Entomopathogenic fungi controlling *Oebalus poecilus* (Hemiptera: Pentatomidae) in rice crop

Maria Vitoria Souza Rocha [1], Lenize Correia de Sousa [1], Márcio da Silva Araújo [2], José Alexandre de Freitas Barrigossi [3], André Cirilo de Sousa Almeida [1] and Flávio Gonçalves de Jesus [1]

[1] *Instituto Federal Goiano - Campus Urutaí, CEP 75790-000, Urutaí, GO, Brasil*
[2] *Universidade Estadual de Goiás, UnU Ipameri, CEP 75780-000, Ipameri, GO, Brasil*
[3] *Embrapa Arroz e Feijão, CEP 75375-000, Santo Antônio de Goiás, GO, Brasil*

* *Corresponding author. E-mail: flavio.jesus@ifgoiano.edu.br*

ABSTRACT. The rice stink bug *Oebalus poecilus* (Dallas 1851) (Hemiptera: Pentatomidae) is one of the most important pests of rice crops. Adults and nymphs feed on spikelets and panicle branches, causing quantitative and qualitative damage to the plant's grains. Entomopathogenic fungi have been studied as agent of biological control of several agricultural insect pests. The objective of this study was to evaluate three entomopathogenic fungi in the control of *O. poecilus* on rice plants. Rice seeds (BRS Esmeralda) were sown in a 0.3 L plastic pot containing soil, sand, and cattle manure (3:1:1). After emergence, the plants were kept in a greenhouse under natural conditions and irrigated daily. *O. poecilus* adults received a topical application of a suspension of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Cordyceps javanica* and 0.01% v/v of Tween 80. In the control, only Tween 80 and water were applied in the dorsum of the insects. After insects treatments, the adults continued feeding on the rice plants until died. The experiments were conducted in a completely randomized design with four replicates (10 insects/replicate). Significant differences in virulence and mortality were observed among the fungi tested. The entomopathogenic fungi were able to infect and kill *O. poecilus* adults on rice plants. *Beauveria bassiana* demonstrated greater virulence and higher mortality after 6 days of application. Furthermore, negative impacts on the development of insects treated with the fungi *B. bassiana*, *C. javanica*, and *M. anisopliae* were observed. Rice plants treated with *B. bassiana* showed lower percentages of damaged grains. Entomopathogenic fungi show promise in controlling rice stink bugs and require further study to determine more effective doses and different developmental stages of the pest.

Key words: Small rice stink bug, entomophatogenic fungi, biological control

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INTRODUCTION

Rice (*Oryza sativa* L.) it is one of the most important cereals for global food security, with Brazil being the largest producer and consumer outside of Asia, with a production of approximately 11 million tons (Barrigosse & Martins, 2015). The rice stalk stink bug *Oebalus poecilus* (Dallas 1851) (Hemiptera, Pentatomidae) is a key pest in South America (Lima et al, 2024). They are found in all regions of Brazil, causing economic damage due to their feeding activity, where nymphs from the second instar onwards and adults feed on the aboveground part of the plants, causing damage during the milky grain stage (Neto et al, 2020). Nymphs and adults also feed on the spikelets and on the primary and secondary branches of the panicle of rice plants (Chaves et al, 2001). Due to the damage caused by this insect, monitoring crop fields starting from the panicle emergence stage is important (Barrigossi & Martins, 2015).

Integrated Pest Management (IPM) seeks alternatives for reducing insect-pest populations in agricultural crops, and reducing the use of synthetic chemical products in agriculture becomes a global goal (Almeida et al., 2025). However, the main control method for this pest is still dependent on the use of synthetic chemical insecticides, which are highly harmful to the environment, along with a growing risk of resistance selection to these insecticides (Resquín-Romero et al., 2020; Vieira et al., 2022).

Biological control is a pest control method compatible with IPM (Integrated Pest Management) practices that does not cause harm to the environment, leaves no residues in food, and exhibits selectivity toward natural enemies. Entomopathogenic fungi have been proven to be biological control agents for stink

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bugs across various agricultural crops. These fungi can directly infect the host through contact via the tegument, causing its death due to the production of toxins following insect colonization (Boaventura & Quintela, 2025).

