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BIOLOGICAL CONTROL

Pathogenicity of the Entomopathogenic Fungi and Nematode on Medfly *Ceratitis capitata* (Wied.) (Diptera: Tephritidae)

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Patogenicidade de Fungos e Nematóide Entomopatogênicos em Mosca-do-mediterrâneo *Ceratitis capitata* (Wied.) (Diptera: Tephritidae)

RESUMO – Os objetivos desta pesquisa foram: avaliar, em laboratório, a patogenicidade de isolados de fungos e nematóides para o controle de pré-pupas e adultos de moscas-das-frutas em laboratório e a aplicação desses entomopatógenos em casa-de-vegetação. Foram testadas diferentes concentrações de *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. sobre pré-pupas de *Ceratitis capitata* (Wied.) em solo estéril e natural sob condições de laboratório. Avaliou-se, em casa-de-vegetação, a eficiência de seis isolados de *B. bassiana* e *M. anisopliae* em pré-pupas de *C. capitata* com a aplicação dos isolados selecionados na concentração de $5x10^8$ conídios/mL em solo de vasos com mudas de citros em casa-de-vegetação. A patogenicidade do nematóide entomopatogênico, *Heterorhabditis* sp. (isolado IBCBn 05) foi avaliada aplicando-se 200 juvenis infectivos (JI)/pré-pupas com a mesma metodologia usada para os fungos. Estudou-se ainda transmissão dos fungos citados em adultos de *C. capitata* sob condições de laboratório. Verificou-se que os fungos *B. bassiana e M. anisopliae* foram patogênicos às pré-pupas de *C. capitata* sendo os isolados IBCB 66 e IBCB 425 os mais virulentos, respectivamente. *Heterorhabditis* sp. também foi patogênico na concentração aplicada. Em casa-de-vegetação o fungo *B. bassiana* obteve eficiência de controle de pré-pupas de 66,6%. Os fungos foram transmitidos na população de adultos de *C. capitata*, mesmo com apenas 10% da população contaminada.

PALAVRAS-CHAVE – Controle biológico, manejo integrado, *Metarhizium anisopliae*, *Beauveria bassiana*, *Heterorhabditis* sp., fruticultura.

ABSTRACT – The objectives of this research were: to evaluate, in laboratory, the pathogenicity of fungi isolates and nematodes in the control of fruit fly prepupae and adults in the laboratory and the application of these entomopathogens in the greenhouse. Different *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. concentrations were tested on *Ceratitis capitata* (Wied.) prepupae in sterilized and natural soil under laboratory conditions. The efficiency of six *B. bassiana* and *M. anisopliae* isolates was evaluated on *C. capitata* prepupae with application this selected isolates at a concentration of 5×10^8 conidia/mL in soil pots containing citrus seedlings in the greenhouse. In addition, the pathogenicity of the entomopathogenic nematode *Heterorhabditis* sp. (isolate IBCBn 05) was evaluated by applying 200 infective juveniles (JI)/ prepupae with the same methodology used for the fungi. Transmission of the above fungi in *C. capitata* adults was also studied under laboratory conditions. It was verified that the fungi *B. bassiana* and *M. anisopliae* were pathogenic to *C. capitata* prepupae, with the isolates IBCB 66 and IBCB 425, respectively, were the most virulent. *Heterorhabditis* sp. was also pathogenic at the concentration applied. In the greenhouse, the *B. bassiana* fungus reached a prepupal control efficiency of 66.6%. The fungi were transmitted among the *C. capitata* adult population even when only 10% of the population was initially contaminated.

KEYWORDS – Biological control, integrated management, *Metarhizium anisopliae*, *Beauveria bassiana*, *Heterorhabditis* sp., fruit crop.

Fruit flies (Diptera: Tephritidae) are considered important agribusiness fruit cropping pests worldwide, due to direct yield damage, great ease of dispersal, and adaptation to several hosts under different edaphic-climatic conditions, in addition to the costs involved in the implementation of control measures.

