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PATHOGENICITY OF ISOLATES OF ENTOMOPATHOGENIC FUNGI TO WORKERS OF *Acromyrmex heyeri* (Forel, 1899) (HYMENOPTERA: FORMICIDAE)

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Resumo

Patogenicidade de isolados de fungos entomopatogênicos a operárias de Acromyrmex heyeri (Forel, 1899) (Hymenoptera: Formicidae). As formigas-cortadeiras se destacam dentre as principais pragas dos cultivos agrícolas e florestais. O controle químico é o método mais utilizado para o controle destes insetos-praga, sendo necessário o desenvolvimento de técnicas menos danosas ao meio ambiente. Assim, o objetivo do presente estudo foi verificar a ação de isolados de *Beauveria bassiana*, *Cordyceps fumosorosea* e *Metarhizium anisopliae* sobre a mortalidade de *Acromyrmex heyeri* (Forel), em laboratório. Para tanto, foram utilizados sete isolados de fungos entomopatogênicos, sendo três isolados de *Beauveria* bassiana (Bals.) Vuill (IBCB 66, IBCB 170 e IBCB 632), um isolado de *Cordyceps fumosorosea* (Wize) (IBCB 130) e três isolados de *Metarhizium anisopliae* (Metsch.) Sorokin (IBCB 348, IBCB 383 e IBCB 425), e cinco concentrações de esporos ($1,0 \times 10^4$; $1,0 \times 10^5$; $1,0 \times 10^6$; $1,0 \times 10^7$ e $1,0 \times 10^8$ esporos.mL^{-1}) para cada isolado fúngico. A testemunha consistiu da aplicação de água destilada autoclavada e Tween 40 0,01%, sem a adição de patógenos fúngicos. Os experimentos foram conduzidos em delineamento inteiramente casualizado com cinco repetições. A estimativa das concentrações letais de CL_{50} e CL_{90} foi realizada utilizando a análise de Probit. Observou-se que os isolados de *B. bassiana*, *C. fumosorosea* e *M. anisopliae* foram patogênicos às formigas-cortadeiras com as cinco concentrações de esporos utilizadas, sendo que, com o aumento da concentração, obteve-se maior mortalidade dos espécimes. Dessa maneira, verificou-se que os fungos entomopatogênicos avaliados neste estudo mostraram-se eficientes como agentes de controle biológico de *Acromyrmex heyeri*.
Palavras-chave: Beauveria bassiana; controle biológico; *Cordyceps fumosorosea;* formigas-cortadeiras; *Metarhizium anisopliae.*

Abstract

Leaf-cutting ants stand out among the main pests of agricultural and forestry crops. Chemical control is the most used method to control these insect pests, requiring the development of techniques that are less harmful to the environment. The present study aimed at verifying the action of *Beauveria bassiana* (Bals.) Vuill, *Cordyceps fumosorosea* (Wize), and *Metarhizium anisopliae* (Metsch.) Sorokin isolates on the *in vitro* mortality of *Acromyrmex heyeri* (Forel). Seven isolates of entomopathogenic fungi were used: three isolates of *Beauveria bassiana* (IBCB 66, IBCB 170, and IBCB 632), one isolate of *Cordyceps fumosorosea* (IBCB 130), and three isolates of *Metarhizium anisopliae* (IBCB 348, IBCB 383, and IBCB 425), and five concentrations of spores (1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 spores mL^{-1}) for each fungal isolate. The control treatment consisted of autoclaved distilled water and 0.01% Tween 40, without fungal pathogens. The experiments were carried out in a completely randomized design with five replications. Estimation of lethal concentrations of LC_{50} and LC_{90} was performed using Probit analysis. The isolates of *B. bassiana*, *C. fumosorosea*, and *M. anisopliae* were pathogenic to leaf-cutting ants with the five tested concentrations of spores. Higher mortality of the specimens was reached with increasing concentration. The entomopathogenic fungi evaluated in this study proved to be efficient agents for the biological control of *Acromyrmex heyeri*.
Keywords: Beauveria bassiana; biological control; *Cordyceps fumosorosea*; leaf-cutting ants; *Metarhizium anisopliae*

INTRODUCTION

Ants belong to the Formicidae family, with more than 16,000 valid species, 17 subfamilies, 39 tribes, and 337 genera, present in all terrestrial environments (BOLTON, 2020). They perform important ecological functions and interact establishing complex food chains. However, any change in this structure can reduce the number of species, their diversity, and the activities of other invertebrates (WAGG *et al*., 2014).

