SELECTION OF *Metarhizium anisopliae* (METSCH.) AND *Beauveria bassiana* (BALS.) ISOLATES TO CONTROL *Alabama argillacea* (HUEBNER) CATERPILLARS

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ABSTRACT: The cotton leafworm, *Alabama argillacea* (Huebner, 1818) (Lepidoptera: Noctuidae), is considered to be one of the key pests in herbaceous cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch) cropping, with constant occurrence in all cotton-growing states of Brazil. In this study *Metarhizium anisopliae* and *Beauveria bassiana* isolates were screened and evaluated for pathogenicity against *Alabama argillaceae*. Initially, a screening of ten isolates of each fungus in a concentracion of 10^8 conidia mL^{-1}, was carried out on 3rd instar larvae of *A. argillacea*. Further studies were conducted to determine the pathogenicity and virulence of six and seven isolates of *M. anisopliae* and *B. bassiana*, respectively, against 3rd instar larvae of *A. argillacea* and using the concentrations of 10^6, 10^7, 10^8, and 10^9 conidia mL^{-1}. The experiments were carried out in Recife, PE, Brazil, at 27 ± 2°C, RH 70 ± 5% and a photophase of 12 hours. Mortalities caused by *M. anisopliae* isolate at the different concentrations ranged from 4.5 to 91.2%, the highest mortality percentage being found for the isolate 1189 at 10^9 conidia mL^{-1}. The isolate 645 of *B. bassiana* caused the highest mortality at the highest concentration, followed by isolates 634, 604, and IPA 198. The lowest lethal time for *B. bassiana* and *M. anisopliae*, was achieved by the isolates 483 (4.1 days) and 1189 (2.0 days), respectively. The isolates 1189, 1022 e 866 of *M. anisopliae* and 483, IPA198 and 604 of *B. bassiana*, at 10^8 e 10^9 conidia mL^{-1} are promissing for use the integrated control of *A. argillacea* larvae, but *M. anisopliae* seems more effective.
Key words: entomopathogenic fungus, cotton leafworm, microbial control

SELEÇÃO DE ISOLADOS DE *Metarhizium anisopliae* (METSCH.) E *Beauveria bassiana* (BALS.) PARA O CONTROLE DE LAGARTAS DE *Alabama argillacea* (HUEBNER)

RESUMO: O curuquerê-do-algodoeiro (*Alabama argillacea*) é considerado uma das principais pragas do algodoeiro herbáceio no Brasil, com ocorrência comum em vários estágios de crescimento da cultura. Este trabalho avaliou a patogenicidade e selecionou isolados dos fungos entomopatogênicos *Metarhizium anisopliae* e *Beauveria bassiana*, para lagartas do curuquerê-do-algodoeiro *Alabama argillacea.* Os experimentos foram conduzidos a 27 ± 2°C, 70 ± 5 % de UR e fotofase de 12 h. Foram utilizados 10 isolados de cada fungo na concentração de 10^8 conídios mL^{-1} sobre larvas de *A. argillacea* no 3º instar. Os isolados selecionados (seis de *M. anisopliae* e sete de *B. bassiana*) foram pulverizados com micropulverizador manual (DeVilbiss) nas concentrações de 10^6, 10^7, 10^8 e 10^9 conídios mL^{-1}, em delineamento inteiramente casualizado. As porcentagens de mortalidade confirmada de lagartas de *A. argillacea* ocasionadas pelos isolados de *M. anisopliae*, variaram de 4,5 a 91,2%, nas diferentes concentrações, sendo que o isolado 1189 de *M. anisopliae*, na concentração 10^9 conídios mL^{-1}, proporcionou a maior mortalidade. O isolado 645 de *B. bassiana*, também proporcionou a mais elevada mortalidade na concentração 10^9 conídios mL^{-1}, seguido dos isolados 634, IPA 198 e 604. O isolado 1189 de *M. anisopliae* apresentou o tempo letal (TL_{50}) igual a 2,0 dias, sendo portanto menor que os demais isolados, na concentração de 10^9 conídios mL^{-1}. Para *B. bassiana*, o menor TL_{50} foi do isolado 483 (4,1 dias). Os isolados 1189, 1022 e 866 de *M. anisopliae* e 483, IPA198 e 604 de *B. bassiana* nas concentrações 10^8 e 10^9 conídios mL^{-1}, mostraram-se promissores para incorporação no manejo integrado de *A. argillacea*, destacando-se aqueles de *M. anisopliae*.
Palavras-chave: fungo entomopatogênico, curuquerê-do-algodoeiro, controle microbiano

