Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF, Brazil); 7M, *M. anisopliae* isolate 7M from the brown stink bug; 9M, *M. anisopliae* isolate 9M from the soybean looper (*Rachiplusia nu*); 11M, *M. anisopliae* isolate 11M from the soybean looper; 12M, *M. anisopliae* isolate 12M from the soybean looper; and 13M, *M. anisopliae* isolate 13M from the soybean looper.

(Bueno et al., 2024), especially those produced on-farm. Due to their slower action, these fungi are particularly suited for an extended pest control. However, field trials are essential to comprehensively evaluate the efficacy of entomopathogenic fungi under such conditions, in order to validate their effectiveness and adapt their use to the needs of soybean growers, contributing to more sustainable and long-term agricultural practices (Lugendo et al., 2025).

Metarhizium anisopliae isolates 11M and 7M are effective in controlling adults and nymphs of the brown stink bug under laboratory conditions, respectively. This result contributes to the scientific advancement in sustainable pest management by proving the effectiveness of native entomopathogenic fungi against one of the main pests of soybean crops in Brazil. The use of native fungal isolates, adapted to local environmental conditions, favors the viability of the production and application of bioinsecticides. Therefore, the findings of the present work provide valuable support for future field trials and for the

potential incorporation of these isolates into integrated pest management strategies aimed at reducing the use of synthetic insecticides.

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