The MedFly *Ceratitis capitata* (Wied.) and 34 species of *Anastrepha*, including the South American fruit flies *A. fraterculus* (Wied.) and *A. obliqua* (Macquart), were related in São Paulo State (Souza Filho *et al.* 2000). The MedFly is exotic specie in Brazil and have 59 host plants registered, in references 21 botanic families (Zucchi 2001).

Fruit fly management depends on surveillance, performed with McPhail-type traps, based on the biological behavior of the insect. Several control methods can be then applied based on surveillance results (Gallo *et al*. 2002). Toxic baiting is the most commonly adopted technique by fruit growers in Brazil. It consists in sprinkling a molasses or hydrolyzed corn protein solution mixed with an insecticide (Raga & Sato 2006).

Few studies have dealt with microbial control application in Tephritidae (Bateman 1972), despite the great development of researches involving fruit fly taxonomy, biology, behavior, ecology, and chemical control.

Garcia *et al*. (1984) evaluated the pathogenicity of the fungus *Metarhizium anisopliae* (Metsch.) Sorok. isolate Standard I to *Ceratitis capitata* (Wied.) under laboratory conditions, and determined a LD_{50} of $8\times10^{6.5664}$ (b = 0.7702) and a LT_{50} = 11.4 days (b = 0.4644). The authors did not observe any Mediterranean fruit fly sensitivity differences to the fungus between sexes.

Carneiro & Salles (1994) verified that *Paecilomyces fumosoroseus* (Bainier) isolate CG 260 caused 100% mortality in *A. fraterculus* (Wied) pupae 20 days after application on third instar larvae, with an LC_{50} of 1.2×10^{6} conidia/mL. According to the authors, the few larvae that were killed showed initial colonization symptoms beginning at the body extremities, with the mycelium expanding through the whole body, while in pupae a concentration of 10^{8} conidia/mL caused 100% mortality, with lesions on the body or mycelium coming out of natural openings, with or without exudation of yellowish liquid (5 to 10 days later). Fungus sporulation on contorted corpses appeared between 15 and 20 days after *P. fumosoroseus* inoculation. Uziel *et al*. (2003) isolated *Entomophtora muscae* (Fresenius) and *E. schizophorae* (Fresenius) from the proboscis of *C. capitata* corpses collected in Israel.

In laboratory and greenhouse experiments, Alves *et al*. (2004) evaluated the effectiveness of the fungi *M. anisopliae, Beauveria bassiana* Bals. (Vuill.), *P. fumosoroseus*, and *Verticillium* sp. on *C. capitata*, via immersion of pupae and prepupae and topical application of a suspension containing 1×10^{8} conidia/mL on adults; in the greenhouse, the solution was applied directly onto the soil containing prepupae in a dark red latosol plus vermiculite. These authors verified that *M. anisopliae* isolates E9 and ESALQ 1037 had an efficiency of up to 90% on pupae. On adults, a maximum efficiency of 60% was obtained for all isolates. In the greenhouse, a prepupal mortality of up to 27% was observed in soil containing *M. anisopliae*.

Mochi *et al*. (2006) evaluated the effect of agrochemicals in the soil on the pathogenicity of *M. anisopliae* to *C. capitata* under laboratory conditions. Conidia of the fungus were applied as a suspension and in the form of dry conidia incorporated into the soil. The authors verified a low influence of these products on the fungus, since it was pathogenic to Mediterranean fruit fly larvae and pupae. The form by which the fungus was applied had no influence on insect survival; however, application in the form of suspension reduced survival at the pupal and adult stages.

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* are considered effective biological control agents of insect pests that spend some stage in the soil (Efron *et al*. 2001) and represent a group with a high potential for use in fruit fly control, favored because they allow the use of an environmentally safe technology. Studies have indicated that tephritid larvae are susceptible to these nematodes, although pupae are more resistant (Beavers & Calkins 1984). *S. carpocapsae* (Mexican isolate) has caused 87% mortality in *C. capitata* larvae at doses of up to 500 infective juveniles/cm^2 (Grewal *et al*. 2001).

