BIOLOGICAL CONTROL

Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in Soil with Different Pesticides

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Patogenicidade de *Metarhizium anisopliae* para *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) no Solo na Presença de Diferentes Agrotóxicos

RESUMO - O presente trabalho objetivou investigar se a presença de agrotóxicos no solo afeta a patogenicidade de *Metarhizium anisopliae* Metsch. (Sorokin) para *Ceratitis capitata* (Wied.). Avaliou-se também o efeito das formas de aplicação dos conídios. Foram utilizados os fungicidas clorotalonil e tebuconazole, o acaricida abamectina, o inseticida triclorfom e o herbicida ametrina, aplicados nas doses recomendadas pelos fabricantes. Porções de solo contidas em frascos de vidro receberam o fungo na forma de suspensão de conídios ou como conídios secos incorporados. Após a aplicação dos agrotóxicos, 20 larvas de 3º ínstar foram colocadas no solo. Os frascos foram então vedados com tecido *voile* e incubados a 27 ± 0,5ºC por nove dias, para iniciar a emergência dos adultos, e mais quatro dias à temperatura ambiente. A sobrevivência total do inseto foi substancialmente afetada. Observou-se a atividade patogênica a partir da fase pupal, com o fungo reduzindo ($P < 0,05$) a sobrevivência, o que também ocorreu na fase de adultos. A fase larval não foi afetada ($P > 0,05$) pelo fungo. A presença de agrotóxicos no solo teve discreta ação na atividade de *M. anisopliae;* apenas com conídios secos incorporados, os fungicidas clorotalonil e tebuconazole reduziram em 86,2% e 82,5%, respectivamente, a sobrevivência total de *C. capitata*, enquanto no controle a redução foi de 95,0%. A forma de aplicação dos conídios não influenciou a sobrevivência total do inseto, mas a aplicação da suspensão de conídios na superfície do solo reduziu a sobrevivência nas fases de pupa e adulto.

PALAVRAS-CHAVE: Controle biológico, controle microbiano, fungo entomopatogênico, mosca-das-frutas

ABSTRACT - This research intended to investigate if the presence of pesticides in the soil could affect the pathogenicity of *Metarhizium anisopliae* Metsch. (Sorokin) for *Ceratitis capitata* (Wied.) and assess the effect of conidia application as suspension or dry conidia. The fungicides chlorothalonyl and tebuconazol, the acaricide abamectin, the insecticide trichlorfon, and the herbicide ametrin were applied at the manufacturer-recommended doses. Soil samples were placed in glass flasks and were given the fungus as conidial suspension or dry. After pesticide application, 20 3rd-instar larvae were placed in the soil. The flasks were sealed with voile fabric and incubated at 27 ± 0.5ºC for nine days, until adult emergence; incubation continued for four more days at room temperature. The total insect survival was significantly affected and pathogenic activity was detected from the pupa stage on. Pupa survival was reduced ($P<0.05$); the same occurred during the adult phase. No effect was observed at the larval stage. The pesticides applied to the soil affected the activity of *M. anisopliae* slightly: only in the dry conidia assay the fungicides chlorothalonyl and tebuconazole reduced (86.2% and 82.5%, respectively) the survival period of *C. capitata* compared to the control (95.0%). The techniques used for conidia application did not influence the total insect survival rate, but conidial suspension applied on soil surface reduced survival during the pupae and adult phases.

KEY WORDS: Biological control, microbial control, entomopathogenic fungus, fruit fly

Entomopathogenic fungi respond for 80% of the insect diseases and have a high genetic variability, which inhibits pest resistance to these pathogens. Among them, *Metarhizium anisopliae* Metsch. (Sorokin) is considered an important microbial control agent and has been studied in several insect species (Alves 1998). The soil is the natural reservoir of insect-infecting fungi, whose stability can be affected by biotic and abiotic factors (Lanza *et al.* 2004).

