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Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with some pesticides

S. Faraji[1], A. Derakhshan Shadmehri[2 and]* and A. Mehrvar[3]
1. Department of Plant Protection, Faculty of Agriculture, University of Maragheh, 2. Department of Plant Protection, Faculty of Agriculture, Shahrood University of Technology, 3. Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, East-Azarbaijan, Tabriz, Iran.
*Corresponding author. E-mail: aderakhshan@shahroodut.ac.ir

Abstract

Entomopathogenic fungi may be affected by pesticides used to protect crop plants. The *in vitro* compatibility of Beauveria *bassiana* and *Metarhizium anisopliae* with three fungicides and 16 insecticides was evaluated. The formulations of pesticides were tested in three concentrations (mean concentration (MC), half MC and twice the MC). All tested fungicides, at three concentrations, were incompatible with these two fungi and significantly inhibited fungal development, germination of spores and spore production. Insecticides, except for spinosad, deltametrin, imidachloprid and abamectin, were incompatible with these two fungi at high concentration and caused complete or strong inhibition of fungal development. The compatible insecticide with *B. bassiana* and *M. anisopliae*, at three concentrations, found to be spinosad. Abamectin, imidachloprid and deltametrin, however at half MC and MC were compatible with *B. bassiana*. Deltametrin, abamectin and hexafloron at half MC were compatible with *M. anisopliae*. We conclude that these pesticides with the defined formulations could be used with the entomopathogenic fungi in integrated pest management programs.
Keywords: *Beauveria*, *Metarhizium*, compatibility, pesticide, entomopathogenic fungus

چکیده

بررسی سازگاری قارچ های بیمارگر حشرات *Beauveria bassiana* و *Metarhizium anisopliae* با تعدادی از آفت‌کش ها

سمیه فرجی، علی درخشان شادمهری و علی مهرور

قارچ‌های بیمارگر حشرات ممکن است به وسیله آفت‌کش‌های شیمیایی که برای مبارزه با آفات استفاده می‌شوند، تحت تاثیر قرار گیرند. سازگاری قارچ‌های *Beauveria bassiana* و *Metarhizium anisopliae* با سه قارچ‌کش و ۱۶ حشره‌کش در آزمایشگاه مورد ارزیابی قرار گرفت. آفت‌کش‌ها در سه غلظت (غلظت میانگین، نصف و دو برابر غلظت میانگین) آزمایش شدند. نتایج آزمایش‌ها نشان داد که قارچ‌کش‌های مورد استفاده در هر سه غلظت با این قارچ‌ها ناسازگار بوده و بازدارندگی کامل روی تندش، رشد میسلیومی، و اسپورزایی قارچ‌ها داشتند. حشره‌کش‌ها به جز اسپینوساد، دلتامترین، ایمیداکلوپرید و آبامکتین در غلظت‌های بالا ناسازگار بودند و یا بازدارندگی شدیدی بر روی رشد قارچ‌ها داشتند. حشره‌کش اسپینوساد در هر سه غلظت با قارچ‌های *B. bassiana* و *M. anisopliae* سازگار بود. آبامکتین، ایمیداکلوپرید و دلتامترین در غلظت میانگین و نصف غلظت میانگین با *B. bassiana* و دلتامترین، آبامکتین و هگزافلومورون در نصف غلظت میانگین با *M. anisopliae* سازگار بودند. بنابراین احتمالا بتوان بیان کرد که این چند آفت‌کش نام‌برده شده با فرمولاسیون‌ها و غلظت‌های مشخص در تحقیق حاضر در صورت استفاده هم‌زمان با این دو قارچ بیمارگر حشرات در برنامه‌های کنترل بیولوژیک، اثرات تداخلی روی قارچ نخواهند داشت.

واژگان کلیدی: *Beauveria* ، *Metarhizium* ، سازگاری، آفت‌کش، قارچ بیمارگر حشرات

Introduction

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin are capable alternative control agents against important agricultural 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-Mazaawi *et al*., 2006). To use these organisms for pest control, commercial products of entomopathogenic fungi have been developed (McCoy & Couch, 1982; McCoy, 1990; Alves & Pereira, 1998). Conidial survival could be affected by interaction with chemical pesticides and environmental factors (Loria *et al*., 1983; Alves & Lecuona, 1998). The pesticides may have antagonistic or synergistic effects on potential insecticidal activity of *B. bassiana* and disrupt natural epizootics of this pathogen. Therefore, the selected isolates of entomopathogenic fungi for use as mycopesticides require compatibility testing with chemical pesticides, for their application in IPM programs.

