

BEHAVIOR AND CYTOLOGICAL ASPECTS OF *Metarhizium anisopliae* and *Metarhizium flavoviride* AFTER PASSAGE IN *Chrysomya albiceps*

Francisco Marlon Carneiro Feijó

Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido – UFERSA. ÁREA, RN, Brasil
E-mail: marlon@ufersa.edu.br

Paulo Moisés Lima

Universidade Federal Rural do Semi-Árido Mossoró, RN, Brasil
E-mail: paulomoises1@yahoo.com.br

Eduardo Henrique de Magalhães Melo

Universidade Federal de Pernambuco - PE, Brasil.
E-mail: eduardo@npd.ufpe.br

Ana Célia Rodrigues Athayde

Universidade Federal de Campina Grande, Campina Grande, PB, Brasil
E-mail: athayd@ecstr.ufcg.edu.br

Elza Áurea de Luna-Alves Lima

Universidade Federal de Campina Grande, Campina Grande, PB, Brasil.
E-mail: marlon@ufersa.edu.br

ABSTRACT - *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* are entomopathogenic fungi with proved action against several species of insects. In this work, the behavior and cytology of the *M. anisopliae* var. *anisopliae* (PL₄₃) and *M. flavoviride* var. *flavoviride* (CG₂₉₁) were evaluated after the passage in eggs, larvae and adults *Chrysomya albiceps*, an important causer of secondary myiasis. The experiment was carried out under an acclimatized environment's humidity and temperature of $60 \pm 10\%$ and $28 \pm 1^\circ\text{C}$. The most expressive results of the biological parameters studied (percentage of germination, quantity of conidia, quantity and diameter of colonies) were reached from re-isolated fungi of larvae. No significant differences were observed in the cytological aspects of the life cycle of the fungi post-passage in eggs, larvae and adults. These results suggest the possibility of the use of the fungi in the control of *C. albiceps* fly.

Keywords: fungi, cytology, fly, biological parameters.

COMPORTAMENTO E ASPECTOS CITOLÓGICOS DE *Metarhizium anisopliae* e *Metarhizium flavoviride* APÓS PASSAGEM EM *Chrysomya albiceps*

RESUMO - *Metarhizium anisopliae* var. *anisopliae* e *Metarhizium flavoviride* var. *flavoviride* são fungos entomopatogênicos com ação comprovada contra várias espécies de insetos. Neste trabalho foram avaliados o comportamento e a citologia de *M. anisopliae* var. *anisopliae* (PL₄₃) e *M. flavoviride* var. *flavoviride* (CG₂₉₁) pós-passage em ovos, larvas e adultos de *Chrysomya albiceps*, uma importante causa de miíase secundária. O experimento foi realizado sob umidade e temperatura de sala climatizada de $60 \pm 10\%$ e $28 \pm 1^\circ\text{C}$. Os resultados mais expressivos dos parâmetros biológicos estudados (percentual de germinação, número de conídios, número e diâmetro de colônias) foram alcançados a partir dos fungos reisolados de larvas. Não foram observadas diferenças significativas nos aspectos citológicos do ciclo de vida dos fungos pós-passage em ovos, larvas e adultos. Esses resultados sugerem a possibilidade do emprego desses fungos no controle de *C. albiceps*.

Palavras-chave: fungos, citologia, mosca, parâmetros biológicos.

INTRODUCTION

Researches about *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* as vectors of pathogens important to public health, as *Musca domestica* (BERNARDI *et al.*, 2006) and *Chrysomya albiceps* fly, are promising (FEIJÓ *et al.*, 2002), as well as against plagues that cause economic losses in cultures of

agronomical interest, such as the *Bemisia tabaci* fly (HERRERA *et al.*, 1999) and the *Vespa vulgaris* wasp (HARRIS *et al.*, 2000).

Many works emphasize the evaluation of the controlling agents in the ability to inactivate plague-insects, other researches are related to the behavior of features inherent to virulence, germination, production of

conidia and the growth of fungi on insects (DURAN *et al.*, 1997).