INTRODUCTION

The cotton leafworm, is considered to be one of the key pests in herbaceous cotton crops, with constant occurrence in all cotton-growing states of Brazil. In the Brazilian Northeast, infestation normally occurs beginning in March, when plant emergence takes place (Bleicher et al., 1990). In other regions, this pest can occur in different seasons, depending on the environmental conditions, especially rainfall and relative humidity (Domiciano & Santos, 1994; Ramalho, 1994). When the attack of cotton leafworms occurs in the first 45 days of crop development, it can cause significant loss of foliage in the main stem, resulting in irreversible damage to the

succeeding phenological stages of the plant (Bleicher et al., 1983).

Leafworm control must start in fields with less than 110 days after germination when 10 medium-sized (larger than 15 mm) caterpillars are found in 10 examined plants, or, in fields with more than 110 days after germination, when 20 medium-sized caterpillars are found in 10 examined plants (Cruz, 1989). Control is done with the use of chemicals (Jesus et al., 1984) and through biological control (Bleicher & Parra, 1990; Almeida, 1996). In spite of the importance of natural biological control for this pest, not many reports can be found on the use of biological control agents to suppress *A. argillacea* caterpillars, either with predators, parasitoids or entomopathogens (Allen, 1977; Bleicher & Parra, 1990; Chocorosqui & Pasini, 2000).

Entomopathogenic fungi, such as *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok., are produced and formulated in several regions in Brazil, in order to control pests belonging to many orders, including Lepidoptera (Ferron, 1978; Marques et al., 1981; Alves, 1998). The vast genetic variability found in these entomopathogens endorse them as important insect microbial control agents. These fungi are completely compatible with the purposes established for the integrated pest management, since isolates obtained from one insect species can be pathogenic to other species that are distant from a taxonomic standpoint and not only to closely-related species (Alves et al., 1984; Alves, 1998).

B. bassiana isolates have proven to be pathogenic to fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Rodrigues & Pratissoli, 1989; França et al., 1989; Faria et al., 1992). At a concentration of 1.64×10^9 conidia mL^{-1}, the fungus *B. bassiana* gave 60% mortality against *Castnia licus* (Drury) larvae (Vilas Boas et al., 1983). High mortality levels of *C. licus* larvae caused by the fungi *B. bassiana* and *Beauveria brongniartii* (Sacc.) Petch, were also observed by Vilas Boas & Alves (1988). The fungi *M. anisopliae* and *B. bassiana*, at a concentration of 10^8 conidia mL^{-1}, caused mortality of *Castnia icarus* (Cramer) larvae on the order of 68.8 and 54.0%, respectively (Silva & Veiga, 1998). The species *Cydia pomonella* (Linnaeus); *Ostrinia nubilalis* (Huebner); *Diatraea saccharalis* (Fabricius); and, *Plutella xylostella* (Linnaeus), are also susceptible to the fungi *M. anisopliae* and *B. bassiana* (Alves, 1998).

Given the importance represented by cotton leafworms to cotton cropping in Brazil and the lack of research on the use of entomopathogens for their control, this project aimed to evaluate their pathogenicity and select isolates of *M. anisopliae* and *B. bassiana* for the control of *A. argillacea* in the laboratory.

MATERIAL AND METHODS

Experiments were performed at the Insect Pathology Laboratory of Universidade Federal Rural de Pernambuco (UFRPE), at a temperature of 27±2°C, 70±5 % RH and 12h photophase.

Insect rearing and maintenance: Initially, *A. argillacea* pupae were obtained from CNPA-EMBRAPA, Campina Grande – PB. After emergence, the adults were transferred into PVC tube cages, measuring 20 cm in height and 19.5 cm in diameter, placed over a paper towel and covered with organza fabric. The internal wall of the cage was lined with a sheet of white paper to allow removal of egg masses. Five couples were maintained per cage. The adults were fed a 30% honey solution, offered on pieces of plastic foam 1.0 cm thick and 3.0 cm in diameter, enclosed in plastic lids. Water was provided by means of a cotton wad inserted into small glass vials (20 mL). Every other day the eggs were collected and transferred to cages identical to those used for the adults. Leaves from the upper part of cotton plants, cultivar CNPA Precoce 1, with their petioles immersed into small glass containers filled with water, were provided daily as food for the caterpillars. Removal of faecal material was also performed daily. This colony was maintained for several generations until the conclusion of the experiments.