Fruit flies are important crop pests in several regions in the world because they attack and cause severe damage to the fruits (Bissolli 2004). The fruit fly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae), a polyphagous insect is the most harmful among the tephritides (Malavasi *et al.* 2000). According to Gallo *et al.* (2002), at the end of the 3rd-instar larval period the larvae are ready to pupate; they bend their bodies and bounce, leaving the fruit and falling on the ground. At this point, the larvae become vulnerable to the action of entomopathogenic fungi in the soil (Bissolli 2004), as observed for other insects (Gutierrez *et al.* 2000).

The use of several pesticides in agriculture can affect the efficacy of entomopathogens. According to Ohashi *et al.* (1983), pesticides can interfere with fungi development and sporulation. The effect of pesticides on entomopathogens can vary according to the nature and concentration of the chemical product, and to the pathogen species and lineage. These products can inhibit vegetative growth, conidiogenesis, fungi sporulation, and cause genetic mutations (Tanzini 2002) that can result in low conidial viability and pathogen virulence to certain pests.

Several studies on the effect of pesticides for entomopathogenic fungi have been reported (Li & Holdom 1994, Todorova *et al.* 1998, Batista Filho *et al.* 2001, Loureiro *et al.* 2002, Tamai *et al.* 2002), but few researchers have performed fungus pathogenicity tests under the impact of pesticides in the soil.

In this study, the main goal was to evaluate the effect of pesticides in the soil on *M. anisopliae* pathogenicity for the fruit fly, *Ceratitis capitata* (Wied.). We assessed the conidial application on soil surface as suspension, similarly to field applications, and as dry conidia incorporated to the soil, as in natural biological control.

Material and Methods

The fungus. The E9 isolate of the fungus *M. anisopliae*, obtained from the spittlebug, *Deois flavopicta* (Stål), from a collection belonging to the Laboratório de Microbiologia da FCAV – Unesp, Jaboticabal, SP, was kept in stock culture at 4ºC in assay tubes containing potato-dextrose-agar (PDA). The isolate was grown in petri dishes with PDA medium, in an acclimatized chamber at 27 ± 0.5ºC, for 15 days.

***C. capitata* rearing.** The artificial diet used for larvae rearing consisted of sugar, Agar, yeast, Nipagin citric acid, and water (Dória 2004). Adults were confined in a metal cage (50 x 50 x 50 cm) lined with nylon screen mesh (70%) and fed on distilled water and honey 20% solution. The insects were kept in an acclimatized room at 27 ± 5 ºC and 12h photophase.

Fungus virulence, mass production, and viability. Prior to mass production, the fungus was submitted to a virulence bioassay with *C. capitata* larvae. Next, the fungus was isolated from the larvae again, placed in petri dishes containing PDA, and reapplied in new bioassays until approximately 100% mortality.

The fungus with recovered virulence was produced in rice, following the methodology described by Alves & Pereira (1998). The conidia so formed were tested for viability, according to the methodology reported by Marques *et al.* (2004). After production, the conidia were dried and removed, following Andrioli (2003). Dry conidia viability was also tested according to Marques *et al.* (2004).

The soil. We used red-yellow podzol of a sandy/medium texture colleted in January 2003, 0-20 cm deep in an environmental protection forest area at a farm located in the municipality Monte Alto, SP (21º 21' 02" S; 48º 31' 17" W). The soil dried at room temperature, and was sieved (1mm mesh) for particle uniformness and stored in plastic bags. Physical and chemical analyses were conducted at the Departamento de Solos e Adubos da FCAV-Unesp, Jaboticabal, SP. The soil field capacity was determined before each bioassay.

The pesticides. The pesticides used were shown effective on *M. anisopliae* in soil in previous work (Mochi *et al.* 2005). Commercial names and other information about the pesticides (active principle, chemical group, recommended dose, crop, classification) were obtained in the Compêndio de Defensivos Agrícolas (1996), as follows: a) Vertimec 18 CE, abamectin, avermectin, 1 L ha^{-1}, tomato, acaricide/insecticide; b) Folicur CE, tebuconazol, triazoles, 0.75 L ha^{-1}, rice, fungicide; c) Daconil 500 SDS, chlorothalonyl, isophthalonitrin, 2.5 L ha^{-1}, rice, fungicide; d) Dipterex, trichlorfon, organophosphorus, 1.4 L ha^{-1}, sugar-cane, insecticide; and e) Gesapax 500, ametrin, triazines, 4 L ha^{-1} with herbicides.