The Anamorphus, like *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride*, in laboratorial conditions, the germination can occur in 12 hours, at a temperature from 23 to 30 °C and relative humidity of 90% (ALVES, 1998) as verified by Milner *et al.* (1997) who observed that the germination of *M. anisopliae* occurred at 96% of relative humidity. The production of conidia is one of the characteristics of fungi lineages, as studied by Sun *et al.* (2003) when it was verified that the sporulation was superior to the control, 11 days after the inoculation in *Coptotermes formosanus*, although the sporulation rate depends on the components of the mean of culture (DOMENECH *et al.*, 2000) as an extract of yeast and urea. Yeo *et al.* (2003) used the radial growth of *M. anisopliae* as a criteria of selection of mycoinsecticides used in the combat against *Myzus persicae*, however, the selection of lineages depends on the temperature (BERLANGA & HERNADEZ, 2000).

The cytology of the fungi is a tool of great importance used to study the taxonomy and the phenomenon of genetic variability demonstrated by the Anamorphus, which are characteristic of presenting the asexual reproduction, or even occurs in fungi that present asexual as well as sexual reproduction, such as the Ascomycota, Basidiomycota e Zigomycota (KENDRICK, 1992). The cytology of *M. anisopliae* var. *anisopliae* referring to the measurement of conidia and nucleus, shape, size of conidia and germination were already approached by Luna-Alves Lima (1985) and Ribeiro (1992). As to the *M. flavoviride* var. *flavoviride*, the studies are still incipient.

The objective of this work was to analyze the behavior of *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride*, related to germination, sporulation, quantity of colonies, diameter of colonies and cytological aspects post-passage in *C. albiceps*.

MATERIAL AND METHODS

Strains used: *M. anisopliae* var. *anisopliae* (PL₄₃) isolated from *Mahavarna posticata*, in Pernambuco (Brazil) and *M. flavoviride* var. *flavoviride* (CG₂₉₁) isolated from *Austracnis guttulosa*, in Australia, is conceded by EMBRAPA/DF, both deposited in Micoteca-URM, Departamento de Micologia-UFPE (Brazil).

Mean of culture used: Potato Dextrose Agar (PDA) (Oxoid).

Radial Growth: a disk of 5 mm of diameter from the fungus colony was put in the center of a Petri plate with PDA. The fungal growth was observed in the period: 3, 6, 9, 12 e 15 days. The readings were made in centimeter, with the help of a millimeter ruler and after the end of the experiment, an arithmetic average was calculated for each evaluated day.

Determination of the quantity of colonies and percentage of germination: culture with 12 days of growth, a disk of 5 mm of diameter was retrieved and

transferred to test tubes containing 10 ml of Tween 80 (0.05% v/v). The suspension was shaken for the desegregation of the conidia. The number of conidia was determined in a Neübauer chamber. The suspension was diluted, reaching 100 conidia.mL⁻¹. From this suspension, 0.1 ml was spread with the help of a Drigalsky handle, throughout the surface of the Petri plates containing PDA. The experiment was accomplished in triplicate for each day of observation. The number of colonies was determined on the pre-established days: 3, 6, 9, 12 and 15, after the inoculation. The percentage of germination was established after 16 hours post-inoculation. A total of 500 conidia per plate were counted after the sowing, it was considered, as a germinated conidia the one whose germinant tube showed to be greater than one third of the size of the conidia.

Determination of the sporulation percentage: from the fungi colonies sowed in an ADP, 5 mm diameter disks were retrieved and inoculated in the center of the Petri plate containing PDA. At 12 days, the 5 mL plates of ethanol solution at 75% were added in order to kill and dry the conidia. Afterwards, the plates were washed ten times with 9.5mL of Tween 80 solution (0.05% v/v). The washed was collected in an Erlenmeyer flask and shaken to promote the desegregation of the conidia. Then, the number of conidia was determined in a Neübauer chamber.

