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Compatibility between Chemical Insecticides and *Metarhizium anisopliae* var. *acridum*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SXS and BPM contributed to the conception and design of the study; in the development of the experiment and data collection; in performing the analysis and interpretation of data; in writing and reviewing writing and in aggregating intellectual content. Author GRG contributed writing, writing review, formatting and adding intellectual content. Author WSDS contributed in writing, reviewing writing, formatting and adding intellectual content. Author DDCC contributed in writing, reviewing the writing, updating bibliographic references, formatting and adding intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

The entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* has been studied in different countries as mycoinsecticide against grasshoppers. One of the utilization strategies considered is the association between entomopathogenic fungi and sublethal dosages of chemical insecticides compatible with the biological control agent. The effect of different chemical insecticides at concentrations varying from 5 to 5000 ppm on the conidial germination and growth of the fungus *M. anisopliae* var. *acridum* (CG 423) was assessed for compatibility evalution. The best

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results with inhibitory concentration (IC_{50}) on germination (liquid medium) and radial colonial growth (solid medium) of *Metarhizium anisopliae* var. *acridum* at 28°C was obtained with Teflubenzuron.

Keywords: Biological control; grasshopper; Rhammatocerus schistocercoides; compatibility.

1. INTRODUCTION

Grasshoppers have been one of the major agricultural pests, causing significant economic losses worldwide [1,2]. The banning of persistent and cumulative chemical insecticides such as Dieldrin has difficulted the control of these pests [3]. An alternative method that has been pursued is the biological control, in which entomopathogenic fungi play a significant role. The entomopathogenic fungi (EPF) *Metarhizium anisopliae* var. *acridum* (former *M. flavoviride* Gams & Rozsypal [4]) is being developed as mycopesticide in different countries for grasshopper control [5,6]. Indeed, laboratory and field trials carried out in Brazil have led to successful results against the acridid *Rhammatocerus schistocercoides* [7,8,9], on what population reductions over 80% have been recorded in those trials, although satisfactory results are dependent on high conidial dosages and, besides, onset of mortality takes at least 10 days.

In many instances, a feasible strategy for pest control is the combination of fungal formulations with low concentrations of chemical insecticides [10,11]. Insecticide concentrations that not lead to mortality can influence mating, host-finding, feeding and other insect behaviors [12]. Studies have shown that insecticide-treated cuticles exhibited diminished resistance to invasion by entomopathogenic fungi [13]. As already noticed a few decades ago, subletal doses of insecticides may act as potent stressors, increasing insect susceptibility to pathogens [14,10].

Previous reports had demonstrated the potential synergism between entomopathogenic fungi and chemicals towards grasshopper mortality [15,16,17]. Observation of synergistic insecticide–pathogen interactions may contribute to programs, allowing use of economic feasible dosages of the fungus and reducing onset of mortality. Other advantages would include reduced environmental contamination, prolonged usage of a particular insecticide and increase human safety [18].

The first step in order to conduct compatibility trials comprises to investigate the toxic potential of insecticides on EPF. The objective of this study was to estimate the toxicity of insecticides commonly used for grasshopper control [16], on germination and mycelial growth of the EPF *M. anisopliae* var. *acridum*.

2. MATERIALS AND METHODS

2.1 Microorganism

The EPF *M. anisopliae* var. *acridum* (CG 423) used for the experiments was obtained from the Fungal Collection kept at the Embrapa Genetic Resources and Biotechnology (Brasília, DF, Brazil), and originally isolated from the acridid *Schistocerca pallens* Thunberg in northeast Brazil. This fungus was cultured on potato dextrose agar at 28°C during 2 weeks. Formed conidia were collected from plates and used in the preparation of conidial suspensions with known concentrations.

2.2 Insecticides

The insecticides tested were fenitrothion (Sumithion 500 CE), malathion (Malathion 500 CE), teflubenzuron (Nomolt 150), chlorpyriphos (Lorsban 480), deltamethrin (Decis 25 CE) and lambdacyhalothrin (Karate 50 CE) (Table 1).

2.3 Assays

Insecticide toxicity was assessed based on conidial germination and mycelial growth. To verify the influence on germination, the fungus was cultivated on liquid culture medium [1% yeast extract; 0.1% antibiotic (Combiotic™); 98.9% solution of Tween 80 at 0.05%] containing different insecticide concentrations [5, 50, 500 or 5000 ppm of the a.i.]. Cultures with 10^6 conidia / ml were kept under orbital shaker at 150 rpm and 28°C. Samples were taken 12, 18 and 24 h following incubation and assessed under light microscope (100x) for percent germinated conidia. Lambdacyhalothrin was not used in this test because the formulated product contains oil-based components, that kept conidia aggregated inside bubbles when in liquid medium and, therefore, it did not allow a precise estimate of conidial germination.