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). Most of these studies were conducted by adding products to the synthetic culture media used for fungal growth. *In vitro* studies indicate inhibition of *B. bassiana* by many pesticides (Olmert & Kenneth, 1974). Neves *et al*. (2001) pointed out the importance of conidial germination in compatibility studies. To develop a successful integrated pest management (IPM) program, it is essential to know the compatibility between entomopathogenic fungi and pesticides (Todorova *et al*., 1994). De Olivera and Neves (2004) evaluated compatibility of *B. bassiana*

with 12 acaricide formulations and showed that abamectin was more compatible with *B. bassiana*. This knowledge should facilitate the choice of chemicals which are less harmful to naturally occurring and/or artificially inoculated beneficial fungi. In this study, we evaluated commonly used pesticides in Iran. The laboratory investigation was conducted to determine the effects of 19 pesticides on conidial germination, mycelial growth as well as sporulation of selected isolates of *B. bassiana* and *M. anisopliae*.

Materials and methods

Fungal isolates

Two fungal isolates, *viz.*, C-IIIA8 of *B. bassiana* and C-IIIM14 isolate of *M. anisopliae* were used in the study. The fungi had initially been isolated from the soil collected from Maragheh, East-Azarbaijan, Iran, by using *Galleria mellonella* (L.) baiting method. The fungi were grown on SDAY (Sabaurod Dexterose Agar with Yeast) medium at 25±1°C and 12 h photoperiods.

Pesticides

The pesticides used for this experiment are shown in Table 1. For compatibility tests, the pesticides were used in three different concentrations, *viz.*, mean recommended concentration (MC), half MC and twice the MC.

Table 1. Pesticides used in the study

Active ingredient	Brand name	Chemical group	Formulation	MC*
Hexaflumuron	Consalt	IGR	10%EC	0.7
Methoxyfenozide	Ronure	IGR	240 SC	0.7
Deltametrin	Asis	Pyrethroid	2.5 EC	0.5
Cypermetrin	Patron	Pyrethroid	40%EC	0.7
Diazinon	Bazudin	Organophosphorus	60%EC	0.5
Chlorpyrifos	Dursban	Organophosphorus	%40.8 EC	2
Carbaryl	Sevin	Carbamates	50%WP	2
Thiodicarb	Larvin	Carbamates	80% WP	2
Imidacloprid	Confidor	Neonicotinoid	35% SE	0.5
Acetamiprid	Mospilan	Neonicotinoid	20% SP	0.5
Tiochloprid	Calipso	Neonicotinoid	480 SC	0.3
Abamectin	Vertimec	Avermectin	1.8% EC	0.2
Spinosad	success	Spinosin	2.5 SC	1
Endosulfan	Thiodan	Chlore	35% EC	1.5
Amitraz	Mitak	Formamidin	20% EC	1
Azadirachtin	Neem	Botanical insecticides	0.09% EC	25
Carbendazim	Bavistin	Benzimidazol	52.5% WP	1.5
Benomyl	Benlat	Benzimidazol	50% WP	1
Mancozeb	Indofil	Dithiocarbamate	75% WP	2.5

* Mean concentration of commercial product for application in 1000 liters of water

Conidial germination

The appropriate concentration (Table 1) of each pesticide was added to 50 ml of cooled SDAY. Treatments were inoculated with 1 ml of a conidial suspension of *B. bassiana* and *M. anisopliae* containing 10^6 conidia/ml that diluted in sterile distilled water and amended with 0.05% Tween 80. The same aliquot of sterile distilled water and 0.05% Tween 80, without the pesticides, was used as control. The treatments were transferred to an incubator (25 ± 1°C; 12 h photoperiods) for 24 hours and the germinated conidia counted (germinated conidia per 100 conidia) to find the conidia viability.

Mycelial growth and spore production

Inoculums of *B. bassiana* and *M. anisopliae* were produced on SDAY for 14 d, at 25°C. The pesticides, at the pre-established concentrations were then added. Approximately 20 ml of each amended media was poured in three 9 cm Petri dishes. 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 SDAY with *B. bassiana* or *M. anisopliae* which were deposited in the center of each plate (Todorova *et al*., 1994). The plates were incubated at 25 ± 1°C and the radial growth in excess of the plugs was measured on the 14th day. Growth was measured on the three radial points from the plug and the mean values were used in the following statistical tests. Each treatment was replicated three times. After 14 d, the conidia from the excess of the plugs were harvested by scraping and then suspended in 1 ml of 0.05% Tween 80 and agitated until conidia were entirely released from the medium surface. The concentration of conidia was calculated by means of a Neubauer hemocytometer.