Acquisition of fungi isolates: The *B. bassiana* and *M. anisopliae* fungi isolates used in the experiments can be found in the collection of the Insect Pathology Laboratory, in the Plant Protection Area - UFRPE (Table 1), where they are stored at temperature 7°C, in glass tubes containing Potato - Dextrose - Agar culture medium plus antibiotic (chloramphenicol) (BDA + A) and Nujol oil. When tests were performed the isolates were subcultured and multiplied on BDA + A or on Complete Medium (CM), the latter containing minerals, glucose, yeast extract and agar, and then placed in a B.O.D. incubator at 26±1°C and 12h photophase.

Selection of the most pathogenic isolates: During this step, 10 *M. anisopliae* and 10 *B. bassiana* isolates were obtained from CM dishes ten days after plating. Then, 20 3rd-instar *A. argillacea* caterpillars were sprayed with 2 mL of suspension for each isolate with a manual microsprayer (DeVilbiss), at the concentration of 10^8 conidia mL^{-1}. Suspensions were prepared with sterile water containing 0.1% Tween 80®. After that, caterpillars were transferred to PVC cages, fed with cotton leaves and observed daily until ten days after inoculation. Concurrently, isolate viability was verified by plating on BDA + A, and making an observation 24h later under the optical microscope, with three counts (replicates) of 100 conidia each.

Dead caterpillars were transferred to Petri dishes lined with slightly moistened filter paper and placed in a B.O.D. incubator at 26±1°C and 12h photophase, in order to confirm the causal agent and to verify conidiogenesis. Only isolates that provided confirmed mortality were selected, i.e., only those *A. argillacea* caterpillars that showed fungal development higher than 50%.

Table 1 - *Metarhizium anisopliae* and *Beauveria bassiana* isolates, origins and hosts.

Isolate	Origin	Host
M. anisopliae		
PL 47	Lab. of Insect Pathology (USP/ESALQ)	*Mahanarva posticata*
1022	"	*Phyllophaga* sp.
1172	"	*Soil sample*
PL 43	"	*Mahanarva posticata*
866	"	*Atta* sp.
UOD	Lab. of Insect Pathology (UFRPE)	*Castnia licus*
E 9	Lab. of Insect Pathology (USP/ESALQ)	*Deois flavopicta*
IPA 207	IPA (PE) Enterprise	*Mahanarva posticata*
IPA 204	IPA (PE) Enterprise	*Mahanarva posticata*
1189	Lab. of Insect Pathology (USP/ESALQ)	*Soil sample*
B. bassiana		
483	Lab. of Insect Pathology (USP/ESALQ)	*Solenopsis invicta*
512	"	*Solenopsis invicta*
561	"	*Solenopsis* sp.
447	"	*Solenopsis invicta*
645	"	*Soil sample*
604	"	*Soil sample*
634	"	*Solenopsis invicta*
635	"	*Solenopsis invicta*
610	"	*Amostra de Solo*
IPA 198	IPA (PE) Enterprise	*Cosmopolites sordidus*

Production of select *M. anisopliae* and *B. bassiana* isolates: Isolate production was started based on cultures obtained from re-isolation of *A. argillacea*. Later, conidia were multiplied in bottles (500 mL) containing rice as culture medium. Bottles were prepared with 140 grams of parboiled rice and 90 mL distilled water, sealed with aluminum foil and a piece of string, and autoclaved at 120°C for 20 minutes, according to an adaptation from the methodology of Marques et al. (1981). After cooling off, the rice was inoculated with 15 mL of a conidia suspension of each isolate, at the concentration of 10^7 conidia mL^{-1}. Bottles were maintained for 10 days in a B.O.D. incubator, at 26 ± 1°C and 12h photophase.

