Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.17, n.2, e1392, 2022
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v17i2a1392 - Protocol 1392
Subm. 08/09/2021 • App. 15/05/2022 • Pub. 14/06/2022

Compatibility levels of fipronil with entomopathogenic fungi

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ABSTRACT: The present study evaluated the compatibility of the insecticide fipronil with entomopathogenic fungi, *in vitro*. The compatibility betwee fipronil and entomopathogenic fungi was based on vegetative growth and sporulation of seven isolates of fungi. The studied isolates were three from *Beauveria bassiana*, one from *Cordyceps fumosorosea*, and three from *Metarhizium anisopliae*, and four concentrations of fipronil (0.2, 0.4, 0.6, and 0.8 g L^{-1}), added in PDA (potato-dextrose-agar) culture medium. After 15 days, the colony diameter was measured and the spores were recorded. There was a reduction in the vegetative growth of the isolates at concentration of 0.8 g L^{-1} of fipronil. For the isolates of *B. bassiana* and *M. anisopliae*, there was a decrease in spore production when added the concentrations above 0.4 g L^{-1} of fipronil; and for *C. fumosorosea*, spore production decreased when fipronil concentrations were greater than 0.6 g L^{-1}. The insecticide fipronil was classified as "compatible" in terms of toxicity in vitro for tested fungi isolates depending on its concentration. The combination of the insecticide fipronil with the entomopathogenic fungi isolates tested is possible at concentrations below 0.6 g L^{-1}.

Key words: *Beauveria bassiana*; *Cordyceps fumosorosea*; *Metarhizium anisopliae*; MIP; insecticide selectivity

Níveis de compatibilidade do fipronil com fungos entomopatogênicos

RESUMO: O presente estudo avaliou a compatibilidade do inseticida fipronil com fungos entomopatogênicos, *in vitro*. A compatibilidade do fipronil foi classificada com base no crescimento vegetativo e esporulação dos isolados dos fungos entomopatogênicos. Foram utilizados sete isolados de fungos entomopatogênicos, sendo três de *Beauveria bassiana*, um de *Cordyceps fumosorosea* e três de *Metarhizium anisopliae*, e quatro concentrações de fipronil (0,2, 0,4, 0,6 e 0,8 g L^{-1}), adicionadas em meio de cultura BDA (batata-dextrose-ágar). Após 15 dias, efetuou-se a mensuração do diâmetro das colônias e a contagem dos esporos. Ao se utilizar a concentração de 0,8 g L^{-1}, verificou-se redução no crescimento vegetativo dos isolados. Para os isolados de *B. bassiana* e *M. anisopliae*, houve diminuição na produção de esporos quando adicionadas concentrações superiores a 0,4 g L^{-1} de inseticida; para *C. fumosorosea*, essa diminuição ocorreu quando adicionadas concentrações superiores a 0,6 g L^{-1}. O inseticida fipronil foi classificado como "compatível" quanto à toxicidade in vitro sobre os isolados testados dependendo da sua concentração. O uso em conjunto do inseticida fipronil com os isolados de fungos entomopatogênicos testados é possível em concentrações menores de 0,6 g L^{-1}.

Palavras-chave: *Beauveria bassiana*; *Cordyceps fumosorosea*; *Metarhizium anisopliae*; MIP; seletividade de inseticidas

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Associate Editor: Jorge Braz Torres

Introduction

In Brazil, the planted forest sector has been an important indicator of economic, social, and environmental development. With the increase in areas planted with these species, local economic changes are encouraged, offering new job opportunities and generating income for the population, as well as contributing to the adaptation and mitigation of climate change and the provision of ecosystem services (IBÁ, 2020).

However, with the gradual growth of areas with homogeneous plantings, there has also been a significant increase in phytosanitary problems, especially with pest insects (Machado & Costa, 2017). There are several methods of controlling pest insects in forests, however, the most commonly used is chemical control through the use of synthetic insecticides, with formulations containing fipronil being widely available for application (Ortiz et al., 2017).