The percentage of inhibition, if any, was calculated using the formula given by Vincent (1927): I= (C-T) ×100/C where I: Percent inhibition, C: Colony diameter (mm) in control, T: Colony diameter (mm) in treatment.

Compatibility calculations

The formula proposed by Alves *et al*. (1998) was used for toxicity classification of chemical products regarding *in vitro* tests with entomopathogenic fungi by calculation of the mycelial growth (VG) and sporulation (SP) in relation to the control (100%). T= 20 (VG) + 80 (SP) / 100

Analysis

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

Results

I- *B. bassiana*

Conidial germination: The effect of pesticides on the germination of *B. bassiana* is shown in Table 2. Among the 16 insecticides tested, chlorpyrifos and carbaryl, at 2MC concentration, completely inhibited (100%) the conidial germination, but abamectin was less effective in fungal inhibition at three concentrations being followed by methoxyfenozide and imidacloprid at MC concentration. Among the three tested fungicides, benomyl and carbendazim showed complete inhibition on the conidial germination while mancozeb, inhibited a range of 90-100% at three concentrations. Endosulfan and azadirachtin induced high reduction of conidial germination (> 50%).

Mycelial growth and spore production

The pesticides significantly inhibited mycelial growth of *B. bassiana* at all tested formulations (Table 2) of which benomyl and carbendazim at all its concentrations, and mancozeb at MC and 2MC concentrations entirely inhibited (100%) the fungal mycelial growth.

All the tested fungicides and the insecticides chlorpyrifos, carbaryl and azadirachtin (at three concentrations), as well as thiodicarb (at 2 MC) totally prevented fungal sporulation (100% reduction) (Table 2). At half MC concentration, minimum inhibition in sporulation was observed by spinosad (13.3%) being followed by imidacloprid (16.6%) and abamectin (26.6%), although only data on spinosad at three concentrations and imidacloprid at half MC were showed little difference from the control treatment.

II- *M. anisopliae*

Conidial germination

Germination of *M. anisopliae* in combination with the pesticides used in the experiment is shown in Table 3. Abamectin and spinosad showed low

inhibition levels on germination rate at the three concentrations. Diazinon completely inhibited *M. anisopliae* at two concentrations (MC and twice MC), and endosulfan induced high reduction on conidial germination (> 80%) at 2MC concentration (Table 3).

Table 2. Effect of pesticides on spore germination, mycelial growth, and sporulation of *Beauveria bassiana*

Treatments	Concentration	Germination*		Mycelial growth*		Sporulation*	
		%	% Reduction	mm	% reduction	$\times 10^7$ spore/ml	% Reduction
Hexaflumuron	half MC	75 E	19.3	37.0 B	7	1.2 DE	60
	MC	70 EF	24.7	36.0 B	10	0.8 F	73.3
	2 MC	70 D	24.7	34.0 C	15	0.05 I	98.3
Methoxyfenozide	half MC	82 B	11.8	37.0 B	7	1.4 D	53.3
	MC	78 BC	16.1 C	35.0 C	12.5	0.1 J	96.6
	2 MC	70 D	24.7	25.0 G	37.5	0.6 G	80
Deltamethrin	half MC	80 BC	13.9	36.0 BC	10	2.0 C	33.3
	MC	76 BCD	18.2	35.0 C	12.5	2.0 D	33.3
	2 MC	72 C	22.5	33.0 D	17.5	1.2 E	60
Cypermethrin	half MC	80 BC	13.9	22.5E	43.7	0.8 F	73.3
	MC	75 CD	19.3	15.0 G	62.5	0.5 I	83.3
	2 MC	65 E	30.1	15.0 K	62.5	0.12 F	96
Diazinon	half MC	78 CD	16.1	37.0 B	7	0.5 F	83.3
	MC	72 DEF	22.5	33.0 D	17	0.3 H	90
	2 MC	70 CD	24.7	5.0 D	17	0.1 H	96.6
Chlorpyrifos	half MC	0 K	100	3.0 F	92	0 G	100
	MC	0 L	100	1.0 J	97.5	0 K	100
	2 MC	0 K	100	0.0 M	100	0 J	100
Carbaryl	half MC	0 K	100	0.0 G	10	0 G	100
	MC	0 L	100	0.0 H	100	0 K	100
	2 MC	0 K	100	0.0 M	100	0 J	100
Thiodicarb	half MC	69 F	25.8	22.5 E	43.7	1.1 E	63.3
	MC	53 H	43	10.0 I	75	0.9 E	70
	2 MC	20 I	78.5	5.0 L	87.5	0 J	100
Imidacloprid	half MC	76 DE	18.27	37.0 BC	7	2.5 B	16.6
	MC	76 BCD	18.27	35.0 C	12.5	2.2 C	26.6
	2 MC	70 D	24.7	35.0 C	12.5	1.8 C	40
Acetamiprid	half MC	70 F	24.7	36.0 BC	10	1.4 D	53.3
	MC	63 G	32.2	28.0 E	30	0.5 G	83.3
	2 MC	57 G	38.8	17.0 J	57.5	0 J	100
Abamectin	half MC	82 B	11.8	36.0 BC	10	2.2 C	26.6
	MC	80 B	13.9	33.0 D	17	2 D	33.3
	2 MC	74 B	20.4	33.0 D	17	1.7 D	43.3
Spinosad	half MC	78 CD	16.1	35.0 BC	12.5	2.6 B	13.3
	MC	74 CDE	20.4	33.0 D	17	2.4 B	20
	2 MC	70 D	24.7	30.0 E	25	2 B	33.3
Endosulfan	half MC	35 I	62.3	30.0 D	25	1.3 DE	56.6