Considered one of the main phytosanitary problems in agriculture and forestry, leaf-cutting ants (family Formicidae, tribe Attini) are insects belonging to the genera *Atta* Fabricius (saúvas), *Acromyrmex* Mayr, and

Amoimyrmex Cristiano, Cardoso & Sandoval (quenquéns) (CRISTIANO *et al*., 2020). Their economic importance is highlighted by the fact that they cut fresh plant material which serves as a substrate for the symbiotic mutualistic fungus *Leucoagaricus gongylophorus* (Basidiomycota: Agaricales), causing severe economic damage (BACCARO *et al*., 2015).

Ants of the genus *Acromyrmex* (34 species) are widely distributed in the Neotropical region and in all the Brazilian territory, foraging throughout the year. *Acromyrmex heyeri* (Forel, 1899), commonly known as the hill-ant, is a species of leaf-cutting ant with distribution restricted to South America, being found in pastures and on the edges of forest fragments (SARUBBI; RAMÍREZ, 2020).

The control of insects with social organization, overlapping generations colonies, parental care, and caste division, associated with the courtship habit, becomes difficult since eliminating the foraging workers is not efficient (DELLA LUCIA *et al*., 2014). Therefore, to prevent the action of leaf-cutting ants from causing significant losses, especially in agricultural areas and forest crops, chemical control methods are commonly used, such as attractive baits. However, this fact is worrying, since Brazil is identified as the largest consumer of chemical products in agriculture, generating resilience of residues in the soil and, consequently, the contamination of the environment (FAN *et al*., 2018).

Currently, there has been a concern in investigating control methods aimed at greater effectiveness and lower environmental impacts. In this sense, the use of biological products is considered a promising alternative since it occurs naturally in any ecosystem. However, man can interfere in this process, facilitating its action through one or more means of manipulating the involved organisms (FONTES *et al*., 2020). Thus, the use of entomopathogenic fungi can be a viable alternative for the biological control of leaf-cutting ants. Thus, effective knowledge about the dynamics related to the horizontal transmission of fungal propagules, from specificity up to the fungal colonization in a target population, will contribute to more effective control of the targeted pest.

Several studies show the effect of microorganisms on biological control, such as *Metarhizium anisopliae* (Metsch.) Sorokin against fire ants *Solenopsis invicta* Buren (QIU *et al*., 2019), and *Beauveria bassiana* (Bals.) Vuill against ant-cutter (FOLGARAIT *et al*., 2020). However, several steps are necessary to obtain a product, including isolating the organism from the environment, selecting efficient isolates for control, optimizing the production of field tests, and testing the effects of these organisms on the soil and other organisms present in the environment (UNFER *et al*., 2019).

Despite being characterized as an insect pest, leaf-cutting ants not only promote damage but also benefits with their actions in important ecological processes, such as nutrient cycling, in the most diverse ecosystems where they are found. Hence, the dispersion of biological control agents has stood out as a control method that aims at maintaining the target species in a lasting and stable way, below the level of economic damage, using natural enemies, which can be predators, parasites, or pathogens (DA SILVA ARAÚJO *et al*., 2015).

Biological products commercialized based on entomopathogenic fungi, e.g., Boveril® based on *Beauveria bassiana* (CEPA ESALQ PL63 - Registration in MAPA 4902) for the control of *Tetranychus urticae* Koch, *Gonipterus platensis* Marelli, *Hypothenemus hampei* Ferrari, and *Bemisia tabaci* Genn., and the Metarril®, based on *Metarhizium anisopliae* (CEPA ESALQ E9 - Registration in MAPA 6605), for the control of *Mahanarva fimbriolata* Stål. Both products are sold by the company Koppert Brasil (KOPPERT, 2020). In the case of *Cordyceps fumosorosea* (Wize), commercial products based on this fungus are already used mainly in North America, Mexico, Colombia, and Europe to control whiteflies, aphids, and thrips (FARIA; WRAIGHT, 2007). However, these microorganisms are not used for the biological control of leaf-cutting ants.