Fipronil is an insecticide from the phenyl pyrazole chemical group (Britto et al., 2016). Fipronil is an aromatic compound, and its toxicological classification is in Class II and has agricultural use with soil and foliar application, seed treatment, seedlings or through irrigation water, depending on the crop (ANVISA, 2021). Fipronil acts on gamma aminobutyric acid (GABA) receptors associated with chloride channels, blocking the passage of chloride ions, thereby eliminating the normal inhibition of nerve impulses, causing an increase in neural activity and thus paralysis and death of the organism (Chaguri, 2016).

Chemical control, although widely used, presents problems due to environmental contamination, low specificity, possibility of pest resurgence, selection of insects resistant to insecticides, and potential risks to human and animal health (Barbosa et al., 2015). Thus, there is a need for research for alternative control measures that impact the environment less, such as the use of biological control (Pessoa et al., 2020a). In this sense, entomopathogenic fungi are employed as a viable alternative for the management of forest pests (Wilcken, 2016), considering that for *Hedypathes betulinus* (cabbage-palm borer) there is a registered biological product based on *B. bassiana*, with a strain cataloged in the collection of Embrapa Recursos Genéticos (CENARGEN) under code CG 716 (registered by Embrapa Florestas and Turfal Ind. e Com. de Produtos Biológicos e Agronômicos) (Borges et al, 2010), showing promising results for the use of these organisms in the biological control of other insect-pests.

In vitro compatibility tests of insecticides with entomopathogenic fungi describe the possible negative or positive effects that may occur when they are applied in association with other products (Alves & Lopes, 2008). In cases where mixing is performed this becomes relevant since the insecticides can act antagonistically on the entomopathogenic fungi including inhibition of vegetative growth, conidiogenesis, and sporulation. These effects can result in mutations that can reduce pathogenicity and virulence (Barbosa Junior, 2020).

The results of *in vitro* compatibility tests can be the basis for the development of Integrated Pest Management (IPM) strategies, and the use of entomopathogenic fungi and insecticides can be combined to control pest insects (Fiedler & Sosnowska, 2017).

Research aiming to verify the synergism of entomopathogenic fungi and insecticides in the control of pest insects has been developed and presented promising results of the association of these two control methods (Ashraf et al., 2017; Shewale & Mohite, 2018). Thus, the possibility of joint use of biological control agents and insecticides for pest insect management is verified (Pessoa et al., 2020b). This association is able to assist in reducing the population and incidence of pests, then optimizing their potential as control agents, due to the presence of substances contained in chemical plant protection products that act as stressors, then favoring the infection of fungi that perform biological control of pests (Wilcken, 2016).

Allowing the action of biological control agents, in a natural or applied way, is of great importance for IPM (Pessoa et al., 2020b). These agents are important tools, used as an alternative to reduce the various problems caused by the indiscriminate use of insecticides (Lopes et al., 2018). Aiming at an alternative for the control of pest insects, the objective of the present study was to evaluate the compatibility of the insecticide fipronil with isolates of *Beauveria bassiana*, *Cordyceps fumosorosea*, and *Metarhizium anisopliae*, *in vitro*, classifying it as to its toxicity on entomopathogenic fungi.

Materials and Methods

The present study was carried out at the Laboratory of Plant Pathology "Dra. Elocy Minussi", belonging to the Department of Plant Protection, Center of Rural Sciences, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.

Preparation and maintenance of the entomopathogenic fungi used

We used seven isolates of entomopathogenic fungi: three isolates of *B. bassiana* (IBCB 66, IBCB 170, and IBCB 632), one isolate of *C. fumosorosea* (IBCB 130), and three isolates of *M. anisopliae* (IBCB 348, IBSP 383, and IBCB 425), which were provided by the Instituto Biológico de São Paulo, Entomopathogenic Fungi Collection "Oldemar Cardin Abreu".

For repotting, the isolates were inoculated onto sterilized Petri dishes containing PDA (potato-dextrose-agar) culture medium. The plates were kept in a B.O.D. type climate-controlled chamber at 25 ± 2 °C under alternating light with a 12 hours photoperiod for a period of seven days for growth and sporulation of the fungi.