	MC	28 J	69.8	25.0 F	37.5	0.8 F	73.3
	2 MC	17 J	81.7	17.5 I	56.2	0.6 G	80
	half MC	60 G	35.4	34.0 C	15	1.1 E	63.3
Amitraz	MC	42 I	54.8	28.0 E	30	0.9 E	70
	2 MC	36 H	61.2	23.0 H	42.5	0.8 F	73.3
	half MC	70 F	24.7	35.0 BC	12.5	0.5 F	83.3
Thiachloprid	MC	68 F	26.8	35.0 C	12.5	0.3 H	90
	2 MC	60 F	35.4	26.0 F	35	0.1 H	96.6
	half MC	38 H	59.13	22.5 E	43.7	0.5 F	83.3
Azadirachtin	MC	30 J	67.7	12.5 H	68.7	0.3 H	90
	2 MC	0 K	100	0.0 M	100	0 J	100
	half MC	0 K	100	0.0 G	100	0 G	100
	MC	0 L	100	0.0 K	100	0 K	100
Carbendazim	2 MC	0 K	100	0.0 M	100	0 J	100
	half MC	0 K	100	0.0 G	100	0 G	100
Benomyl	MC	0 L	100	0.0 K	100	0 K	100
	2 MC	0 K	100	0.0 M	100	0 J	100
	half MC	8 J	91.3	5.0 F	87.5	0 G	100
Mancozeb	MC	7 L	92.4	0.0 K	100	0 K	100
	2 MC	0 K	100	0.0 M	100	0 J	100
Control		93 A	0	40.0 A	0	3 A	0

* Means followed by the same letter in a column are not significantly different (p≥0.05) by DMRT (Duncan's multiple range tested).

Table 3. Effect of pesticides in three different concentrations on conidial germination, mycelial growth, and sporulation of *Metarhizium anisopliae* in studies conducted on formulation-amended SDAY media at 25 ± 1^{0}C and 12 h photoperiod

Treatments	**Concentration**	**Germination***		**Mycelial growth***		**Sporulation***	
		%	**% reduction**	**mm**	**% reduction**	**×10^5 (spore/ml)**	**% reduction**
	half MC	75 E	19.3	40 A	0	4.5 C	25
Hexaflumuron	MC	70 F	24.7	40 B	0	1.2 G	80
	2 MC	70 D	24.7	40 B	0	0.8 G	86.6
	half MC	77 DE	11.8	38 AB	5	0.4 I	93.3
Methoxyfenozide	MC	75 D	19.3	42 A	0	0.1 J	98.3
	2 MC	70 D	24.7	42 A	0	0.1 I	98.3
	half MC	80 C	13.9	35 CD	12.5	3.2 E	46.6
Deltamethrin	MC	74 E	18.2	35 E	12.5	2.6 D	56.6
	2 MC	68 E	24.5	32 D	20	2.2 D	63.3
	half MC	66 G	18.5	38 BC	5	2G	66.6
Cypermethrin	MC	62 I	31.9	38 C	5	0.8 H	86.6
	2 MC	50 H	45.1	35 C	12.5	0.5 H	91.6
	half MC	78 CD	16.1	15 F	62.5	0 J	100
Diazinon	MC	0 M	100	7.5 K	81.25	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100

