BIOLOGICAL CONTROL

Action of Pesticides to *Metarhizium anisopliae* in Soil

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Ação de Pesticidas em *Metarhizium anisopliae* no Solo

RESUMO - Este trabalho objetivou analisar a ação de alguns acaricidas, fungicidas, inseticidas e herbicidas contendo diferentes princípios ativos, sobre *Metarhizium anisopliae* Metsch. (Sorokin)*,* inoculado em solo autoclavado. A ação dos pesticidas foi analisada por meio da atividade respiratória do fungo. A primeira avaliação foi realizada 48h após a inoculação, em seguida foram adicionados os pesticidas e a atividade respiratória avaliada por nove vezes a cada 48h e mais cinco vezes a cada quatro dias. Com exceção dos fungicidas, não se observou efeito significativo (P > 0,05) dos pesticidas sobre *M. anisopliae*. Houve redução da produção de CO_2 nos tratamentos mancozebe do 4º ao 6º dia de incubação, tebuconazole nos intervalos entre o 4º e 6º, 8º e 10º e 32º e 36º, oxicloreto de cobre do 10º ao 12º, 32º ao 36º e clorothalonil do 8º ao 10º, 10º ao 12º e 32º ao 36º dias de incubação. O mesmo ocorreu com os acaricidas abamectina e óxido de fenibutatina, observando-se redução da produção de CO_2 entre o 20º e 24º dia de incubação. Os herbicidas glifosato, trifluralina e ametrina reduziram a atividade respiratória do fungo entre o 10º e 12º dia de avaliação e o inseticida triclorfon, apenas do 32º ao 40º dias de incubação. Os resultados indicam que a ação tóxica dos pesticidas sobre o fungo no solo foi pequena, sugerindo que esse bioagente de controle de pragas possa ser usado em conjunto ou associado aos pesticidas, sem comprometimento de sua atividade.

PALAVRAS-CHAVE: Atividade respiratória, fungo entomopatogênico, manejo integrado, controle biológico

ABSTRACT - The present study aimed to analyze the action of some acaricides, fungicides, insecticides and herbicides containing different active ingredients on *Metarhizium anisopliae* Metsch. (Sorokin) inoculated into autoclaved soil. The action of the pesticides was evaluated based on the fungal respiratory activity. The first assessment was done at 48h after inoculation. The pesticides were then added and respiratory activity was determined nine times every 48h and an additional five times every 4 days. Except for the fungicides, no significant effect (P > 0.05) of the pesticides on *M. anisopliae* was observed. A reduction in CO_2 production was observed for the mancozeb treatment from day 4 to day 6 of incubation, and for tebuconazol between days 4 and 6, 8 and 10, and 32 and 36. The same was observed for copper oxychloride between days 10 and 12 and 32 and 36, and for chlorothalonyl between 8 and 10, 10 and 12, and 32 and 36 days of incubation. Identical effect occurred for the acaricides abamectin and fenbutatin oxide, with a reduction in CO_2 production between 20 and 24 days of incubation. The herbicides glyphosate, trifluralin and ametrin reduced the respiratory activity of the fungus between days 10 and 12, while the insecticide trichlorfon reduced respiratory activity only from 32 to 40 days of incubation. The results indicate that the toxic action of pesticides on the fungus in soil is small, suggesting that this pest control bioagent can be used in combination with pesticides without compromising its activity.

KEY WORDS: Biological control, entomopathogenic fungus, integrated management, respiratory activity

The increasing use of chemical products necessary for the implantation and maintenance of agriculture characterized by healthy crops and high productivity has generated negative aspects for the biotic complex of nature, affecting plants, animals and humans.

In addition to a toxic action on the organism to which

they are applied, when reaching the soil chemical products may affect non-target organisms, thus damaging the environment. One possible medium- and long-term solution to minimize this effect is the use of integrated management, which emerged as a prudent option in terms of economic and ecological aspects. One strategy of integrated management is the combined use of chemical products and entomopathogens. This combined control can be very important for cultures where the use of chemical products is necessary, such as fruit trees in a temperate climate, citrus, coffee, cotton and soybean (Alves 1998a), since this approach reduces the application of pesticides, with a consequent decrease in environmental impact and expenses with these materials.

Entomopathogenic fungi are present in soil as an integrating part of a complex ecosystem characterized by a wide variety of microorganisms important for agricultural production. Among these microorganisms, *Metarhizium anisopliae* (Metsch.) Sorokin can be emphasized, due to its ability to promote biological control in nature or when applied to agricultural cultivations.

Several studies have analyzed the effects of pesticides on entomopathogenic fungi in order to determine their compatibility for pest control (Poprawski & Majchrowicz 1995, Neves *et al.* 2001, Loureiro *et al.* 2002). Most of these reports were conducted by adding products to the synthetic culture media used for fungal growth. However, when reporting the effect of these products on entomopathogenic fungi in soil, the results obtained with synthetic culture media may not reflect the field situation. The action of pesticides on entomopathogenic microorganisms on plants in field and on culture medium in the laboratory was analyzed by Almeida *et al.* (2003), who observed that the toxicity of the products was lower in the field than in the laboratory assays. However, few studies with this objective have been performed, probably due to the complexity of the analysis, since a precise determination of the impact of pesticides on the microbiota present in soil is difficult in view of the nature, heterogeneity, dynamics and adaptive responses of the microbial population (Frighetto 1997).