The objectives of this study were to evaluate the pathogenicity of fungal isolates and nematodes applied in the laboratory and in the greenhouse to *C. capitata* prepupae and adults.

Material and Methods

C. capitata rearing

The laboratory and greenhouse experiments were conducted at the Biological Control Laboratory of Centro Experimental Central do Instituto Biológico - CEIB, Campinas-SP, Brazil.

The *C. capitata* individuals used in the experiments were obtained from the stock rearing at the Economic Entomology Laboratory of CEIB, maintained since 1993 with annual introductions of wild populations and reared on artificial diet (Raga *et al*. 1996).

Production of entomopathogenic fungi isolates

The isolates were plated onto Petri dishes by the three-point method with a platinum wire loop. *M. anisopliae* was plated onto PDA medium (potato, dextrose, and agar) and *B. bassiana* was plated onto ME medium (sporulation medium prepared with 0.36g KH_2PO_4, 1.05g NaH $PO_4.7H_2O$, 0.60g $MgSO_4$. $7H_2O$, 1.0g KCl, 10.0g dextrose, 5g yeast extract, 20g agar,

and 1000 mL distilled water), sterilized by autoclaving for 20 minutes at 120ºC.

After inoculation, the isolates were placed in a incubator, at 25ºC and a 12-hour photophase for 7 days. After that period, the dishes containing already sporulated fungi were scraped and the corresponding material thus obtained was diluted in distilled water and counted in a Neubauer chamber to obtain the desired concentration.

Selection of entomopathogenic fungi isolates for prepupae

Mediterranean fruit fly prepupae were taken to a freezer for 10 minutes to allow them to enter a dormancy state to facilitate handling. Ten prepupae were placed in each plastic container (7 cm diameter × 8 cm depth) containing 200g of soil. After the prepupae penetrated into the soil, the isolates were applied using a pipette containing 10 mL of each suspension tested, and the entire area of the container was covered. Two soil types were used, natural and sterilized. The latter was previously autoclaved for 20 minutes at 120ºC.

Concentrations of 5×10^7, 1×10^8, and 5×10^8 conidia/mL of each isolate were compared against a control; the treatments were replicated five times. Isolates evaluated were: IBCB 425 *M. anisopliae* (soil from Iporanga, SP) and IBCB 66 *B. bassiana* [*Hypothenemus hampei* (Ferrari) – São José do Rio Pardo, SP].

The same methodology used was employed to prepare the concentrations and apply them onto the *C. capitata* prepupae. The fungi *M. anisopliae* (IBCB 323, IBCB 348, IBCB 425, and ESALQ 1037) and *B. bassiana* (IBCB 04, IBCB 14, IBCB 15, IBCB 28, IBCB 35, and IBCB 66) were used in the experiments.

The applied isolates are stored at the "Odemar Cardim Abreu" Pathogen Bank, maintained by the Biological Control Laboratory of CEIB in Campinas, SP (Table 1).

Test involving entomopathogenic nematodes

In this experiment, nematodes of the genus *Heterorhabditis* sp. (isolate IBCBn 05) were tested on *C. capitata* prepupae. These microorganisms were distributed at a rate of 200 infective juveniles/insect in plastic containers (7 cm diameter × 8 cm depth) containing 200g of soil and ten Mediterranean fruit fly prepupae.

Greenhouse experiment

In this step, it was used the fungus and nematode isolates selected in the previous step, plated onto Petri dishes, and the corresponding inocula were prepared according to the methodology described in step of laboratory.

The isolates were prepared in a suspension consisting of 30mL distilled water for each replicate, at a concentration of 5×10^8 conidia/mL, applied individually on the soil surface of plastic bags containing sweet orange seedlings (*Citrus sinensis* L. Osbeck). Next, ten *C. capitata* prepupae were placed in the plastic bag containing a soil volume of approximately 5 kg (20 cm diameter × 35 cm depth). Ten replicates were used for each isolate and compared against a control. The nematode *Heterorhabditis* sp. (IBCBn 05) was applied at a concentration of 200 infective juveniles per prepupa.