Biological control with the use of entomopathogenic fungi is a promising research area. However, currently, there is a clear need for biological knowledge so that strategies to control leaf-cutting ants can be applied. The perspective of the real pathogenicity of entomopathogenic fungi against leaf-cutting ants and the development of new techniques for applying these biological products can contribute to more effective control and reduce the damage caused by leaf-cutting ants in agricultural and forestry crops. The objective of the present study was to verify, in the laboratory, the action of isolates of *Beauveria bassiana, Cordyceps fumosorosea*, and *Metarhizium anisopliae* on the mortality of *Acromyrmex heyeri*.

MATERIAL AND METHODS

The present study was carried out at the Laboratory of Forest Entomology and the Laboratory of Phytopathology "Drª Elocy Minussi", Department of Phytosanitary Defense, Center of Rural Sciences of the Federal University of Santa Maria.

Collection and identification of target insects

The material was collected in a pasture area, between a young black wattle crop (*Acacia mearnsii* De Wild.) and a eucalyptus plantation (*Eucalyptus* spp.), belonging to the State Center for Forestry Diagnosis and Research (Santa Maria, Rio Grande do Sul), on February 23, 2021. The area close to the nest was in intense

foraging activity by the ants. Specimens of workers from a colony were collected. The biological material was obtained between 2:00 pm and 3:00 pm, a period of intense foraging activity by the workers (SANTOS et al., 2020).

The leaf-cutting ant specimens were collected with tweezers and placed in a sterilized container and sent to the Laboratory of Forest Entomology. For the identification procedure, some specimens were mounted and identified at the genus level by means of morphological characters observation in a spectroscope microscope. Identification at the species level was confirmed by a specialist in the area.

Origin and maintenance of entomopathogenic fungal isolates

For this study, seven isolates of entomopathogenic fungi provided by the Biological Institute of São Paulo, Collection of Entomopathogenic Fungi "Oldemar Cardin Abreu" were used: three from *Beauveria bassiana* (IBCB 66, IBCB 170, and IBCB 632), one from *Cordyceps fumosorosea* (IBCB 130), and three from *Metarhizium anisopliae* (IBCB 348, IBCB 383, and IBCB 425).

To subculture the entomopathogenic fungal isolates, Petri dishes (90 mm) containing PDA (potato-dextrose-agar) culture medium were used. A small amount of fungal mycelium was subcultured in the center of each plate. After inoculation, the plates were kept in a B.O.D. at 25 ± 2 °C in a light regime alternating with a 12-hour photoperiod, for ten days for fungal growth and sporulation.

Preparation of spore suspensions

After ten days of mycelial growth in the Petri dishes containing the isolates, suspensions with the spores were prepared by adding 10 mL of sterile distilled to the Petri dish and scrapping the fungal mycelium from the surface of the medium with the aid of a Drigalski loop. The suspension was filtered through a double layer of gauze into a beaker. An adhesive diffuser (0.01% Tween 40) was added to count the spores in a Neubauer chamber. After spore counting, the suspension was calibrated for concentrations of 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 spores mL^{-1} for each fungal isolate.

Pathogenicity test

For each fungal isolate, an experiment was carried out with 250 specimens of leaf-cutting ants, corresponding to five repetitions of 10 ants for each treatment (T1 = suspension of 1.0×10^4 spores mL^{-1}; T2 = suspension of 1.0×10^5 spores mL^{-1}; T3 = suspension of 1.0×10^6 spores mL^{-1}; T4 = suspension of 1.0×10^7 spores mL^{-1}; T5 = suspension of 1.0×10^8 spores mL^{-1}). The control was made using only autoclaved distilled water and 0.01% Tween 40, without the addition of fungal pathogens.

The ants were individually transferred to gerbox-type boxes (11 x 11 x 3.5 cm), previously sterilized with 70% alcohol, containing filter paper with 1 mL of the spore suspensions, and cotton balls moistened with a10% honey solution. The boxes were kept at a temperature of 25 ± 2 °C, relative humidity of 70 ± 10%, and evaluated every 24 hours for ten days.

To confirm whether the ant mortality was caused by the studied entomopathogenic fungi, the dead ants were removed daily from the gerbox boxes and stored in a humid chamber at a temperature of 25 ± 2 °C and relative humidity of 70 ± 10 % for seven days, to verify the possible extrusion of the fungal pathogen.