Because *M. anisopliae* is an important microbial agent of a wide variety of insects pest in nature or when applied to crops, it is essential to know the effect of chemical products on this fungus in soil in order to adequate techniques to the management of agroecosystems that permit the compatible use of this pathogen with agricultural defense strategies. The objective of the present study was to analyze a possible toxic action of some active ingredients present in acaricides, fungicides, insecticides and herbicides, used at the doses recommended by the manufacturers, on the entomopathogenic fungus *M. anisopliae* in soil based on the measurement of respiratory activity.

Material and Methods

Fungus. Isolate E9 of *M. anisopliae* (Metsch.) Sorokin obtained from the spittlebug *Deois flavopicta* (Stål) and maintained in a stock culture at 4ºC in test tubes containing potato-dextrose agar (PDA) was used. For the assays, the isolate was cultured on petri dishes containing PDA at 27ºC for 15 days. The conidial suspension used for soil inoculation was obtained from young colonies. The spores were removed from the surface of these colonies and transferred aseptically to a tube containing a 1:1 mixture of 0.89% (w/v) NaCl and 0.1% (v/v) Tween 80®. After vigorous shaking in an electrical tube shaker, the suspension was standardized at a concentration of 1.8×10^8 conidia ml^{-1} by counting the spores in a Neubauer chamber.

Soil. The soil used was a Yellow Red Podzol of a sandy/medium texture collected in January 2003 at a depth of 0 to 20 cm in an environmentally preserved forest located on an agricultural property at the following geographic coordinates: 21º 21' 02" S and 48º 31' 17" W. The chemical characteristics of the soil were: pH (CaCl$_2$) 5.5; organic matter = 26 g dm^{-3}; P (resin) = 6 mg dm^{-3}; K = 3.5 mmol$_c$ dm^{-3}; Ca = 38 mmol$_c$ dm^{-3}; Mg = 15 mmol$_c$ dm^{-3}; H+Al = 58 mmol$_c$ dm^{-3}; SB = 56.5 mmol$_c$ dm^{-3}; CEC = 76.5 mmol$_c$ dm^{-3}; V = 74%. After collection, the soil was dried at ambient temperature, sifted through a 1-mm mesh to obtain particles of uniform size and stored in plastic bags until the time for use. The soil with 65% water holding capacity saturation was determined before each bioassay. Portions (100 g) of this soil were transferred to 250-ml Erlenmeyer flasks and the flasks were sealed and autoclaved at 121ºC, at a pressure of 1 kgf cm^{-2}, for 1h.

Pesticides. Table 1 lists the commercial name and active ingredients of the pesticides.

Bioassays. A separate assay was conducted for each pesticide category, i.e., acaricide, fungicide, insecticide and herbicide, used at the doses recommended by the manufacturers. Each assay consisted of treatments with one of the four pesticides in each category (see Table 1) and two controls, one consisting of soil only and the other of soil inoculated with the fungus but without pesticide addition. The assays were performed in five replicates for each treatment and the controls.

After rigorous disinfection with alcohol, 1700-ml glass pots received 100 g portions of autoclaved soil in Erlenmeyer flasks under a laminar air flow. Sterile distilled water was then added to these soil samples at a quantity sufficient to reach 65% of its saturation capacity, subtracting the amount of fluid to be added in the form of inoculum and pesticide, and the samples were left to stand for 1h. After this period, 2 ml of the conidial suspension were spread over the whole soil surface (124.68 cm$^{2)}$) in each flask with a pipette. One 40-ml beaker containing 20 ml distilled water to maintain the ambient humidity and other containing 20 ml 0.3 M NaOH, were placed over the soil surface. The flasks were hermetically sealed with Parafilm, covered with a lid and kept in a climatized chamber at 27 ± 0.5ºC for 48h. After this period, the first measurement of respiratory activity was performed using an adaptation of the method described by Jenkinson & Powlson (1976). Briefly, the beaker containing 0.3 M NaOH was removed and titrated with 0.3 M HCl to determine the amount of CO_2 accumulated during the

Table 1. Pesticides used in the compatibility assays with *M. anisopliae* in soil[1].