The greenhouse was held at 25ºC±2ºC and 60±10% of relative humidity. The soil temperature of the plastic bags was 22ºC and the humidity near of saturation.

"In vitro" study of entomopathogenic fungi transmission in *C. capitata* adults

The isolates were plated according to the methodology described in the previous steps. The isolates were placed in B.O.D. incubators at 25ºC. After a 7-day period, the dishes containing the fungus were scraped and the material thus obtained was diluted in distilled water and counted in a Neubauer chamber to produce a concentration of 5×10^8 conidia/mL for both isolates.

Table 1. Entomopathogenic fungi isolates and entomopathogenic nematode used in selection experiments, from the "Oldemar Cardim Abreu" Collection of entomopathogenic microorganisms at the Biological Control Laboratory, Instituto Biológico, Campinas-SP.

Isolate	Species	Host	Origin
IBCB 04	*Beauveria bassiana*	Soil	Cascavel – PR
IBCB 14	*B. bassiana*	Soil	Cascavel – PR
IBCB 15	*B. bassiana*	Soil	Aral Moreira – MS
IBCB 28	*B. bassiana*	*Cosmopolites sordidus*	Miracatu – SP
IBCB 35	*B. bassiana*	*C. sordidus*	Cruz Almas – BA
IBCB 66	*B. bassiana*	*Hypothenemus hampei*	S.J. Rio Pardo – SP
IBCB 323	*Metarhizium anisopliae*	Soil	Jundiaí – SP
IBCB 348	*M. anisopliae*	*Mahanarva fimbriolata*	Sertãozinho – SP
IBCB 425	*M. anisopliae*	Soil	Iporanga – SP
ESALQ 1037	*M. anisopliae*	*Solenopsis* sp.	Porto Alegre – RS
IBCBn 05	*Heterorhabditis* sp.	Soil	Itapetininga – SP

The suspensions were applied onto Petri dishes (9 cm diameter) using a Potter spray tower, at a rate of 1 mL/dish. Eight dishes (14 cm diameter) were used in the experiment (n=8) plus one control for each isolate. Adult insects were infected and distributed among dishes according to the percentage of insects infected with the fungus, at 10, 20, 30, 40, 50, 60, 70, and 80%. Later, uninfected adult insects were added to each dish until a number of 10 insects per dish was reached. Each dish received a cotton wad moistened with water and a small amount of fly diet. The dishes were placed in an incubator for ten days. Upon dying, the insects were placed in a humid chamber for one week.

All data were submitted to analysis of variance (ANOVA), and the means were compared using Tukey test ($P > 0.05$). Original data were transformed to $\sqrt{X + 0.5}$.

Results and Discussion

Selection of entomopathogenic fungi isolates for prepupae

A reduction in emergence of *C. capitata* adults was observed at the three concentrations tested, for both entomopathogenic fungi species and in both soil types used, with the exception of *M. anisopliae* at 1×10^8 conidia/mL tested in sterilized soil, whose observed emergence was similar to the control. The fungi *B. bassiana* (IBCB 66) and *M. anisopliae* (IBCB 425), at the concentrations tested, reduced Mediterranean fruit fly emergence by up to 80% (Table 2).

Table 2. Adult emergence from *C. capitata* prepupae inoculated with different concentrations of *B. bassiana* and *M. anisopliae* in natural and sterilized soil (Temperature 25ºC; Relative Humidity 70%).

Treatment (n=5)	*B. bassiana* natural soil[1, 2]	*B. bassiana* sterilized soil[1, 2]	*M. anisopliae* natural soil[1, 2]	*M. anisopliae* sterilized soil[1, 2]
5×10^7 con./mL	7.6±1.1 ab	6.8±1.6 ab	4.0±2.8 a	5.0±3.0 ab
1×10^8 con./mL	7.8±1.3 ab	8.0±1.0 ab	2.8±1.3 a	8.6±1.5 c
5×10^8 con./mL	6.4±2.2 a	5.6±2.3 a	2.0±1.0 a	2.8±0.8 a
Control	10.0±0.0 b	8.6±1.1 b	8.0±1.8 b	7.4±1.3 bc
CV (%)	23	26	31	22

[1]Means (±EP) followed by the same letter are not different by Tukey test ($P > 0.05$).
[2] Original data transformed to $\sqrt{X + 0.5}$.