Statistical analysis

The experiments were conducted in a completely randomized design with five replications. The data set was submitted to the normality and homogeneity tests and when meeting the assumptions, submitted to the analysis of variance (ANOVA), and the averages to the Tukey test ($p < 0.05$), using the SISVAR software, version 5.6.86 (FERREIRA, 2014), to obtain the percentage of control of entomopathogenic fungal isolates in the different concentrations of spores.

To assess the susceptibility of leaf-cutting ants to different isolates of entomopathogenic fungi, the concentration-mortality data were subjected to a chi-square test (χ^2) to verify whether they fit the assumptions of the Probit model. The lethal concentrations of LC_{50} and LC_{90} and the respective 95% confidence intervals were estimated based on Probit analysis (SAS INSTITUTE, 2002).

RESULTS

At the beginning of the bioassay, the ants were agitated, walking quickly in different directions. Over the days, they began to cut the filter papers present on the plates.

The isolates of *B. bassiana*, *C. fumosorosea*, and *M. anisopliae* were pathogenic to leaf-cutting ants with the five spore concentrations (1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 spores mL^{-1}). The confirmation of the mortality of leaf-cutting ant specimens by the entomopathogenic fungi was based on the observation of the extrusion of the pathogen in the corpses (FIGURE 1).

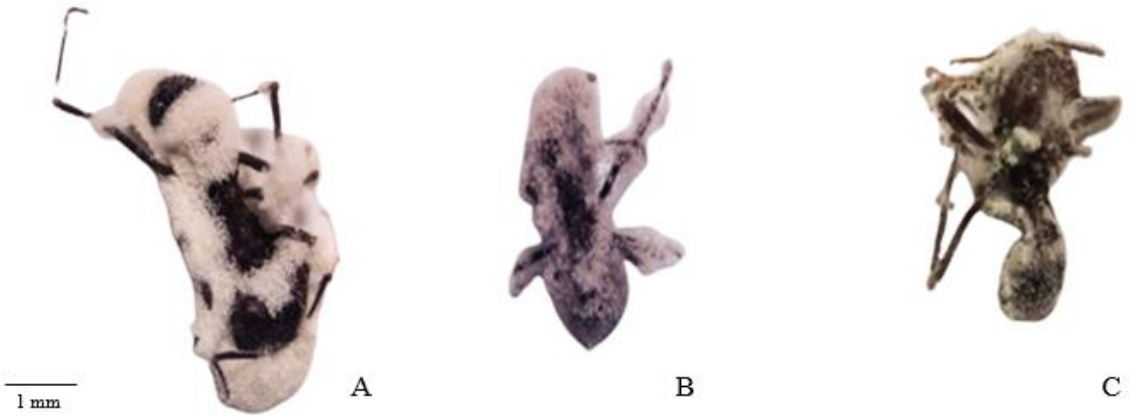

Figure 1. Extrusion of entomopathogenic fungi in corpses of *Acromyrmex heyeri* (Forel). (A) *Beauveria bassiana* (Bals.) Vuill. (B) *Cordyceps fumosorosea* (Wize). (C) *Metharizium anisopliae* (Metsch.) Sorokin.
Figura 1. Extrusão de fungos entomopatogênicos em cadáveres de *Acromyrmex heyeri* (Forel). (A) *Beauveria bassiana* (Bals.) Vuill, (B) *Cordyceps fumosorosea* (Wize). (C) *Metharizium anisopliae* (Metsch.) Sorokin.

Concerning the *B. bassiana* isolates, the highest mortalities of leaf-cutting ants occurred when higher concentrations of entomopathogenic fungal spores were used (Table 1). The isolate IBCB 632 showed a mortality rate of 92% when subjected to a concentration of 1.0×10^8 spores mL^{-1}. However, it statistically did not differ from the value found when using a concentration of 1.0×10^7 spores mL^{-1} (78%). There was no significant difference when using the concentration of 1.0×10^4 spores mL^{-1} and the control, with values of 30 and 24%, respectively.

Table 1. Mortality percentage (%) of *Acromyrmex heyeri* (Forel) specimens by *Beauveria bassiana* (Bals.) Vuill isolates at different spore concentrations.
Tabela 1. Percentual de controle (%) de espécimes de *Acromyrmex heyeri* (Forel) por isolados de *Beauveria bassiana* (Bals.) Vuill em diferentes concentrações de esporos.

