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Susceptibility of *Atta sexdens rubropilosa* (Forel) (Hymenoptera: Formicidae) to *Metarhizium anisopliae* (Metsch.) Sorokin in laboratory conditions

Suscetibilidade de *Atta sexdens rubropilosa* (Forel) (Hymenoptera: Formicidae) a *Metarhizium anisopliae* (Metsch.) Sorokin em condições de laboratório

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Abstract
This study aimed to evaluate the pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* on leaf-cutting ants *Atta sexdens rubropilosa*, under laboratory conditions. It was inoculated suspensions containing different concentrations of conidia on specimens of soldiers and workers, kept under controlled conditions. The mortality was verified daily until 15 days after inoculation. The experimental delineation was entirely random. Sporulation data were submitted to variance analysis and the average of the treatments were compared to each other by the Scott-Knott test. Probit analysis was performed to obtain the values of LT_{50} (in days). All isolates were pathogenic to *A. sexdens rubropilosa*, being more virulent for workers than for soldiers. Isolates IBCB 348, IBCB 410 and IBCB 425 were the most efficient in the mortality of the ants. Isolates IBCB 425 and UFGD 03 caused high percentages of confirmed mortality. The results showed that the tested isolates have the potential for using them as control agents of *A. sexdens rubropilosa*.
Keywords: Leaf-cutting ants; Microbial control; Entomopathogenic fungi.

Resumo
O objetivo deste trabalho foi avaliar a patogenicidade do fungo entomopatogênico *Metarhizium anisopliae* sobre formigas cortadeiras *Atta sexdens rubropilosa*, em condições de laboratório. Foi realizada a inoculação de suspensões contendo diferentes concentrações de conídios em exemplares de soldados e operárias, mantidos sob condições controladas. A mortalidade foi verificada diariamente até 15 dias após a inoculação. O delineamento experimental utilizado foi o inteiramente casualizado. Os dados de esporulação foram submetidos à análise de variância e as médias de tratamentos comparadas pelo teste de Scott-Knott. Foi realizada análise de Probit para obtenção dos valores de

TL50 (em dias). Todos os isolados foram patogênicos a *A. sexdens rubropilosa*, sendo mais virulentos para operárias do que para soldados. Os isolados IBCB 348, IBCB 410 e IBCB 425 foram os mais eficientes na mortalidade das formigas, IBCB 425 e UFGD 03 causaram altas porcentagens de mortalidade confirmada. Portanto, os isolados testados apresentam potencial para utilização como agentes de controle de *A. sexdens rubropilosa*.
Palavras-chave: Formigas cortadeiras; Controle microbiano; Fungos entomopatogênicos.

Resumen
El objetivo de este trabajo fue evaluar la patogenicidad del hongo entomopatógeno *Metarhizium anisopliae* sobre la hormiga cortadora de hojas *Atta sexdens rubropilosa*, en condiciones de laboratorio. Se inocularon suspensiones que contenían diferentes concentraciones de conidios en especímenes de soldados y trabajadores, mantenidos en condiciones controladas. La mortalidad se controló diariamente hasta 15 días después de la inoculación. El diseño experimental utilizado fue completamente al azar. Los datos de esporulación se sometieron a análisis de varianza y las medias de los tratamientos se compararon mediante la prueba de Scott-Knott. Se realizó análisis probit para obtener valores de TL50 (en días). Todos los aislados fueron patógenos para *A. sexdens rubropilosa*, siendo más virulentos para los trabajadores que para los soldados. Los aislados IBCB 348, IBCB 410 e IBCB 425 fueron los más eficientes en la mortalidad de hormigas, IBCB 425 y UFGD 03 provocaron altos porcentajes de mortalidad confirmada. Por lo tanto, los aislamientos probados tienen potencial para su uso como agentes de control de *A. sexdens rubropilosa*.
Palabras clave: Hormigas cortadoras de hojas; Control microbiano; Hongos entomopatógenos.

1. Introduction

The leaf-cutting ants are among the main pests from the Neotropical regions and are responsible for great damage in mixed-farming and forest systems (Hernández & Jaffé, 1995; Matrangolo et al., 2010). *Atta sexdens rubropilosa* (Forel), popularly known as leaf-cutting ant, are widely distributed species through Brazil (Motta & Luxnich, 2014) and attacks several cultures, pastures and reforestations, influencing any vegetal material (Boaretto & Forti, 1997). The ants cut and transport the vegetal material to the nests to cultivate a symbiotic fungus, which they use as food (Della Lucia & Souza, 2011).