Brazil has the largest microbial control program in the world, with the fungi *M. anisopliae* and *B. bassiana* being the most widely used for insect pest control (Mascarin et al., 2019; Almeida et al., 2025). These fungi have a broad spectrum of activity and application, capable of infecting a wide variety of arthropod pest species, serving as an alternative to chemical control (Boaventura & Quintela, 2025).

Entomopathogenic fungi have shown promise in controlling stink bugs in important agricultural crops: *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) showed efficiency in controlling the stink bugs *Euschistus heros*, *Oebalus poecilus*, *O. ypsilongriseus*, and *Thyanta perditor* (Hemiptera: Pentatomidae), causing mortality ranging from 75 to 97.5%. *Beauveria* sp. (BRM 67744) caused 75% mortality, and *C. javanica* (BRM 27666 and BRM 14526) also caused mortality ranging from 17.5 to 57.3% in *O. poecilus* (Sousa et al., 2023). The objective of this study was to evaluate three entomopathogenic fungi in the control of *O. poecilus* on rice plants.

MATERIAL AND METHODS

Insects rearing

Adults of *O. poecilus* obtained from the Entomology Laboratory of Embrapa Arroz e Feijão were used. The insects were transported to the Instituto Federal Goiano, Campus Urutaí, in containers containing rice panicles. To initiate insect rearing on rice plants, *O. poecilus* adults were kept in cages, where they were fed on rice plants to promote oviposition. The produced eggs were collected and transferred to Gerbox plastic containers (11×11×3,5 cm), lined with moistened paper towels. These containers were maintained in the laboratory under controlled conditions of temperature (25±2°C), relative humidity (70±10%), and photoperiod (14 h) until the eggs hatched. The nymphs remained under these same conditions until reaching the second instar, at which point they were transferred back to rice plants of the cultivar BRS Esmeralda, where they developed into the adult stage, completing the oviposition cycle."

Experimental setup

To obtain plants, seeds of rice cultivar BRS Esmeralda were sown in 3.0 L pots containing substrate (soil, sand, and cattle manure - 3:1:1). The plants received the recommended fertilization for rice crops (Sousa & Lobato, 2004) and were irrigated periodically according to water demand. The pots remained in a greenhouse (under natural conditions) to protect the plants against pest attacks, as no insecticides were applied. The experiment was conducted during the R5 and R6 phenological stages of the rice plants.

Rice plants were infested fifty days after emergence. For infestation, ten treated and untreated stink bugs were allocated per pot, using four pots per treatment. The insects were handled with the aid of a fine paintbrush and the plants were covered with a voile fabric cage, remaining in the greenhouse. Commercial products provided by Lallemand Plant Care Brazil (Patos de Minas, MG, Brazil) were used: *Cordyceps javanica* (Lalguard Java), *Metarhizium anisopliae*, and *Beauveria bassiana*. Each product constituted a treatment, at the recommended dosages per concentration (conidia mL^{-1}), as follows: *C. javanica* (5×10^6 = 0,65g/L), *M. anisopliae* ($6{,}15 \times 10^8$ = 8,13g/L) and *B. bassiana* (10g/L). The products were diluted in a glass container containing 1 L of water and 100 µL/L of Tween 80 solution. Tween 80, or polysorbate 80 ($C_{64}H_{124}O_{26}$), is a non-ionic surfactant used to emulsify oil in water. The mixture was stirred until completely dissolved, without lump formation. The stink bugs were individualized in Petri dishes, and then, using a micropipette, 2 µL of the solution was applied to the dorsum of the insects. Dead stink bugs were isolated in Petri dishes and kept in a climate controlled chamber (BOD) to confirm fungal induced mortality by observing sporulation in a humid chamber. The experiment was concluded 20 days after insect infestation, and the evaluated variables were: insect survival, body size (mm^2), total body mass, and insect dry mass (mg).

