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Compatibility of *Beauveria bassiana* (Bals.) Vuill. with Several Pesticides

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ABSTRACT

The entomopathogenic fungus *Beauveria bassian*a (Bals.) Vuill. is one of the facultative insect pathogens with significant host range and host specifity. Conidia survival may be effected by environmental factors or by bio-pesticides and chemical products used to protect crop plants. In this research compatibility of mentioned fungi with imidaclopride, flufenoxuron, teflubenzuron+ phuzalon, endosulfane and amitraz and effect of these pesticides on conidial germination, vegetative growth and sporulation of the fungus were studied. The formulations of pesticides were tested in tree concentration (mean concentration-MC, half MC & twice the MC). The results indicated that flufenoxuron is not compatible with *B. bassiana* and it caused complete or strong inhibition in its development. The compatible formulation with *B. bassiana* (isolate DEBI008) was imidacloprid. This formulation could be used simultaneously with this entomopathogenic in integrated pest management.

Key Words: *Beauveria bassian*a; Entomopathogenic; Compatibility; Pesticides; Fungi

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is a capable alternative control agent against the important pests (Boiteau 1988; Todorova *et al.,* 1994; Van Der Geest *et al*., 2000; Liu *et al.* 2002; Hatting *et al.,* 2004; Leland *et al.,* 2005; Quesada-Moraga *et al.,* 2006; Al-maza awi *et al.,* 2006). Seeking to use the potential of these organisms for pest control, commercial products have been developed with entomopathogenic fungi (McCoy & Couch 1982; McCoy, 1990; Alves & Pereira, 1998). Conidial survival can be effected by interaction with agrochemicals, environmental factor (Benz, 1987) or by bio-pesticide and/or chemical product used to protect plants (Anderson & Roberts, 1983; loria *et al.,* 1983; Alves & Lecuona; 1998). The pesticides and herbicides may antagonize or synergize efficacy and potential insecticidal activity of *B. bassiana* and may disrupt natural epizootics of this pathogen (Benz, 1987).

Many experiments have been carried out aiming to detect pesticides side effects on entomopathogenic fungi (Clark *et al.,* 1982; Gardner & Storey, 1995; Neves *et al.,* 2001). Olmert and kennth (1974) have studied the necessity of descriptive the effects of pesticides on a wide rang of entomopathogenic fungi. *In vitro* studies indicate inhibition of *B. bassiana* by many pesticdes (Ramarajah *et al*., 1967; Olmert & Kenneth 1974). Neves *et al*. (2001) pointed out the importance of conidial germination in compatibility studies. Todorove *et al*. (1998) reinforced the importance reinforced of pesticides influence on conidial germination, since those fungal structures are responsible the occurrence of the first disease foci in the field. Integrated pest management (IPM) programs it is essential to know the influence of the compatibility between entomopathogenic fungi and pesticide used in crop protection (Todorova *et al.,* 1998). De Olivera and Neves (2004) evaluated compatibility of *B. bassiana* whit 12 acaricides formulation and showed that the formulations more compatible with *B. bassiana* were Avermectin and the pyrethroids.

This knowledge should facilitate the choice of chemicals compatible with or less harmful to naturally occurring or artificially inoculated beneficial fungi. If *B. bassiana* is to be incorporated into a pest management program, it is necessary to determine the effects of pesticides on it. In this study, we selected pesticide commonly used in plant protection. Our laboratory investigation was conducted to determine the effects of five pesticides on conidial germination, vegetative growth and sporulation of a selected isolate of *B. bassiana*.

MATERIALS AND METHODS

Fungus. *B. bassiana* isolate (DEBI008) was used in this study the isolate had initially been isolated from *Chorthippus brunneus* (Ort: Acaridae) in Iran (Tehran). The fungus was grown on PDA (Potato-Dexterose-Agar) medium (25 ± 1°C; 12 h photophase) and conidial produced were used for studies.

Pesticides. The pesticides selected for these experiments are shown on Table I. For compatibility tests, the pesticides used in three different concentrations, mean concentration (MC), half MC and twice the MC (De Olivera & Neves, 2004).