In a laboratory study, Alves *et al.* (2004) concluded that a higher conidial concentration ($1x10^8$ conidia/mL) of *B. bassiana* and *M. anisopliae* is needed to prevent *C. capitata* emergence. The immersion technique used in the present study for infecting prepupae and pupae provided 90% mortality. Although we used similar concentrations, those authors applied the fungus by broadcast spray in Potter Tower.

In the *B. bassiana* selection of isolates, it was observed that the emergence of *C. capitata* adults in different isolates was similar among themselves and different from the control. This fact leads us to continue our studies with *B. bassiana* isolate IBCB 66 (Table 3). The *M. anisopliae* isolate IBCB 425 was different from the others and was the most effective in reducing *C. capitata* emergence when applied to the soil (Table 4).

These results also agree with those by Alves *et al.* (2004), who observed *C. capitata* pupal mortality values of up to 90% caused by *M. anisopliae* isolates. Selecting entomopathogenic fungal isolates is one of the most important steps in a microbial control program, because the process allows verifying which isolates are more virulent to the pest, as well as their behavior in relation to pest mortality, sporulation, and production on artificial culture medium (Garcia *et al.* 1984, Alves 1998, Rhode *et al.* 2006).

Garcia *et al.* (1989) observed that *M. anisopliae* was highly pathogenic to *C. capitata* prepupae and pupae, but was less pathogenic to larvae of this insect. The authors also commented that it is possible to develop a fruit fly management strategy with the application of pathogens to the soil, especially to reach prepupae and pupae. Mochi *et al.* (2006) also verified the pathogenicity of the fungus *M. anisopliae* to *C. capitata* larvae, prepupae, and pupae under laboratory conditions, causing a survival decrease of up to 95% in adults emerged from the soil, with the fungus applied in the form of a conidial suspension. These authors also verified that the agrochemicals chlorthalonil, tebuconazole, abamectin, trichlorfon, and amethrin when applied to the soil at the dose recommended by the manufacturer in the presence of *M. anisopliae* did not influence pathogenicity. These results demonstrate the potential for *M. anisopliae* application to the crown projection area of fruit trees for fruit fly management.

Table 3. Mean number of adults emerged from *C. capitata* of prepupae inoculated with different *B. bassiana* isolates in natural soil 10 days after application (Temperature 25ºC; Relative Humidity 70%).

Isolates (n=5)	Adults emerged[1, 2]
IBCB 04	7.0±2.9 a
IBCB 14	6.2±3.5 a
IBCB 15	7.0±1.0 a
IBCB 18	7.8±2.1 a
IBCB 35	6.2±1.4 a
IBCB 66	5.6±3.3 a
Control	10.0±0.0 b
CV (%)	21

[1]Means (±EP) followed by the same letter are not different by Tukey test at 5%.
[2] Original data transformed to $\sqrt{X + 0.5}$.

Table 4. Mean number of adults emerged from *C. capitata* prepupae inoculated with different *M. anisopliae* isolates in natural soil 10 days after application (Temperature 25ºC; Relative Humidity 70%).

Isolates (n=5)	Adults emerged[1, 2]
IBCB 323	3.4±1.5 b
IBCB 348	3.2±1.3 b
IBCB 425	0.8±0.8 a
ESALQ 1037	6.2±0.8 c
Control	10.0±0.0 d
CV (%)	36

[1]Means (±EP) followed by the same letter are not different by Tukey test at 5%.

[2] Original data transformed to $\sqrt{X + 0.5}$.

