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General Entomology

Biological Control of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) with fungi from Brazilian Amazon

Nárcya Trindade de Souza, Bruno Leite Beltrão Frederico, Gleison Rafael Queiroz Mendonça, Atilon Vasconcelos de Araujo, Adalberto Hipólito Sousa & Clarice Maia Carvalho

Universidade Federal do Acre, Rio Branco, AC, Brazil.

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Abstract. Stored grains are attacked by insect pests, causing various types of damage and potentially generating economic losses for producers. *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), popularly known as the red flour beetle, is one of these secondary pests. Thus, the objective of this study was to evaluate the potential of entomopathogenic fungi from the Brazilian Amazon for the control of *T. castaneum*. To evaluate the control of *T. castaneum*, different conidial suspensions (10^5, 10^6, 10^7 and 10^8 conidia/mL) of *Beauveria* sp. (4.438) and *Paecilomyces* sp. (4.658) were used, placing 1 mL of each concentration in the center of Petri dish and keeping the insects for 10 min. After that, they were transferred to another Petri dish, incubated for 7 days at room temperature, and Tween 0.01% and NaCl 0.9% solutions were used as negative controls. *T. castaneum* mortality was obtained only at the highest dilution, 10^8 conidia/mL, being 22% for the fungus *Beauveria* sp. (4.438) and 5% for *Paecilomyces* sp. (4.658). Susceptibility to exposure to abiotic factors was measured with suspensions of *Beauveria* sp. (4.438) conidia at a concentration of 10^6 conidia/mL exposed to ultraviolet light for 0, 30, 60 and 120 seconds, and at temperatures of 20, 26 and 32 °C for 30, 60 and 90 min. The highest germination rate was at 20 °C for 90 minutes, with 92%. The entomopathogenic fungi from the Amazon showed low potential for controlling *T. castaneum*, however, they may present a higher mortality rate for other insect pests.

Keywords: Amazonia*; Beauveria*; entomopathogenic; *Paecilomyces*; red flour beetle.

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Corresponding author:
Clarice Maia Carvalho
clarice.carvalho@ufac.br

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Storage pests, as their name suggests, are insects that attack stored grains, such as corn, soybeans and wheat, and bring a series of damages and economic losses, such as contamination with parts of their bodies, proliferation of fungi and bacteria, decrease in germination rate and use of grains for food (Mokhtar 2021).

A well-known storage pest is the red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), an insect that usually infests ground grains such as bran, flour, feed, cornmeal, and its presence in broken grains indicates that primary pests infested the site (Magalhães *et al.* 2015). To control these pests, fumigant insecticides are used, but they are not fully effective and have a residual effect on the grains, causing contamination in humans and the soil (Da Silva *et al.* 2015). Furthermore, they are not fully effective and some insects have become resistant to their use (Galzer & Azevedo-Filho 2016).

Therefore, alternative ways to control these pests are essential, such as the use of entomopathogenic fungi, which are not toxic to humans or animals, are not polluting, do not cause imbalance in nature, and will not develop in production, being specific to insects, among other qualities (Pimentel & Ferreira 2012).

Fungi have the potential for biocontrol, because depending on climatic conditions, they can penetrate the body of insects, increasing their mortality (Amatuzzi *et al.* 2018). In addition, some of these fungi have a high endophytic potential, associating with plants without causing damage and may also serve as plant defense (Mantzoukas & Eliopolus 2020).

Fungi of the genus *Beauveria* have great potential for pest control, but their use in commercial products for this area is still restricted (Erler & Ates 2015). In addition, fungi of the genus *Paecilomyces,* which can be found in soils, can be used to control nematodes and have potential for biological control (Mora *et al.* 2016).

Despite its high biodiversity, the Amazon is little studied on the research of entomopathogenic fungi for pest control. For *T. castaneum*, entomopathogenic fungi from other regions of Brazil were studied (Zettler 1991; Sawada *et al.* 2020; Duarte *et al.* 2022) however, no study was done with fungi from the Amazon.