Conidial germination. The appropriate concentration of

Table I. Pesticide used in this study

Active ingredient	Brand name	Chemical group	Formulation	MC[1]
amitraz	Mitak	Formamidin	EC 20%	1 lit
flufenoxuron	Cascaid	IGR[2]	EC 5%	0.5 lit
teflubenzuron+phuzalon	Darton	IGR	EC 21.7%	0.5 lit
imidachlopride	Konfidur	Chloronicotinly	SE 35%	0.5 lit
endosulfane	Tiodan	Cyclodiene	EC 35%	1.5 lit

[1] Mean concentration of commercial product for application in 1000 liters of water

[2] Insect growth regulators; EC- Emulsion concentration; SE- Solution concentration

each pesticide was added to 50 mL of Cooled (45°C) PDB (Potato-Dexterose-Broth). This treated directly inoculated with 1 mL of a conidial suspension of *b. bassiana* containing 10^6 spore/mL that diluted in sterile distilled water amended with 0.01% Tween 80. The seam aliquot of sterile distilled water standard spore suspension and 0.01% Tween 80, without the pesticides, was used as control. The treatments were randomly transferred to an incubator (24 ± 1°C; 12 h photophase) for 24 h.

After incubation, by hemocytometer approximately 200 germinated or non-germinated conidial/quadrant were counted under light microscope. Data were used to calculate % germinated or non-germinated spore.

Vegetative growth and spore production. Inoculums of *B. bassiana* was produced on PDA for 20 d, at 24°C. Autoclaved PDA and cooled to 40 ± 5°C. The pesticides, in the pre-established concentration were then added. Approximately 20 mL of each one of these amended media was poured in to four 8 cm culture Petri dish. The same amount of medium without the pesticide was used as control (De Olivera & Neves, 2004). After media solidification, each plate was inoculated with a small plug (1 mm deep, 7 mm diameter) of PDA with *B. bassiana*, was deposited in the center of each plate containing the mixture of PDA and pesticide (Todorova *et al.,* 1998). The plates were incubated at 24 ± 1°C and the liner growth in excess of the plugs was measured on the 14th day following the treatment. Growth was measured on the four conidial points from the plug and the mean value was used in the following statistical tests. Each pesticide concentration combination with fungus and corresponding control was replicated four times.

After 14 d, the conidia from the excess of the plugs were harvested by scraping and suspended in 1 mL of 0.01% Tween 80. The concentration of conidial was estimated using a hemocytometer.

Analysis. A completely randomized design (CRD) was used in all experiments. Data were submitted to ANOVA and means were compared by Duncan multiple range test ($P < 0.05$) using SPSS 2004.

RESULTS

Conidial germination. The effect of pesticides on the germination of *B. bassiana* in dependently of concentration is shown in Table II. Of the pesticides tested, flufenoxuron showed strongly complete inhibition of *B. bassiana*, whereas imidaclopride showed relatively little fungal inhibition and it's in 0.5 x similar to that of the control. The formulation with flufenoxuron, at tree concentration (MC, half MC & twice MC) and amitraz, teflubenzuron+ phuzalon and endosulfane induced high reduction (> 85%).

Of the pesticide tested, amitraz, teflubenzuron+ phuzalon and endosulfane caused, respectively 51%, 65% and 65% reduction on conidial germination at half MC concentration. The reduction on percent germination for imidaclopride formulation was lower than 27% and flufenoxuron was higher than 96%.

Vegetative growth and spore production. Effects of the pesticides on *B. bassiana* vegetative growth in concerned results have shown that almost all formulations studies significantly inhibition fungal development (Table II). The vegetative growth inhibition induced by imidaclopride formulation with the half MC and MC was not significantly different from the control treatment. However, the formulation with amitraz, flufenoxuron, endosulfane (at MC & twice the MC) and teflubenzuron + phuzalon (at twice the MC) induced fungal growth inhibition higher than 70%. Flufenoxuron, even if inducing % growth inhibition higher than 95% at tree concentrations.

The formulation with teflubenzuron + phuzalon (at tree concentrations), amitraz and endosulfane (at MC & twice the MC), induced levels of sporulation inhibition higher than 78%. Whereas formulation with flufenoxuron totally prevented sporulation (100% reduction) at an concentration used. However, only data on imidaclopride at MC and half MC were not significantly different from the control treatment. The formulation with flufenoxuron and imidaclopride highest and lowest levels of inhibition on germination, vegetative growth and sporulation conformed, respectively.