Observation of the fungal microstructures: part of the fungal culture was put aseptically in strategic points of the Petri plate, containing ADP, covered with a laminule, previously flambéed. The plates were left in environmental temperature, waiting for the time of analysis (24, 48, 72, 96, 120h). At the time established, a laminule with the microstructures was retrieved, and these were colored by the Amann blue and observed under immersion objective.

Nuclear condition: for the observation of the nuclei, a Giemsa-HCl. coloration technique was used (LUNA-ALVES LIMA & AZEVEDO, 1983).

Statistical analysis: the statistical techniques used were: t-Student test with equal or unequal variances in the comparison between the re-isolates of both fungi and the F test (ANOVA) in the comparison between the re-isolates for each fungus. It is stressed that in the existence of a significant difference between the re-isolates, comparison paired Tukey tests were used an the verification of the hypothesis of variance equality was carried out through the Levene F test. The level of significance considered in the statistical decisions was of 5.0% and the software used for the obtainment of the statistical tests was SPSS (Statistical Package for the Social Sciences) in the version 11 (ZAR, 1999).

RESULTS AND DISCUSSION

Metarhizium anisopliae var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* re-isolated of

egg, larvae and adult of *Chrysomya albiceps* post-experimental infection

The percentage of germination varied between the fungi studied, being the greatest indexes observed in the re-isolated *M. anisopliae* var. *anisopliae* (98.33%) and *M. flavoviride* var. *flavoviride* (96.53%) larvae differing from the control (Table 1). The effects on germination of *M.*

anisopliae var. *anisopliae* were shown by Vélez *et al.* (1999) who reported 80% of germination in the *M. anisopliae* lineages re-isolated from *Hypothenemus hampei*, demonstrating also high germination. On the other hand, Prior (1992) recommends the selection of lineages for each use, bearing the fact that the same ones depend on the specific interaction like the host as Hajaek & ST. Leger (1994) established.

Table 1 – Percentage of *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* germination after re-isolation of egg, larvae and adult of *Chrysomya albiceps*.

	<i>M. anisopliae</i> var. <i>anisopliae</i>	<i>M. flavoviride</i> var. <i>flavoviride</i>
	Average ± S.D.	Average ± S.D.
Control	77.60 ± 6.09 ^(a,AB)	74.60 ± 2.11 ^(a, A)
Egg	85.80 ± 1.64 ^(a,AC)	83.73 ± 2.23 ^(a,B)
Larvae	98.33 ± 1.03 ^(a,B)	96.53 ± 1.92 ^(a,C)
Adult	89.87 ± 5.87 ^(a,BC)	92.40 ± 1.11 ^(a,C)

Averages followed by different small letters, on the line and different capital letters, on the column differ statistically between each other by the Tukey test at a 5% level of probability error.

The number of *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* conidia from egg, larvae and adult is on Table 2. It was observed that the larvae

reisolates presented greater rates of sporulation, with a statistical difference between the fungi studied. The increase in the number of conidia after the passage by the insect was also observed by Maniana (2002) when the *M. anisopliae* sporulation was verified after passage in the *Glossina fuscipes* fly.

Table 2 – Sporulation of *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* after re-isolation of egg, larvae and adult of *Chrysomya albiceps*.

	<i>M. anisopliae</i> var. <i>anisopliae</i>	<i>M. flavoviride</i> var. <i>flavoviride</i>
	Average ± S.D.	Average ± S.D.
Control	2.07 ± 0.25 x 10 ⁸ ^(a,A)	1.51 ± 0.23 x 10 ⁸ ^(a,A)
Egg	2.43 ± 0.94 x 10 ⁸ ^(a,B)	1.70 ± 0.11 ^(a,A)
Larvae	3.27 ± 0.36 x 10 ⁸ ^(a,C)	3.33 ± 0.68 ^(a,B)
Adult	2.91 ± 0.95 x 10 ⁸ ^(a,D)	2.14 ± 0.85 x 10 ⁸ ^(a,A)

Averages followed by different small letters, on the line and different capital letters, on the column differ statistically between each other by the Tukey test at a 5% level of probability error.