The leaf-cutting ants present a high social complexity with populous colonies and a high level of polymorphism, which allows efficient foraging with great quantities of vegetal material, characteristics that make them herbivores that stand out in the Neotropical region (Holldobler & Wilson, 1990). These complex features presented by these insects, combined with a protective system for the queen, production of antimicrobial substances, "grooming" (cleansing) and recognition and removal of contaminated materials (Marinho et al., 2006), complicate their control. The control of leaf-cutting ants is carried out with great quantities of chemical products, mainly with the use of granulated baits. However, these products are highly toxic, costly and have reduced efficiency, since they lead to a feigned extermination of ants' colonies and selection of stronger populations (Diehl-Fleig et al., 1993; Silva & Diehl-Fleig, 1988).

Entomopathogenic fungi act in the natural and microbial control of insects, constituting an important factor in pest deletion. The occurrence of these pathogens may be noticed through epizooties and enzooties on insect's populations under natural conditions (Leite et al., 2003; Lacey et al., 2015). *Beauveria bassiana* and *Metarhizium anisopliae* have been found contaminating *A. sexdens rubropilosa* and *A. biospheric* queens (Jaccoud et al., 1999; Travaglini et al., 2016; Loureiro et al., 2022). Despite the defense and recognition mechanisms against pathogenic agents presented by the leaf-cutting ants, promising results have been gotten in the microbial control of these insects (Wilcken & Berti Filho, 1994). However, these results vary according to the type of studied castes and the manner of application of these agents (Loureiro & Monteiro, 2004, 2005; Dornelas et al., 2016). Therefore, this work aimed to evaluate the efficiency of isolates of *M. anisopliae* entomopathogenic fungus for controlling the soldiers and workers ants of *A. sexdens rubropilosa*, under laboratory conditions.

2. Methodology

Soldiers and workers from *A. sexdens rubropilosa* were collected from nests at the UFGD campus and placed into recipients with screened lids, separated by castes. In the laboratory, the ants were put into a freezer for some seconds for

contention of them.

For this study, we used five isolates of *M. anisopliae*: CG 423, IBCB 348, IBCB 410, IBCB 425 and UFGD 03). The fungus was sowed at Petri dishes containing solid and sterilized culture medium potato-dextrose-agar (PDA) by the three points method and then incubated in a chamber (B.O.D.) at 25 ±1°C, 70% RH, and under a 12h photoperiod for 7 to 15 days. Posteriorly, the conidia formed on the Petri dishes were collected with a nickel-chrome loop, previously sterilized with flame, and transferred to tubes containing 10mL of sterile distilled water and 0.1 mL of Tween 80, from which serial dilutions were prepared and standardized in concentrations of 1.0 × 107, 1.0 × 108, 1.0 ×109 and 1.0 × 1010 conidia mL-1 (Diehl-Fleig et al., 1993).

For ant infection, 1mL of each suspension was applied in Petri dishes. After the application of the suspension, groups of 10 were placed on the dishes. After infection, the insects were maintained without food in a chamber (B.O.D) at 25±1°C, 70% RH, and under a 12h photoperiod, for 15 days (Loureiro & Monteiro, 2005). Petri dishes were observed every day, for 15 days, to verify the mortality. The dead insects were immersed in a solution of alcohol at 70% and transferred to new chambers at 25±1°C, 70% RH and under a 12h photoperiod, to confirm mortality by the pathogen.

The experimental design was randomized, using five replicates per concentration (1.0x107, 1.0x108, 1.0x109 and 1.0x1010 con mL-1) for six different strains (CG 423, IBCB 348, IBCB 410, IBCB 425 and UFGD 03), each replicate containing 10 ants (soldiers and workers). Data of confirmed mortality were calculated as total accumulated mortality. The data concerning the sporulation were submitted for analysis of variance and the mean values were compared by the Scott-Knott test at a 5% level of probability. To obtain the values of median lethal times (LT_{50}) in days, Probit analysis was achieved for different treatments (Loureiro & Monteiro, 2004).

3. Results and Discussion

All tested isolates of *M. anisopliae* fungus were pathogenic to soldiers and workers of *A. sexdens rubropilosa* (Tables 1 and 2). Silva & Diehl- Fleig (1988) tested different isolates of *B. bassiana* and *M. anisopliae* for controlling the soldiers and leaf-cutting ants of *A. sexdens piriventris*. Through bioassays in the laboratory and tests in the field, the pathogenicity of both fungi to the insect was proved, obtaining LT_{50} of 2 and 3 days for the two isolates of *M. anisopliae* tested. According to the authors, the results obtained in the field indicate that there are possibilities for using these fungi for controlling the insect.