Concentration (spores mL^{-1})	**Mortality percentage (%)**		
	IBCB 632	**IBCB 170**	**IBCB 66**
1 x 10^4	30 c*	34 cd	36 cd
1 x 10^5	46 bc	58 bc	50 bc
1 x 10^6	64 ab	56 bc	68 b
1 x 10^7	78 a	82 ab	74 ab
1 x 10^8	92 a	90 a	100 a
Control	24 c	24 d	24 d
M.G.	55,7	57,7	58,7
CV (%)	26,75	25,97	31,96

*Means followed by the same letter on the line do not differ by Tukey's test at 5% probability of error. CV= Coefficient of variation.

The isolate IBCB 170 showed a mortality percentage of 90% when subjected to a concentration of 1.0×10^8 spores mL^{-1}, statistically differing from the other values. Similarly, isolate IBCB 66 promoted 100% mortality of the specimens when using the maximum concentration of spores.

For *M. anisopliae* isolates, the highest percentages of control of leaf-cutting ant specimens occurred when there was an increase in the concentration of spores of entomopathogenic fungi. The isolate IBCB 387 showed the highest mortality (100%) when using the concentration of 1.0×10^8 spores mL^{-1} (Table 2).

Table 2. Mortality percentage (%) of *Acromyrmex heyeri* (Forel) specimens by *Metarhizium anisopliae* (Metsch.) Sorokin isolates at different spore concentrations.
Tabela 2. Percentual de controle (%) de espécimes de *Acromyrmex heyeri* (Forel) por isolados de *Metarhizium anisopliae* (Metsch.) Sorokin em diferentes concentrações de esporos.

Concentration (spores mL^{-1})	**Mortality percentage (%)**		
	IBCB 348	**IBCB 425**	**IBCB 387**
1 x 10^4	28 cd*	36 c	26 d
1 x 10^5	42 cb	62 bc	44 cd
1 x 10^6	56 bc	56 bc	64 bc
1 x 10^7	82 ab	80 ab	92 ab
1 x 10^8	96 a	98 a	100 a
Control	24 d	24 c	24 d
M.G.	54,7	59,4	58,4
CV (%)	26,4	34,95	25,14

*Means followed by the same letter on the line do not differ by Tukey's test at 5% probability of error. CV= Coefficient of variation.

The IBCB 348 and IBCB 425 isolates showed mortality rates of 96 and 98%, respectively, for the concentration of 1.0×10^8 spores mL^{-1}, statistically differing from the other concentrations. As with the *B. bassiana* isolates, no significant differences were found between the concentration of $1.0\text{x}10^4$ spores mL^{-1} and the control.

Regarding the isolate of *C. fumosorosea*, the highest mortality of leaf-cutting ants occurred when the concentration of 1.0×10^8 spores mL^{-1} was used, statistically differing from the other treatments. For this species, a decrease in specimen mortality was also observed when lower concentrations of spores were used (Table 3).

Table 3. Mortality percentage (%) of *Acromyrmex heyeri* (Forel) specimens by *Cordyceps fumosorosea* (Wize) isolate at different spore concentrations.
Tabela 3. Percentual de controle (%) de espécimes de *Acromyrmex heyeri* (Forel) por isolado de *Cordyceps fumosorosea* (Wize) em diferentes concentrações de esporos.

Concentrations (spores mL^{-1})	**Mortality percentage (%)**
1 x 10^4	32 cd*
1 x 10^5	50 cd
1 x 10^6	58 bc
1 x 10^7	88 ab
1 x 10^8	100 a
Control	24 d
M.G.	58,7
CV (%)	26,86

*Means followed by the same letter on the line do not differ by Tukey's test at 5% probability of error. CV= Coefficient of variation.

According to the Probit analyses, the average lethal concentrations of mortality were estimated from the different concentrations of spores used. The needed concentration to promote 50% mortality in *A. heyery* workers after 10 days of exposure to entomopathogenic fungal isolates ranged from 1.58×10^5 spores mL^{-1} (IBCB 170 isolate) to 6.94×10^6 spores mL^{-1} (IBCB 425 isolate). Regarding the concentrations necessary to kill 90% of the leaf-cutting ant specimens, the values ranged from 1.84×10^6 spores mL^{-1} (IBCB 130 isolate) to 1.10×10^8 spores mL^{-1} (isolate IBCB 632) (Table 4).