Select isolate virulence evaluation: Two experiments with a completely randomized factorial design were carried out for the isolate virulence tests; the first experiment consisted of 6 *M. anisopliae* isolates in 4 concentrations; the second was performed 15 days later, with 7 *B. bassiana* isolates in 4 concentrations. Three replicates consisting of 10 3rd instar *A. argillacea* caterpillars were utilized for each treatment, totaling 30 caterpillars per treatment. The caterpillars were placed on Petri dishes and sprayed with 2 mL of the *M. anisopliae* isolate suspensions in the first experiment, and the *B. bassiana* in the second, at concentrations of 10^6; 10^7; 10^8; and, 10^9 conidia mL^{-1}, by using a manual microsprayer (DeVilbiss), akin to the initial step. Controls were sprayed with distilled water plus Tween 80^R at 0.1%. Then, caterpillars were transferred to the PVC cages, as previously described, and maintained according to the rearing procedures adopted for them. The numbers of dead caterpillars were recorded daily for 10 days, and the larvae were transferred to humid chambers for confirmation of mortality by the pathogens. Isolate efficiency was evaluated by means of the confirmed mortality percentage, caterpillars that showed fungus development, and corrected in relation to the control by Abbott's (1925) formula. Lethal time (LT_{50}) determination was performed through Probit analysis, by using the software MOBAE (Haddad et al., 1995). Caterpillar mortality percentages were submitted to analysis of variance and the means compared by the Tukey test ($P \leq 0,05$).

RESULTS AND DISCUSSION

Selection of the most pathogenic isolates: Conidia viability for the *M. anisopliae* and *B. bassiana* isolates was higher than 95% in all experiments. The confirmed mortality of *A. argillacea* caterpillars, 10 days after inoculation, ranged from 10 to 70% and from 25 to 70%, for *M. anisopliae* and *B. bassiana*, respectively (Table 2). Differences in mortality for caterpillars treated with different isolates demonstrated the importance of the selection. Isolates 1189; 1022; PL 47; IPA 204; 1172; and 866 of *M. anisopliae* and 610; 561; 634; 604; IPA 198; 483; and 645 of *B. bassiana* were selected after the first bioassay, since they caused confirmed caterpillar mortalities above 50%. This selection criterion was the chosen option, due to the likelihood of obtaining isolates with a higher potential for a possible utilization in the management of the pest.

Isolate virulence evaluation: At the concentration 10^9 conidia mL^{-1}, a variation occurred ranging from 56.7 to 91.2% as confirmed mortality (Table 3). All selected *M. anisopliae* isolates caused mortality of *A. argillacea* above 50%, indicating that isolate 1189 caused the highest mortality, at both concentrations of 10^9 and 10^8 conidia mL^{-1} (Table 3). At a concentration of 10^8 conidia mL^{-1}, isolates 1189 and 1022 caused mortality of *A. argillacea* caterpillars above 70%, which are values comparable to those observed by Silva & Veiga (1998) when they used this fungus against *C. icarus* larvae. On the other hand, at a concentration of 10^7 conidia mL^{-1}, only isolates 1189 and 866 caused mortalities higher than 50%, with isolate 1189 killing 60.1% of the caterpillars. Using a similar concentration, Rodrigues & Pratissoli (1989) verified that *M. anisopliae* was not pathogenic to *S. frugiperda* caterpillars. At the concentration of 10^6 conidia mL^{-1}, all *M. anisopliae* isolates caused mortalities below 25%.

Table 2 - Confirmed mortality of *Alabama argillacea* caterpillars, by *Metarhizium anisopliae* and *Beauveria bassiana* isolates at a concentration of 10^8 conidia mL^{-1}, ten days after inoculation. Temperature: 27 ± 2°C, RH: 70 ± 5% and photophase: 12h.

M. anisopliae	Mortality[1]	*B. bassiana*	Mortality[1]
	---- % ----		---- % ----
1189	70	604	70
1022	65	561	60
866	65	IPA 198	60
PL 47	60	634	60
IPA 204	60	610	55
1172	55	645	55
E9	30	483	55
PL 43	30	635	40
IPA 207	25	512	30
UOD	10	447	25

[1]Confirmed mortality: insects with conidiogenesis.

For *B. bassiana*, at the concentration of 10^9 conidia mL^{-1}, mortalities obtained with all isolates were higher than 60%; however, the isolates did not differ statistically from each other, and the highest mortality of *A. argillacea* caterpillars was observed with isolate 645 (80.7%) (Table 3). These results are similar to those obtained by França et al. (1989) and Faria et al. (1992), when they worked with other *B. bassiana* isolates and verified high mortalities of *S. frugiperda* caterpillars. At a concentration of 10^8 conidia mL^{-1}, according to what was verified during the initial selection phase, isolates caused mortalities over 50%, with isolate 604 being prominent, since it was not different at concentrations of 10^9 and 10^8 conidia mL^{-1}. At concentrations of 10^7 and 10^6 conidia mL^{-1}, *B. bassiana* isolates caused caterpillar mortalities below 40.0 and 25.0%, respectively, which are levels similar to those recorded by Rodrigues & Pratissoli (1989) when they applied *B. bassiana* at the concentration of 10^7 conidia mL^{-1} against *S. frugiperda* caterpillars, observing mortality levels of 40.0%.