According to Probit analysis, there was no significant difference among the concentrations for the most tested isolates. In general, the lowest values of median lethal times were obtained with the concentrations that contained the highest quantities of conidia. In some cases, the isolates did not adapt to the Probit model, since a significant χ^2 and high heterogeneity of data occurred (Tables 1 and 2).

The isolates IBCB 348 and IBCB 410 were the most virulent for *A. sexdens rubropilosa* workers, with LT_{50} of 1.28 and 1.29 days respectively, at a concentration of 1.0×10^{10} conidia mL-1 (Table 2). Concerning the soldiers, IBCB 410 was the more virulent isolate, with values of LT_{50} varying between 1.87 and 2.99 days, followed by IBCB 425 (2.15 to 2.75 days) (Table 2).

The tested isolates were more virulent for workers than for soldiers. These results agree with those obtained by Alves and Sosa-Gomes (1983), which also proved a higher susceptibility of workers compared to the soldiers of *A. sexdens rubropilosa* for the isolates of *B. bassiana* and *M. anisopliae*. However, the highest median lethal times were verified while using *M. anisopliae* fungus (2.8 and 4.05 days for leaf cutting and soldiers' ants, respectively). The workers' ants perform a fundamental role in the maintenance of the fungal sponge, contributing to ensuring the development of symbiotic fungus, and assuring the nest keeps healthy (Bass & Cherrett, 1994). Thus, this caste may be considered a potential target for the control of the nests.

Table 1. Median Lethal Times (LT_{50}) in days, confidence intervals (CI) (P< 0.05), equations of linear regression, and values of χ^2 obtained by Probit analysis for the pathogenic activity of *M. anisopliae* on *Atta sexdens rubropilosa* soldiers.

Isolate/Concentration	LT_{50} (in days)	Confidence Interval (CI)	Linear Regression Equation	χ^2
CG 423				
1.0×10^{10}	7.24	(5.56; 9.43)	Y= 1.92 + 3.57*logx	93.79*
1.0×10^{9}	5.98	(4.65; 7.68)	Y= 2.36 + 3.39*logx	63.10*
1.0×10^{8}	6.42	(5.51; 7.49)	Y= 2.01 + 3.69*logx	27.24*
1.0×10^{7}	4.25	(3.42; 5.28)	Y= 3.63 + 2.17*logx	13.18*
IBCB 348				
1.0×10^{10}	2.28	(1.75; 2.96)	Y= 3.71 + 3.57*logx	9.63*
1.0×10^{9}	2.97	(2.53; 3.48)	Y= 3.35 + 3.47*logx	8.50
1.0×10^{8}	2.48	(2.11; 2.92)	Y= 3.70 + 3.28*logx	6.92
1.0×10^{7}	2.71	(2.16; 3.41)	Y= 3.69 + 3.00*logx	16.75*
IBCB 410				
1.0×10^{10}	1.87	(1.33; 2.62)	Y= 4.12 + 3.20*logx	11.11*
1.0×10^{9}	2.30	(2.05; 2.59)	Y= 3.89 + 3.03*logx	2.92
1.0×10^{8}	2.08	(1.68; 2.56)	Y= 4.01 + 3.07*logx	6.66
1.0×10^{7}	2.99	(2.45; 3.63)	Y= 3.67 + 2.78*logx	12.38
IBCB 425				
1.0×10^{10}	2.15	(1.61; 2.88)	Y= 3.77 + 3.66*logx	11.74*
1.0×10^{9}	2.39	(1.93; 2.96)	Y= 3.56 + 3.79*logx	7.31
1.0×10^{8}	2.75	(1.99; 3.80)	Y= 3.74 + 2.85*logx	25.27*
1.0×10^{7}	2.29	(1.90; 2.75)	Y= 3.86 + 3.14*logx	5.83
UFGD 03				
1.0×10^{10}	5.59	(4.58; 6.82)	Y= 2.26 + 3.66*logx	51.90*
1.0×10^{9}	5.29	(4.28; 6.53)	Y= 2.53 + 3.41*logx	17.79*
1.0×10^{8}	6.01	(4.62; 7.81)	Y= 2.34 + 3.40*logx	38.09*
1.0×10^{7}	6.55	(5.51; 7.79)	Y= 1.88 + 3.81*logx	45.02*

* Significant x^2 (P < 0,05). Source: Authors (2022).

