Pathogenicity of *Beauveria bassiana* to *Atta sexdens* rubropilosa (Hymenoptera: Formicidae) in laboratory conditions

Patogenicidade de *Beauveria bassiana* sobre *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) em condições de laboratório

Patogenicidad de *Beauveria bassiana* a *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) en condiciones de laboratorio

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Abstract
This research, under laboratory condition, evaluated the efficiency of different strains of entomopatogenic fungus *Beauveria bassiana* to control leaf-cutting ant *Atta sexdens rubropilosa*. Soldiers and workers were inoculated with suspensions containing 1.0×10^7, 1.0×10^8, 1.0×10^9 and 1.0×10^{10} con mL^{-1} and maintained in B.O.D, at 25±1°C, 70% RH and 12 hours of photophase, without food. The mortality was evaluated daily, with 15 days after inoculation. Experimental design was entirely random. The sporulation data were submitted to the analysis of variance and the LT_{50} values (in days) were obtained through the Probit analysis. All strains of *B. bassiana* were pathogenic, being workers more susceptible than soldiers. The strains IBCB 21 and IBCB 07 caused high percentages of confirmed mortality showing potential for use in the control of *A. sexdens rubropilosa*. Strain IBCB 21 was more virulent for workers, whereas isolated IBCB 07 was more virulent for soldiers. Both isolates presented no significant difference among tested concentrations.
Keywords: Leaf-cutting ants; Entomopathogenic fungi; Microbial control.

Resumo
Este trabalho avaliou a eficiência de diferentes isolados do fungo entomopatogênico *Beauveria bassiana* no controle de formigas cortadeiras *Atta sexdens rubropilosa*, em condições de laboratório. Suspensões contendo $1,0 \times 10^7$, $1,0 \times 10^8$, $1,0 \times 10^9$ e $1,0 \times 10^{10}$ con mL^{-1} foram inoculadas em soldados e operárias, mantidos em câmara B.O.D, a 25±1°C, 70% UR e fotofase de 12 horas, sem alimentação. A mortalidade foi verificada diariamente, até 15 dias após a inoculação. O delineamento experimental utilizado foi inteiramente casualizado. Os dados referentes à esporulação foram submetidos à análise de variância e os valores de TL_{50} (em dias) foram obtidos através da análise de Probit. Todos os isolados de *B. bassiana* foram patogênicos, sendo as operárias mais suscetíveis que os soldados. Os isolados IBCB 21 e IBCB 07 foram os mais virulentos para operárias e soldados, respectivamente. O isolado IBCB 21 também

causou altas porcentagens de mortalidade confirmada, apresentando potencial para utilização no controle de *A. sexdens rubropilosa*.

Palavras-chave: Formigas cortadeiras; Fungo entomopatogênico; Controle microbiano.

Resumen

Este trabajo evaluó la eficiencia de diferentes aislamientos del hongo entomopatógeno *Beauveria bassiana* en el control de la hormiga cortadora de hojas *Atta sexdens rubropilosa*, en condiciones de laboratorio. Las suspensiones conteniendo $1{,}0 \times 10^7$, $1{,}0 \times 10^8$, $1{,}0 \times 10^9$ e $1{,}0 \times 10^{10}$ con mL^{-1} fueron inoculadas en soldados y trabajadores, mantenidos en un B.O.D, a $25 \pm 1°C$, 70% UR e fotofase de 12 horas, sem alimentación. La mortalidad se controló diariamente, hasta 15 días después de la inoculación. El diseño experimental utilizado fue completamente al azar. Los datos relativos a la esporulación se sometieron a análisis de varianza y los valores de LT_{50} (em días) se obtuvieron mediante análisis Probit. Todos los aislamientos de *B. bassiana* fueron patógenos, siendo los trabajadores más susceptibles que los soldados. Los aislamientos IBCB 21 y IBCB 07 fueron los más virulentas para trabajadores y soldados, respectivamente. El aislado IBCB 21 también causó altos porcentajes de mortalidad confirmada, mostrando potencial para su uso en el control de *A. sexdens rubropilosa*.

Palabras clave: Hormigas cortadoras de hojas; Hongos entomopatógenos; Control microbiano.