Thus, this study aimed to evaluate the potential of entomopathogenic fungi from the Brazilian Amazon to control *T. castaneum*.

MATERIAL AND METHODS

Fungus reactivation. Two fungi isolated from Amazonian soils stored in the Microbiology Laboratory Collection of the Universidade Federal do Acre (UFAC), using the technique in distilled water, were reactivated by inoculation in Petri dishes with Potato-Dextrose-Agar (PDA) culture medium and transferred into tubes containing the PDA medium.

Virulence bioassay. Insects of the *T. castaneum* species used in the bioassay were donated by the UFAC Entomology Laboratory, kept in a plastic bottle with a lid that allowed for gas exchange. Twenty adult, unsexed insects were used for each replication (Pimentel & Ferreira 2012), with ground corn being used as substrate (Magalhães *et al.* 2015).

For the test, dilutions of 10^5, 10^6, 10^7 and 10^8 conidia/mL were used, with 1 mL of each concentration being added to the center of the Petri dish and the insects kept for 10 min. Subsequently, the insects were transferred to another Petri dish, incubated for 7 days at room temperature, with five repetitions of each dilution. As a negative control, 0.01% Tween solution and 0.9% NaCl solution were used (Remadevi *et al.* 2010).

Conidia sensitivity assays to abiotic factors. The fungus with the highest virulence rate was tested for resistance to abiotic factors, being cultivated in a Petri dish containing PDA medium and incubated for 14 days at room temperature. After this period, the conidia were scraped and diluted in 10 mL of 0.1% Tween 80 solution. The conidial suspension was homogenized in a vortex shaker and the conidial count was done in a Neubauer chamber. The 10^6 dilution was standardized and used in resistance tests to abiotic factors (Remadevi *et al.* 2010).

The temperature sensitivity test was done at temperatures of 20 °C, 26 °C and 32 °C. Glass tubes with 1 mL of the 10^6 conidia/mL solution closed with cotton were used, at times of 0, 30, 60 and 90 minutes for each evaluated temperature (Oliveira *et al.* 2016).

For the ultraviolet radiation test, 1 mL of the 10^6 conidia/mL solution was placed in Petri dishes at 40 cm from the UV lamp for times of 0, 30, 60 and 120 seconds (Oliveira *et al*. 2016).

To determine the viability of conidia exposed to abiotic factors, 10 µL was inoculated in a Petri dish with PDA medium and incubated at 28 °C for 12 h. Three repetitions were done for each test. After incubation, lactophenol blue dye was added and a coverslip was placed to count viable (forming germ tube) or non-viable conidia. For each point, 100 conidia were counted, totaling 300 conidia per treatment (Oliveira *et al.* 2016).

Statistical analysis. The data obtained the bioassay with *T. castaneum* and in the viability tests in relation to abiotic factors were statistically analyzed according to normality. Analysis of variance was used, followed by Tukey's test. For data processing, BioEstat 5.0 software was used.

RESULTS

Virulence bioassay. The virulence potential of the Amazonian fungi *Beauveria* sp. 4.438 and *Paecilomyces* sp. 4.658 against *T. castaneum* at concentrations of 10^5, 10^6, 10^7 and 10^8 conidia/mL (Figure 1).

Mortality rates of 22% were observed for the fungi *Beauveria* sp. 4.438 and 5% for *Paecilomyces* sp. 4.658. Both evaluated fungi had a higher *T. castaneum* mortality rate at conidial concentration 10^8 (Figure 1).

Conidia sensitivity assays to abiotic factors. The fungus *Beauveria* sp. 4.438 had the best results in the bioassay of virulence and was used for tests of conidial sensitivity to abiotic factors.

In the resistance test of the fungus *Beauveria* sp. 4.438, temperature and time variations influenced the germination index of the fungi, with an average germination index between 92 and 74% (Table 1). In addition, the influence of the temperature of 32 ºC was recorded, and it was observed that as the exposure time increased, there was a decrease in the germination rate.