Jaccoud et al. (1999) investigated the effects of *M. anisopliae*, applying spores of the fungus in foraging arenas of mini-nests from *A. sexdens rubropilosa*. All treated nests suffered an increase in ants' mortality during the first ten days after application. Besides, the reduction of foraging activity was verified as well as the effect on the garden health of the fungus. The ants' mortality was particularly high for the median workers, which performed a great role in trying to clean the spores. Therefore, being a group more exposed, it is expected that this class suffer the most elevated indexes of mortality.

For the isolate CG 423, when applied to a lower concentration of conidia in soldiers (1.0×10^{7} conidia mL-1), occurred a higher rate of mortality, and on the 10^{th} day after the inoculation, it was obtained 100% of accumulated mortality, while for the higher concentration (1.0×10^{10} conidia mL^{-1}), the total mortality of the soldiers was obtained at the 13^{th} day (Fig 1). This indicates an interesting feature, since it becomes more advantageous to use a lower quantity of propagules to occur the development of the disease. On the other hand, the results using a high potential of inoculation may be unexpected, since a great number of fungus conidia on insect tegument can influence negatively their germination or can favor the penetration of bacteria, generating septicemia and killing the insect quickly (Alves & Lecuona, 1998).

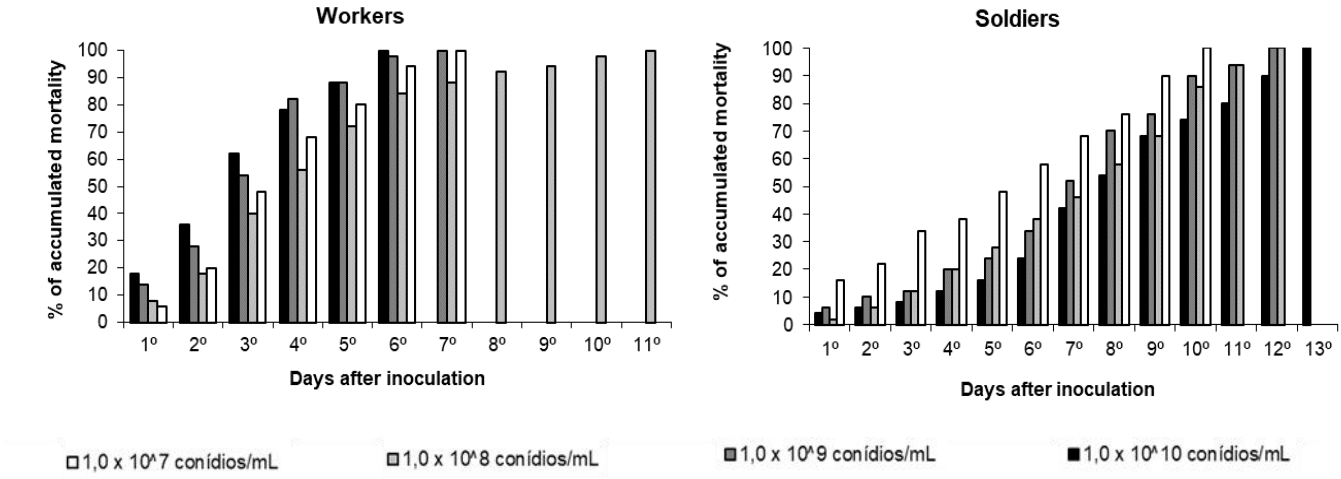
Table 2. Median Lethal Times (LT_{50}) in days, confidence intervals (CI) (P< 0.05), equations of linear regression and values of $\chi 2$ obtained by Probit analysis for pathogenic activity of *M. anisopliae* on *Atta sexdens rubropilosa* workers.

