Journal of Entomological Society of Iran
2012, 31(2), 29-37



# Compatibility of the entomopathogenic fungus *Beauveria bassiana* with the insecticides fipronil, pyriproxyfen and hexaflumuron

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**Abstract**

The compatibility of *Beauveria bassiana* isolate DEBI 002 (Atashgah) with fipronil, pyriproxyfen and hexaflumuron insecticides was assayed. To determine the impact of the insecticides on the germination of the fungal spore, different concentrations of the compounds were added to the culture medium (malt agar). In order to calculate the mycelial growth in different treatments, each colony diameter was measured and the spores were counted in the surface area to assess its sporulation. The results showed that pyriproxyfen at 1500 ppm and hexaflumuron at 80 ppm completely inhibited mycelial growth, while the inhibitory effect of fipronil at 1600 ppm remained at 76.6%. The inhibitory effect of lower concentrations of all three insecticides was between 10 and 20%. All tested insecticides inhibited the spore production between 80 to 100% at the highest concentrations without any significant differences. Pyriproxyfen at 400 ppm and hexaflumuron at all concentrations completely inhibited spore germination, with significant difference, comparing with the rest of treatments. The results indicate that hexaflumuron has the highest inhibitory effect on the spore germination and is not recommended to be used simultaneously with *B. bassiana* against the insect pests.
**Key words:** *Beauveria bassiana*, compatibility, fipronil, hexaflumuron, pyriproxyfen

**چکیده**

سازگاری سه حشره‌کش فیپرونیل، پایری‌پروکسی‌فن و هگزافلومورون با جدایه‌ی DEBI 002 (آتشگاه) قارچ *Beauveria bassiana* مورد بررسی قرار گرفت. برای تعیین اثر این حشره‌کشها روی جوانه‌زنی اسپور قارچ، غلظت‌های مختلف آن‌ها به محیط کشت مالت آگار اضافه شد. به‌منظور محاسبه‌ی میزان رشد میسیلیوم در تیمارهای مختلف، قطر کلنی اندازه‌گیری شد و ارزیابی میزان اسپورزایی با شمارش اسپورها در واحد سطح صورت گرفت. نتایج نشان داد که پایری‌پروکسی‌فن در غلظت ۱۵۰۰ پی‌پی‌ام و هگزافلومورون در غلظت ۸۰۰ پی‌پی‌ام به‌طور کامل مانع رشد میسیلیوم شدند، درحالی‌که میزان بازدارندگی فیپرونیل در غلظت ۱۶۰۰ پی‌پی‌ام، ۷۶/۶٪ بود. تأثیر بازدارندگی هر سه حشره‌کش در غلظت‌های پایین‌تر، ۲۰-۱۰٪ بود. میزان مهار اسپورزایی هر سه حشره‌کش در بالاترین غلظت تفاوت معنی‌داری با یکدیگر نداشت و ۱۰۰-۸۰٪ بود. پایری‌پروکسی‌فن در غلظت ۴۰۰ پی‌پی‌ام و هگزافلومورون در تمام غلظت‌ها مانع جوانه‌زنی اسپور شدند و درصد جوانه‌زنی این تیمارها با سایر تیمارها تفاوت معنی‌داری داشت. براساس نتایج، هگزافلومورون بیشترین اثر بازدارندگی را روی جوانه‌زنی اسپورها داشت، و بنابراین، کاربرد هم‌زمان آن با قارچ بیمارگر *B. bassiana* در کنترل آفات توصیه نمی‌شود.

**واژگان کلیدی:** *Beauveria bassiana*، سازگاری، فیپرونیل، هگزافلومورون، پایری‌پروکسی‌فن

## Introduction

Biological control, particularly using entomopathogenic fungi, is an integral part of Integrated Pest Management (IPM) strategies for reducing the population density of many pests. Therefore, preservation of naturally found or introduced entomopathogens for insect

control should be considered in plant protection programs (De Oliveira *et al.*, 2003). In addition, it is necessary to ensure the compatibility of entomopathogenic fungi with other crop protection methods such as chemical control that may inhibit the development and reproduction of the pathogens (Neves, 2001).