The number of *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* recovered colonies re-isolated from eggs, larvae and adults in the period of time: 3, 6, 9, 12, 15, was statistically different between both fungi and the most expressive results were observed in *M. anisopliae* var. *anisopliae* reisolated from larvae, with a statistical difference in relation to the control (Table 3). In works conducted about the parameter of behavior, a relevant increase on the number of *Paecilomyces fumosoroseus* colonies re-isolated from *Boophilus microplus* engorged females (SANTOS *et al.*, 2002) was observed.

Averages followed by different small letters, on the line and different capital letters, on the column differ

statistically between each other by the Tukey test at a 5% level of probability error.

The larvae re-isolates of the fungi studied, in the previously established period of time (3, 6, 9, 12, 15) presented the greatest increase when the diameter of colonies was compared with a difference in relation to the control and to the egg and adult re-isolates (Table 4). These results were different from the data obtained by Nascimento *et al.* (2002) when they studied the radial growth of *M. anisopliae* re-isolate of *Rhipicephalus sanguineus*, however these results probably occurred due to the humidity used (64%), from according to Fargues *et al.* (1997), the humidity is an essential feature for the growth of entopathogenic fungi.

Table 3 – Number of *Metarhizium anisopliae* and *Metarhizium flavoviride* colonies after reisolation of egg, larvae and adult of *Chrysomya albiceps*.

	Origin	<i>M. anisopliae</i> var. <i>anisopliae</i>	<i>M. flavoviride</i> var. <i>flavoviride</i>
		Average ± S.D.	Average ± S.D.
3 days	Control	3.67 ± 0.58 ^(a,A)	0.33 ± 0.58 ^(b,A)
	Egg	6.33 ± 0.58 ^(a,B)	0.60 ± 1.15 ^(b,A)
	Larvae	7.00 ± 1.00 ^(a,B)	2.00 ± 1.00 ^(b,A)
	Adult	6.00 ± 0.00 ^(a,B)	1.33 ± 1.53 ^(b,A)
6 days	Control	8.33 ± 1.5 ^(a,A)	4.00 ± 0.00 ^(b,A)
	Egg	9.00 ± 1.00 ^(a,A)	4.67 ± 0.58 ^(b,A)
	Larvae	10.00 ± 1.00 ^(a,A)	5.33 ± 0.60 ^(b,A)
	Adult	9.33 ± 1.0 ^(a,A)	5.00 ± 1.00 ^(b,A)
9 days	Control	9.33 ± 0.58 ^(a,A)	5.00 ± 1.00 ^(b,A)
	Egg	11.33 ± 1.15 ^(a,AB)	5.66 ± 1.50 ^(b,A)
	Larvae	12.33 ± 0.58 ^(a,BC)	7.30 ± 0.50 ^(b,A)
	Adult	10.33 ± 0.58 ^(a,BC)	7.00 ± 0.08 ^(b,A)
12 days	Control	10.33 ± 0.58 ^(a,A)	6.33 ± 0.58 ^(b,A)
	Egg	11.67 ± 0.58 ^(a,B)	7.00 ± 0.08 ^(b,A)
	Larvae	13.00 ± 0.00 ^(a,C)	8.00 ± 0.08 ^(b,A)
	Adult	12.00 ± 0.00 ^(a,B)	7.33 ± 1.50 ^(b,A)
15 days	Control	12.00 ± 0.10 ^(a,A)	8.33 ± 0.58 ^(b,A)
	Egg	12.33 ± 0.58 ^(a,A)	9.00 ± 0.50 ^(b,A)
	Larvae	16.33 ± 0.58 ^(a,B)	9.33 ± 0.50 ^(b,A)
	Adult	14.33 ± 0.58 ^(a,C)	9.30 ± 1.50 ^(b,A)

Table 4 – Diameter of *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* colony after reisolation of egg, larvae and adult of *Chrysomya albiceps*.