Commercial name	Active ingredient	Chemical group	Dose	Culture	Category
Vertimec 18 CE	Abamectin	Avermectin	1 L ha^{-1}	Tomato	Acaricide
Kumulus	Sulfur	Sulfur	500 g ha^{-1}	Citrus	Acaricide
Torque 500 SC	Fenbutatin oxide	Organostanic	2,7 L ha^{-1}	Citrus	Acaricide
Dicofol	Dicofol	Organoclorine	3 L ha^{-1}	Cotton	Acaricide
Manzate 800	Mancozeb	Dithiocarbamate	4,5 kg ha^{-1}	Coffee	Fungicide
Folicur CE	Tebuconazol	Triazoles	0,75 L ha^{-1}	Rice	Fungicide
Cuprogarb 500	Copper-oxychloride	Copper compounds	3,5 kg ha^{-1}	Coffee	Fungicide
Daconil 500 SDS	Chlorothalonyl	Isophthalonitrin	2,5 L ha^{-1}	Rice	Fungicide
Decis 50 SC	Deltametrin	Pyrethroide	50 ml 100 L[-1 (2)]	Citrus	Insecticide
Dipterex	Trichlorfon	Organophosphorus	1,4 L ha^{-1}	Sugar cane	Insecticide
Ethion 500	Phosphorodithioate	Organophosphorus	2 L ha^{-1}	Citrus	Insecticide
Confidor	Imidacloprid	Chloronicotine	400 g ha^{-1}	Sugar cane	Insecticide
Roundup	Glyphosate	Glycine	4 L ha^{-1}	Sugar cane	Herbicide
Gramoxone	Paraquate	Bipyridyl compounds	2,25 L ha^{-1}	Sugar cane	Herbicide
Premerlin 600 CE	Trifluralin	Dinitroanilines	4 L ha^{-1}	Sugar cane	Herbicide
Gesapax 500	Ametrin	Triazines	4 L ha^{-1}	Corn	Herbicide

[1]Compêndio de Defensivos Agrícolas 1996
[2]Citrus: 50 ml 100 L-1. In half cup applications add 3 L to 5 L molasses 100 L -1 water, using 1L to 1.5 L solution.

period analyzed. Next, 2.5 ml pesticide solution were spread over the whole soil surface with a pipette at the amount and concentration calculated to obtain the dose (per mm^2 soil surface) recommended by the manufacturer. The second measurement was made 48h after application of the pesticide, followed by an additional eight measurements every 48h and five measurements every four days, for a total of 40 days of incubation.

Statistical Analysis. The assays were organized according to a design in plots subdivided according to time, with the treatments in the plots and time in the subplots (days after inoculation). The results were submitted to analysis of variance using the F test and means were compared by the Tukey test at 5% of probability. The respiratory activity curves of the fungus as a function of time were fitted using the Origin program, employing an exponential growth curve according to the equation $Y = y^0 + A_1 e^{(x+x0)/t1}$.

Results and Discussion

In all assays, the highest respiratory activity was observed during the first 48h after inoculation of autoclaved soil with *M. anisopliae* (Figs. 1 and 2; Tables 2-5), suggesting rapid germination of the conidia and saprophytic growth of the fungus due to the absence of competitors and easy utilization of the mineral nutrients and organic matter available.

According to Alves (1998b), in deuteromycetes grown under laboratory conditions germination occurs in a minimum period of 12h. Thus, 48h after inoculation the conidia had already germinated and the fungus showed high soil colonization activity. In each assay, in the treatment in which the soil sample was inoculated with the fungus but no pesticide was applied (control), a high respiratory activity upon subsequent evaluations was expected. However, this was not the case since on the fourth day after inoculation a
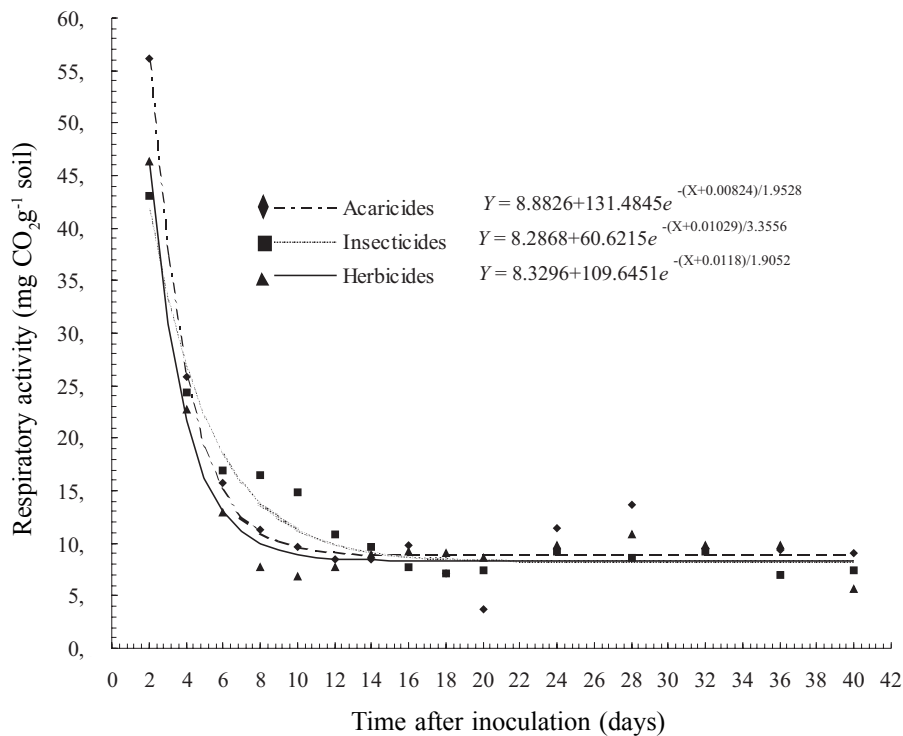
Figure 1. Fitted curves of the total amount of CO_2 produced by *M. anisopliae* in autoclaved soil containing different acaricides, insecticides and herbicides. The control values were included to obtain the curve for each pesticide, since significant difference was observed between treatments and control ($P > 0.05$).

significant decline ($P < 0.05$) in CO_2 production by *M. anisopliae* was observed, suggesting that in the second evaluation the fungus had already colonized the soil.