Entomopathogenic fungi are important natural control agents of many insect pests (Carruthers & Hural, 1990), of which *Beauveria bassiana* (Balsamo) Vuillemin is known as one of the capable alternative control agents against a number of key pests (Van der Geest *et al.*, 2000; Liu *et al.*, 2002; Leland *et al.*, 2005; Al-Mazra'awi *et al.*, 2006; Quesada-Moraga *et al.*, 2006). The commercially produced entomopathogenic fungi have been available for being used in biological control of insect pests (McCoy & Couch, 1982; McCoy, 1990). Little is known about the compatibility of these fungi with the recommended insecticides and this study is intended to improve the current knowledge to facilitate the future use of these products in IPM programs (Neves *et al.*, 2001). In pest control programs, interaction between different factors such as environmental conditions, agrochemicals and biopesticides can affect conidial survival of entomopathogens (Anderson & Roberts, 1983; Loria *et al.*, 1983; Benz, 1987). Utilization of selective insecticides in combination with entomopathogens can increase the efficiency of pest control, resulting in reduction of the amount of applied insecticides, minimizing environmental contamination risk and the expression of pest resistance to insecticide (Quintela & McCoy, 1998).

Many studies have been carried out to investigate the inhibition and side effects of insecticides on a wide range of entomopathogenic fungi including *B. bassiana* (Ramaraje *et al.*, 1967; Olmert & Kenneth, 1974; Clark *et al.*, 1982; Gardner & Storey, 1995; Batista Filho *et al.*, 2001; Neves *et al.*, 2001; Alizadeh *et al.*, 2007). However, using sublethal doses of some pesticides in combination with some entomopathogens can act in a positive manner, in which the target insect pests become more susceptible to the action of pathogens (Batista Filho *et al.*, 2001).

Todorova *et al.* (1998) underscored the pesticide effects on conidial germination that are responsible for the occurrence of the first disease foci in the field. They also emphasized that the germination inhibition lowers the efficiency of the pathogen in the agro-ecosystems and in those applied by overflow. These studies underline the possibility of application of less harmful insecticides together with entomopathogenic fungi in the IPM programs.

Hexaflumuron, fipronil and pyriproxyfen insecticides have been recommended against subterranean termites (Remmen & Su, 2005). The combination of insecticides and

entomopathogenic fungi will have beneficial effects on the human life and the environment. The *in vitro* effects of three insecticides (fipronil, pyriproxyfen and hexaflumuron) on different developmental stages of *B. bassiana* were here evaluated to determine the most compatible insecticide with *B. bassiana*.

## Materials and methods

### Entomopathogenic fungus and insecticides

*Beauveria bassiana* isolate Atashgah (DEBI002) was used in this study. The fungus had initially been isolated from soil in the city of Karaj, Iran. Conidia were grown on SDA (Sabouraud Dextrose Agar) medium (25 ± 1°C; 12 h photophase) and the harvested conidia used for conidial germination, vegetative growth and sporulation studies.

The tested insecticides were fipronil, pyriproxyfen and hexaflumuron with three different concentrations (table 1).

**Table 1.** Insecticides and their concentrations used in compatibility tests.

| Trade name | Entry name | Formulation | Chemical group | Concentration: ppm (A. I.)[1] | Concentration: ppm (A. I.)[2] |
|---|---|---|---|---|---|
| Consult | Hexaflumuron | EC[3] 10% | IGR[4] | 15, 45, 80 | 50, 100, 400 |
| Admiral | Pyriproxyfen | EC 10% | IGR | 10, 500, 1500 | 50, 100, 400 |
| Agenda | Fipronil | EC 2.5% | Phenylpyrazole | 20, 800, 1600 | 50, 100, 400 |

1. Concentrations used in vegetative growth and spore production tests; 2. Concentrations used in germination tests; 3. Emulsifiable Concentration; 4. Insect Growth Regulator.

### Conidial germination

The mentioned concentrations (table 1) of each insecticide were added to 50 ml of cooling (40 ± 5°C) malt agar medium and inoculated with 100 µl of suspension containing $10^7$ spores/ml. The control group was treated with an aqueous solution of 0.05% Tween 80. The treatments were kept in an incubator (25 ± 1°C; 12 h photophase) for 24 hours and the germinated spores counted (germinated spores per 100 spores) to find the spore viability.