	Origin	<i>M. anisopliae</i> var. <i>anisopliae</i>	<i>M. flavoviride</i> var. <i>flavoviride</i>
		Average ± S.D.	Average ± S.D.
3 days	Control	1.50 ± 0.10 ^(a,A)	1.40 ± 0.00 ^(a,A)
	Egg	1.60 ± 0.10 ^(a,AC)	1.53 ± 0.06 ^(a,B)
	Larvae	1.80 ± 0.10 ^(b,BC)	1.97 ± 0.06 ^(a,C)
	Adult	1.70 ± 0.10 ^(a,AC)	1.83 ± 0.06 ^(b,C)
6 days	Control	2.23 ± 0.12 ^(b,A)	2.63 ± 0.06 ^(a,A)
	Egg	2.47 ± 0.06 ^(b,B)	2.73 ± 0.06 ^(a,AC)
	Larvae	2.90 ± 0.00 ^(b,C)	3.20 ± 0.20 ^(a,B)
	Adult	2.63 ± 0.06 ^(b,B)	3.00 ± 0.00 ^(a,BC)
9 days	Control	4.53 ± 0.06 ^(a,A)	4.10 ± 0.10 ^(b,A)
	Egg	5.00 ± 0.10 ^(a,B)	4.20 ± 0.00 ^(b,A)
	Larvae	5.83 ± 0.06 ^(a,C)	4.87 ± 0.06 ^(b,B)
	Adult	5.53 ± 0.06 ^(a,D)	4.53 ± 0.06 ^(b,C)
12 days	Control	5.43 ± 0.32 ^(a,A)	5.27 ± 0.06 ^(b,A)
	Egg	6.00 ± 0.10 ^(a,B)	5.40 ± 0.10 ^(b,A)
	Larvae	6.90 ± 0.00 ^(a,C)	6.00 ± 0.00 ^(b,B)
	Adult	6.70 ± 0.00 ^(a,C)	5.87 ± 0.06 ^(b,B)
15 days	Control	6.80 ± 0.10 ^(a,A)	6.70 ± 0.00 ^(a,A)
	Egg	6.50 ± 0.10 ^(b,AC)	6.87 ± 0.06 ^(a,A)
	Larvae	7.20 ± 0.10 ^(a,B)	7.30 ± 0.10 ^(a,B)
	Adult	6.80 ± 0.00 ^(b,AC)	7.03 ± 0.06 ^(a,C)

Averages followed by different small letters, on the line and different capital letters, on the column differ statistically between each other by the Tukey test at a 5% level of probability error.

Analysis on light microscopy of *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium flavoviride* var. *flavoviride* after the reisolation of *Chrysomya albiceps* egg, larvae and adult post-experimental infection.

The vegetative and reproductive structures present in *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* before and after passage into egg, larvae, and *C. albiceps* adult were analyzed in the period of time previously determined of 24, 48, 72, 96 and 120h.

In the analysis of the *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride*, just the formation of the mycelium was observed in the first 48 hours in the *C. albiceps* egg, larvae and adult reisolate, as well as the control. At 72 hours, *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* anastomosis were observed, which permit the intercommunication between the segments, allowing the passage into cytoplasmic organelles, including the nuclear migration. This phenomenon is related to the variability that through parasexuality (MESSIAS & AZEVEDO, 1980), allows the mitotic products to equal themselves to the mitotic products (LUNA-ALVES LIMA, 1985), allowing the recombination and genetic improvement in economically important fungi that do not present a sexual cycle (AZEVEDO, 1998). Also, appressoria on both strains were observed. These structures are related to the capacity of entomopathogenic fungi on hosts (ALVES, 1998). *M. anisopliae* var. *anisopliae* conidiophores were observed from 96 hours, on the egg, larvae and adult reisolates and at 120 hours, abundant *M. flavoviride* var. *flavoviride* ramified conidiophores reisolated from adults were also observed. The vegetative and reproductive structures reisolated from egg, larvae and adult did not differ from the control.