1. Introduction

The leaf-cutting ants, that belong to *Atta* genus, Attini tribe (Hymenoptera: Formicidae), are considered herbivores which stand out in the Neotropical region (Holldobler & Wilson, 1990) and are responsible for great damages in vegetal production (Hernández & Jaffé, 1995). With an intense activity during all the year, they attack practically all cultures, pastures, and the reforestations (Boaretto & Forti, 1997; Britto et al., 2016), cutting and transporting it to their nests, where they cultivate a symbiotic fungus, using it as food. The damage caused is more prominent in the pre-cut phases (reform areas or forest management) and immediately after planting or at the beginning of sprouting conduction (Giesel et al., 2020).

The leaf-cutting ants have several biological features and complex behavior, such as architecture, size, and place of the nests; system of protection for the queen; production of anti-microbial substances and "grooming" (cleansing), which complicate their control (Marinho et al., 2006). Most of their control methods are based on application of great quantities of chemical products. However, these products are expensive and generally not effective since they only apparently exterminate the colony or change the place of the nest. Furthermore, these methods lead to selection of resistant populations, and cause damages to the environment and human health, since they are extremely toxic (Diehl-Fleig et al., 1993; Meirelles et al., 2015).

Entomopathogenic fungi are important agents that act in natural and microbial control of the insects, through the epizooties and enzooties, which represent an important factor of pests' deletion (Jaccoud et al., 1999). The efficiency of *B. bassiana* for controlling the leaf-cutting ants has been demonstrated in several works, showing encouraging perspectives for the problem (Alves & Sosa-Gomes, 1983; Wilcken & Berti Filho, 1994; Cantú-Ruiz et al., 2017). Thus, this present work aimed to evaluate the pathogenicity of different strains from the entomopathogenic fungus *B. bassiana* on the soldiers and workers of *Atta sexdens rubropilosa*, under laboratory conditions.

2. Methodology

The specimens of soldiers and workers from *Atta sexdens rubropilosa* were collected from nests at the UFGD campus and placed in recipients with screened lids, separated by castes. In the laboratory they were taken into a freezer for some seconds for contention of them.

We used the following *Beauveria bassiana* strains: IBCB 07, IBCB 21 and IBCB 66, belonging to the entomopathogenic bank from the Biologic Institute of Campinas; the strain ESALQ 447, which belongs to the superior school of agriculture Luiz de Queiroz (ESALQ/USP); and the UFGD 02 and UFGD 11 strains, that belongs to the entomopathogenic bank of Federal University of Mato Grosso do Sul (UFMS/CPCS). The fungal cultures used for infection were obtained by

increasing the fungus on Petri dishes containing solid and sterilized culture medium potato-dextrose-agar (PDA). After sowing by the three points methods, the Petri dishes were incubated in a chamber (B.O.D.) at $25\pm1°C$, 70% RH, and under a 12h photoperiod for 7 to 15 days. Subsequently, the conidia formed on the surface of *B. bassiana* colonies were collected with nickel-chrome loop, previously sterilized with flame, and transferred to tubes containing 10 mL of sterile distilled water and 0.1 mL of Tween 80. From these tubes containing 10 mL of conidia suspension, serial dilutions were prepared and standardized in concentrations of 1.0×10^7, 1.0×10^8, 1.0×10^9 and 1.0×10^{10} con mL^{-1} (Diehl-Fleig et al., 1993).

Ants' infection was performed according to Loureiro and Monteiro (2005). After infection, the insects were maintained without food in a chamber (B.O.D.) at $25\pm1°C$, 70% RH, and under a 12h photoperiod, for 15 days. During this period, water was replaced for the maintenance of the humidity when necessary.

Petri dishes were observed every day to verify the mortality. The corpses of dead ants were immersed in a solution of alcohol at 70% and transferred to new chambers at $25\pm1°C$, 70% RH and under a 12h photoperiod, to confirm mortality by the pathogen.

The experimental design was randomized, using five replicates per concentration (1.0×10^7, 1.0×10^8, 1.0×10^9 and 1.0×10^{10} con mL^{-1}) for six different strains (IBCB 07, IBCB 21, IBCB 66, ESALQ 447, UFGD 02 and UFGD 11), each replicate containing 10 ants (soldiers and workers). Data of the confirmed mortality and total accumulated mortality were calculated. The data concerning the sporulation were submitted to analysis of variance and the mean values were compared by the Scott-Knott test at a 5% level of probability. For obtaining the values of Median Lethal Times (LT$_{50}$) in days, Probit analysis was achieved for different treatments (Loureiro & Moino Júnior, 2008).