Isolate/Concentration	LT_{50} (in days)	Confidence Interval (CI)	Linear Regression Equation	$\chi 2$
CG 423				
1,0 x 10^{10}	2,27	(1,88; 2,75)	Y= 3,91 + 3,02.logx	2,21
1,0 x 10^{9}	2,44	(1,92; 3,10)	Y= 3,54 + 3,75.logx	9,22
1,0 x 10^{8}	3,31	(3,00; 3,66)	Y= 3,16 + 3,52.logx	5,83
1,0 x 10^{7}	2,97	(2,59; 3,41)	Y= 3,10 + 3,99.logx	3,84
IBCB 348				
1,0 x 10^{10}	1,28	(0,77; 2,11)	Y= 4,66 + 3,10.logx	2,8
1,0 x 10^{9}	1,62	(1,07; 2,44)	Y= 4,30 + 3,28.logx	8,17*
1,0 x 10^{8}	2,16	(1,33; 3,50)	Y= 4,02 + 2,90.logx	13,01*
1,0 x 10^{7}	2,08	(1,68; 2,58)	Y= 3,79 + 3,76.logx	6,44
IBCB 410				
1,0 x 10^{10}	1,29	(0,84; 2,01)	Y= 4,63 + 3,22.logx	2,33
1,0 x 10^{9}	1,74	(0,89; 3,39)	Y= 4,25 + 3,07.logx	7,66*
1,0 x 10^{8}	1,59	(1,16; 2,17)	Y= 4,19 + 3,98.logx	2,32
1,0 x 10^{7}	2,03	(1,56; 2,64)	Y= 3,85 + 3,72.logx	5,29
IBCB 425				
1,0 x 10^{10}	1,77	(1,15; 2,70)	Y= 4,30 + 2,78.logx	12,89*
1,0 x 10^{9}	2,58	(1,96; 3,39)	Y= 3,78 + 2,94.logx	13,53*
1,0 x 10^{8}	2,49	(2,14; 2,89)	Y= 3,65 + 3,38.logx	1,75
1,0 x 10^{7}	2,78	(2,43; 3,17)	Y= 3,23 + 3,97.logx	1,79
UFGD 03				
1,0 x 10^{10}	4,08	(3,63; 4,60)	Y= 2,55 + 4,00.logx	15,71
1,0 x 10^{9}	3,46	(3,04; 3,95)	Y= 3,00 + 3,68.logx	7,29
1,0 x 10^{8}	3,62	(3,22; 4,08)	Y= 3,12 + 3,34.logx	11,18
1,0 x 10^{7}	3,77	(3,56; 3,99)	Y= 2,85 + 3,71.logx	1,84

* Significant x^2 (P < 0,05). Source: Authors (2022).

For isolates IBCB 348 and IBCB 410, the accumulated mortality of workers and soldiers occurred at 100%, between 5 and 7 days after inoculation, respectively, using the concentration of 1.0×10^{10} conidia mL^{-1} (Figures 2 and 3). The speed the pathogen kills its host is a desirable feature for the control of many pests, but it does not have to be considered unique. The isolate must be able to provide high final mortality (Tamai et al., 2002). For social insects such as ants, the fast effect of the insecticide is not a desirable feature, as these insects present behavior colony protection, which involves the isolation of sick individuals from the rest of the colony, preventing the transmission and/or the spread of the fungus between the healthy individuals.

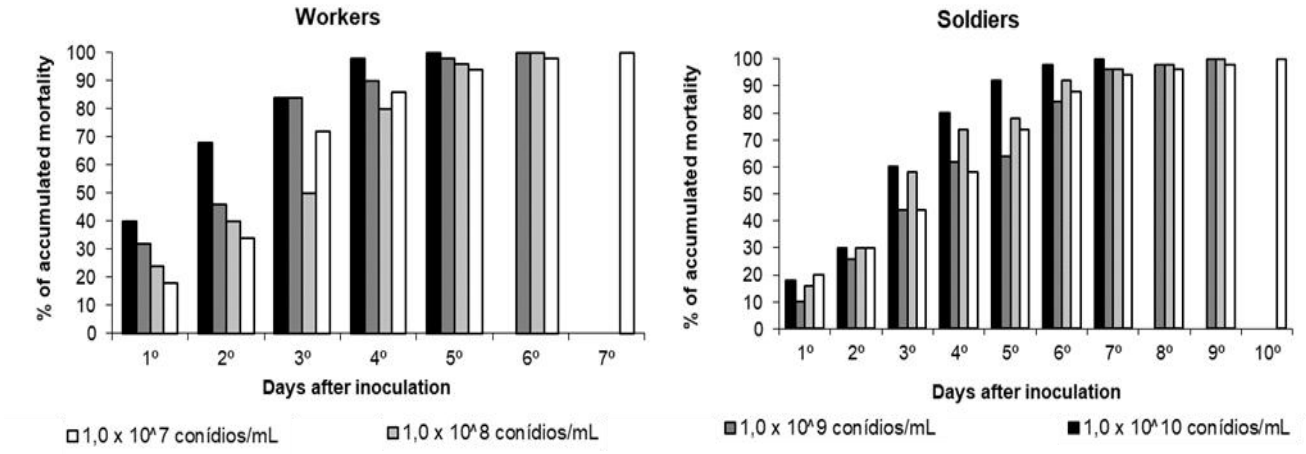
Figure 1 – Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *M. anisopliae*, isolate CG 423 (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).