To test the influence of the insecticides on mycelial growth, 0.5 μl of a conidial suspension (10^5 conidia / ml) was peaked to Petri dish center, which contained solid culture medium (0.001% $FeSO_4$; 0.05% KCl; 0.15% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 0.6% $NaNO_3$; 0.001% $ZnSO_4$; 0.15% hydrolyzed casein; 0.05% yeast extract; 1% glucose; 0.2% peptone; 1.5% agar and 97.7% distilled water), containing different insecticide concentrations (5, 50, 500 or 5000 ppm of the a.i.). Plates were kept at 28°C and 12 h light. Growth was calculated by daily measurements of grown colony. For both experiments, the fungus was seeded on medium without insecticide as a control. All experiments were carried out using a completely random design with three replicates. Averages were compared.

3. RESULTS AND DISCUSSION

All of tested insecticides showed some inhibitory effect on fungal development. Inhibition of growth varied according to insecticide, concentration and incubation period. Germination was less compromised by teflubenzuron, followed by chlorpyriphos and then malathion, deltamethrin and fenitrothion. As insecticide concentration increased, the inhibition of the germination also increased. However, the deleterious insecticide effects were minimized after long incubation periods, suggesting that, depending on concentration, some insecticides caused a delay in germination but did not inactivated the conidia. For concentrations 5 and 50 ppm, there was germination in all treatments, although germination was faster in the control. At 500 ppm, germination did not occur only in the deltamethrin treatment, whereas at 5000 ppm germination was observed only in teflubenzuron treated plates (Fig. 1).

The insecticides showing less inhibitory effects on EPF mycelial growth when used at 5 ppm. At higher concentrations, colonies showed a slower growth, except for lambdacyhalotrhin and deltamethrin at 5000 ppm, in which there was no detectable growth even after 16 days incubation. The least inhibition level observed was by teflubenzuron, followed by malathion and lambdacyhalothrin, and finally, chlorpyriphos, deltamethrin and fenitrothion with similar inhibition levels (Fig. 2).

In treatments with malathion (5000 ppm), chlorpyriphos (50 to 5000 ppm) and fenitrothion (500 to 5000 ppm), sporulated colonies presented lighter color than colonies grown on insecticide-free medium. Similar observations were reported by [2] when studying the effects of different fungicides and herbicides on *M. anisopliae, Beauveria bassiana, Paecilomyces farinosus* and *P. fumosoroseus*. It seems that changes in colony color may be related to deleterious effect of a particular concentration on physiological traits of the fungal pathogen. In fact, this observation was usually correlated to treatments in which conidial germination or radial growth was impacted.

Table 1. Chemical insecticides assayed with *Metarhizium anisopliae* var. *acridum*

Insecticide	Commercial name	Producer	Chemical group	Toxicity to Mammalian [a]
Fenitrothion	Sumithion500 CE	Iharabras S/A	organophosphorate	Moderately toxic
Malathion	Malathion500 CE	Action S/A	organophosphorate	Unlikely to harm
Chlorpyriphos	Lorsban480	Dowelanco Industrial LTDA	organophosphorate	Moderately toxic
Teflubenzuron	Nomolt 150	Cyanamid LTDA	growth regulator	Unclassified
Lambdacyhalothrin	Karate50 CE	ZenecaBrasil S/A	synthetic pyrethroid	Low toxic
Deltamethrin	Decis25 CE	Hoeschst Shering Agrevo do Brasil	synthetic pyrethroid	Low toxic

[a]Toxicologic class accord to Ministry of Agriculture, Brazil, AGROFIT (http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons).