After seven days of mycelial growth, the spore suspensions were prepared. Ten mL of sterile distilled water were added to the Petri dish and, with the aid of a Drigalski loop, the fungal mycelium was scraped from the surface of the medium, the suspension being filtered through a double layer of gauze into a beaker and an adhesive spreader was added to the surface

of the medium. 0.01% (Tween 40) to perform the spore count in a Neubauer chamber. After counting spores the suspension was calibrated to a concentration of 1×10^6 spores mL^{-1}.

Cultivation of entomopathogenic fungi in culture medium containing fipronil

PDA culture medium was prepared and autoclaved at 121 °C for approximately 20 minutes. After cooling for a few minutes at room temperature, the antibiotic penicillin was added to the medium. Then, four different concentrations of fipronil-based insecticide (0.01% pp) were added (0.2, 0.4, 0.6, and 0.8 g L^{-1}), which was previously filtered using 0.22 µm diameter filters in order to remove contaminants. The negative treatments were done by growing the isolates on PDA medium containing penicillin alone, without the insecticide. The flasks containing the PDA medium, penicillin, and the insecticide were shaken manually for one minute to homogenize these components and then poured into sterile Petri dishes.

Using a pipettor, 10 µL of the previously prepared spore suspension (1×10^6 spores mL^{-1}) was transferred to the center of each Petri dish. Subsequently, the plates were incubated in B.O.D. type chambers under controlled temperature and light (25 ± 2 °C and 12 hours photoperiod) for a period of 15 days.

Evaluation of mycelial growth and sporulation of entomopathogenic fungi

The evaluation of mycelial growth and sporulation of entomopathogenic fungal isolates after 15 days of incubation. The mycelial growth of each isolate on the PDA culture medium containing the different concentrations of the insecticide fipronil was measured with the help of a digital pachymeter in two diametrically opposite directions of the colony to obtain the means of the mycelial growth values.

To evaluate the sporulation of the isolates, 10 mL of sterile distilled water was added to each Petri dish, and then the colony was scraped off with a Drigalski loop to release the spores. The suspension was pipetted and filtered through a double layer of gauze to retain the mycelial fragments and culture medium. Spore counts were performed in a Neubauer chamber, with the aid of an optical microscope, at 40× magnification.

Classification of the insecticide fipronil for toxicity on different isolates of entomopathogenic fungi

To classify the insecticide as to its toxicity on isolates of *B. bassiana*, *C. fumosorosea*, and *M. anisopliae*, *in vitro*, we used the model proposed by Alves et al. (1998), by which the percentage values are calculated in relation to the control (100%), according to the Equation (1):

$$T = \frac{20(CV) + 80(ESP)}{100} \quad (1)$$

where:

T - corrected value of vegetative growth and sporulation for product classification;

CV - percentage of vegetative growth with respect to the witness; and,

ESP - percentage of sporulation in relation to the witness.

From the "T" values, the insecticide was classified as *very toxic* (0 to 30), *toxic* (31 to 45), *moderately toxic* (46 to 60), or *compatible* (> 60) (Alves et al., 1998).

Statistical analysis

The experiments were conducted in an entirely randomized design with four repetitions. For the isolates of *B. bassiana* and *M. anisopliae*, a two-factor composite was used: four concentrations × three isolates. Data were subjected to analysis of variance (ANOVA), and averages to Tukey test ($p < 0.05$), using SISVAR software version 5.6.86 (Ferreira, 2014).

Results and Discussion

From the *in vitro* trial, the isolates of *B. bassiana* treated with fipronil showed a reduction in vegetative growth at all concentrations analyzed, compared to the respective controls (Table 1). The mycelial growth of the isolate IBCB 66 had the greatest reduction at the concentration of 0.8 g L^{-1}. This value, when compared to the respective control, had a reduction in growth of 21.87%. The same occurred with isolates IBCB 170 and IBCB 632, where there was a greater reduction in mycelial growth at the 0.8 g L^{-1} concentration, with 19.75 and 21%, respectively.