Table 3 - Confirmed mortality (%) of *Alabama argillacea* caterpillars, by *Metarhizium anisopliae* and *Beauveria bassiana* isolates and conidia concentrations. Temperature: 27 ± 2°C, RH: 70 ± 5%, photophase: 12h.

Treatment isolate	Mortality[1]			
	10^9 conidia mL^{-1}	10^8 conidia mL^{-1}	10^7 conidia mL^{-1}	10^6 conidia mL^{-1}
		Metarhizium anisopliae		
1189	91.2 ± 1.53 Aa	71.6 ± 2.00 ABa	60.1 ± 1.00 Ba	16.3 ± 0.58 Ca
1022	73.5 ± 0.58 Aab	70.8 ± 1.73 ABa	36.6 ± 0.58 Bab	4.5 ± 0.58 Ca
866	73.5 ± 0.58 Aab	66.7 ± 0.58 Aa	53.3 ± 2.08 Aab	6.7 ± 1.00 Ba
PL 47	71.6 ± 2.00 Aab	53.3 ± 0.58 Aa	35.1 ± 2.08 ABab	11.6 ± 1.53 Ba
IPA 204	64.0 ± 2.08 Aab	63.4 ± 0.58 Aa	19.3 ± 1.00 Bb	15.7 ± 1.15 Ba
1172	56.7 ± 0.58 Ab	50.1 ± 1.73 ABa	20.0 ± 0.00 ABb	22.1 ± 1.53 Ba
		Beauveria bassiana		
645	80.7 ± 1.00 Aa	50.0 ± 0.00 ABa	18.3 ± 1.73 BCa	4.5 ± 0.58 Ca
634	77.8 ± 1.53 Aa	60.6 ± 2.00 ABa	39.8 ± 1.00 BCa	19.3 ± 1.00 Ca
IPA 198	76.2 ± 2.89 Aa	67.2 ± 1.53 Aa	29.7 ± 1.00 Ba	23.8 ± 0.58 Ba
604	73.8 ± 1.15 Aa	73.5 ± 0.58 Aa	32.9 ± 1.15 Ba	1.1 ± 0.58 Ca
610	65.1 ± 3.06 Aa	53.3 ± 1.15 ABa	26.5 ± 0.58 BCa	4.5 ± 0.58 Ca
483	63.4 ± 0.58 Aa	54.0 ± 2.31 Aa	6.7 ± 1.00 Ba	4.5 ± 0.58 Ba
561	60.0 ± 1.73 Aa	60.1 ± 1.00 Aa	32.8 ± 1.53 ABa	6.7 ± 1.00 Ba

[1]Means (± DP) followed by the same upper case letter, in the row, and lower case letter, in the column, are not different by Tukey test at 5%.
[1]Transformation as arcsine of square root of x/100.
CV = 23.20% (*M. anisopliae*) - CV = 26.02% (*B. bassiana*)

A positive relationship was recorded between mortality percentages and concentrations among the *M. anisopliae* and *B. bassiana* isolates, just as expected. Concurrently, with the increase in conidia concentration, a reduction in LT_{50} was observed. Isolate 1189 from *M. anisopliae*, at the concentrations 10^8 and 10^9 conidia mL^{-1}, presented the shortest lethal time (Table 4). These low values are probably associated to the presence of enzymes that aid in the process of penetration of the fungi (St. Leger et al. 1988), as well as toxins such as the destruxins, which cause variability among isolates, endowing this isolate with higher virulence against *A. argillacea* caterpillars. For *B. bassiana* at a concentration of 10^9 conidia mL^{-1}, variation in LT_{50} ranged from 4.1 to 6.4 days. Isolate 483 presented the shortest LT_{50} for this rate, whereas at a concentration of 10^8 conidia mL^{-1}, the isolates showing the shortest LT_{50} were 634 and 604, with an LT_{50} of 5.7 days (Table 5). Isolates of the fungi *M. anisopliae* and *B. bassiana* at concentrations of 10^8 and 10^9 conidia mL^{-1} utilized in this research demonstrated to be promising for incorporation to an integrated management scheme against *A. argillacea*.