Total body mass was determined by weighing the stink bug on an analytical balance. Subsequently, the insects were placed in an oven at 60 °C for 24 hours to obtain dry mass, being weighed again after this period. The measurement of total body surface area was performed using a digital caliper by measuring the length and width of the insects. Grain damage on rice plants was evaluated at the end of the experiment, for each treatment with and without application of entomopathogenic fungi. The evaluated variables were: number of grains per plant, grain weight, and the percentage of healthy and damaged grains.

Statistical analysis

Residual normality and homoscedasticity were verified using by the Shapiro-Wilk and Bartlett tests. The data were then used to fit the analysis of variance models and the means were compared by the Tukey test ($\alpha = 0.05$) (R Core Team 2026 – Agricolae package).

RESULTS AND DISCUSSION

Confirmed mortality of *O. poecilus* was evaluated six and 12 days after treatment application (Table 1). In the first evaluation ($F = 3.35$, $P = 0.0250$), it was observed that *B. bassiana* provided the highest mortality rates, although without differing from *Cordyceps javanica* and

M. anisopliae. In the second evaluation (F = 8.14, P < 0.0001), the fungi differed statistically from the control treatment, causing confirmed mortality ranging from 25 to 45%, respectively.

Table 1: Mortality rate (±SE) of *Oebalus poecilus* (Hemiptera: Pentatomidae) after treatment with *Beauveria bassiana*, *Cordyceps javanica* and *Metarhizium anisopliae*. Urutai. GO. Brazil.

Treatments[1]	Mortality – 6 days[1]	Mortality – 12 days[1]
Beauveria bassiana	20.0±2.5 a	45.0± 3.3 a
Cordyceps javanica	15.0±1.5 ab	30.0± 2.0 a
Metarhizium anisopliae	15.0±1.5 ab	25.0±1.7 a
Control	-[2]	-[2]
F treatment	3.35	8.14
p-value	0.0250	0.00001

[1]Means followed by the same letter in the column do not differ statistically from each other according to the Tukey test at 0.05% probability. [2]Data were not included in the statistical analysis due to zero variance.

Beauveria bassiana presented a higher mortality rate compared with *C. javanica* and *M. anisopliae*, respectively. *B. bassiana* is a fungus widely recognized for its virulence against a broad spectrum of insect pests, including the rice stalk stink bug. Its potential as a biological control agent reinforces its relevance in integrated pest management in rice crops. Santos et al. (2002) demonstrated the pathogenicity of *B. bassiana* infecting *O. poecilus* under natural conditions. In laboratory, these authors observed *O. poecilus* mortality exceeding 84% at a conidial concentration of 1.25×10^9. *M. anisopliae* (BRM 2335) and *Beauveria* sp. (BRM 67744 at 1×10^8 conidia.mL^{-1}) were more virulent to *O. poecilus* adults, causing confirmed mortality of 78.6% and 52.5%, respectively (Sousa et al., 2023).

These entomopathogenic fungi have a broad spectrum of action, being capable of colonizing various insect species and causing epizootics under natural conditions. They colonize insects primarily through the cuticle, and once inside the host body, they multiply rapidly, with death caused by tissue destruction and produced toxins (Boaventura & Quintela, 2025)

Cordyceps javanica and *M. anisopliae* resulted in *O. poecilus* insects with the lowest total body mass, although not differing from insects treated with *B. bassiana* (Table 2). Insects treated with *B. bassiana*, *M. anisopliae*, and *C. javanica* presented the lowest body dry mass when compared to the control treatment. Meanwhile, insects treated with *C. javanica* and *B. bassiana* exhibited the lowest total body surface area, though without differing from *M. anisopliae*.

The insect tegument is an effective physico-chemical barrier against entomopathogenic agents. Its cuticle, composed of chitin, proteins, polyphenols, water, and lipids, can be degraded by proteases and chitinases, which are enzymes synthesized by fungi. This degradation occurs through hydrophobic interaction and causes alterations in the insect integument, allowing the fungus to obtain nourishment and enhance its germination capacity through the assimilation of these substances (Silva, 2023).