The pesticides were applied to the soil according to the amounts and concentrations recommended per square millimeter of soil surface by the manufacturer.

Bioassay design. We conducted two bioassays with fungicides and two with acaricides, insecticides, and herbicides. The fungus was applied to one of the fungicide assays and to all other pesticide assays on soil surface, in 2 ml suspension with 1.8 x 10^8 conidia ml^{-1}; in the remaining assay, the fungus was incorporated to the soil as dried conidia. The amounts of suspension and dried conidia were the same.

Each bioassay treatment consisted of one of the chemical products and the fungus, the chemical product only, or still the fungus only. In conidia suspension assays, the control consisted of soil and suspension vehicle. In the assay with soil-incorporated conidia, only soil control was conducted. Four replications were conducted for each treatment or control.

After a rigorous asepsis with alcohol, 100 g non-autoclaved soil was placed in 1.7 L pots. Sterile distilled

water was delivered to the soil until it reached 65% field capacity minus the amount of liquid to be delivered as inoculants and/or pesticide, depending on the treatment; the soil was let to rest for 1h. Next, pesticides were added and the twenty 3rd-instar *C. capitata* larvae were placed in each flask containing the soil. The flasks were taped with voile tissue held on the edges by latex stripes; the flasks were kept in an acclimatized chamber at 27 ± 0.5ºC for nine days. This period was needful for beginning adult emergence. To collect the emerged adults, the pots were removed from the chamber and kept at room temperature (26-28ºC), for four days.

Bioassay evaluation. Insect emergence was evaluated on the 9th, 10th, 11th and 12th days after pupation, which occurred on the day of the bioassay installation. On the 5th day after the first emergence, we turned the soil to locate dead pupae and larvae, which were washed in sterile distilled water, placed in a moisture chamber, and kept in a acclimatized chamber at 27 ± 0.5ºC, for observation of pathogen extrusion.

The adults were separated according to treatment and replication, for longevity evaluation. The cages were kept in an acclimatized chamber at 27 ± 0.5ºC and the insects were daily fed on distilled water and honey 20% solution. The dead adults were removed and placed in a moisture chamber and kept in acclimatized chambers 27 ± 0.5ºC, for observation of fungal extrusion. Evaluations were conducted until the 20th day after the last emergence day.

Statistical analysis. Data on experiments with fungicides and the other pesticides were analyzed according to the factorial designs 2 x 3 x 2 and 2 x 4 x 2, respectively. The factors were determined by fungal presence or absence, by three fungicide application categories (no fungicides, fungicide 1, fungicide 2), or by four categories of application of the other pesticides (no pesticides, acaricide, insecticide, herbicide). Two fungi application techniques (in suspension or as dry conidia) were tested in all treatments. Data collected from both experiments were submitted to analysis of variance by the F-test and the treatment means were compared by the Tukey test ($P < 0.05$). The ESTAT program was used for the statistical analysis.

Insect survival during larva, pupa, and adult stages were analyzed. Survival was calculated as number of insects starting each stage, independently of the previous one. Total survival was the quantity of live insects at the end of the experiment.

Results

The high viability of conidia produced in rice (98.7%) was not significantly affected by removal and drying (97.5%).

Larva survival. In the fungicide experiment, *M. anisopliae* conidia in the soil did not affect the larvae ($P > 0.05$); there was no difference between larva survival in the soil with or without the fungus (Table 1). However, the fungal application technique did; the lowest larva survival rate occurred in suspension-applied fungus treatments. Survival was not affected by the fungicides chlorothalonyl and tebuconazol in the soil; no difference ($P > 0.05$) was observed among treatments with the fungicides nor in relation to the control.

Similar results were obtained for the pathogenicity of *M. anisopliae* for *C. capitata* larvae after application of acaricides, insecticides, and herbicides. These pesticides did not affect larva survival and no differences were found among pesticide treatments, or between these and the control (Table 2).