Entomopathogenic nematodes

C. capitata emergence from soil containing the nematode *Heterorhabditis* sp. applied directly to the soil in the presence of *C. capitata* prepupae was reduced in relation to Control treatments in natural and sterilized soil. However, emergence was not different between natural soil and sterilized soil within the soil treated or not with the nematode (Table 5).

Table 5. Mean number of adults emerged from *C. capitata* prepupae inoculated with the entomopathogenic nematode *Heterorhabditis* sp. in natural and sterilized soil 10 days after application (Temperature 25°C; Relative Humidity 70%).

Treatment (n=5)	Adults emerged[1, 2]
Nematode in natural soil	2.4±1.3 a
Nematode in sterilized soil	4.2±1.3 a
Control in natural soil	7.6±1.1 b
Control in sterilized soil	8.8±0.8 b
CV (%)	36

[1]Means (±EP) followed by the same letter are not different by Tukey test (P > 0.05)

[2] Original data transformed to $\sqrt{X + 0.5}$.

The results presented in this research are in agreement with those by Gazit *et al*. (2000), who tested the pathogenicity of the nematodes *Steinernema riobrave* (Cabanillas, Poinar and Raulston) and *Heterorhabditis* sp. on *C. capitata* prepupae under natural conditions and obtained mortalities of up to 80%, with a five-day persistence of the nematodes in the soil. These authors suggested that entomopathogenic nematodes are potential fruit fly control agents, as also suggested by Grewal *et al*. (2001).

Greenhouse experiment

It was observed that the fungus *B. bassiana* (IBCB 66) caused an adult emergence reduction in relation to the control, although similar to the fungus *M. anisopliae* (IBCB 425) and the nematode *Heterorhabditis* sp. Only *B. bassiana* differed from the control, with significantly reduction of emergency rate (Table 6).

Table 6. Mean number of adults emerged from *C. capitata* of prepupae inoculated with the entomopathogenic nematode *Heterorhabditis* sp. and the fungi *M. anisopliae* and *B. bassiana* in the soil 10 days after application in the greenhouse.

Isolates (n=5)	Adults emerged[1, 2]
Metarhizium anisopliae – IBCB 425	5.6±1.1 ab
Beauveria bassiana – IBCB 66	4.2±1.3 a
Heterorhabditis sp. – IBCBn 05	6.0±1.2 ab
Control	7.0±0.7 b
CV (%)	25

[1]Means (±EP) followed by the same letter are not different by Tukey test (P > 0.05).

[2] Original data transformed to $\sqrt{X + 0.5}$.

In the present research, the *B. bassiana* treatment efficiency was 66.7%, while the *M. anisopliae* efficiency was 25%, demonstrating the *M. anisopliae* and *B. bassiana* potential to control *C. capitata*, from suspension applications directly to the soil on the plants crown projection area. Alves *et al*. (2004) observed mortalities of approximately 27% caused by *M. anisopliae* at a concentration of 1×10^7 conidia/mL, i.e. 10% of the concentration tested in the present study, consequently demanding more quantity of inoculums.

Garcia *et al*. (1989) mentioned that the application of entomopathogens to the soil is a viable fruit fly management alternative in orchards and coffee groves. However, the use of traps and baits containing chemical insecticides should be taken into consideration, in addition to cultural management via cleaning the orchards by collecting fruits from the ground, and a light soil harrowing to expose fruit fly prepupae and pupae and to facilitate the infection of fungi applied for biological control purposes.

Beavers & Calkins (1984) obtained 82.4% and 84.4% infection in *Anastrepha suspensa* (Loew) adults by *Steinernema feltiae* (Filipjev) and *Heterorhabditis heliothidis* (Poinar), respectively, when both were applied on filter paper in Petri dishes in laboratory. The adults were infected for nematodes by walking on the paper filter infected with juvenile nematodes. Lindegren *et al*. (1990) achieved excellent mortality levels on *C. capitata* prepupae caused by infective juveniles of *S. feltiae* in the field, starting at concentrations of 150 juvenile/individuals. Our results were according to these authors, because the efficiency of in *Heterorhabditis* sp reduction of *C. capitata* emergence were about 80% and 40%, in laboratory and greenhouse, respectively.