A reduction in fungal activity with increasing time of permanence in the soil was observed for the treatments with pesticides. However, as shown in Fig. 1, this decrease was not due to the action of acaricides, insecticides and herbicides, but was probably the result of the depletion of carbon sources in the soil organic matter, maturation of the fungal population and sporulation or of senescence, a natural characteristics of the hyphae forming mycelium. In the fungicide assay, a marked influence of the pesticides on the decline of fungal respiratory activity was observed, with CO_2 production being significantly lower in the treatments with copper oxychloride and chlorothalonyl compared to control (Fig. 2). According to Li & Holdom (1994) *M. anisopliae* isolates showed more tolerance to insecticides and herbicides than to fungicides.

No significant effect of acaricides on the survival of *M. anisopliae* in soil was observed. The addition of these pesticides two days after soil inoculation with the fungus did not have any deleterious effect on fungal respiratory activity. During the period from 16 to 18 days after inoculation, fungal respiratory activity was higher in the treatments with abamectin and fenbutatin oxide than in the control. From day 20 to 24, a significant reduction ($P < 0.05$) in fungal activity was observed for the same treatments, but only during this interval, with the activity remaining stable during the other periods analyzed (Table 2).

Fenbutatin oxide was classified as pathogen selective by Alves *et al.* (1992), because it was one of the active ingredients that caused less damage to the conidia of some entomopathogenic fungi. Loureiro *et al.* (2002) reported that *M. anisopliae* colonies grown in the presence of abamectin showed morphological alterations and reduced production of conidia.

The vegetative growth and reproduction of *Beauveria bassiana* (Bals.) Vuill. on synthetic medium was analyzed by Tamai *et al.* (2002) who classified sulfur as compatible and dicofol as highly toxic to this entomopathogen. In the present assay, neither sulfur nor dicofol altered the activity of *M. anisopliae* in soil. Thus, these pesticides exerting a non toxic effect, different from that observed for *B. bassiana* on synthetic medium containing dicofol.

Survival of *M. anisopliae* was affected by the action of some of the fungicides tested during the assessments. In the treatments with mancozeb and tebuconazol CO_2 production was significantly lower ($P < 0.05$) between day 4 and day 6 after inoculation compared to control (Table 3). In the treatment with mancozeb the decline in CO_2 production was only observed during this period. Fungal activity remaining stable thereafter until the end of the assay and the mean production CO_2 total did not differ from control. In soil receiving tebuconazol additional significant reductions ($P < 0.05$) in CO_2 production were observed between 8 and 10 days and between 32 and 36 days, but again the mean production CO_2 total did not differ from control. Analyzing the

compatibility between the active ingredients tebuconazol, mancozeb and copper oxychloride and *B. bassiana* based on the determination of the vegetative growth and reproduction of the fungus, Tamai *et al.* (2002) classified the fungicides as highly toxic to the pathogen. A similar result was reported by Loureiro *et al.* (2002) who observed that the growth of *M. anisopliae* colonies was inhibited by the same fungicides. Although a toxic action of these products on *M. anisopliae* in soil was observed in some evaluations of the present study, this effect cannot be considered marked since the pathogen recovered its respiratory activity almost immediately after the decline and the mean production CO_2 total in the treatments with the fungicides did not differ from control.

In the treatment with chlorothalonyl, respiratory activity of the fungus declined from day 8 to day 10 and, as observed for copper oxychloride, from days 10 to 12 and from days 32 and 36 of incubation. In both treatments the mean production CO_2 total was significantly lower than that observed from the control. The growth of *B. bassiana* was completely suppressed by chlorothalonyl that caused an 88% to 100% reduction in sporulation (Todorova *et al.* 1998). Determining the effect of fungicides on the survival of *B. bassiana* in the field, Jaros-Su *et al.* (1999) concluded that copper hydroxide might be less deleterious to the fungus than chlorothalonyl or mancozeb. In an *in vitro* experiment studying the effect of chlorothalonyl on soil total microbiota, Chen *et al.* (2001) observed that the fungicide abolished 30% to 50% of peak respiration compared to control. Tamai *et al.* (2002) observed that copper oxychloride is highly toxic to *B. bassiana*.

The insecticides analyzed showed no toxic effect on the fungus in soil. An action was only observed for the trichlorfon treatment between 32 and 36 days and between 36 and 40 days of incubation (Table 4). Tamai *et al.* (2002) classified imidacloprid, deltametrin and trichlorfon as compatible with *B. bassiana* and only phosphorodithioate as highly toxic in culture medium assays. In the present study, imidacloprid, deltametrin and phosphorodithioate did not affect *M. anisopliae*, whereas trichlorfon caused a significant reduction ($P < 0.05$) in fungal respiratory activity at the end of the study period.