Table 2: Average of body mass (ABM - g), dry mass (DM - g) and body surface area (BS - mm^2) of *Oebalus poecilus* (Hemiptera: Pentatomidae) adults after treatment with *Beauveria bassiana*, *Cordyceps javanica* and *Metarhizium anisopliae*. Urutai. GO. Brazil.

Treatments	ABM	DM	BS
Beauveria bassiana	0.021 0.026 ab	0.006 0.0027 b	0.483 0.064 a
Cordyceps javanica	0.012 0.003 b	0.008 0.0019 b	0.360 0.037 b
Metarhizium anisopliae	0.011 0.002 b	0.006 0.0025 b	0.380 0.020 ab
Control	0.025 0.004 a	0.011 0.002 a	0.483 0.217a
F treatment	4.08	20.14	20.14
p-value	0.0124	< 0.0005	< 0.0005

[1]Means followed by the same letter in the column do not differ statistically from each other according to the Tukey test at 0.05% probability.

These entomopathogenic fungi synthesize metabolites that are toxic to insects. Depsipeptides are among the most thoroughly studied: destruxin is produced by *M. anisopliae* and beauvericin by *B. bassiana*. These toxins exert a wide range of biological effects on insects, such as inducing cell membrane depolarization through the opening of calcium channels, leading to tetanic paralysis and insect death. Furthermore, they cause morphological alterations in the cytoskeleton and plasmatocytes of insects, compromising immune responses such as encapsulation and phagocytosis. They also induce structural changes in epithelial cells, triggering membrane disruption and oxidative stress. Finally, they inhibit the fluid transfer rate in Malpighian tubules, compromising insect homeostasis (Mora, 2016).

These characteristics explain why the insects exhibited changes in their body development. The production and release of secondary metabolites by entomopathogenic fungi likely interfere with metabolic processes and the normal development of hosts. These toxins directly impact energy balance, digestion, and nutrient absorption, compromising growth and energy accumulation. Furthermore, secondary metabolites also interfere with the hormonal regulation of insects. This can destabilize essential endocrine processes, such as molting and reproduction, which also contributes to alterations in body mass and development (Alves, 1998).

Rice plants treated with *C. javanica* and *M. anisopliae* showed the highest number of grains per panicle (Table 3). Furthermore, plants treated with *B. bassiana* presented higher grain mass values, whereas the percentage of grains damaged by *O. poecilus* was higher in the control treatment. Subramanian (2025)

observed higher rice grain production in treatments using biopesticides for *O. poecilus* control. The strategy of using entomopathogenic fungi is a promising approach to battle the stink bugs in IPM of rice crop (Quintela et al., 2013).

Table 3: Average number of grains (ANG), grain mass (GM) and Percentage of damaged grains (PDG) damaged by *Oebalus poecilus* (Hemiptera: Pentatomidae) adults on rice plants after treatment with *Beauveria bassiana*, *Cordyceps javanica* and *Metarhizium anisopliae*. Urutai. GO. Brazil.

Treatments	ANG	GM	PDG
Beauveria bassiana	245.0 50.09 a	4.60 1.07 a	70.0 13.02 a
Cordyceps javanica	231.0 0.54 ab	3.75 0.02 b	41.3 15.79 b
Metarhizium anisopliae	222.3 94.2 b	3.46 0.38 b	40.0 7.87 b
Control	106.5 36.1 c	1.07 1.09 c	30.3 11.5 b
F treatment	23.59	79.88	15.87
p-value	< 0.0005	< 0.0005	< 0.0005

[1]Means followed by the same letter in the column do not differ statistically from each other according to the Tukey test at 0.05% probability.

CONCLUSION

The entomopathogenic fungi were capable of infecting and killing *O. poecilus* adults on rice plants. *Beauveria bassiana* demonstrated higher virulence and higher mortality at six days after application. Furthermore, a negative interference was observed in the development of insects treated with the fungi *B. bassiana*, *C. javanica*, and *M. anisopliae*. Entomopathogenic fungi show promise in controlling stink bug pests in rice crops and need to be studied to determine more efficient doses and their effects across different developmental stages of this pest.

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CONFLICT OF INTEREST

We have no conflict of interest to declare

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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