Chlorpyrifos	half MC	0 J	100	0 H	100	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Carbaryl	half MC	0 J	100	5 G	87.5	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Thiodicarb	half MC	69 F	25.8	30 E	25	1.5H	75
	MC	53 J	43	20 J	50	0.5 I	91.6
	2 MC	20 J	78.5	3 J	82.5	0 J	100
Imidacloprid	half MC	76 DE	18.3	38 AB	5	2.5 F	58.3
	MC	76 C	18.3	35 E	12.5	2.2 E	63.3
	2 MC	70 D	24.7	35 C	12.5	1.8 E	70
Acetamiprid	half MC	70 F	24.7	35 CD	12.5	1.4 H	76.6
	MC	63 H	32.3	35 E	12.5	0 K	100
	2 MC	57 G	38.8	27 G	32.5	0 J	100
Abamectin	half MC	86 B	5.5	36 BCD	10	4.2D	30
	MC	83 B	8.8	32 G	20	3.7 C	38.3
	2 MC	77 B	15.4	31 E	22.5	3.2 C	46.6
Spinosad	half MC	80 C	12.8	38 AB	5	4.8 D	20
	MC	76 C	16.5	36 D	10	3.9 B	35
	2 MC	71 C	22	35 C	12.5	3.7 B	38.3
Endosulfan	half MC	35 I	62.3	31 E	22.5	2.3 F	61.6
	MC	28 L	69.8	25 I	37.5	2 F	66.6
	2 MC	17 K	81.7	19 I	52.5	1.6 F	73.3
Amitraz	half MC	60 H	35.4	34 CD	15	1.9 G	68.3
	MC	42 K	54.8	26 H	35	1.2 G	80
	2 MC	36 I	61.2	21 H	47.5	0.8 G	86.6
Thiachloprid	half MC	70 F	24.7	34 D	15	0.5 I	91.6
	MC	68 G	26.8	33 F	17.5	0.1 J	98.3
	2 MC	60 F	35.4	29 F	27.5	0 J	100
Azadirachtin	half MC	0 J	100	7.5 E	81.5	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Carbendazim	half MC	0 J	100	0 H	100	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Benomyl	half MC	0J	100	0 H	100	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Mancozeb	half MC	0 J	100	0 H	100	0 J	100
	MC	0 M	100	0 L	100	0 K	100
	2 MC	0 L	100	0 K	100	0 J	100
Control		91 A	0	40 A	0	6 A	0

* Means followed by the same letter in a column are not significantly different (p≥0.05) by DMRT (Duncan's multiple ranges tested).

Mycelial growth and spore production

The mycelial growth inhibition induced by methoxyfenozide formulation with the MC and 2MC; but spinosad and imidacloprid were not significantly different from the control treatment. Diazinon and azadirachtin at half MC concentration induced at least 80% fungal growth inhibition and at MC and 2MC showed complete inhibition on fungal mycelial growth. Chlorpyrifos entirely inhibited the mycelial growth at the three concentrations and carbaryl was highly effective at all concentrations.

Diazinon, chlorpyrifos and carbaryl at the three concentrations as well as acetamiprid, thiodicarb and azadirachtin at 2MC concentration induced complete inhibition in sporulation. All the fungicides completely inhibited the spore germination of the fungus (Table 3).

Compatibility calculations

The data concerning sporulation and mycelia growth were subjected to the formula for the determination of 'T' values. Spinosad at all the three concentrations was classified as compatible, but abamectin was compatible at half MC and MC concentrations and was moderately toxic when was used at 2 MC with the both fungi *B. bassiana* and *M. anisopliae*. Imidacloprid was compatible at half MC and MC concentrations and was moderately toxic at 2 MC for the fungus *B. bassiana*, while it was moderately toxic at all the concentrations with *M. anisopliae*. All the fungicides at all the three concentrations were identified as incompatible with the fungi (Table 4).