Source: Authors (2022).

About the isolate IBCB 425, it was obtained accumulated mortality of 100% for soldiers and workers at 7 and 8 days after the inoculation, respectively (Fig 4). High percentages of confirmed mortality were also obtained in soldiers (92 to 98%) and workers (96 to 99%) (Table 3). For the isolate UFGD 03, it was obtained 100% of accumulated mortality of the soldiers and workers 9 days after the inoculation, using the concentration of 1.0×10^9 conidia mL^{-1} (Fig 5).

Figure 2 - Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *M. anisopliae*, isolate IBCB 348 (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).



Source: Authors (2022).

The variation of pathogenicity among the isolates may be related to factors such as low virulence of the isolate, classification, tolerance of the host and others (Alves, 1998; Loureiro et al., 2022). According to Paccola-Meirelles & Azevedo (1990), this variability is the result of differences in enzymes and toxins production (amylase, protease, lipase), speed of conidia germination, mechanical activity in the cuticle penetration, and capacity of colonization of the isolates. The confirmed mortality, represented by the number of cadavers in which the sporulation of the fungus was observed, varied between 46 to 98% for workers and 52 to 98% for soldiers, and no significant difference occurred between the values of sporulation for

workers and soldiers (Table 3). There was also no significant difference between the tested concentrations regarding the confirmed mortality, except for the isolate UFGD 03, in the concentration 1.0×10^7 conidia mL^{-1} (Table 3).

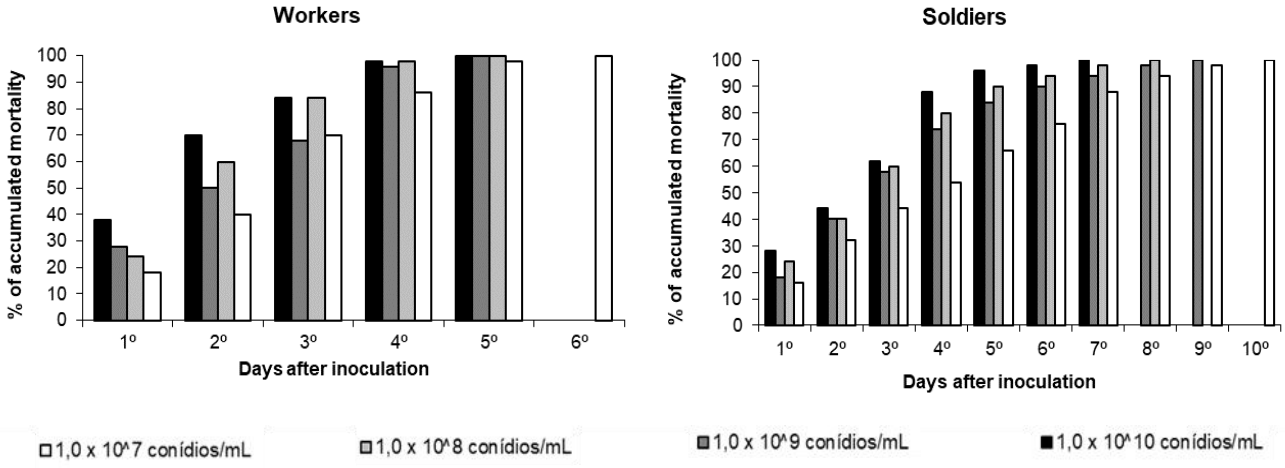
Table 3. Confirmed mortality (%) (± SE) from *A. sexdens rubropilosa* workers and soldiers after inoculation with *M. anisopliae* (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).