The effect of the insecticide fipronil at the concentration of 0.8 g L^{-1} resulted in reduction in vegetative growth and sporulation of different isolates of entomopathogenic fungi (*B. bassiana*, *B. brongniartii*, *M. anisopliae*, *Paecilomyces* sp., and *Aspergilus* sp.) (Bezerra, 2018), with the *B. bassiana* isolate showing the highest reduction in vegetative growth (80.9 ± 0.98%), among all entomopathogenic fungal isolates

Table 1. Mycelial growth (mm) of *B. bassiana* isolates on potato-dextrose-agar (PDA) culture medium, treated with different concentrations (g L^{-1}) of the insecticide fipronil, at 15 days of incubation.

Isolated	Control	Fipronil concentrations (g L^{-1})			
		0.2	0.4	0.6	0.8
IBCB 66	54.14 ± 2.19 a*	44.67 ± 1.17 b	42.99 ± 1.58 b	44.32 ± 0.87 b	42.30 ± 0.93 b
IBCB 170	52.80 ± 2.09 a	48.48 ± 1.09 ab	46.21 ± 1.18 b	43.13 ± 1.47 c	42.37 ± 1.99 c
IBCB 632	52.94 ± 2.73 a	46.94 ± 5.70 b	44.85 ± 1.02 bc	43.55 ± 0.87 bc	41.82 ± 1.42 c
Overall average	53.29	46.69	44.68	43.67	42.16
CV (%)			5.31		

* Averages followed by the same letter in the row do not differ by Tukey test at 5% probability of error. CV = Coefficient of variation.

tested. This value was higher than that found in the present study, which for this same fipronil concentration, an average vegetative growth reduction of 20.88% was obtained. The better compatibility observed in the present study can be explained by the different characteristics of the isolates used in the studies, since each isolate has different characteristics and responses to fipronil compatibility.

The lowest sporulation occurred at the 0.8 g L^{-1} fipronil concentration, with 28.18, 32.81, and 33.58%, respectively, for isolates IBCB 66, IBCB 170, and IBCB 632 (Table 2). The concentrations of 0.2, 0.4, and 0.8 g L^{-1} did not cause significant differences in relation to the sporulation of the isolates. The values of 4.66 × 10^6 and 3.42 × 10^6 mL^{-1} at a concentration of 0.6 g L^{-1} for isolates IBCB 170 and IBCB 642, respectively, demonstrate a difference in sporulation between isolates, with the highest sporulation occurring in isolate IBCB 170 and the lowest in isolate IBCB 632.

Analyzing the toxicity of fipronil incorporated into PDA culture medium on B. bassiana soleus, Fregonesi et al. (2016) observed, at 15 days of incubation, mean mycelial growth values ranging between 44.25 and 50.75 mm, and sporulation ranging between 1.40 × 10^9 and 4.97 × 10^9 spores mL^{-1}. Such values are similar to the results obtained in the present research, where the overall average mycelial growth of *B. bassiana* was 46.09 mm, and average sporulation was 4.67 × 10^6 mL^{-1}.

The mycelial growth of *M. anisopliae* when submitted to different concentrations of fipronil was observed to be lower for the isolate IBCB 348 at 0.8 g L^{-1} of fipronil (Table 3). This effect resulted in a reduction of 15.98% in relation to the respective control. The mycelial growth of the *M. anisopliae* isolate IBCB 383 was lower at the 0.6 g L^{-1} fipronil concentration, which represented a reduction of 18.49% when compared to the respective control. However, there was no significant difference when compared to the average mycelial growth at the 0.8 g L^{-1} concentration (Table 3).

Sporulation was reduced compared to the respective controls for *M. anisopliae* isolates IBCB 348 and IBCB 425 submitted to concentrations of the insecticide fipronil (Table 4).

The isolate IBCB 348 showed no significant differences between the fipronil concentrations used. For this same isolate, it was found that the concentration of 0.4 g L^{-1} presented the lowest spore production, representing a reduction of 22.61% when compared to the respective control.