The *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* conidia presented a cylindrical shape post-passage into *C. albiceps* egg, larvae and adult. However, as to the nuclear condition, *M. anisopliae* var. *anisopliae* presented 100% of uninucleate conidia on the egg and larvae reisolates and 0.9% de binucleate conidia on the adult reisolate, though *M. flavoviride* var. *flavoviride* presented 0.72% of binucleate conidia on the larvae reisolate.

The present work adds some data about the action of *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *flavoviride* and the register of its prevalence after the passage into *C. albiceps* eggs, larvae and adults. The results of the biological parameters demonstrate the action of the entomopathogenic fungi, after the passage in *C. albiceps*

CONCLUSIONS

The percentage of germination, quantity of conidia, quantity and diameter of colonies of fungi was **greater** post-passage in *C. albiceps*. No differences were observed in the cytological aspects of the fungi post-passage in cytological aspects of fungi post-passage in *C. albiceps*. This way, future researches should be carried out, aiming to contribute to the study of the biological control of this fly important to public health.

REFERENCES

- ALVES, S.B. **Controle Microbiano de Insetos**. São Paulo: FEALq, 1998. 1163p.
- AZEVEDO, J.L. **Genética de Microorganismos**. Goiânia:UFG, 1998. 478p.
- BERLANGA, P.A.M.; HERNANDEZ, V.V.M. Efecto de la temperatura sobre el crecimiento y la virulencia de *Metarhizium anisopliae*, y *Beauveria bassiana* en *Schistocerca piceifrons piceifrons*. **Manejo Integrado de Plagas**, v. 63, p.51-55, 2000.
- BERNARDI, E.; PINTO, D.M.; NASCIMENTO, J.S.; RIBEIRO, P.B.; SILVA, C.I. Efeito dos fungos entomopatogênicos *Metarhizium anisopliae* e *Beauveria bassiana* sobre o desenvolvimento de *Musca domestica* L. (Diptera Muscidae) em laboratório. **Arquivos do Instituto Biológico de São Paulo**, São Paulo, v.73, n.1, p.127-129, 2006.
- DOMENECH, F.; RODRIGUEZ, J.A.; LEON, M.; RODRIGUEZ, D.E.; SANCHEZ, D.; ALFONSO, A. Influence of nitrogen source the C/N ratio in obtaining spores of *M. anisopliae* cepa n.11 by solid state fermentation. **Revista do Desenvolvimento da Cana de Açúcar**, v.34, p.2-19, 2000.
- DURAN, F.; SANABRIA, M.E.; ACOSTA, S.; ZAMBRANO, C. Germination y formation de apressorios del hongo entomopatogeno y especificidade del hongo entomopatogeno *Metarhizium anisopliae*. In: CONGRESSO VENEZUELANO DE FITOPATOLOGIA, 15., 1997, Maracaibo. **Anais...** Maracaibo: Sociedade Venezuelana de Fitopatologia, 1997.
- FARGUES, J.; GOETTEL, M.S.; SMITS, N.; OUEDRAOGO, A.; ROUGIER, M. Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. **Mycologia**, v.89, p.383-392, 1997.
- FEIJÓ, F.M.C.; MELO, E.H.M.; MACIEL, M.V.; ATHAYDE, A.C.R.; LUNA-ALVES LIMA, E.A. Efeito de *Metarhizium anisopliae* var. *anisopliae* em adultos de *C. albiceps*, Manaus, AM, 2002. In: CONGRESSO