Table 4. Lethal concentration (LC_{50} and LC_{90}; spores mL^{-1}) of *Beauveria bassiana* (Bals.) Vuill, *Cordyceps fumosorosea* (Wize), and *Metarhizium anisopliae* (Metsch.) Sorokin isolates against *Acromyrmex heyeri* (Forel) specimens.

Tabela 4. Concentração - letal (CL_{50} e CL_{90}; esporos.mL^{-1}) de isolados de *Beauveria bassiana* (Bals.) Vuill, *Cordyceps fumosorosea* (Wize) e *Metarhizium anisopliae* (Metsch.) Sorokin contra espécimes de *Acromyrmex heyeri* (Forel).

Isolate	*n*	Incline ± EP	LC_{50} (95% IC)	LC_{90} (95% IC)	χ^2	*df*
IBCB 632	250	0.54 ± 0.21	4.71×10^5 (2.53×10^3-7.27×10^6)	1.10×10^8 (6.85×10^6 – 1.53×10^9)	6.43	3
IBCB 170	250	0.40 ± 0.14	1.58×10^5 (1.74×10^4 – 9.89×10^5)	9.01×10^7 (2.57×10^6 – 8.00×10^8)	7.77	3
IBCB 66	250	0.89 ± 0.25	1.92×10^6 (3.21×10^3 – 4.44×10^7)	5.22×10^7 (1.99×10^7 – 3.03×10^8)	2.60	3
IBCB 348	250	0.86 ± 0.22	1.60×10^6 (1.00×10^5 – 5.09×10^6)	4.98×10^7 (1.84×10^7 – 2.97×10^8)	9.69	3
IBCB 425	250	1.38 ± 0.52	6.94×10^6 (2.31×10^5 – 1.64×10^7)	2.39×10^7 (4.25×10^6 – 2.88×10^8)	7.31	3
IBCB 383	250	1.11 ± 0.27	7.42×10^5 (9.46×10^4 – 1.89×10^6)	1.06×10^7 (4.72×10^6 – 3.76×10^7)	9.23	3
IBCB 130	250	1.32 ± 0.50	1.95×10^6 (1.53×10^4 – 4.90×10^6)	1.84×10^6 (8.35×10^6 – 2.29×10^8)	6.88	3

LC50: concentration of entomopathogenic fungi (spores mL^{-1}) required to kill 50% of leaf-cutting ants under observation for 10 days. Similarly, LC_{90} is the concentration of entomopathogenic fungi required to kill 90% of the leaf-cutter ants tested. $\chi 2$ = P > 0.05 in the quality fit-test. df = Degrees of freedom.

DISCUSSION

Initially, the act of cutting the filter papers present in the gerbox boxes was verified. This behavior may be related to the biology of leaf-cutting ants, which have the routine of cutting leaves to feed the symbiotic fungus they cultivate inside the nests when in their natural environment (HAEDER *et al*., 2009).

No fungal growth on the corpses of leaf-cutting ants was observed in the controls, indicating that mortality in these groups was not the result of fungal infection, but likely due to the stress caused by social isolation or, due to the ants' senescence.

As can be seen in Table 1, there was a reduction in the mortality of leaf-cutter ant specimens when using lower concentrations of spores. In the control, mortality did not differ statistically from the values found when using the concentration of 1.0×10^4 spores mL^{-1}. Similarly, increasing the number of spores of *M. anisopliae* and *B. bassiana* caused a higher percentage of infection by entomopathogenic fungi in specimens of *A. heyery* (SANTOS *et al*., 2020)

All spore concentrations used in this study were able to ensure infection and disease progression in leaf-cutting ant specimens. The spore concentration had a positive linear response with mortality since a higher concentration caused higher mortality. This may be related to the number of viable spores that manage to position themselves in the insect's cuticle and that will later germinate, having a larger opportunity to infect the insect (RAMÍREZ-SÁNCHEZ *et al*., 2019).

It can be highlighted that entomopathogenic fungi have a wide spectrum of action, being able to colonize several species, as well as being used as agents in the biological control of insect species potentially causing economic losses. These microorganisms can be bio-prospected from different environments, such as the soil, as well as from the insect that is desired to be controlled (GOFFRÉ *et al*., 2018).