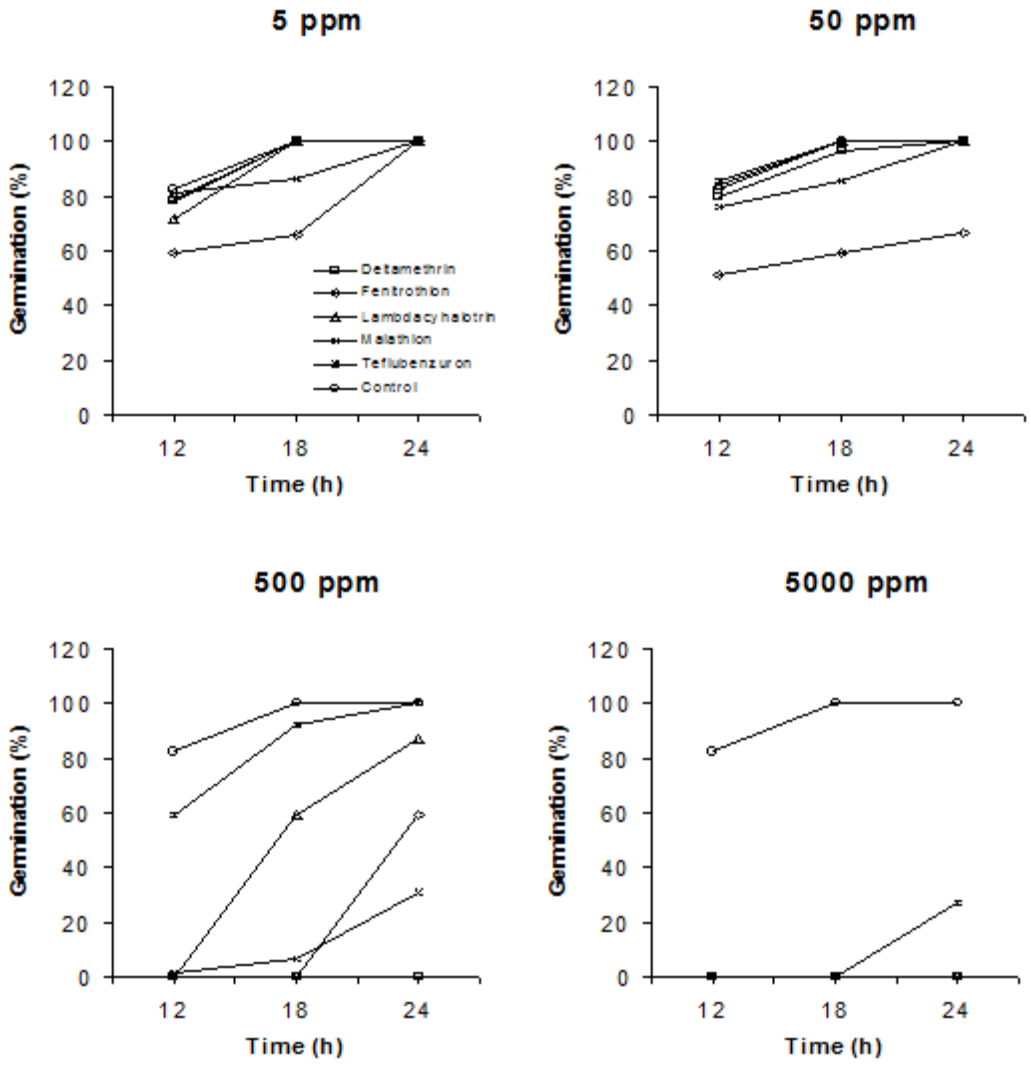

Fig. 1. Effect of different concentrations of chemical insecticides on germination of Metarhizium anisopliae var. acridum (CG 423) in liquid medium

Table 2. Inhibitory Concentration (IC_{50}) of different chemical insecticides on germination (liquid medium) and radial colonial growth (solid medium) of *Metarhizium anisopliae* var. *acridum* (CG 423) at 28°C

Inseticide	Inhibitory concentration (ppm)	
	Germination (24 h)	Colonial growth (16 days)
Deltamethrin	275.0 (±0) a	28.4 (±0) a
Malathion	410.5 (±71.51) a	313.3 (±33.51) b
Fenitrothion	270.3 (±70.72) a	26.8 (±1.90) a
Chlorpyriphos	1832.7 (±402.77) b	32.5 (±1.33) a
Teflubenzuron	3622.8 (±207.43) c	4335.6 (±83.18) c
Lambdacyhalothrin	-	171.4 (±5.72) b

Average for three replicates. Values in a given column with the same letter do not significantly differ using the Student-Newman-Keuls method (α=0.05)