The germination of the fungus of the genus *Beauveria* sp. 4.438 was influenced by the time of exposure to UV radiation, with a mean germination of 47.6% after 30 seconds and 12% after 120 seconds (Figure 2).

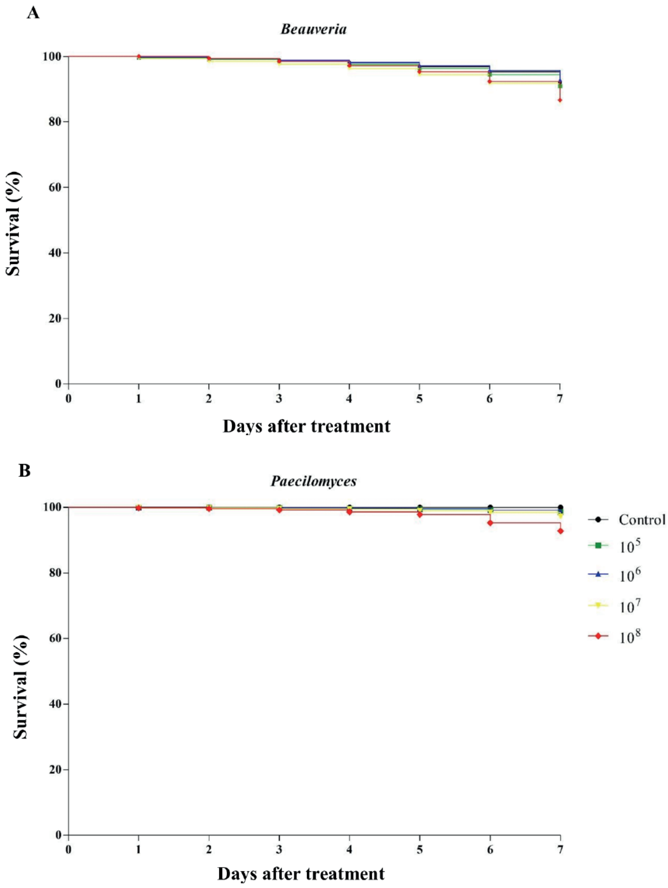

Figure 1. Percent survival of *Tribolium castaneum* within seven days after exposure to different conidial concentrations of *Beauveria* sp. 4.438 (A) and *Paecilomyces* sp. 4.658 (B).

Table 1. Germination percentage of fungal conidia of *Beauveria* sp. 4.438 after exposure thermal stress conditions at different times.

Fungi	Control	Germinated conidia %								
		ºC (30')			ºC (60')			ºC (90')		
		20	26	32	20	26	32	20	26	32
Beauveria sp. 4.438	86 ab	82 ab	85 ab	90 a	84 ab	87 ab	88 ab	92 a	74 b	85 ab

Means followed by the same letter do not differ in Tukey's test (p ≥0.05).

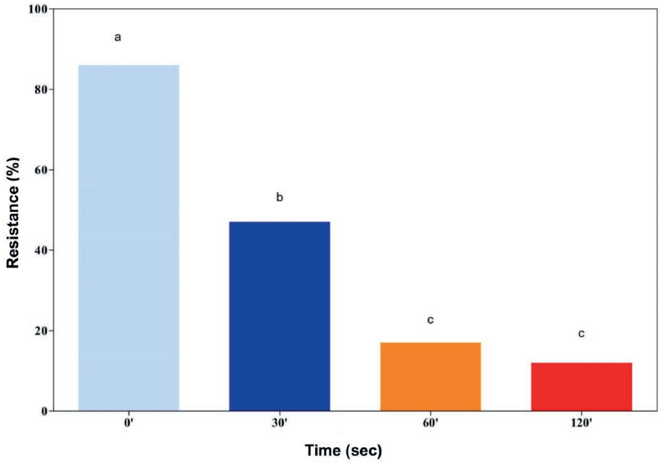

Figure 2. Percentage of conidia germination of the fungus *Beauveria* sp. (4.438) after exposure to UV radiation at different time periods.