Regarding the isolate IBCB 383, the highest production of conidia occurred at the concentration of 0.2 g L^{-1}, which was similar to the control. In contrast, the greatest reduction in conidia production occurred with this isolate submitted to 0.8 g L^{-1}, which represented a reduction of 41.03% when compared to its respective control (Table 4). The isolate IBCB 425 at the concentration of 0.8 g L^{-1} showed the lowest

Table 2. Sporulation (× 10^6 mL^{-1}) of *B. bassiana* isolates on potato-dextrose-agar (PDA) culture medium, treated with different concentrations (g L^{-1}) of the insecticide fipronil, at 15 days of incubation.

Isolated	Control	Fipronil concentrations (g L^{-1})			
		0.2	0.4	0.6	0.8
IBCB 66	6.21 ± 0.46 Aa*	5.02 ± 0.69 Aa	4.35 ± 0.98 Ba	4.35 ± 0.22 Bab	4.46 ± 0.78 Ba
IBCB 170	6.43 ± 0.92 Aa	5.26 ± 0.45 Aa	4.75 ± 0.37 Ba	4.66 ± 0.31 Ba	4.32 ± 0.40 Ba
IBCB 632	5.33 ± 0.39 Aa	4.35 ± 0.64 Ab	3.96 ± 0.54 ABa	3.42 ± 0.38 Bb	3.54 ± 0.73 Ba
Overall average	5.99	4.87	4.35	4.14	4.11
CV (%)			14.78		

* Averages followed by the same capital letter in the row and lower case in the column do not differ by Tukey test at 5% error probability. CV = Coefficient of variation.

Table 3. Mycelial growth (mm) of *M. anisopliae* isolates on potato-dextrose-agar (PDA) culture medium, treated with different concentrations (g L^{-1}) of the insecticide fipronil, at 15 days of incubation.

Isolated	Control	Fipronil concentrations (g L^{-1})			
		0.2	0.4	0.6	0.8
IBCB 348	55.48 ± 3.21 a*	49.71 ± 0.83 ab	48.56 ± 1.01 b	49.10 ± 1.91 b	46.61 ± 3.28 b
IBCB 383	54.67 ± 3.32 a	48.85 ± 4.38 ab	48.53 ± 1.83 ab	44.56 ± 0.63 b	45.02 ± 1.26 b
IBCB 425	50.24 ± 2.39 ab	54.03 ± 2.40 a	52.42 ± 3.75 ab	46.70 ± 1.94 b	46.45 ± 2.22 b
Overall average	54.13	50.86	49.83	46.78	46.02
CV (%)			6.35		

* Averages followed by the same letter in the row do not differ by Tukey test at 5% probability of error. CV = Coefficient of variation.

Table 4. Sporulation (× 10^6 mL^{-1}) of *M. anisopliae* isolates on potato-dextrose-agar (PDA) culture medium, treated with different concentrations (g L^{-1}) of the insecticide fipronil, at 15 days of incubation.

Isolated	Control	Fipronil concentrations (g L^{-1})			
		0.2	0.4	0.6	0.8
IBCB 348	7.12 ± 0.94 Aa*	5.77 ± 1.36 Aa	5.51 ± 0.73 Aa	6.11 ± 0.33 Aa	5.84 ± 0.76 Aa
IBCB 383	5.63 ± 0.17 Aa	6.29 ± 0.58 Aa	4.52 ± 0.74 ABa	5.19 ± 0.88 Aa	3.32 ± 0.73 Bb
IBCB 425	6.43 ± 1.61 Aa	5.65 ± 0.50 Aa	5.00 ± 0.41 Aa	5.76 ± 0.10 Aa	3.63 ± 0.73 Bb
Overall average	6.39	5.90	5.01	5.69	4.26
CV (%)			16.79		

* Averages followed by the same capital letter in the row and lower case in the column do not differ by Tukey test at 5% error probability. CV = Coefficient of variation.

production of conidia, statistically different from the other treatments, and presenting a reduction of 56.45% when compared to the respective control. It was found that there were significant differences at the 0.8 g L^{-1} concentration, since isolate IBCB 348 showed the highest spore production, differing statistically from isolates IBCB 383 and IBCB 425. For the other treatments (0.2, 0.4, and 0.6 g L^{-1}, and the control) no significant differences were found between them and the isolates.