Treatments		***Metarhizium anisopliae***		
Isolate	**Concentration**	**Soldiers**	**Workers**	**VG (%)**
Control	Without Tween®	0.00 ± 0.00 dA	0.00 ± 0.00 cA	0.00
Control	With Tween®	0.00 ± 0.00 dA	0.00 ± 0.00 cA	0.00
CG 423	107	54.00 ± 5.09 cA	54.00 ± 8.12 bA	31.23
	108	52.00 ± 7.34 cA	50.00 ± 3.16 bA	27.32
	109	62.00 ± 3.74 cA	58.00 ± 3.74 bA	16.29
	1010	70.00 ± 5.47 cA	64.00 ± 5.09 bA	21.45
IBCB 348	107	58.00 ± 5.83 cA	50.00 ± 4.47 bA	24.17
	108	52.00 ± 6.63 cA	48.00 ± 3.74 bA	26.62
	109	52.00 ± 3.74 cA	52.00 ± 3.74 bA	17.76
	1010	58.00 ± 3.74 cA	52.00 ± 3.74 bA	17.28
IBCB 410	107	58.00 ± 5.83 cA	56.00 ± 5.09 bA	24.30
	108	54.00 ± 6.78 cA	46.00 ± 5.09 bA	29.18
	109	60.00 ± 3.16 cA	56.00 ± 5.09 bA	18.63
	1010	68.00 ± 5.83 cA	62.00 ± 3.74 bA	20.41
IBCB 425	107	98.00 ± 2.00 aA	96.00 ± 2.44 aA	15.71
	108	96.00 ± 2.44 aA	98.00 ± 2.00 aA	15.71
	109	92.00 ± 3.74 aA	98.00 ± 2.00 aA	18.34
	1010	92.00 ± 2.00 aA	96.00 ± 2.44 aA	17.34
UFGD 03	107	64.00 ± 5.09 cB	96.00 ± 4.00 aA	21.52
	108	80.00 ± 4.47 bA	90.00 ± 7.74 aA	28.29
	109	84.00 ± 6.78 bA	86.00 ± 8.71 aA	35.77
	1010	90.00 ± 5.47 aA	92.00 ± 3.74 aA	25.47
VG (%)		**24.57**	**25.74**	

* Significant x^2 ($P < 0,05$). Source: Authors (2022).

The isolate IBCB 425 caused the highest percentage of confirmed mortality, varying between 92 and 98% for soldiers and 96 and 98% for workers, followed by the isolate UFGD 03, varying between 64 and 90% for soldiers and 86 and 96% for workers; both differing significantly from the others. The elevated confirmed mortality is an important feature since the capacity to produce propagules from the pathogen may unleash the epizooties in the field, through the dispersion of them in the environment and the contamination of healthy individuals (Alves & Lecuona, 1998). Furthermore, the confirmed mortality may be chosen as a parameter to study the behavior of the best concentration because the fungi, as biological control agents, differ from the chemical products through the capacity of increasing the pathogen density by the dispersion of secondary inoculum, repeating the cycle through the host population (Hajek & St. Leger, 1994).

Figure 3 - Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *M. anisopliae*, isolate IBCB 410 (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).

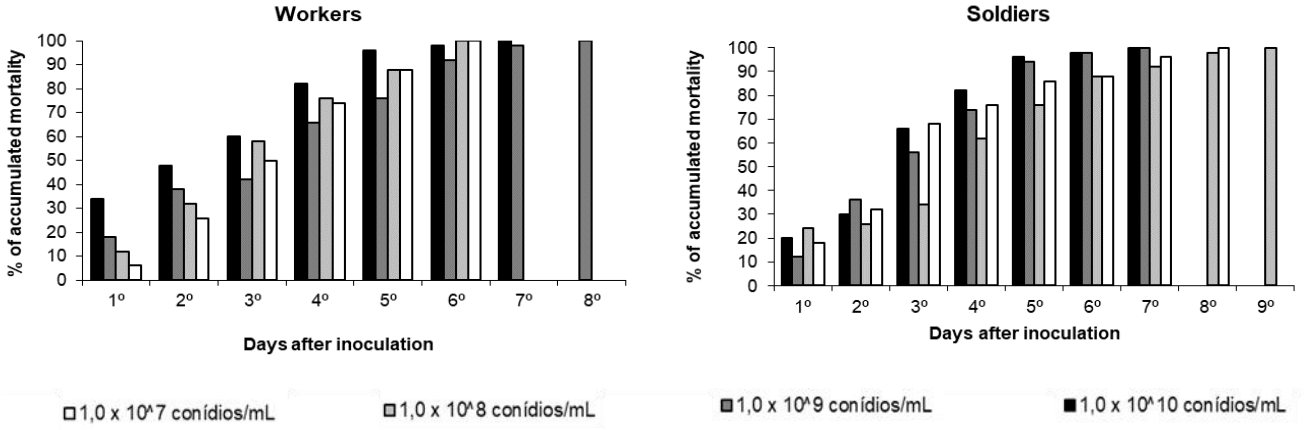


Source: Authors (2022).