A higher larval survival rate occurred in the treatment with soil-applied conidia suspension. This can be better understood when the significant result of the interaction between fungus and application technique (A x C) was analyzed by decomposition (analysis not shown). In the control with soil-incorporated dry conidia, 3.8% of the larvae died due to causes other than the presence of the fungus or pesticides in the soil. This led to a lower larva survival rate that produced the significant interaction effect.

Pupa survival. In the treatment with fungicides, pupa survival in the soil containing the fungus was significantly reduced ($P < 0.01$) to 22.3%, as opposed to pupa survival in the soil without fungus (89.5%) (Table1). Fungicides affected fungal activity, as shown by the fungus and fungicide interaction (A x B). Treatments with the fungus in the soil and receiving fungicides had higher pupa survival than the control (Table 3). The isolated action of fungicides in the soil did not affect pupa survival. No significant difference between the treatments with and without fungicides was found (Table 1).

In the decomposition of the interaction fungicide and application technique (B x C) (analysis not shown), tebuconazol enhanced pupa survival when fungal dry conidia were used. Probably, this fungicide was more toxic to the fungus, spread faster in the soil, and inactivated the soil-mixed conidia. The conidia applied as suspension probably remained on soil surface, allowing for higher conidia survival and leading to higher pupa mortality. Pupa survival was not affected by fungus application techniques. However, the decomposition of the interaction fungus and application technique (A x C) (analysis not shown) demonstrates that the conidia applied on soil surface as suspension caused higher pupa mortality. The larvae were probably contaminated by the soil surface conidia and at pupation, the conidia had already germinated and began the infectious process that led to pupa death.

In the experiment with different pesticides (acaricides, insecticides, and herbicides) fungus presence in the soil diminished pupa survival. In the treatment with the fungus, only 12.7% of the pupae survived; in the control, 85.8% did (Table 2). However, a small impact of some pesticides was observed among the pupae. The decomposition of the interaction fungus and pesticides (A x B) showed that the insecticide trichlorfon was toxic for the insect and reduced pupae survival in the treatment without fungus in the soil (Table 4). The acaricide abamectin and the herbicide ametrin

Table 1. Survival of *C. capitata* in soil with *M. anisopliae* conidia applied dry or as suspension followed by addition of different fungicides.

Analyzed factors	Survival (%)			
	Larvae	Pupae	Adults	Total
Fungus (A)				
Absence	99.7a	89.5a	80.6a	72.5a
Presence	97.5a	22.3b	17.8b	6.45b
F-test	3.99NS	278.83**	172.26**	280.88**
msd (5%)	2.11	6.05	8.58	7.30
Fungicides (B)				
Control	97.8a	50.6a	48.2a	38.7a
Chlorothalonyl	100.0a	59.3a	46.3a	37.5a
Tebuconazol	98.1a	57.8a	53.0a	42.1a
F-test	1.82NS	1.82NS	0.74NS	0.60NS
msd (5%)	3.11	8.93	12.66	10.77
Application techniques (C)				
Suspension	97.2b	52.3a	44.0b	39.7a
Dry conidia	100.0a	59.5a	54.4a	39.1a
F-test	6.97*	2.89NS	10.32**	2.18NS
msd (5%)	2.11	6.05	8.58	7.30
Interaction				
A X B	1.07NS	6.35**	0.41NS	0.91NS
A X C	3.99NS	14.40**	26.07**	17.02**
B X C	1.82NS	4.94*	0.42NS	0.38NS
A X B X C	1.07NS	0.13NS	0.39NS	0.14NS
C.V. (%)	6.31	20.65	29.80	29.10

Means in original values; analysis of variance conducted with data transformed into root (x/100). Means followed by at least one letter in common, in the column, do not differ by the Tukey-test (P>0.05). NS = not significant; **significant at 1% probability; *significant at 5% probability.

exerted a toxic effect on the insect that was not enough to be statistically different in mortality as compared with the control (Table 4).