3. Results and Discussion

According Probit analysis, there was a significant difference among almost all of the tested concentrations for the strain UFGD 11, on both studied castes. Only the concentration 1.0×10^{10} for workers was not different. It was also observed a variation among the most effective concentrations for different strains. In some cases, the strains did not adapt to the Probit model, since a significant χ^2 and high heterogeneity of data occurred (Tables 1 and 2).

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 *Beauveria bassiana* on *Atta sexdens rubropilosa* workers.

Strain/ Concentration	LT_{50} (in days)	Confidence Interval (CI)	Linear Regression Equation	χ^2
IBCB 07				
1.0×10^{10}	1.86	(0.99; 3.49)	$Y= 4.04 + 3.52*logx$	9.12*
1.0×10^{9}	1.72	(1.33; 2.22)	$Y= 4.35 + 2.72*logx$	2.47
1.0×10^{8}	2.19	(1.63; 2.96)	$Y= 3.83 + 3.39*logx$	6.40
1.0×10^{7}	2.54	(1.94; 3.32)	$Y= 3.41 + 3.91*logx$	6.95
IBCB 21				
1.0×10^{10}	1.70	(1.02; 2.83)	$Y= 4.17 + 3.56*logx$	5.51
1.0×10^{9}	1.57	(1.17; 2.10)	$Y= 4.42 + 2.92*logx$	3.24
1.0×10^{8}	1.74	(1.27; 2.40)	$Y= 4.24 + 3.10*logx$	4.90
1.0×10^{7}	1.73	(1.19; 2.51)	$Y= 4.00 + 4.16*logx$	3.82
IBCB 66				
1.0×10^{10}	3.22	(3.02; 3.43)	$Y= 2.63 + 4.64*logx$	3.72
1.0×10^{9}	3.80	(3.37; 4.28)	$Y= 2.90 + 3.61*logx$	9.80
1.0×10^{8}	2.68	(2.48; 2.91)	$Y= 3.00 + 4.64*logx$	1.46
1.0×10^{7}	2.95	(2.86; 3.04)	$Y= 2.87 + 4.52*logx$	0.11
ESALQ 447				
1.0×10^{10}	2.52	(2.02; 3.13)	$Y= 3.41 + 3.94*logx$	8.37
1.0×10^{9}	1.93	(1.35; 2.74)	$Y= 4.03 + 3.37*logx$	7.76
1.0×10^{8}	2.69	(2.07; 3.50)	$Y= 3.36 + 3.79*logx$	6.49
1.0×10^{7}	2.21	(1.80; 2.72)	$Y= 3.69 + 3.76*logx$	6.25
UFGD 02				
1.0×10^{10}	3.09	(2.92; 3.26)	$Y= 2.86 + 4.35*logx$	0.38
1.0×10^{9}	2.92	(2.19; 3.88)	$Y= 3.17 + 3.91*logx$	16.02*
1.0×10^{8}	2.93	(2.34; 3.66)	$Y= 3.19 + 3.85*logx$	9.46
1.0×10^{7}	2.06	(1.85; 2.30)	$Y= 4.00 + 3.14*logx$	2.45
UFGD 11				
1.0×10^{10}	3.02	(2.53; 3.59)	$Y= 2.88 + 4.41*logx$	10.57
1.0×10^{9}	3.89	(3.20; 4.73)	$Y= 1.84 + 5.34*logx$	28.21*
1.0×10^{8}	3.66	(3.05; 4.39)	$Y= 1.99 + 5.33*logx$	17.08*
1.0×10^{7}	3.70	(3.12; 4.38)	$Y= 2.63 + 4.16*logx$	22.76*