DISCUSSION

The two Amazonian fungi used in the virulence assay, *Beauveria* sp. 4.438 and *Paecilomyces* sp. 4.658 demonstrated low pathogenicity against *T. castaneum*, even when used in higher conidial concentrations.

However, previous studies record the pathogenicity of the fungus of the genus *Beauveria*, considered a promising entomopathogen for the control of insect pests, and can also be used in conjunction with natural pesticides, being an alternative for producers and with action proven to control several Hemiptera, however, there are few works with the *T. castaneum* pest (Souza *et al.* 2019).

Despite the low virulence potential registered for the fungus *Paecilomyces* sp. 4.658 for *T. castaneum*, previous studies describe the potential use of fungi of this genus to control pests, such as the bovine tick (Angelo *et al.* 2012), and the production of hydrolytic enzymes associated with virulence factors for insect pests, indicating the potential use of these fungi for biological control (Khan *et al.* 2003).

Results show low virulence of *Beauveria* for the control of *T. castaneum*, as the tests done on larvae of this beetle (Zamani *et al.* 2016). Studies report the use of vegetable oils to control this pest, such as Indian neem oil (*Azaradicta indica*, A. Juss.), chrysanthemum (*Chisantemum cinerariaefolium* Sch.Bip.) (Da Silva *et al*. 2015), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), thyme (*Thymus vulgaris* L.) (Pauliquevis & Favero 2015), *Myracrodruon urundeuva* M.Allemão (Magalhães *et al.* 2015) and garlic (*Allium sativum* L.) (Sileem *et al.* 2019). Vegetable oils with potential to control *T. castaneum* can be used in formulations to increase the virulence of entomopathogenic fungal species.

Temperature directly influences the development of microorganisms. Study recorded variations in the temperature considered optimal for the development and germination of different fungal species, requiring prior evaluation to determine the species most adapted to the environmental condition, the possible abiotic factors of its habitat (Mastore *et al.* 2019).

In addition, different species of entomopathogenic fungi have evolved in a way that they manage to generate mechanisms to protect themselves from abiotic factors, such as temperature and UV, increasing their germination potential and the potential for virulence (Marques *et al.* 2016), being important studies to assess different species.

The Amazonian fungus *Beauveria* sp. 4.438 had a conicial germination rate that ranged from 74 to 92% at exposure to different temperatures and a mean germination of 47.6% after 30 seconds of exposure to UV radiation.

Studies record the sensitivity of the genus *Beauveria* to UV exposure and reduced conidial viability, with rates below 50% after exposure to this stress condition, which are associated with direct DNA damage, mutations and errors in the transcription process of the evaluated fungus (Zorzetti *et al*. 2014).

Tolerance to UV radiation varies according to the fungal species evaluated, indicating the susceptibility of the genus *Beauveria* to conidial inactivation due to UV exposure (Ottati-de-Lima *et al*. 2021). Thus, measures for conidial protection are indicated, such as encapsulation and formulation with natural substances, including sodium alginate and vegetable oils, for the use of this microorganism as an agent for pest control (Oliveira *et al*. 2016).

The Amazonian fungi *Beauveria* sp. 4.438 and *Paecilomyces* sp. 4.438 had low virulence against insect pests of the species *T. castaeum*. However, these fungi may be more virulent to other insect pests and can be used for their biological control.

Environmental conditions directly influence the development of fungi, where high temperatures have become a limiting factor for conidial germination, as well as exposure to UV light for a long period.

This is the first report of a virulence assay of Amazonian entomopathogenic fungi against *T. castaeum*.

AUTHORS CONTRIBUTION

NTS: development of experiments and initial writing of the article; BLBF: development of experiments; GRQM: development of experiments and statistical analysis; AVA: development of experiments and statistical analysis; AHS: Final Article Review; CMC: development of experiments, final review of the article and research coordination

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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