### Vegetative growth and spore production

Standard SDA medium was autoclaved at 121°C and 1 atm for 21 min, cooled to 40 ± 5°C and amended with 0.8 g/L streptomycin and 0.3 g/L penicillin. The insecticides were filtered with millipore filters (0.2 µm) to remove contaminant microorganisms. The formulated insecticides were added and mixed by hand to homogeneity with SDA media. Approximately 50 ml of each one was poured into three 9 cm culture plates. The same amount

of streptomycin - penicillin was added to the control insecticide-free medium (De Olivera & Neves, 2004). After the solidification of the medium, the fungi were inoculated with spatula in one point per plate (three dishes / treatment). The dishes were then incubated at 25 ± 1°C and 12 h photophase. After nine days, the colony diameters were assessed by measuring in two directions with a common ruler (Batista Filho *et al.*, 2001). To quantify the conidia production, three central colony disks (2.84 $cm^2$) were drawn from each treatment and placed in glass tubes, where the conidia suspended in 10 ml of water containing 0.05 % Tween 80. Conidial concentration was measured, using a hemocytometer.

#### Statistical analysis

A completely randomized design was used in all experiments, data submitted to ANOVA and means compared by using Duncan's Multiple Range Test ($P < 0.05$).

## Results

#### Conidial germination

Significant reduction in the germination of *B. bassiana* was observed in all three concentrations of hexaflumuron (50, 100 and 400 ppm) and in the highest concentration of pyriproxyfen (400 ppm) (table 2). Only hexaflumuron showed an absolutely complete inhibition of *B. bassiana*. Hexaflumuron, fipronil and pyriproxyfen, at 50 ppm concentration, reduced the conidial germination by respectively 100, 12.80 and 25.98%. There was no significant difference between control and three concentrations of fipronil (table 2).

**Table 2.** Mean (± SE) reduction percentage of conidial germination caused by different insecticide concentrations. The entomopathogenic fungus *B. bassiana* (isolate DEBI 002) grown at 25 ± 10°C and 12 h photophase, on malt agar medium amended with insecticides.

| Treatment | Concentration: ppm (A. I.) | Germination reduction (%) (n = 9) |
|---|---|---|
| Pyriproxyfen | 50 | 25.98 ± 19.68 a |
| Pyriproxyfen | 100 | 45.37 ± 15.55 a |
| Pyriproxyfen | 400 | 100 ± 0.0 b |
| Hexaflumuron | 50 | 100 ± 0.0 b |
| Hexaflumuron | 100 | 100 ± 0.0 b |
| Hexaflumuron | 400 | 100 ± 0.0 b |
| Fipronil | 50 | 12.80 ± 7.26 a |
| Fipronil | 100 | 16.18 ± 5.99 a |
| Fipronil | 400 | 46.04 ± 12.64 a |

Means within a column followed by the same letter are not significantly different at the % level ($P < 0.05$; Duncan's Multiple Range Test).

**Vegetative growth and spore production**

All concentrations of the insecticides had negative effects on the vegetative growth and conidia production of *B. bassiana*. However, the mean vegetative growth reduction caused by hexaflumuron and pyriproxyfen at 15 and 10 ppm concentrations respectively was not significantly different from the control. All insecticides at the lowest concentrations induced at most 21% fungal growth inhibition (table 3). At their highest concentrations, hexaflumuron, fipronil and pyriproxyfen caused 100, 76.64 and 100% reduction on vegetative growth respectively. Hexaflumuron, fipronil and pyriproxyfen, at their highest concentration, induced at least 88% sporulation inhibition (table 3). All three concentrations of pyriproxyfen had highly reduced the sporulation production (64-93%). Only the data of hexaflumuron at 15 ppm concentration was equal to the control. Hexaflumuron, fipronil and pyriproxyfen caused 13.67, 41.31 and 75.21% reduction on sporulation at their lowest concentration respectively (table 3). Hexaflumuron induced highest level of inhibition on germination, vegetative growth and sporulation.