Batista Filho *et al.* (2001) reported that imidacloprid did not affect *M. anisopliae* at the maximum dose recommended for the field but inhibited fungal growth at the minimum dose, thus classifying this insecticide as moderately toxic to the fungus at the maximum dose and incompatible at the minimum dose. However, no toxicity of imidacloprid to *M. anisopliae* was observed by Loureiro *et al.* (2002), and Neves *et al.* (2001) showed that the product did not inhibit germination, radial growth or fungal reproduction. Nevertheless, according to the last authors, deltametrin presented marked toxicity to *M. anisopliae* and other entomopathogenic fungi. Similar results have been reported by Oliveira *et al.* (2003), who observed a strong toxic effect of this product on the germination, vegetative growth and spore production of *B. bassiana*. When we analyzed the toxicity of deltametrin to *M. anisopliae* in soil, no significant difference in fungal respiratory activity was observed between this treatment and the control.

The respiratory activity of the fungus was little affected by the herbicides evaluated. Only glyphosate, trifluralin and ametrin reduced CO_2 production between 10 and 12 days of incubation (Table 5). The *in vitro* effect of two trifluralin-based herbicides on total microbial respiration of four agricultural soils was analyzed by Marzocca *et al.* (1994). The authors found that respiration was not affected by these products in any of the soils. Min *et al.* (2001) observed that
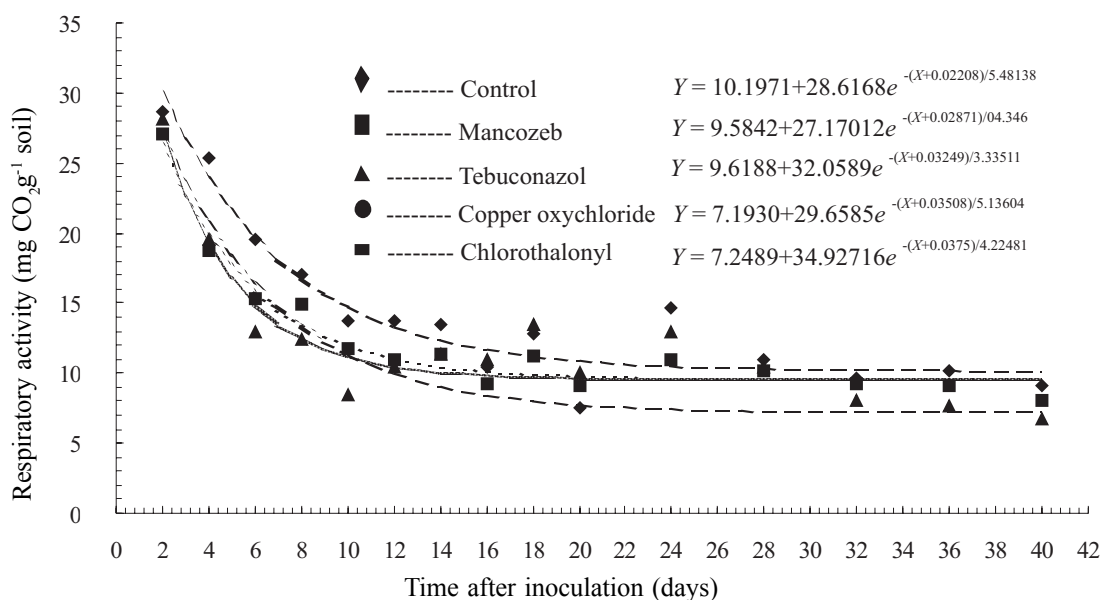
Figure 2. Fitted curves of the total amount of CO_2 produced by *M. anisopliae* in autoclaved soil containing different fungicides.

Legend/equations in figure:

Control: $Y = 10.1971 + 28.6168e^{-(X+0.02208)/5.48138}$

Mancozeb: $Y = 9.5842 + 27.17012e^{-(X+0.02871)/04.346}$

Tebuconazol: $Y = 9.6188 + 32.0589e^{-(X+0.03249)/3.33511}$

Copper oxychloride: $Y = 7.1930 + 29.6585e^{-(X+0.03508)/5.13604}$

Chlorothalonyl: $Y = 7.2489 + 34.92716e^{-(X+0.0375)/4.22481}$

Table 2. Respiratory activity (mg CO_2 100g^{-1} soil) of *M. anisopliae* in autoclaved soil submitted to the action of acaricides.[1,2]