Fungus application techniques influenced pupa survival with significant differences (P < 0.01) among treatments. When the dry conidia were incorporated to the soil, survival was 54.3%; when the fungus was applied as conidia suspension, 44.2% of the pupae survived, indicating that the latter application technique led to higher mortality (Table 2). These results can be related to insect contamination during the larva stage because conidia application to soil surface can have enhanced the attachment of a larger number of conidia to larva integument, thus shortening the infection process as compared to conidia incorporated to the soil.

Adult survival. In the fungicide bioassay, the survival of adult fruit flies was affected by the fungus but not by the fungicides. The fungus application technique influenced insect survival because the number of adult survivors was larger when dry conidia were incorporated to the soil (Table 1).

The decomposition of the interaction fungus and application technique (A x C) (analysis not shown), showed a higher efficiency of fungal action when conidia in suspension were applied, which can be a consequence of the higher insect contamination during the larva stage. It can also be due to the insect biological behavior because the

Table 2. Survival of *C. Capitata* in soil with *M. anisopliae* conidia applied as suspension or dry conidia with addition of pesticides.

Analyzed factors	Survival (%)			
	Larvae	Pupae	Adults	Total
Fungus (A)				
Absence	97.1a	85.8a	87.0a	72.8a
Presence	98.9a	12.7b	4.7b	0.6b
F-test	3.92NS	429.89**	349.39**	1025.20**
msd (5%)	1.82	4.81	7.86	4.87
Pesticides (B)				
Control	98.1a	52.4a	46.2a	42.1a
Abamectin (acaricide)	98.7a	46.8a	40.6a	32.8a
Trichlorfon (insecticide)	98.4a	46.4a	43.8a	31.5a
Ametrin (herbicide)	96.8a	51.5a	52.6a	40.3a
F-test	0.60NS	1.00NS	1.66NS	3.08*
msd (5%)	3.41	9.02	14.72	9.12
Application techniques (C)				
Suspension	99.0a	44.2b	46.1a	35.0a
Dry conidia	97.0b	54.3a	45.5a	38.4a
F-test	6.21*	10.52**	0.05NS	2.13NS
msd (5%)	1.82	4.81	7.86	4.87
Interaction				
A X B	0.62NS	3.55*	1.03NS	5.42**
A X C	12.37**	0.00NS	1.06NS	2.13NS
B X C	1.39NS	0.93NS	0.80NS	2.93*
A X B X C	0.35NS	1.00NS	2.45NS	0.63NS
C.V. (%)	7.00	22.35	36.69	23.22

Means in original values; analysis of variance conducted with data transformed into root (x/100). Means followed by at least one letter in common, in the column, do not differ by the Tukey-test (P>0.05). NS = not significant; **significant at 1% probability; *significant at 5% probability.

adult walked for approximately 1h before flying for the first time.

In the acaricide, insecticide, and herbicide treatment, adult survival was affected only by the presence of fungus (4.7%), as compared with 87% survival in the control (Table 2). This data indicates that although most of the contamination probably occurred during the larva or pupa stages, the fungal pathogenic action extended until the adult stage.

Total survival (larvae, pupae and adults). In the experiment with fungicides, *C. capitata* was significantly affected by *M. anisopliae* in the soil; only 6.4% of the insects survived (Table 1). Presence of fungicides and application technique did not affect total survival. However, the interaction fungus and application technique (A x C) influenced insect survival (Table 1). Treatment with the fungus with both application techniques reduced *C. capitata* survival but application of conidia suspension on soil surface had a more important effect on the insect's biological cycle, reducing insect total survival (Table 5).

As in the previous experiment, the fungus in the soil significantly reduced total insect survival in the experiment with the acaricide, the insecticide, and the herbicide; only 0.6% of the insects survived (Table 2). The interaction fungus and pesticides (A x B) showed that the insecticide trichlorfon and the acaricide abamectin directly reduced total fly survival (Table 6). The decomposition of the interaction pesticides

Table 3. Decomposition of the interaction fungus and fungicides, for the survival of *C. capitata* pupae in evaluating the effect of fungicides on *M. anisopliae* in the soil.