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

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

| Strain/ Concentration	LT_{50} (in days)	Confidence Interval (CI)	Linear Regression Equation	χ^2
IBCB 07				
1.0×10^{10}	2.15	(1.62; 2.86)	$Y= 4.01 + 2.96*logx$	4.62
1.0×10^{9}	2.46	(2.00; 3.04)	$Y= 3.91 + 2.75*logx$	6.74
1.0×10^{8}	2.68	(2.19; 3.28)	$Y= 3.54 + 3.39*logx$	12.35
1.0×10^{7}	2.50	(2.18; 2.88)	$Y= 3.85 + 2.85*logx$	4.27
IBCB 21				
1.0×10^{10}	2.78	(2.53; 3.06)	$Y= 3.17 + 4.09*logx$	2.73
1.0×10^{9}	3.48	(2.96; 4.10)	$Y= 2.61 + 4.38*logx$	10.23
1.0×10^{8}	3.06	(2.75; 3.39)	$Y= 2.31 + 5.53*logx$	4.96
1.0×10^{7}	3.84	(3.27; 4.52)	$Y= 2.11 + 4.92*logx$	12.64*
IBCB 66				
1.0×10^{10}	4.10	(3.52; 4.78)	$Y= 2.07 + 4.77*logx$	19.85*
1.0×10^{9}	6.77	(5.72; 8.01)	$Y= 1.65 + 4.02*logx$	63.41*
1.0×10^{8}	4.30	(4.05; 4.55)	$Y= 2.61 + 3.76*logx$	4.18
1.0×10^{7}	3.09	(2.63; 3.63)	$Y= 2.17 + 5.76*logx$	8.42
ESALQ 447				
1.0×10^{10}	3.27	(2.39; 4.48)	$Y= 2.83 + 4.20*logx$	22.71*
1.0×10^{9}	3.13	(2.76; 3.56)	$Y= 3.49 + 3.03*logx$	4.85
1.0×10^{8}	4.08	(3.35; 4.96)	$Y= 2.80 + 3.58*logx$	22.52*
1.0×10^{7}	2.29	(2.00; 2.63)	$Y= 3.92 + 2.98*logx$	5.87
UFGD 02				
1.0×10^{10}	3.72	(2.41; 5.75)	$Y= 2.97 + 3.53*logx$	33.69*
1.0×10^{9}	3.09	(2.69; 3.56)	$Y= 3.10 + 3.85*logx$	8.00
1.0×10^{8}	3.79	(3.42; 4.21)	$Y= 3.21 + 3.08*logx$	4.85
1.0×10^{7}	3.71	(3.06; 4.48)	$Y= 3.27 + 3.02*logx$	15.67*
UFGD 11				
1.0×10^{10}	3.95	(3.15; 4.97)	$Y= 1.88 + 5.20*logx$	27.49*
1.0×10^{9}	4.03	(3.30; 4.94)	$Y= 2.44 + 4.20*logx$	35.40*
1.0×10^{8}	5.51	(4.90; 6.20)	$Y= 2.03 + 3.99*logx$	20.17*
1.0×10^{7}	5.51	(4.29; 7.07)	$Y= 2.02 + 4.01*logx$	63.16*

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

In general, all tested strains were more virulent for workers than for soldiers, once LT_{50} varied among 1.57 to 3.89 days for workers, and 2.15 to 6.77 for soldiers. Similar results were found in other studies that used different concentrations of *B. bassiana*. Alves and Sosa-Gomes (1983) confirmed a higher susceptibility of the workers in relation to the soldiers. They obtained LT_{50} of 3.56 and 3.93 days for leaf-cutting ants' workers and soldiers, respectively. Silva and Diehl-Fleig (1988) tested *B. bassiana* for another subspecies of *A. sexdens* (*A. sexdens piriventris*, a species of wide occurrence in Rio Grande do Sul) and obtained LT_{50} of 2.72 and 3.33. In the field, inoculated colonies with the fungi presented a total reduction of the external activity 60 days after the application (Silva & Diehl-Fleig, 1988). According to Bass and Cherret (1994) the soldiers played an important role in the maintenance of the fungal sponge, which contributes to ensuring the development of symbiotic fungus and assuring the nest keeps healthy. Thus, this caste may be considered as a potential target for the control of the nests.

Strain IBCB 21 was the most virulent for workers of *A. sexdens rubropilosa*, with LT_{50} varying between 1.57 and 1.74 days for different concentrations, with no significant difference among them (Table 1). For the soldiers, the most virulent strain was IBCB 07, with values of LT_{50} varying between 2.15 and 2.68 days and no significant difference among concentrations (Table 2). For strains IBCB 07 and IBCB 21, 100% mortality of workers occurred at five days after the inoculation using the concentration of $1.0x10^{10}$ con mL^{-1} (Figures 1 and 2). The speed which the pathogen kills its host is a desirable feature for

many pests control but it does not have to be considered unique. The strain must be able to provide high final mortality, which demands less frequent sprays when applied in the field, with the possibility of reducing the costs of control (Tamai et al., 2002). For social insects such as ants, the quick effect of chemical or biological insecticide is not desirable since these insects present the behavior of colony protection, which involves the isolation of sick individuals from the rest of the colony, preventing transmission and/or the spread of the fungus between healthy individuals (Marinho et al., 2006).