The mechanism of fungus infection in the insect can be direct or driven by the action of secondary metabolites. Toxic metabolites include extracellular enzymes, proteins, and low molecular weight compounds, such as destruxins, produced by *M. anisopliae* and *B. bassiana* (FAN et al., 2013). However, some fungi, which lack toxins, cause the death of the insect by consuming all its nutrients (RAMÍREZ *et al*., 2014). After being infected by the fungus, the insects show symptoms such as spots, paralysis, and altered behavior. The insect stops feeding, becoming debilitated, and the death occurs in a few days due to a combination of factors such as nutrient depletion, fungal toxins, physical obstruction of circulation, invasion of organs, and other physical damage due to vegetative growth of the fungus (GIMENES *et al*., 2014). This explains the rapid mortality of the leaf-cutting ants observed in the present study, since in the first days of incubation, insect corpses were found, later confirmed by the infection of entomopathogenic fungal isolates.

The fungi of the genera *Beauveria* and *Metarhizium* are among the main fungal genera acting as regulatory mechanisms of insect populations in several ecosystems. *B. bassiana* and *M. anisopliae*, in turn, are considered two fungal species widely employed in studies of insect pest control in several agricultural and forestry

crops (CASTILLO *et al*., 2012). The fungus *Cordyceps fumosorosea*, on the other hand, is in the initial phase of studies, and there are no reports in the literature about its use aiming at controlling leaf-cutting ants. However, considering the results of the present study, its use is promising, considering that it caused high mortality of the specimens in the different concentrations of spores used, as well as revealing the lowest estimated value of CL_{90} (Table 4).

This study presents promising results regarding the biological control of *A. heyeri* with the use of entomopathogenic fungi, considering that data on this interaction is already found in the literature (SANTOS *et al*., 2020). However, studies referring to lethal concentration values, which are a good indication of the fungi's efficiency, are missing. Moreover, it is worth noting the potential of *C. fumosorosea*, which resulted in high mortality rates of specimens of leaf-cutting ants at different spore concentrations, reaching 100% mortality when using the concentration of 1.0×10^{8} spores mL^{-1}.

Other studies explored different concentrations of entomopathogenic fungal spores to control leaf-cutting ants (SANTOS *et al*., 2020; LOUREIRO; MONTEIRO, 2005), using spore concentrations that reached 1.0×10^{9} spores mL^{-1}. However, in the present research, lower concentrations of spores were used, which caused high mortality of leaf-cutting ant specimens, indicating a more economical alternative for the management of these insects.

Leaf-cutter ants exhibit a large amount of behavioral and immunological defenses to avoid contamination in the colony. Therefore, struggle using parasitic fungi becomes difficult under field conditions, even though satisfactory results are obtained in the laboratory (CASTILHO *et al*., 2012). Thus, some studies tested parasitic fungi associated with traditional methods to circumvent these defenses. Lopez and Orduz (2003) carried out tests using granulated baits together with entomopathogens and obtained encouraging results. Galvanho *et al*. (2012), in turn, conducted experiments associating *B. bassiana* with the insecticide imidacloprid, verifying that this neonicotinoid act on the nervous system, alters the behavior of the insect, and leads to a reduction in activities such as self-grooming, allowing the infection to spread more easily through the colony.

It is worth noting that the action of entomopathogenic fungi differs depending on the studied group, as well as the form of application. As stated by Loureiro and Monteiro (2005), individuals of a particular caste may be more susceptible than those of another, or a species of fungus may be more pathogenic for soldiers than for workers. This study demonstrated the potentiality of these control agents in *A. heyeri* workers, by application via direct contact of the pathogen with the insect, making possible the feasibility of this application in the field.

New laboratory and field studies, involving different species and isolates, spore concentrations, ant castes, and forms of application need to be conducted to better assess the efficiency of fungi as controlling agents of these insects in field conditions, enabling their use in programs of integrated pest management.

CONCLUSIONS

Based on the results of this study, it can be concluded that:

• Fungal isolates from *Beauveria bassiana* (Bals.) Vuill, *Cordyceps fumosorosea* (Wize), and *Metarhizium anisopliae* (Metsch.) Sorokin offer control of *Acromyrmex heyeri* (Forel) at levels from 26% to 100%.

• The use of the entomopathogenic fungus *C. fumosorosea* as a biological control agent for *A. heyeri* is promising, considering that it caused high mortality of specimens in all tested spore concentrations.

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