Evaluating the effect of fipronil with isolates of *M. anisopliae*, Bezerra (2018) found that the isolate URM 4920, in contact with fipronil at a concentration of 0.8 g L^{-1} did not show a reduction in conidia production when compared to the control. This variation may be related to the fact that these are different isolates, which tend to develop differently, even when subjected to similar environmental conditions.

For *C. fumosorosea* (IBCB 130), there was a reduction in mycelial growth with increasing fipronil concentration (Table 5). The lowest mean mycelial growth was obtained when the isolate was subjected to a concentration of 0.8 g L^{-1}, which represented a reduction of 18.24% when compared to the control.

Regarding sporulation, there were differences among treatments, and the lowest spore production occurred at 0.8 g L^{-1}, which represented a 40.86% reduction when compared to the respective control (Table 5). As noted by Rojas (2015), in Brazil there are no registered products based on *C. fumosorosea* and few studies have been conducted with this entomopathogenic fungus species. However, the results of this study show the prospect of compatibility of *C. fumosorosea* and the insecticide fipronil, taking into account the concentrations to be used, so that they do not interfere significantly in the development of the fungal isolate, and consequently, in its pathogenic action on insects.

The seven entomopathogenic fungal isolates treated using the 0.8 g L^{-1} fipronil concentration showed a reduction in vegetative growth. A possible explanation for these results is cited by Oliveira et al. (2002). These authors suggest that metabolization of the medium containing the formulation by the fungi may generate toxic residues that, when accumulating, can block pathways of compounds important for fungal growth.

Table 5. Mycelial growth (mm) and sporulation (× 10^6 mL^{-1}) of *C. fumosorosea* isolates on potato-dextrose-agar (PDA) culture medium, treated with different concentrations (g L^{-1}) of the insecticide fipronil, at 15 days of incubation.

Fipronil concentrations (g L^{-1})	Mycelial growth (mm)	Sporulation (× 10^6 mL^{-1})
0.0 (control)	58.88 ± 1.86 a*	6.24 ± 0.70 a
0.2	53.05 ± 1.25 a	5.47 ± 0.40 ab
0.4	49.59 ± 1.18 a	5.03 ± 0.53 b
0.6	48.86 ± 3.26 b	4.75 ± 0.47 bc
0.8	48.14 ± 1.51 b	3.69 ± 0.43 c
Overall average	51.71	5.04 × 10^6
CV (%)	4.40	11.98

* Averages followed by the same letter do not differ by Tukey test at 5% probability of error. CV = Coefficient of variation.

The results of mycelial growth and sporulation of isolates of *B. bassiana* (IBCB 66, IBCB 170, and IBCB 632) and *M. anisopliae* (IBCB 348, IBCB 383, and IBCB 425), demonstrate variability in the values found, when compared to isolates of the same species. Such variability may be related to the fact that the toxicity of chemicals on entomopathogenic fungi varies by virtue of the fungal species or strain (Silva et al., 2013).

From the results obtained, regarding vegetative growth and sporulation, fipronil can be classified as to it is *in vitro* toxicity on each of the entomopathogenic fungal isolates at the different concentrations used (Table 6). These results corroborate with other studies such as those of Fregonesi et al. (2016), who classified fipronil as compatible with isolates IBCB 66, IBCB 170, and IBCB 632 of *B. bassiana*, stating that it can be used in association; and Bezerra (2018), who found similar T-factor values for *B. bassiana* and *M. anisopliae*, classifying fipronil as compatible with isolates of entomopathogenic fungi.

The compatibility results of entomopathogenic fungi with fipronil found in the present study can be justified by the statements of Pessoa et al. (2020a), where they comment that the fungus, in an activity comparable to what occurs with living beings in general, makes use of its entire reproductive

Table 6. Calculated "T" index values and classification of the insecticide fipronil at different concentrations for *in vitro* toxicity on isolates of *B. bassiana* (IBCB 66, IBCB 170, and IBCB 632), *C. fumosorosea* (IBCB 130), and *M. anisopliae* (IBCB 348, IBCB 383, and IBCB 425).