Figure 1. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana* strain IBCB 07 ($25\pm1°C$; $70\pm10\%$ RH; 12h photoperiod).
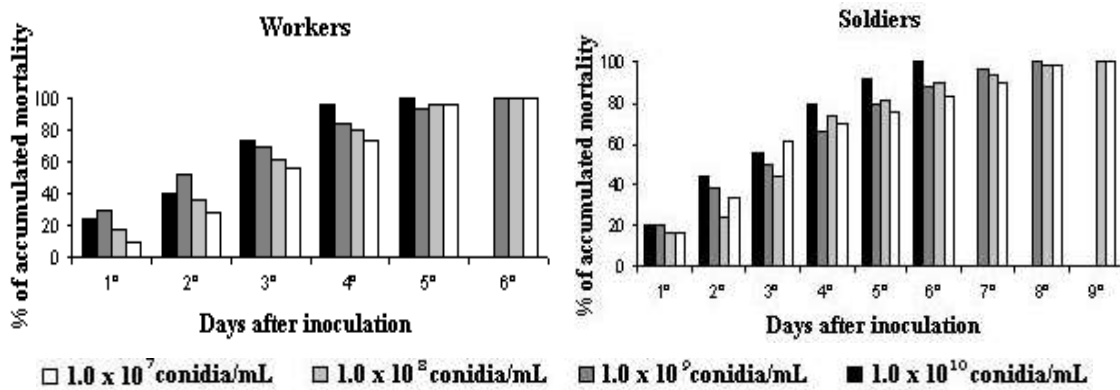
Source: Authors (2022).

Figure 2. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana* strain IBCB 21 ($25\pm1°C$; $70\pm10\%$ RH; 12h photoperiod).
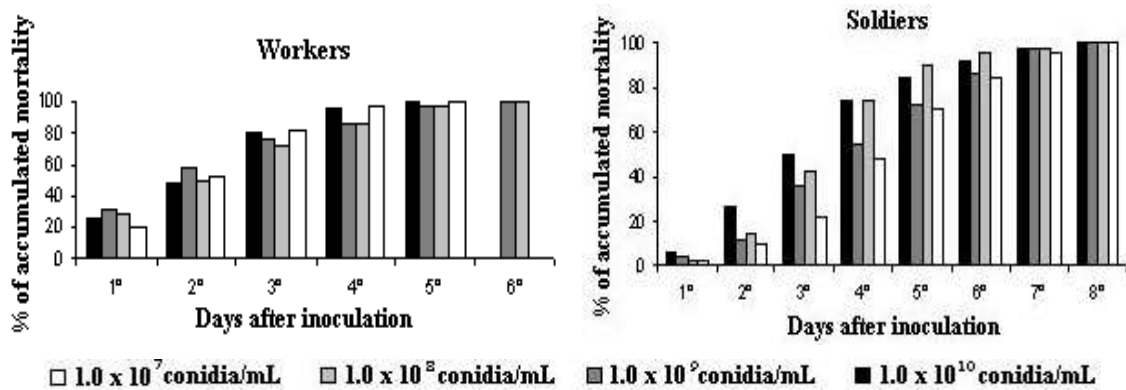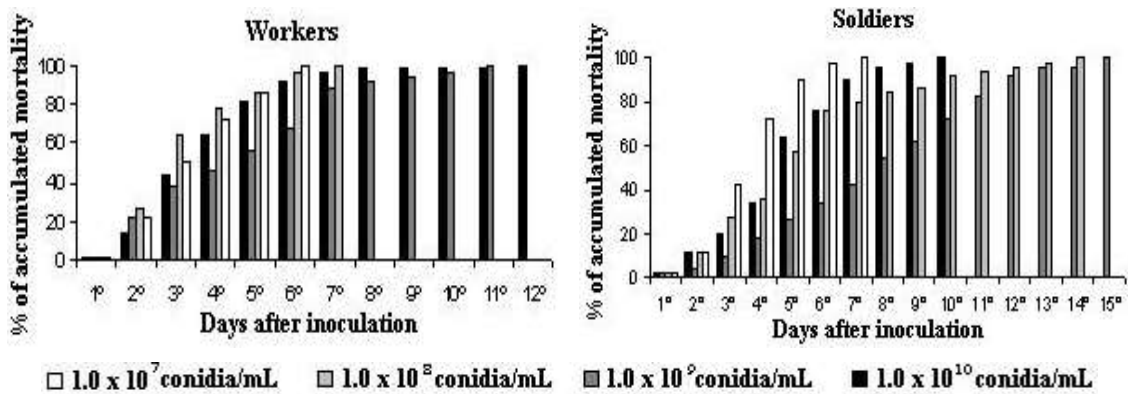

Source: Authors (2022).