B x A	No fungus	Fungus	F-test
Control	92.4aA	8.8bB	152.12**
Chlorothalonyl	86.2aA	32.5aB	53.87**
Tebuconazol	90.0aA	25.6aB	85.52**
F-test	0.84NS	7.33**	

Means followed by the same small letter in the column or capital letter in the line, do not differ by the Tukey-test at 5% probability. NS = not significant; **significant at 1% probability; *significant at 5% probability.

Table 4. Decomposition of the interaction fungus and pesticide for the survival of *C. capitata* pupae in evaluating pesticide effect on *M. anisopliae* in the soil.

B x A	No fungus	Fungus	F-test
Control	93.6aA	11.3aB	149.54**
Abamectin	81.6abA	12.0aB	90.54**
Trichlorfon	77.0bA	15.7aB	66.15**
Ametrin	90.9abA	12.0aB	134.31**
F-test	3.98*	0.57NS	

Means followed by the same small letter in the column or capital letter in the line do not differ by the Tukey-test at 5% probability. NS = not significant; **significant at 1% probability; *significant at 5% probability.

and application technique (B x C) (analysis not shown) showed that the herbicide ametrin did not affect the insect. The insecticide trichlorfon used in the treatment with conidia applied as suspension reduced survival as compared with conidia incorporated to the soil; the acaricide abamectin reduced insect survival when dry conidia were incorporated in the soil.

Discussion

M. anisopliae was pathogenic for *C. capitata* and reduced its survival significantly. This activity was small during the larval stage; for most of the analyzed factors, no differences were found among treatments.

In a field experiment, Quintela *et al.* (1994) found that soil treatment with conidia of *Beauveria bassiana* (Bals.) Vuill. and *M. anisopliae*, controlled 30-50% of the *Chalcodermus bimaculatus* (Fiedler) larvae. Bissolli (2004) observed that the E9 isolate of *M. anisopliae* caused 66.25% mortality of *C. capitata* larvae bathed in the fungal conidia suspension. The larvae probably could not find an appropriate environment to pupate because they were placed in moisture chambers and not in the soil, leading to an extended 3rd-instar larva stage. Consequently, the larvae were more exposed to the pathogen and had a high mortality rate.

The situation described above did not occur in our study. The larvae remained on soil surface for approximately 1h; then, they entered the soil and pupated. Therefore, larvae exposure to fungi and to pesticides was short, which can explain the high survival rates during this stage.

The most important activity of the fungus occurred during the pupa stage and during conidia application to soil surface as suspension. According to Bissoli (2004), the mortality of pupae. This result was probably due to the soil-application of a suspension with lower conidia concentration (10^7 conidia/ml). For Bissolli (2004), the mortality of pupae bathed in suspension of several entomopathogenic fungi in the laboratory was low; the same occurred with the control. In our experiments, insect contamination occurred during larva stage. At the time larvae reached pupa stage, the fungus had probably started its infectious process and reaching the new stage was not enough to interrupt the fungus' active mechanism. The result was a higher insect mortality during pupa stage, differently from the results obtained by Bissoli (2004).

Our data support the results obtained by Ekesi *et al.* (2003), who studied the infectivity of *M. anisopliae* isolates for *C. capitata* after inoculating the fungus in the soil and stirring the flasks for 2 min. for uniformness, similarly to conidia incorporation to the soil, as conducted in our study. Those authors found a significantly lower adult emergence in the treatment with *M. anisopliae*, although fungal survival and pathogenicity were affected by temperature and humidity.

Laboratory studies showed that *M. anisopliae* is pathogenic for *C. capitata* eggs (Castilho 2000), larvae (Gutiérrez *et al*. 2000), and adults (Garcia *et al*. 1984, 1985).

Table 5. Decomposition of the interaction fungus and application technique for the total survival of *C. capitata* in evaluating fungicide effect on *M. anisopliae* in the soil.

A x C	Suspension	Dry conidia	F-test
No fungus	78.7aA	66.2aA	3.50NS
Fungus	0.8bB	12.0bA	15.69**
F-test	218.10**	79.80**	

Means followed by the same small letter in the column or capital letter in the line do not differ by the Tukey-test at 5% probability. NS = not significant; **significant at 1% probability; *significant at 5% probability.