The low percentage of confirmed mortality presented for some isolates may be related to some factors. It did not discard the possibility that the insects had been dead by the fungus. In some cases, the alcohol used for the external disinfection may become unviable to the fungus after its entrance into the interior of the cadaver, through casual fissures on the tegument, caused during the handling of them (Tamai et al., 2002). Furthermore, the external contamination of the insects also may be higher when they are handled during the installation of the bioassays. The fungi penetration, mainly when used in high concentrations, may cause the appearance of "orifices" on the tegument of the insects, which may be attacked by other microorganisms. In this way, because bacteria grow much faster than fungi, they can settle in the host's body, causing septicemia, which obstructs the growth of the entomopathogen fungi. This fact can interfere with the results of confirmation death of the insect through the fungus (Alves & Pereira, 1998). According to Jaccoud et al. (1999), the stress caused by fungus presence is sufficient to change the social homeostasis of the colony and kill it. From the control perspective, this feature is particularly interesting, suggesting that it is not necessary to kill all the ants directly with the control agent. In the control treatments did not occur fungus sporulation over the cadavers, which indicates the mortality that occurred in this group was not a result of the infection by *M. anisopliae* fungus (Table 3).

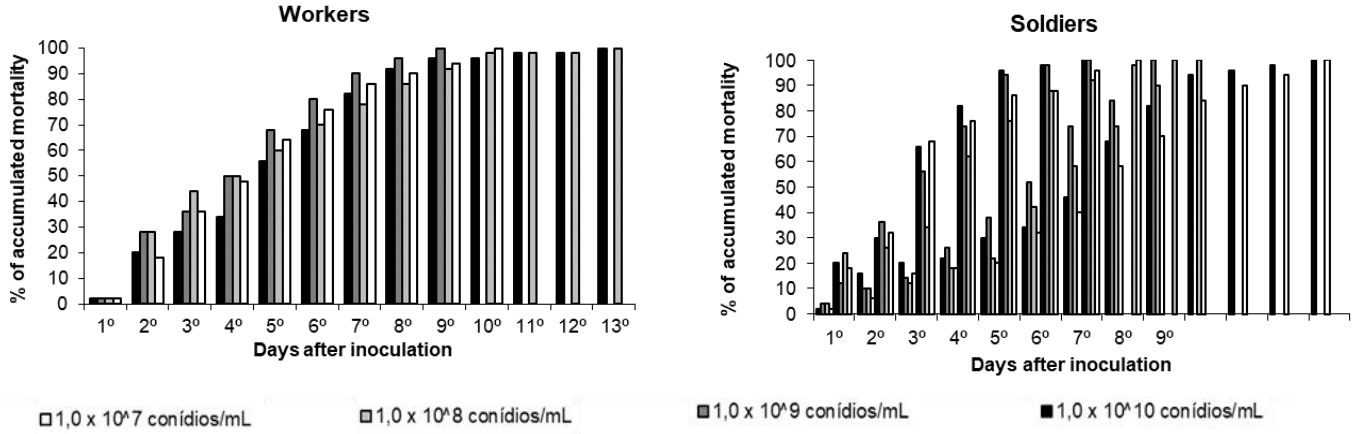
Alves & Lecuona (1998) confirm that for each pest is possible to select one efficient entomopathogen for its control. However, for being possible the acquisition of new isolates of the fungi that may fight against *A. sexdens rubropilosa*, it is necessary to continue the selection, programs and carry out new tests with a higher number of isolates. In this work, it was used isolates that have never been tested for controlling leaf-cutting ants, and the results indicate a good potential of them as control agents. For controlling social insects, it is very important to consider the lethal time and the capacity of sporulation from the isolate over the cadavers. Thus, the lower the lethal time associated with fast sporulation, the higher the chances of the fungus achieving an elevated potential of inoculum into the nests before they manage to remove all the cadavers (Stimac et al., 1987). From that perspective, the isolate IBCB 425 obtained good results and must be considered to control the leaf-cutting ants, since it presented an elevated percentage of sporulation over the cadavers.

Figure 4 - Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *M. anisopliae*, isolate IBCB 425 (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).



Source: Authors (2022).

Figure 5 - Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *M. anisopliae*, isolate UFGD 03 (25 ± 1°C; 70 ± 10% RH; 12h photoperiod).



Source: Authors (2022).

4. Conclusion

All the tested isolates of *M. anisopliae* were pathogenic to *A. sexdens rubropilosa*, which were more virulent for the workers than for the soldiers. The isolates IBCB 348 and IBCB 410 were the most efficient for the workers' mortality. For the soldiers, the most virulent isolates were IBCB 410 and IBCB 425. The isolates UFGD 03 and IBCB 425 caused high percentages of confirmed mortality. The tested isolates have the potential for using them as control agents of *A. sexdens rubropilosa*.

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