The lowest values of LT_{50} were not always attributed to the highest concentration. Strains IBCB 66 and ESALQ 447 obtained the highest rate of mortality when applied at the lowest concentration of conidia. At the 6th and 7th days after the inoculation, it was obtained 100% of accumulated mortality for workers and soldiers, respectively (Figures 3 and 4). This indicates an interesting feature, since the smaller the number of propagules of the pathogen bound to the insects' body needed to develop a disease, the more virulent the strain. On the other hand, when using a high inoculum potential, the results may be unexpected because a large number of fungal conidia on the insect's tegument can negatively influence its germination and favor the penetration of bacteria, generating septicemia and consequently rapid death of the insect (Alves & Lecuona, 1998).
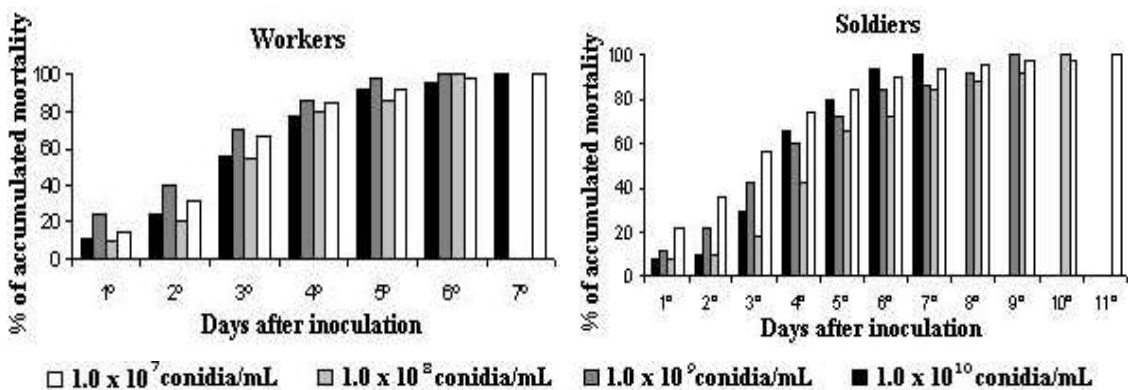
Figure 3. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana* strain IBCB 66 (25±1°C; 70±10% RH; 12h photoperiod).
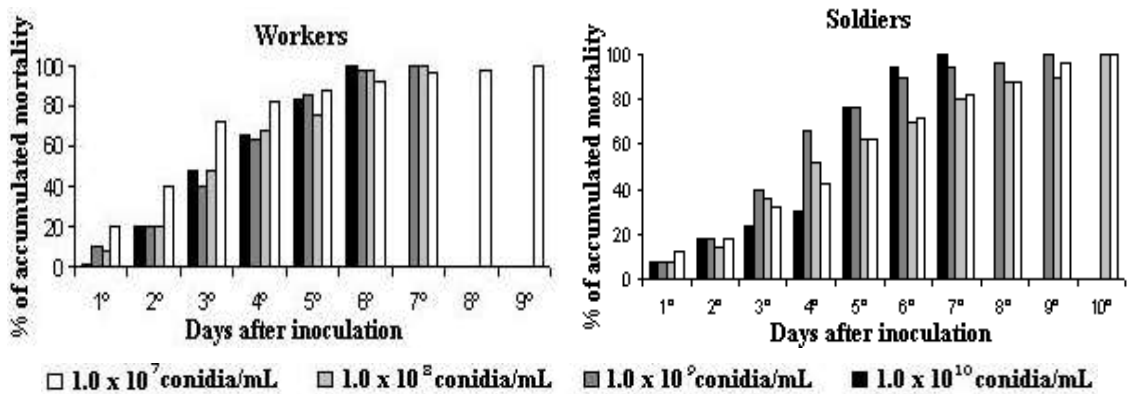

Source: Authors (2022).