"In vitro" study of entomopathogenic fungi transmission in *C. capitata* adults

In this study, a high mortality of adults contaminated by the fungi *M. anisopliae* and *B. bassiana* was observed in all treatments, demonstrating

that only 10% of the adult population contaminated by the fungus in these confinement conditions (Petri dishes with 14 cm diameter) may transmit the disease to other individuals of the population (Table 7). Pest habits such as foraging and aggregation of males for copulation are factors that may facilitate the transmission of fungi to the rest of the population. Another possibility is the use of food baits containing entomopathogens (Cruz *et al*. 1999).

The use of toxic baits against fruit fly adults is an effective technique to reduce the pest in orchards and coffee groves; however, environmental contamination (Nascimento & Carvalho 1999) and attraction to parasitoids and beneficial insects are aspects that must be taken into consideration.

Our results demonstrated the potential of the use of microbial control for medfly using the entomopathogenic fungi *B. bassiana, M. anisopliae* and nematode *Heterorhabditis* sp. The use of traps for controlling *C. capitata* adults is viable because the probability of infected adults contaminate the remainder natural population is considerable.

Table 7. Mean number of confirmed *C. capitata* adults killed, inoculated with the fungus *B. bassiana* at different percentages of contaminated insects, after 10 days (Temperature 25°C and Relative Humidity 70%).

Treatment (n=5) Contaminated: Non-contaminated	*Metarhizium anisopliae*[1]	*Beauveria bassiana*[1]
Control 0:0	5.5±0.7 c	7.0±0.0 a
1:9	10.0±0.0 a	10.0±0.0 a
2:8	9.5±0.7 a	10.0±0.0 a
3:7	9.0±0.0 ab	7.0±0.0 a
4:6	10.0±0.0 a	8.0±0.0 a
5:5	7.5±0.7 b	9.5±0.7 a
6:4	10.0±0.0 a	8.0±2.8 a
7:3	10.0±0.0 b	6.5±0.7 a
8:2	7.5±0.7 a	6.5±0.7 a
CV (%)	17%	19%

[1]Means (±EP) followed by the same letter in the column are not different by Tukey test (P > 0.05).

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References

Alves, S.B. 1998 (ed.) Controle Microbiano de Insetos. Piracicaba: FEALQ. 1163 p.

Alves, S.B., L.S. Rossi, J.M.M. Walder, S.A. Vieira. 2004. Avaliação de fungos entomopatogênicos para *Ceratitis capitata*. Man. Integ. Plagas Agroecol. 72: 31-38.

Bateman, M.A. 1972. The ecology of fruit flies. Annu. Rev. Entomol. 17: 493-518.

Beavers, J.B., C.O. Calkins. 1984. Susceptibility of *Anastrepha suspensa* (Diptera: Tephritidae) to Steinernematid and Heterorhabidit nematodes in laboratory studies. Environ. Entomol. 13: 137-139.

Carneiro, R.M.D.G., L.A.B. Salles. 1994. Patogenicidade de *Paecilomyces fumosoroseus*, isolado CG 260 sobre larvas e pupa de *Anastrepha fraterculus* Wied. An. Soc. Entomol. Bras. 23: 341-343.

Cruz, I.B.M., J.C. Nascimento, M. Taufer, A.K. Oliveira. 1999. p. 55-66. Morfologia do aparelho reprodutor e biologia do desenvolvimento. In Malavasi, A., R.A. Zucchi. (eds.). Moscas-das-frutas de importância econômica no Brasil. Ribeirão Preto: Ed. Holos. 327 p.

Efron, D., D. Nestel, I. Glazer. 2001. Spatial analysis of entomopathogenic nematodes and insect hosts in a citrus grove in a semi-arid region in Israel. Environ. Entomol. 30: 254-261.