Period analyzed (days)	Control	Sulfur	Dicofol	Abamectin	Fenbutatin oxide	Mean[3]	F test[4]	msd (5%)	CV (%)
0 - 2	28.1 a	28.3 a	28.1 a	27.6 a	28.0 a	28.0 A	0.04 n.s.	5.630	10.61
2 - 4	12.4 a	12.6 a	12.5 a	14.2 a	12.1 a	12.8 B	1.45 n.s.	2.928	12.09
4 - 6	8.3 a	7.8 a	7.4 a	7.9 a	6.9 a	7.7 C	0.70 n.s.	2.583	17.68
6 - 8	5.6 a	5.8 a	5.5 a	5.6 a	5.5 a	5.6 DE	0.31 n.s.	0.832	7.81
8 - 10	5.8 a	6.2 a	6.0 a	5.6 a	5.6 a	5.9 D	0.70 n.s.	1.324	11.86
10 - 12	5.2 a	4.7 a	4.6 a	4.8 a	4.7 a	4.8 EFG	1.92 n.s.	0.689	7.54
12 - 14	4.2 a	4.3 a	4.0 a	4.3 a	4.2 a	4.2 FGH	0.30 n.s.	0.855	10.61
14 - 16	5.1 a	5.0 a	4.8 a	4.6 a	4.6 a	4.8 EF	2.45 n.s.	0.672	7.30
16 - 18	2.0 b	3.4 ab	4.0 ab	4.8 a	4.7 a	3.8 GH	4.79 **	2.194	30.31
18 - 20	2.1 a	1.9 a	1.7 a	1.8 a	1.8 a	1.9 J	0.92 n.s.	0.754	20.98
20 - 24	3.1 a	3.0 a	3.6 a	1.5 b	1.4 b	2.5 IJ	23.34 **	0.884	18.13
24 - 28	3.3 a	3.2 a	3.6 a	3.3 a	3.2 a	3.3 HI	1.87 n.s.	0.512	8.02
28 - 32	2.3 a	2.4 a	2.6 a	2.1 a	2.3 a	2.3 IJ	1.93 n.s.	0.518	11.44
32 - 36	2.4 a	2.1 a	2.5 a	2.2 a	2.3 a	2.3 J	2.17 n.s.	0.452	10.14
36 - 40	2.3 a	2.1 a	2.4 a	2.2 a	2.2 a	2.2 J	0.86 n.s.	0.403	9.38
Means	6. 1 a	6.2 a	6.2 a	6.2 a	6.0 a				

F test for treatments = 0.87NS. F test for days after inoculation = 1008.51**. F test for interaction treatments x days = 1.14NS. CV for plots = 14.83. CV for subplots = 16.96. Msd: minimum significant difference. CV: coefficient of variation.

[1] Daily means obtained during the period analyzed.

[2] Means on the same line followed by the same lower case letter did not differ from one another (Tukey test, 5% probability).

[3] Means in the same column followed by the same upper case letter did not differ from one another (Tukey test, 5% probability).

[4] F test for comparison between treatments in each period.

n.s. non significant; * significant at the 5% level of probability; ** significant at the 1% level of probability

Table 3. Respiratory activity (mg CO_2 100g^{-1} soil) of *M. anisopliae* in autoclaved soil submitted to the action of fungicides.[1,2]

Period analyzed (days)	Control	Mancozeb	Tebuconazol	Copper oxycechloride	Chlorothalonyl	Means[3]	F test[4]	msd (5%)	CV (%)
0 - 2	14.2 a	13.5 a	14.0 a	13.8 a	14.1 a	14.0 A	0.41 n.s.	1.820	6.87
2 - 4	12.7 a	9.4 a	9.1 a	9.8 a	11.6 a	10.6 B	2.13 n.s.	4.490	22.48
4 - 6	9.8 a	7.6 bc	6.5 c	8.7 ab	7.9 abc	8.1 C	6.36**	2.078	13.49
6 - 8	8.5 a	7.4 a	6.2 a	7.3 a	6.3 a	7.1 C	2.95*	2.385	17.53
8 - 10	6.9 a	5.9 ab	4.2 b	5.2 ab	4.0 b	5.2 DE	6.53**	2.001	20.04
10 - 12	6.8 a	5.4 ab	5.2 ab	4.8 b	4.0 b	5.2 DE	4.82**	2.007	20.10
12 - 14	6.7 a	5.6 a	5.6 a	4.8 a	5.4 a	5.7 D	1.20 n.s.	2.680	24.85
14 - 16	5.2 a	4.6 a	5.5 a	4.1 a	3.6 a	4.6 EF	1.62 n.s.	2.552	28.97
16 - 18	6.4 a	5.5 a	6.7 a	3.8 a	3.4 a	5.2 DE	2.71 n.s.	3.795	38.38
18 - 20	3.7 ab	4.4 ab	5.0 a	3.1 b	3.8 ab	4.0 F	3.73*	1.601	20.75
20 - 24	3.6 a	2.7 a	3.2 a	2.1 a	2.2 a	2.8 G	2.86 n.s.	1.649	31.05
24 - 28	2.7 a	2.5 a	2.6 a	1.9 a	1.8 a	2.3 G	2.20 n.s.	1.202	27.25
28 - 32	2.4 a	2.3 a	2.0 a	1.9 a	1.6 a	2.0 G	1.88 n.s.	0.923	23.59
32 - 36	2.5 a	2.2 ab	1.9 b	1.9 b	2.0 b	2.1 G	4.70**	0.524	12.85
36 - 40	2.2 a	2.0 a	1.7 a	1.4 a	1.7 a	1.8 G	2.35 n.s.	0.831	23.74
Means	6.3 a	5.4 ab	5.3 ab	5.0 b	4.9 b				

F test for treatments = 4.06*. F test for days after inoculation = 268.08**. F test for interaction treatments x days = 2.33**. CV for plots = 43.89. CV for subplots = 19.33.
Msd: minimum significant difference. CV: coefficient of variation.
[1]Daily means obtained during the period analyzed.
[2]Means on the same line followed by the same lower case letter did not differ from one another (Tukey test, 5% probability).
[3]Means in the same column followed by the same upper case letter did not differ from one another (Tukey test, 5% probability).
[4]F test for comparison between treatments in each period.