DISCUSSION

Conidial germination is very important step in pest management with fungi, because the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host. The entomopathogenic fungus success, however, depends on conidial viability (Batista Filho *et al.,* 1998; De Olivera & Neves, 2004). Our research showed that, in general the agrochemicals tested (except imidaclopride) significantly effected *B. bassiana* germination, vegetative growth and sporulation *in vitro* (Table II). The effect of amitraz on *B. bassiana* had been tested earlier by De Olivera and Neves (2004). The results of our experiments were in agreement with those previously reported and showed strong or complete inhibition of *B. bassiana* in laboratory testes. These results might be explained by a different sensitivity to a given fungicide among given fungal isolate (Olmert & Kenneth, 1974). Further more, inhibition of vegetative growth is not

Table II. Effect of pesticides in three different concentration spore germination, vegetative growth, and sporulation of the entomopathogenic fungus *B. bassiana* (DEBI008 isolate) in studies conducted on formulation-amended PDA media at 24 ± 2°C and 12 h photophase

Treatments	Concentration	Germination(%) %reduction N = 30		Vegetative growth(mm) %reduction N = 4		Sporulation ($\times 10^4$spore/mli) %reduction N = 10	
		Mean ± SE		Mean ± SE		Mean ± SE	
amitraz	0.5x	40.13±1.21 D	51	14.63±0.8 D	70	37.5±7.5 C	59
	1x	11.23±1.36 F	86	9.64±0.56 E	80	20±4 DE	78
	2x	10.8±0.41 F	86	2.77±0.42 FG	94	17.5±4.7 DEF	81
flufenoxuron	0.5x	2.33±0.41GH	97	2.3±0.4 FG	95	0.0±0.0 F	100
	1x	2.57±0.33 GH	96	0.2±0.1G	99.5	0.0±0.0 F	100
	2x	0.53±0.34 H	99	0.0±0.0 G	100	0.0±0.0 F	100
teflubenzuron+phuzalon	0.5x	28.05±2.83 E	65	27.8±2.2 C	42.7	15±5 DEF	83
	1x	8.44±1.77 FG	90	24.3±1.4C	50	8±0.5 EF	91
	2x	5.55±0.25 FGH	92	9.71±1.12 C	80	6.5±0.8 EF	93
imidaclopride	0.5x	80.1±1.16 A	1	48.7±0.9 A	0	90±7.7 A	3
	1x	70.15±2.6 B	14	48±0.77 A	1.2	80±12.2 A	13.5
	2x	59±5.6 C	27	37.3±3.2 B	23	55±5 B	46
endosulfane	0.5x	28±2.9 E	65	16±1.62 D	67	30±8.2 CD	67
	1x	11.5±1.52 F	85	14.64±0.8 D	70	12.5±2.5 EF	86
	2x	7.77±0.55 FG	90	5.2±0.56 F	89	11.5±2.8 EF	87
Control	0	81.12 ± 2.76 A	0	48.69 ± 0.89 A	0	92.5 ± 4.8A	0

necessarily an indication of reduction in sporulation or conidial viability and vice versa (Zimmerman, 1975). According to James and Elzen (2001), imidaclopride had no negative effect on *B. bassiana*. Synergistic interaction of imidacloprid with fungal agents in insect control have been reported previously (Kaakeh *et al.,* 1997; Quintela & McCoy, 1998; Lacey *et al*., 1999; Ramakrishnan *et al*., 1999; Furlong & Groden, 2001; Ying *et al.*, 2003).

Concerning the effect of the products presently used on vegetative growth and sporulation, a significant reduction was found in relation to the control treatment. Alves *et al.* (1998) however pointed out that what happens under field conditions to the chemical compounds. Thus, when the innocuousness of a given product is determined in the laboratory, no doubts that its selectivity under filed condition will stand. On the other hand, the high toxicity *in vitro* of a given formulation may suggest similar toxicity under filed conditions. However for filed studies the inhibition of conidial germination should be the key factor to be considered, as discusses by Neves *et al.* (2001).

Our results demonstrated that flufenoxuron is not compatible with *B. bassiana* and it caused complete or strong inhibition in its development. Our testes *in vitro* suggest that the flufenoxuron, teflubenzuron + phuzalon, amitraz and endosulfane are harmful and not compatible with isolate DEBI008 of *B. bassiana*. Only imidaclopride formulation could be used simultaneously with this entomopathogenic in integrated pest management. However, filed evaluation of the interactions between *B. bassiana* and these pesticides should be under taken to evaluate their effect on pest and beneficial insects.

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