Isolated	Fipronil concentration (g L^{-1})	"T" values	Classification*
IBCB 66	0.2	81	Compatible
	0.4	75	Compatible
	0.6	72	Compatible
	0.8	73	Compatible
IBCB 170	0.2	83	Compatible
	0.4	76	Compatible
	0.6	74	Compatible
	0.8	69	Compatible
IBCB 632	0.2	84	Compatible
	0.4	77	Compatible
	0.6	69	Compatible
	0.8	68	Compatible
IBCB 130	0.2	125	Compatible
	0.4	82	Compatible
	0.6	78	Compatible
	0.8	64	Compatible
IBCB 348	0.2	83	Compatible
	0.4	80	Compatible
	0.6	86	Compatible
	0.8	82	Compatible
IBCB 383	0.2	107	Compatible
	0.4	82	Compatible
	0.6	90	Compatible
	0.8	64	Compatible
IBCB 425	0.2	92	Compatible
	0.4	83	Compatible
	0.6	90	Compatible
	0.8	64	Compatible

* Classification according to Alves et al. (1998).

mechanism when in the presence of a toxic principle that alters its environment, and impairs its development, thus resulting in increased vegetative growth and conidiogenesis.

Alves & Lopes (2008) state that the microorganism, in a physiological resistance mechanism, can metabolize the toxic principles of the active ingredient, using the molecules resulting from this process, released in the culture medium, as secondary nutrients, promoting its vegetative growth and conidiogenesis. This information justifies the fact that in the present study, although a decrease in sporulation was observed in some isolates treated with fipronil compared to their respective controls, these values were not statistically significant.

In vitro studies have the advantage of exposing the microorganism as much as possible to the action of the chemical, which does not occur under field conditions, where various factors act as obstacles to this exposure. Thus, once a product is found to be harmless in the laboratory, it is expected to be compatible in the field (Oliveira et al., 2018). On the other hand, the high toxicity of a product *in vitro* does not always indicate its high toxicity in the field, but rather the possibility of damage of this nature (Moino Junior & Alves, 1998). Thus, studies aiming at the applicability of chemical insecticides with entomopathogenic fungi under field conditions are necessary.

In the IPM context, all control tactics are used, and the strategy of applying entomopathogenic fungi together with insecticide requires compatibility between them. Entomopathogenic fungi can adapt to the environment, subsequently presenting enzootic and epizootic action contributing to natural biological control (Costa et al., 2018). Studies concerning the effect of the insecticide fipronil on entomopathogenic fungi are scarce in the literature (Bezerra, 2018; Fregonesi et al., 2016; Oliveira et al., 2002) especially when dealing with *B. bassiana*, *C. fumosorosea*, and *M. anisopliae* species. Thus, other researches aiming to verify the compatibility between this insecticide and entomopathogenic fungi are necessary, in view of a more adequate management of insect-pest control, considering the biology of the organisms involved, being more effective and less harmful to the environment.

Conclusions

The vegetative growth and sporulation of the entomopathogenic fungal isolates studied are reduced using concentrations between 0.4 and 0.8 g L^{-1} of fipronil.

Fipronil can be classified as "compatible" based on the *in vitro* toxicity index "T" on the entomopathogenic fungal isolates *B. bassiana*, *C. fumosorosea*, and *M. anisopliae* studied.

Compliance with Ethical Standards

Author contributions: Conceptualization: MAS, ECC, ACQ; Data curation: MAS; Formal analysis: MAS; Investigation: MAS, ACQ; Methodology: MAS; Project administration: MFBM; Resources: MFBM; Supervision: ECC; Validation: MAS; Visualization: MAS; Writing – original draft: MAS, ECC, ACQ; Writing – review & editing: MAS, ECC, MFBM, ACQ.

Conflict of interest: Os autores declaram não haver conflitos de interesse.

Financing source: The Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) – Finance Code 001.

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