Gallo, D., O. Nakano, S. Silveira Neto, R.P. Carvalho Lima, G.C. Batista, E. Berti Filho, J.R.P. Parra, R.A. Zucchi, S.B. Alves, J.D. Vendramim, L.C. Marchini, J.R.S. Lopes, C. Omoto. 2002. Manual de Entomologia Agrícola, Piracicaba: Ed. FEALQ. 920 p.

Garcia, A. S., C.L. Messias, H.M.L. Souza, A.E. Piedrabuena. 1984. Patogenicidade de *Metarhizium anisopliae* var. *anisopliae* a *Ceratitis capitata* (Wied) (Diptera; Tephritidae). Rev. Bras. Entomol. 28: 421-424.

Garcia, A.S., H.M.L. Souza, C.L. Messias, A.E. Piedrabuena. 1989. Patogenicidade de *Metarhizium anisopliae* nas diferentes fases de desenvolvimento de *Ceratitis capitata* (Wied.) (Diptera, Tephritidae). Rev. Bras. Entomol. 33: 17-23.

Gazit, Y., Y. Rossler, I. Glazer. 2000. Evaluation of entomopathogenic nematodes for the control of Mediterranean Fruit Fly (Diptera; Tephritidae). Biocontrol Sci. Technol. 10: 157-164.

Grewal, P.S., E.A.B. De Nardo, M.M. Aguillera. 2001. Entomopathogenic nematodes: potential for exploration and use in South America. Neotrop. Entomol. 30: 191-205.

Lindegren, J.E., T.T. Wong, D.O. McInnis. 1990. Response of Mediterranean fruit fly (Diptera: Tephritidae) to the entomogenous nematode

Steinernema feltiae in field tests in Hawaii. Environ. Entomol. 19: 254-383-386.

Mochi, D.A., A.C. Monteiro, S.A. Bortoli, H.O.S. Dória, J.C. Barbosa. 2006. Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. Neotrop. Entomol. 35: 382-389.

Nascimento, A.S., R. Carvalho. Manejo integrado de moscas-das-frutas. 1999. p.169-173. In Malavasi, A., R.A. Zucchi. (eds.). Moscas-das-frutas de importância econômica no Brasil. Ribeirão Preto: Ed. Holos. 327 p.

Raga, A., S.T. Yasuoka, E.A. Amorim, M.E. Sato, N. Suplicy Filho & J.T. Faria. 1996. Sensibilidade de ovos de *Ceratitis capitata* (Wied., 1824) irradiados em dieta artificial e em frutos de manga (*Mangifera indica* L.). Sci. Agric. 53: 114-118.

Raga, A. & M.E. Sato. 2006. Time-mortality for fruit flies (Diptera: Tephritidae) exposed to insecticides in laboratory. Arq. Inst. Biol. 73: 73-77.

Rhode, C., L.F.A. Alves, P.M.O.J. Neves, S.B. Alves, E.R.L. Silva, J.E.M. Almeida. 2006. Seleção de isolados de *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. contra o cascudinho *Alphitobius diaperinus* (Panzer) (Coleoptera; Tenebrionidae). Neotrop. Entomol. 35: 231-240.

Souza Filho, M.F., A. Raga, R.A. Zucchi. 2000. p. 277-283. São Paulo. In Malavasi, A., R.A. Zucchi. (eds.) Moscas-das-frutas de importância econômica no Brasil: conhecimento básico e aplicado. Ribeirão Preto: Ed. Holos. 327 p.

Uziel, A., K. Levy, B. Yuval. 2003. Infection of *Ceratitis capitata* by two species of the *Entomophtora muscae* species complex (Zygomycetes: Entomophtorales) in the field. Phytoparasitica 31: 1-3.

Zucchi, R.A. 2001. Mosca-do-mediterrâneo, *Ceratitis capitata* (Diptera: Tephritidae). In Vilela, E.F.; Zucchi, R.A.; Cantor, F. (Eds.). Pragas introduzidas no Brasil. Ribeirão Preto: Holos Editora. p. 15-2