On the opposite way, the highest concentration of strain UFGD 02 obtained 100% of accumulated mortality for workers and soldier, at the 6th and 7th days after the inoculation, respectively (Figure 5), result similar to that found by Loureiro & Monteiro (2004, 2005). They evaluated the pathogenicity of different strains of *B. bassiana*, *M. anisopliae* and *Cordyceps* (= *Isaria*) *farinosus* on workers and soldiers of *A. sexdens sexdens*, verifying the highest efficiencies for the biggest concentrations of fungi (1.0×10^8 and 1.0×10^9 con mL^{-1}). The confirmed mortality varied from 74 to 88% (Table 4). When a higher quantity of conidia germinates, the invasion and the colonization of the insect body is faster and more effective, becoming difficult the proliferation of other competitive microorganisms which might damage the fungus sporulation (Neves & Hirose, 2005). However, the concentrations with higher quantities of conidia did not always present a higher percentage of sporulation. The fungi penetration, mainly when used in high concentration, causes the appearance of "orifices" on the tegument of the insects, which may be attacked by other microorganisms. In this case, due the fact of the bacteria grows faster than fungi, they finally colonize the host body, causing septicemia, characterized by the aspect and odor, obstructing the growth of the primary pathogen, that is, the entomopathogen with the capacity of penetration and interfering in the confirmation results of the death of the insect through the fungus (Alves & Pereira, 1998).

Figure 4. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana* strain ESALQ 447 (25±1°C; 70±10% RH; 12h photoperiod).
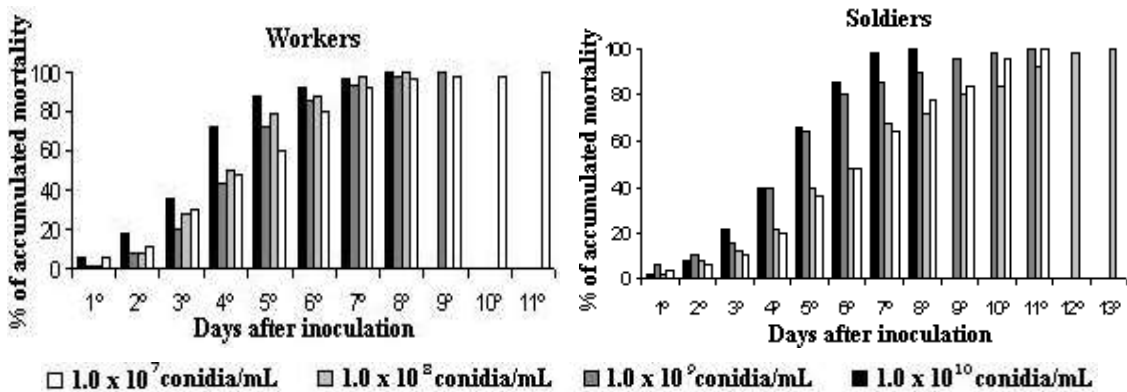

Source: Authors (2022).

Figure 5. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana*, strain UFGD 02 (25±1°C; 70±10% RH; 12h photoperiod).

Source: Authors (2022).

The strain UFGD 11 obtained a high percentage of confirmed mortality (84 to 96% for workers and 82 to 90% for soldiers), despite high LT_{50} values (Table 3). On the 1.0×10^{10} con/mL concentration, it was obtained 100% of accumulated mortality for soldiers and workers eight days after the inoculation (Figure 6).

Figure 6. Accumulated Mortality (%) of workers and soldiers of *A. sexdens rubropilosa*, after the inoculation with *B. bassiana* strain UFGD 11 (25±1°C; 70±10% RH; 12h photoperiod).

Source: Authors (2022).