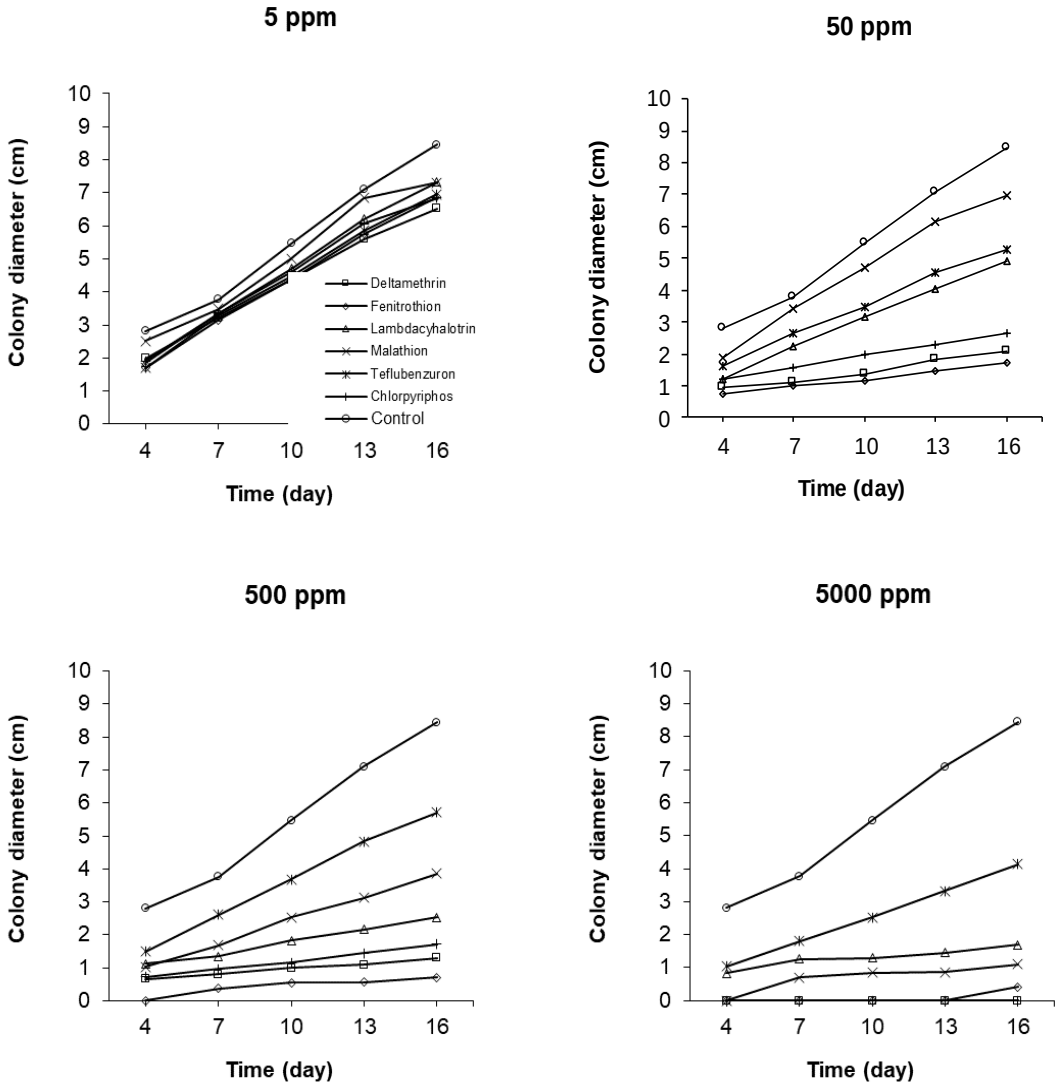

Fig. 2. Effect of different concentrations of chemical insecticides on radial growth of *Metarhizium anisopliae* var. *acridum* (CG 423) on solid medium

A few colonies developed in solid medium amended with fenitrothion (50 ppm) showed areas with remarkable growth, suggesting some degree of insecticide tolerance. "Tolerant" colonies were re-isolated and preserved for future genetic and biochemical analyses.

Colony growth was more affected than germination as revealed by its lower IC_{50} values, except for teflubenzuron (Table 2). A likely explanation is that in the colony growth bioassay, EPF was insecticide exposed longer than germination assay. It is possible that conidia able to germinate in the presence of the insecticide may be unable to develop and produce mycelium under the same condition.

On the other hand, under field conditions the effect of insecticides on fungal germination seems to be more significant than its effect on fungal growth [19,20,21]. Just after germination and penetration on the host cuticle, the influence of the insecticide on fungal pathogenesis would be minimized. In addition, a variety of degradation factors would act on insecticide persistence in the field, reducing its half-life.

Among the tested insecticides, germination and colonial growth of *M. anisopliae* var. *acridum* were least affected by teflubenzuron. Although the level of germination inhibition by chlorpyriphos was not as high, colony growth was severely reduced. Malathion inhibited

moderately both germination and colonial growth. Fenitrothion and deltamethrin were the most severe inhibitors.

4. CONCLUSION

The insecticides showing less inhibitory effects on EPF mycelial growth when used at 5 ppm and the best results with inhibitory concentration (IC_{50}) on germination (liquid medium) and radial colonial growth (solid medium) of *Metarhizium anisopliae* var. *acridum* (CG 423) at 28°C was obtained with Teflubenzuron.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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