Table 4 - Lethal time (LT_{50}) for *Metarhizium anisopliae* isolates against *Alabama argillacea* caterpillars. Temperature: 27 ± 2°C, RH: 70 ± 5% and photophase: 12h.

Isolate	Conidia concentration mL^{-1}	LT_{50} and IC* (Day)	Equation	X^2
1189	10^9	2.0 (1.9 - 2.2)	Y= 4.02 + 3.22 logx	0.02*
	10^8	3.6 (2.9 - 4.4)	Y= 2.57 + 4.38 logx	1.09*
	10^7	4,9 (4.0 - 6.2)	Y= 2.97 + 2.91 logx	1.41*
1022	10^9	4.3 (3.7 - 5.1)	Y= 3.41 + 2.49 logx	0.70*
	10^8	6.9 (5.9 - 7.9)	Y= 0.23 + 5.70 logx	3.12*
PL 47	10^9	6.6 (5.8 - 7.4)	Y= 0.39 + 5.65 logx	4.13*
	10^8	7.7 (6.8 - 8.7)	Y= 2.35 + 3.00 logx	1.55*
IPA 204	10^9	6.3 (5.6 - 7.1)	Y= 2.45 + 3.18 logx	0.68*
	10^8	6.6 (5.7 - 7.6)	Y= 2.91 + 2.55 logx	1.38*
1172	10^9	7.6 (6.4 - 8.9)	Y= 0.15 + 5.52 logx	5.71*
	10^8	8.5 (7.4 - 9.7)	Y= 2.22 + 2.99 logx	0.81*
866	10^9	4.8 (4.6 - 5.1)	Y= 0.56 + 6.47 logx	0.17*
	10^8	5.8 (5.1 - 6.6)	Y= 1.30 + 4.85 logx	2.59*
	10^7	7.3 (6.3 - 8.5)	Y= 1.58 + 3.95 logx	0.92*

*Confidence interval (Significance at the 5%).
Mortality did not reach 50% for all isolates at a concentration of 10^6 conidia mL^{-1}, and for isolates 1022; PL 47; IPA 204; and 1172 at a concentration of 10^7 conidia mL^{-1}.

Table 5 - Lethal time (LT_{50}) for *Beauveria bassiana* isolates over *Alabama argillacea* caterpillars. Temperature: 27 ± 2°C, RH: 70 ± 5% and photophase: 12h.

Isolate	Conidia concentration mL^{-1}	LT_{50} and IC* (Day)	Equation	X^2
610	10^9	6.4 (5.9 - 7.1)	Y= 0.78 + 5.21 logx	1.76*
	10^8	6.8 (6.3 - 7.4)	Y= -1.02 + 7.21 logx	0.01*
561	10^9	5.3 (4.6 - 6.1)	Y= 2.83 + 3.00 logx	0.87*
	10^8	6.5 (5.9 - 7.2)	Y= 1.39 + 4.44 logx	1.34*
634	10^9	5.3 (4.8 - 5.8)	Y= -0.05 + 6.97 logx	0.60*
	10^8	5.8 (4.7 - 7.0)	Y= 2.06 + 3.87 logx	0.77*
604	10^9	4.5 (4.0 - 5.2)	Y= 0.96 + 6.13 logx	0.92*
	10^8	5.7 (5.0 - 6.6)	Y= -2.16 + 9.44 logx	2.01*
IPA 198	10^9	4.4 (3.6 - 5.2)	Y= 2.75 + 3.52 logx	1.89*
	10^8	5.9 (5.3 - 6.6)	Y= 1.67 + 4.32 logx	2.16*
645	10^9	4.9 (4.4 - 5.5)	Y= 1.93 + 4.42 logx	1.91*
	10^8	7.9 (7.2 - 8.8)	Y= -0.82 + 6.46 logx	0.67*
483	10^9	4.1 (3.5 - 4.9)	Y= 2.61 + 3.89 logx	0.03*
	10^8	6.9 (6.1 - 7.8)	Y= 2.78 + 2.65 logx	0.74*

*Confidence interval (Significance at the 5%).
Mortality did not reach 50% for all isolates at concentrations of 10^7 and 10^6 conidia mL^{-1}

ACKNOWLEDGEMENTS

To CNPq, for granting a Master's degree scholarship to the first author; to Prof. Jorge Braz Torres and Prof. Irene Maria Ramos Marques, for the suggestions and collaboration.

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Received April 27, 2001