n.s. non significant; * significant at the 5% level of probability; ** significant at the 1% level of probability

Table 4. Respiratory activity (mg CO_2 $100g^{-1}$ soil) of *M. anisopliae* in autoclaved soil submitted to the action of insecticides.[1,2]

Period analyzed (days)	Control	Imidacloprid	Phosphoro-dithioate	Trichlorfon	Deltametrin	Means [3]	F test [4]	msd (5%)	CV (%)
0 - 2	22.3 a	20.7 a	22.3 a	20.4 a	22.0 a	21.5 A	0.57 n.s.	5.030	12.31
2 - 4	12.7 a	11.2 a	12.0 a	12.4 a	12.4 a	12.2 B	0.44 n.s.	3.712	16.08
4 - 6	7.9 a	7.6 a	8.7. a	9.2 a	8.5 a	8.4 C	1.03 n.s.	2.653	16.61
6 - 8	7.6 a	7.9 a	8.3 a	8.9 a	8.1 a	8.2 C	0.70 n.s.	2.500	16.07
8 - 10	7.1 ab	6.6 b	7.7 ab	8.3 a	7.4 ab	7.4 C	2.87*	1.549	10.98
10 - 12	5.5 ab	4.6 b	7.0 a	4.8 ab	5.0 ab	5.4 D	3.34*	2.210	21.44
12 - 14	4.6 a	4.4 a	5.1 a	4.8 a	5.0 a	4.8 DE	0.76 n.s.	1.333	14.51
14 - 16	3.8 a	3.8 a	4.1 a	4.0 a	3.6 a	3.9 EF	1.01 n.s.	0.749	10.10
16 - 18	3.5 a	3.8 a	3.9 a	3.2 a	3.4 a	3.6 F	2.83 n.s.	0.738	10.84
18 - 20	3.6 a	3.4 a	4.0 a	3.4 a	4.0 a	3.7 F	0.97 n.s.	1.288	18.17
20 - 24	2.5 a	2.1 a	2.2 a	2.2 a	2.3 a	2.3 G	1.43 n.s.	0.507	11.63
24 - 28	2.0 a	1.9 a	2.2 a	2.2 a	2.1 a	2.1 G	1.38 n.s.	0.472	11.64
28 - 32	2.3 ab	2.3 ab	2.4 a	2.0 b	2.5 a	2.3 G	6.98**	0.286	6.48
32 - 36	1.8 a	1.7 ab	1.8 a	1.5 b	1.8 a	1.7 G	5.55**	0.253	7.64
36 - 40	2.0 a	1.8 ab	2.0 a	1.6 b	1.9 a	1.8 G	7.85**	0.246	6.94
Means	6.0 a	5.6 a	6.2 a	5.9 a	6.0 a				

F test for treatments = 1.99NS. F test for days after inoculation = 601.83**. F test for interaction treatments x days = 0.84NS. CV for plots = 23.58. CV for subplots = 17.91.
Msd: minimum significant difference. CV: coefficient of variation.
[1]Daily means obtained during the period analyzed.
[2]Means on the same line followed by the same lower case letter did not differ from one another (Tukey test, 5% probability).
[3]Means in the same column followed by the same upper case letter did not differ from one another (Tukey test, 5% probability).
[4]F test for comparison between treatments in each period.
n.s. non significant; * significant at the 5% level of probability; ** significant at the 1% level of probability

Table 5. Respiratory activity (mg CO_2 100g^{-1} soil) of *M. anisopliae* in autoclaved soil submitted to the action of herbicides.[1,2]

Period analyzed (days)	Control	Glyphosate	Trifluralin	Ametrin	Paraquat	Means[3]	F test[4]	mds (5%)	CV (%)
0 - 2	23.1 a	22.6 a	23.5 a	23.8 a	22.9 a	23.2 A	0.34 n.s.	3.504	7.98
2 - 4	11.6 a	10.4 a	11.3 a	11.8 a	11.6 a	11.3 B	1.41 n.s.	1.980	9.20
4 - 6	6.6 a	6.3 a	6.6 a	6.2 a	6.6 a	6.4 C	0.72 n.s.	1.055	8.59
6 - 8	3.9 a	3.6 a	3.9 a	3.8 a	3.9 a	3.8 EF	0.31 n.s.	1.088	14.83
8 - 10	3.5 a	3.1 a	3.6 a	3.4 a	3.1 a	3.4 F	1.37 n.s.	0.873	13.51
10 - 12	5.3 a	3.1 b	3.1 b	3.0 b	4.8 a	3.9 EF	50.12**	0.666	9.02
12 - 14	4.6 a	4.1 a	4.6 a	4.2 a	4.6 a	4.4 DE	1.02 n.s.	0.987	11.66
14 - 16	4.6 a	4.5 a	4.9 a	4.7 a	4.2 a	4.6 D	2.42 n.s.	0.666	7.58
16 - 18	4.7 a	4.6 a	4.6 a	4.4 a	4.2 a	4.5 D	1.98 n.s.	0.578	6.72
18 - 20	4.4 a	4.2 a	4.2 a	4.5 a	4.0 a	4.3 DE	2.64 n.s.	0.551	6.75
20 - 24	2.0 bc	1.8 c	2.5 ab	2.7 a	3.1 a	2.4 G	11.94**	0.661	14.11
24 - 28	2.8 ab	2.7 ab	2.9 a	2.6 ab	2.5 b	2.7 G	4.04*	0.338	6.53
28 - 32	2.5 a	2.5 a	2.5 a	2.4 a	2.2 a	2.4 G	0.98 n.s.	0.400	8.66
32 - 36	2.4 a	2.4 a	2.4 a	2.5 a	2.4 a	2.4 G	0.51 n.s.	0.363	7.77
36 - 40	1.4 a	1.4 a	1.4 a	1.4 a	1.3 a	1.4 H	0.29 n.s.	0.227	8.43
Means	5.6 a	5.1 a	5.5 a	5.4 a	5.4 a				