Table 6. Decomposition of the interaction fungus and acaricide, insecticide, and herbicide for total survival of *C. capitata* when evaluating the effect of pesticides on *M. anisopliae* in the soil.

B x A	No fungus	Fungus	F-test
Control	84.3aA	0.0aB	346.19**
Abamectin	65.0bA	0.6aB	210.21**
Trichlorfon	61.8bA	1.2aB	186.07**
Ametrin	80.0aA	0.6aB	299.00**
F-test	8.26**	0.24NS	

Means followed by the same small letter in the column or capital letter in the line, do not differ by the Tukey-test at 5% probability. NS = not significant; **significant at 1% probability; *significant at 5% probability.

Most studies report the pathogen inefficiency for pupae. We observed high pupa mortality. Differences in results can be due to the different methods used: most authors submitted the pupa to pathogen action directly whereas in our study pupa death was a consequence of larva exposure to the pathogen.

The fungal pathogenic action lasted until adult stage: several insects were infected and died, causing a low survival rate. Bissolli (2004) also found that the mortality of adult *C. capitata* subject to *M. anisopliae* was higher than for the control.

Total mortality data showed that the fungus was more effective for *C. capitata*; on average no more than one insect survived to the end of the experiment. The pesticides had a small influence on the action of *M. anisopliae*; the fungicides attacked fungal conidia only during the pupa stage, thus decreasing the pathogenic action on the insects and consequently, lowering the fly mortality in these stages. Chandler *et al*. (1998) studied the occurrence of entomopathogenic fungi in soils treated with the fungicides triadimefon and benomyl using larvae of *Galleria mellonella* (L.) as bait. Both fungicides inhibited fungi growth in i*n vitro* assays. However, *B. bassiana* was the dominant species on the field, showing a lower fungicide effect.

Mietkiewski *et al*. (1997) studied the effect of two fungicides, two insecticides, and one herbicide on naturally occurring entomopathogenic fungi, by introducing *G. mellonella* larvae in the field. They found that the larvae were infected by five fungi species. The same authors argued that even though fungi pathogenicity was lower when pesticides were in the soil, pesticides did not stop the microbiota of entomopathogenic fungi in each treatment area.

Quintela & McCoy (1998) analyzed the effect of imidacloprid and two entomopathogenic fungi on the survival of *Diaprepes abbreviatus* (L.) larvae in the soil. They found that larval mortality and mycosis development in the insect increased synergically when sub-lethal doses of imidacloprid with *M. anisopliae* or *B. bassiana* conidia were applied. However, treatments with 5 x 10^3, 10^4 and 10^5 conidia of *B. bassiana* or *M. anisopliae* caused only a very low larval mortality. We did not find any synergic effect associating *M. anisopliae* and pesticides because the fungus caused high insect mortality both in the control and in the pesticide combination experiments. This may be due to the high concentration of conidia suspension and to pesticide application at manufacturer-recommended doses.

Application technique had a small effect on fungal activity, affecting the larva stage in both experiments, and the adult and pupa stages in experiments with fungicides and other pesticides, respectively, but techniques did not affect total survival in any of the experiments. Conidia applied as suspension on soil surface probably enhanced larvae-fungi contact, promoting a more important adhesion of conidia to the integument and eventual development of the infectious process. This process resulted in lower insect survival rate in some of their life cycle stages. However, as total survival was not affected by application techniques, we suggest that the conidia applied on soil surface or those soil-incorporated are able to efficiently control fruit flies in the soil. Our findings can foster the development of management techniques in agro-ecosystems that enhance the survival of *M. anisopliae* in the soil.

Our results show that pesticide application affected the pathogenic activity of *M. anisopliae* on *C. capitata* in the soil to a low extent. Only fungicides reduced fungal activity significantly. *M. anisopliae* was pathogenic for *C. capitata* when soil-applied, mainly during the pupa and adult stages, and its capacity to reduce fruit fly survival was not affected by fungus application techniques in the soil. In the field, pesticides are usually applied on the aerial plant parts with a significant amount reaching the soil. Therefore, the evaluation methodology used in our research can become a new approach to the analysis of pesticide effects on entomopathogenic fungi.

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