**Table 3.** Effect of insecticides in different concentrations on vegetative growth and sporulation of the entomopathogenic fungus, *B. bassiana* (DEBI002 isolate), grown at 25 ± 10°C and 12 h photophase, on SDA medium amended with insecticides, based on mean percentage ± SE.

| Treatment | Concentration: ppm (A. I.) | Vegetative growth (mm) (%) reduction (n = 9) | Sporulation ($\times 10^7$ spor/ml) (%) reduction (n = 9) |
|---|---|---|---|
| Pyriproxyfen | 10 | 10.73 ± 5.65 a | 75.21 ± 2.56 dc |
| Pyriproxyfen | 500 | 29.94 ± 3.91 bc | 64.10 ± 0.0 bc |
| Pyriproxyfen | 1500 | 100 ± 0.0 e | 92.82 ± 0.51 d |
| Hexaflumuron | 15 | 14.49 ± 1.64 a | 13.67 ± 4.27 a |
| Hexaflumuron | 45 | 35.59 ± 12.43 c | 41.88 ± 0.0 b |
| Hexaflumuron | 80 | 100 ± 0.0 e | 100 ± 0.0 d |
| Fipronil | 20 | 20.90 ± 4.70 ab | 41.31 ± 12.25 b |
| Fipronil | 800 | 43.50 ± 2.60 c | 91.45 ± 3.24 d |
| Fipronil | 1600 | 76.64 ± 3.28 d | 88.14 ± 0.82 dc |

Means within a columns followed by the same letter are not significantly different at the % level ($P < 0.05$; Duncan's Multiple Range Test.

## Discussion

This study proves that *B. bassiana* is affected by the insecticides hexaflumuron, pyriproxyfen and fipronil and also they inhibit the development and reproduction of the entomopathogen *B. bassiana* that would be a limiting factor in their future applications in the IPM programs (Anderson & Roberts, 1983). The insecticides caused different levels of

inhibition on germination, vegetative growth and sporulation of *B. bassiana* that were mainly dependent on the chemical property as well as concentrations of each compounds.

Fungal germination is an important factor in compatibility evaluation of pesticides with entomopathogenic fungi in pest management because the beginning of epizootics is conditioned by the capacity of conidia to germinate on the host (Anderson & Roberts, 1983; Alizadeh *et al.*, 2007).

The insecticide hexaflumuron showed the highest inhibitory effect on conidial germination, whereas fipronil had the lowest negative effect on the germination (table 2). The highest concentrations of all three insecticides had the highest inhibitory effect on the sporulation (table 3). The survival of the entomopathogenic fungus inoculums in the field depends primarily on the conidia, which are responsible for the first foci of the disease (Neves *et al.*, 2001; De Olivera & Neves, 2004). It was reported that chemical products may counteract the electrostatic charge of the surface and possibly remove the mucous layer covering conidia, as well as metabolic blockage and inhibition of the enzyme in cell wall (St. Leger & Cooper, 1987; Boucias *et al.*, 1988; St. Leger *et al.*, 1991).

Almost all the insecticides significantly inhibited the fungal vegetative growth, especially when the highest concentrations were applied (table 3). According to Feng *et al.* (1994), inhibition of vegetative growth is not the most important index of compatibility for fungi and insecticides. Under field conditions, inhibition of vegetative growth is not a good indication of fungicidal effects, but conidial germination seems to be the key factor (Loria *et al.*, 1983; Neves *et al.*, 2001), so the effect of insecticides on conidial germination should be considered as one of the most important factors (Anderson & Roberts, 1983).

In conclusion, hexaflumuron and fipronil caused the highest and lowest levels of in vitro inhibition on the germination, vegetative growth and sporulation of *B. bassiana* respectively. The usual high level of in vitro toxicity is not expected necessarily to happen during the field experiments (Alizadeh *et al.*, 2007). However, it is necessary to do experiments under the semifield and field conditions for evaluating the efficacy of this entomopathogenic fungus when mixed with chemical insecticides to control insect pests.

**Acknowledgements**

We are grateful to Mr. Ali Mohammadipour for providing entomopathogenic fungus and for his generous support. We wish to thank Mrs. Fatemeh Saberfar for her assistance in

performing the tests. This work was supported by Iranian Research Institute of Plant Protection.

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*Received*: 31 May 2010
*Accepted*: 1 February 2011