F test for treatments = 2.39NS. F test for days after inoculation = 1856.32**. F test for interaction treatments x days = 1.86**. CV for plots = 15.90. CV for subplots = 11.57. Msd: minimum significant difference. CV: coefficient of variation.

[1] Daily means obtained during the period analyzed.

[2] Means on the same line followed by the same lower case letter did not differ from one another (Tukey test, 5% probability).

[3] Means in the same column followed by the same upper case letter did not differ from one another (Tukey test, 5% probability).

[4] F test for comparison between treatments in each period.

n.s. non significant; * significant at the 5% level of probability; ** significant at the 1% level of probability

the soil microbial population used the pesticide as a carbon and nitrogen source for their growth, thus suggesting that trifluralin had been easily decomposed by them.

Although in the present study trifluralin, glyphosate and ametrin affected microbial activity, this effect was only observed over a short period of time, a fact not sufficient to consider these products toxic to the fungus. Haney *et al* (2000) concluded that glyphosate seems to be rapidly and directly degraded by soil microorganisms. In another study (Haney *et al.* 2002), these authors demonstrated that the product significantly stimulated soil microbial activity and biomass, in addition to being easily degraded irrespective of the amount of organic matter or soil type. According to Accinelli *et al.* (2002), application of six herbicides, including glyphosate, at the doses normally used in agriculture, had no significant effect on soil microbial activity, whereas application of the pure compounds and their commercial formulations at a dose of 20 mg active ingredient per gram soil markedly stimulated microbial activity.

Fungal activity was higher in the treatments with ametrin and paraquat compared to control from 20 to 24 days of evaluation (Table 5). Harrison and Gardner (1992), studying the influence of some herbicides such as glyphosate and paraquat on *B. bassiana* in sterile soil, observed no significant difference when comparing these treatments to controls, indicating that these two products are compatible with the fungus, in agreement with the present results.

Many pesticides used in agriculture can have at least a temporary impact on the soil microorganism population, with rapid recovery of the microbiota being sometimes observed. This might have occurred in some of the present treatments (herbicides); in these cases the cellular material of dead microorganisms becomes a readily available substrate for the survivors which, due of the lack of competition, proliferate abundantly (Moreira & Siqueira 2002).

Comparison of the *in vitro* test results shows that the toxicity of the chemical products to the fungus was higher in the culture medium assays than in the soil assays. After administration of the pesticide the synthetic medium was vigorously shaken to permit efficient distribution of the product. In addition, the homogeneity of the culture medium probably facilitates the distribution of the pesticide, promoting a rapid and effective action on the fungus, a fact that probably does not occur in soil. Since soil is a heterogenous environment, it impairs the distribution of the pesticide and may retain it partially adsorbed to some soil components, thus restricting the action of the pesticide on the fungus. Soil organic matter is the main component responsible for the adsorption of pesticides through hydrophobic interaction (Green & Karickhoff 1990). However, according to Monteiro (1997), the more clayish a soil, the greater the adsorption of pesticides. Studies performed by Upchurch (1966), Blanco (1979) and Alves *et al.* (2004) have shown that iron present in soil can influence adsorption and reduce the efficiency of herbicides. Since soil is the natural habitat of entomopathogenic fungi, these aspects are important for the survival of the fungus and for biological control. Another factor to be considered is that microorganisms are the main

organisms responsible for the degradation of pesticides in soil. Thus, the action of these products on *M. anisopliae* tends to be even lower in non-autoclaved soil. However, this aspect still needs to be investigated.

In the present study, we analyzed pesticides of different categories, with each product presenting its own characteristic chemical composition, mode of action and potential effect on fungi. Despite this fact, the results showed that fungicides most effectively influenced the respiratory activity of *M. anisopliae*, with copper oxychloride and chlorothalonyl being the active ingredients that most affected CO_2 production by the fungus. The herbicides, acaricides and insecticides tested had only a small effect on the respiratory activity of *M. anisopliae*. These findings indicate that the action of pesticides on the fungus in soil was highly discrete, suggesting that these agents can be used in combination in agroecosystems without compromising fungal activity. It is possible that a chemical pesticide could be fungistatic to *M. anisopliae*, resulting in lowered CO_2 production, while the fungus remains pathogenic to its insect host. Thus, bioefficacy studies of *M. anisopliae* in insect control are necessary in order to determine whether the use of these pesticides in combination with the fungus is really appropriate.

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