- BRASILEIRO DE MEDICINA VETERINÁRIA, 30., 2002. **Anais...**, Manaus: Sociedade Brasileira de Medicina Veterinária, 2002, CD ROM.
- HAJAEK, A.K.; ST. LEGER, R.J. Interactions between fungal pathogens and insect hosts. **Annual Review of Entomology**, v.39, p.293-322, 1994.
- HARRIS, R.J.; HARCOURT, S.J.; GLARE, T.R.; ROSE, E.A.; NELSON, T.J. Susceptibility of *Vespa vulgaris* (Hymenoptera: vespidae) to generalist entomopathogenic fungi and their potential for wasp control. **Journal of Invertebrate Pathology**, v.75, p.251-258, 2000.
- HERRERA, F.; CARBALLO, M.; SHANNON, P. Efficacy of native entomopathogenic fungal strains against *Bemisia tabaci* in the laboratory. **Manejo Integrado de Plagas**, v.54, p.37-43, 1999.
- KENDRICK, B. **The Fifth Kingdom**. Canadá. Mycologue Publications, 1992. 406p.
- LUNA-ALVES LIMA, E.A. **Características citológicas e genéticas de linhagens selvagens, mutantes e diplóides de *Metarhizium anisopliae* (Mtsch.) Sorokin**. 1985. PhD Thesis. Universidade Federal do Rio de Janeiro, Brasil.
- LUNA-ALVES LIMA, E.A.; AZEVEDO, J.L. Técnicas para coloração nuclear em estruturas vegetativas de *Metarhizium anisopliae* (Metsch.) Sorokin. **Boletim do Grupo de Pesquisadores de Controle Biológico**, v.4, p.20-21, 1983.
- MANIANIA, N.K. A low cost contamination device for infecting adult tsetse flies, *Glossina spp.*, with the entomopathogenic fungus *Metarhizium anisopliae* in the field. **Biocontrol Science and Technology**, v.12, p.59-66, 2002.
- MESSIAS, C.L.; AZEVEDO, J.L. Parasexuality in the deuteromycete *Metarhizium anisopliae*. **Transactions of the British Mycological Society**, v.75, p.473-477, 1980.
- MILNER, R.J. et al. The effect of humidity on germination and infection of termites by the hyphomycete, *Metarhizium anisopliae*. **Journal of Invertebrate Pathology**, v.69, p.64-69, 1997.
- Nascimento, F.S.B.; Santos, G. F.; Athayde, A.C.R.; Luna Alves-Lima, A.E. Viability de *Metarhizium anisopliae* após infecção experimental de *Rhipicephalus sanguineus*. In: CONGRESSO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 12., 2002, Rio de Janeiro, RJ. **Anais...** Rio de Janeiro, 2002. CD-ROM.
- PRIOR, C. Discovery and characterization of fungal pathogens for locust and grasshopper control. In: LOMER, C.J.; PRIOR, C. **Biological control of locusts and grasshoppers**. Redwood Press, 1992. p. 230-238.
- RIBEIRO, S.M.A.; LUNA-ALVES LIMA, E.A.; ASSUNÇÃO, W.T.G.; LIMA, D.M.M. Behavior and characteristics of a wild strain of *Metarhizium anisopliae*. **Revista de Microbiologia**, v.23, p.97-100, 1992.
- SANTOS, G.F.; NASCIMENTO, F.S.B.; ATHAYDE, A.C.R.; LUNA ALVES-LIMA, E.A. Viabilidade de *Peacilomyces lilacinus* após infecção experimental de *Boophilus microplus*. In: CONGRESSO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 12., 2002, Rio de Janeiro, RJ. **Anais...** Rio de Janeiro, 2002, CD-ROM.
- SUN, J.Z.; FUXA, J.R., HERDERSON, G. Sporulation of *Metarhizium anisopliae* and *Beauveria bassiana* on *Coptotermes formosonus*. **Journal of Invertebrate Pathology**, v.81, p.78-85, 2003.
- VELEZ, P.E.A.; GONZALES, M.T.G.; RIVERA, A.M.; ESTRADA, M.N.V.; MONTOYA, E.C.R. Caracterización de aislamientos de *Beauveria bassiana* y *Metarhizium anisopliae* de la colección de Cenicafe. **Revista Colombiana de Entomología**, v.25, p.191-207, 1999.
- Yeo, H.; Pell, J.K.; Alderson, P.G.; Clark, S.J.; Pye, B.J. Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species. **Pest Management Science**, v.59, p.156-165, 2003.
- ZAR, J.H. **Bioestatistical Analysis**. New Jersey. New Prentice Hall. 1999. 663p.