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

Treatments		*Beauveria bassiana*		
Strain	Concentration	Soldiers	Workers	VC(%)
Control	Without Tween	0.00 ± 0.00 fA	0.00 ± 0.00 eA	0.00
Control	With Tween	0.00 ± 0.00 fA	0.00 ± 0.00 eA	0.00
IBCB 07	10^7	38.00 ± 6.63 eA	34.00 ± 6.78 dA	41.67
	10^8	70.00 ± 10.95 bA	56.00 ± 11.66 cA	40.16
	10^9	26.00 ± 6.00 eA	28.00 ± 5.83 dA	49.00
	10^{10}	58.00 ± 5.83 cA	20.00 ± 5.47 dB	32.43
IBCB 21	10^7	94.00 ± 4.00 aA	98.00 ± 2.00 aA	7.37
	10^8	92.00 ± 3.74 aA	84.00 ± 6.78 bA	13.92
	10^9	58.00 ± 7.34 cB	88.00 ± 7.34 bA	22.51
	10^{10}	88.00 ± 3.74 aA	96.00 ± 2.44 aA	7.69
IBCB 66	10^7	64.00 ± 10.29 bB	92.00 ± 5.83 aA	23.98
	10^8	88.00 ± 3.74 aB	98.00 ± 2.00 aA	7.21
	10^9	44.00 ± 5.09 dA	56.00 ± 9.27 cA	33.47
	10^{10}	60.00 ± 7.07 cA	78.00 ± 10.19 bA	28.44
ESALQ 447	10^7	62.00 ± 6.63 bA	68.00 ± 5.83 cA	21.48
	10^8	68.00 ± 3.74 bA	68.00 ± 5.83 cA	16.11
	10^9	68.00 ± 3.74 bA	78.00 ± 3.74 bA	11.46
	10^{10}	84.00 ± 2.44 aA	80.00 ± 3.16 bA	7.71
UFGD 02	10^7	86.00 ± 5.09 aA	82.00 ± 3.74 bA	11.90
	10^8	88.00 ± 3.74 aA	84.00 ± 2.44 bA	8.22
	10^9	74.00 ± 4.00 bB	86.00 ± 2.44 bA	9.27
	10^{10}	88.00 ± 2.00 aA	90.00 ± 3.16 aA	6.65
UFGD 11	10^7	82.00 ± 5.83 aA	84.00 ± 5.09 bA	14.76
	10^8	90.00 ± 5.47 aA	88.00 ± 3.74 bA	11.78
	10^9	86.00 ± 5.09 aA	94.00 ± 2.44 aA	9.94
	10^{10}	82.00 ± 3.74 aB	96.00 ± 2.44 aA	7.95
VC (%)		20.20	19.15	

Values followed by a small letter (in the column) and a capital letter (in the line) do not differ significantly by the Scott-Knott test at a 5% level of probability. Data transformed to arcsen $(x/100)^{1/2}$ Source: Authors (2022).

The confirmed mortality varied from 20 to 98% for workers and 26 to 94% for soldiers, occurring a significant difference among the concentrations (Table 3). In general, there were no significant differences between the values of sporulation for workers and soldiers. The low percentage of confirmed mortality presented by some strains does not discard the possibility of these 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 in the interior of the corpses through casual fissures on the tegument, caused during their handling (Tamai et al., 2002).

The high confirmed mortality is an important characteristic, since the ability of the pathogen to produce propagules can trigger epizootics in the field, through their dispersion in the environment and contamination of healthy individuals (Alves & Lecuona, 1998). In addition, confirmed mortality can be chosen as a parameter to study the behavior of the best concentration, since fungi, as biological control agents, basically differ from chemical products in their ability to increase the pathogen density through secondary inoculum dispersion, repeating the cycle through the host population (Hajek & St. Leger, 1994; Dornelas et al., 2016).

The variability among the strains is result of differences in the enzymes and toxins production (amylase, protease, lipase), on the speed of conidia germination, on the mechanical activity in the cuticle penetration, and in the capacity of colonization of the strains (Paccola-Meirelles & Azevedo, 1990). In this work, it was used strains that have never been tested for the control of leaf-cutting ants, and the obtained results indicate a good potential for them as control agents. The selection of the best strains must take into account some factors such as a high index of mortality in a short period and elevated sporulation on the corpses. For the control of social insects, it is important to consider the sporulation capacity of the strain on corpses. Thus, the faster the sporulation occurs, the greater the chances of the fungus reaching a high potential of the inoculum in the nests, before the insect manages to remove all the corpses (Stimac et al., 1987).

4. Conclusion

1. Workers of *A. sexdens rubropilosa* were more susceptible than soldiers to the tested strains of *B. bassiana*.

2. The strain IBCB 21 was more virulent for workers, presenting LT_{50} of 1.57 days, no significant difference among tested concentrations and high percentages of confirmed mortality, highlighting its potential for use in the control of *A. sexdens rubropilosa.*

3. For soldiers, the more virulent strain was IBCB 07, with LT_{50} of 2.15 days and no significant difference among the concentrations.

4. Future works must be realized to evaluate the pathogenicity of different strains of *B. bassiana*, as well, other entompathogenic fungi.

Acknowledgments

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