Table 4. "T" values and compatibility classification of pesticides, in term of toxicity towards *Beauveria bassiana* and *Metarhizium anisopliae*

Treatments	Concentration	*B. bassiana*		*M. anisopliae*	
		"T"values*	Classification#	"T"values	Classification
Hexaflumuron	half MC	50.50	MT	80.00	C
	MC	39.30	I	36.00	I
	2 MC	18.33	I	30.60	I
Methoxyfenozide	half MC	83.55	C	24.33	I
	MC	44.16	MT	21.33	I
	2 MC	28.50	I	21.33	I
Deltamethrin	half MC	71.28	C	60.16	C
	MC	70.78	C	52.16	MT
	2 MC	48.50	MT	45.33	MT
Cypermethrin	half MC	24.53	I	45.66	MT
	MC	10.70	I	29.66	I
	2 MC	9.58	I	23.66	I
Diazinon	half MC	31.78	I	7.50	I
	MC	24.50	I	3.75	I
	2 MC	19.14	I	0.00	I
Chlorpyrifos	half MC	1.40	I	0.00	I
	MC	0.50	I	0.00	I
	2 MC	0	I	0.00	I
Carbaryl	half MC	0	I	2.50	I
	MC	0	I	0.00	I
	2 MC	0	I	0.00	I
Thiodicarb	half MC	40.52	I	35.00	I
	MC	31.00	I	16.66	I

Imidacloprid	2 MC	2.5	I	1.50	I
	half MC	85.16	C	52.33	MT
	MC	68.66	C	47.83	MT
Acetamiprid	2 MC	55.33	MT	42.55	MT
	half MC	55.28	MT	36.16	I
	MC	52.14	MT	17.50	I
Abamectin	2 MC	41.5	I	13.50	I
	half MC	76.64	C	74.00	C
	MC	69.78	C	65.30	C
Spinosad	2 MC	58.14	MT	57.60	MT
	half MC	81.36	C	83.00	C
	MC	74.22	C	70.00	C
Endosulfan	2 MC	61.6	C	66.80	C
	half MC	49.6	MT	46.16	MT
	MC	33.7	I	39.16	I
Amitraz	2 MC	24.7	I	30.80	I
	half MC	46.28	MT	42.33	MT
	MC	38	I	29.00	I
Thiachloprid	2 MC	32.7	I	21.16	I
	half MC	30.78	I	32.00	I
	MC	25.5	I	17.30	I
Azadirachtin	2 MC	15.64	I	10.60	I
	half MC	24.52	I	3.75	I
	MC	12.24	I	0.00	I
Carbendazim	2 MC	0	I	0.00	I
	half MC	0	I	0.00	I
	MC	0	I	0.00	I
Benomyl	2 MC	0	I	0.00	I
	half MC	0	I	0.00	I
	MC	0	I	0.00	I
Mancozeb	2 MC	0	I	0.00	I
	half MC	2.5	I	0.00	I
	MC	0	I	0.00	I

* The formula proposed by Alves *et al*. (1998).
# T= toxic; MT= moderately toxic; C= compatible; I= incompatible

Discussion

Pesticides have capacities to affect the developmental stages of entomopathogenic fungi. Therefore, the selected isolates of fungi for use in IPM programs require compatibility testing with pesticides (De Olivera & Neves, 2004; Rashid *et al*. 2012). In this study, all tested insecticides and fungicides displayed varying degrees of viability to inhibit conidial germination, growth and sporulation of *B. bassiana* and *M. anisopliae*.

The pesticides tested, except spinosad, abamectin and imidacloprid at low concentration significantly affected *B. bassiana* and *M. anisopliae* germination, mycelial growth and sporulation *in vitro* (Tables 2 and 3). In some cases, fungal species showed different reactions to insecticides. Acetamiprid was moderately compatible with *B. bassiana* at half MC and MC

concentrations but was incompatible with *M. anisopliae* at all three concentrations (Table 4).

According to James & Elzen (2001), Alizadeh *et al.* (2007) and Singh *et al*. (2014) imidacloprid had no negative effect on *B. bassiana*. Results of this study revealed that imidacloprid was compatible with *B. bassiana* at half MC and MC concentrations and incompatible in 2MC concentration but was incompatible with *M. anisopliae* in all three concentrations.

The fungicides significantly or totally inhibited growth of hyphomycetes entomopathogenic fungi. The effects of mancozeb on *B. bassiana* had been tested by Clark *et al*. (1982), Loria *et al*. (1983), Hassan *et al*. (1991), Majchrowicz & Poprawski (1993) and Todorova *et al*. (1994). The harmful effect of benomyl was mentioned by Olmert & Kenneth (1974). The results demonstrated that spinosad, abamectin, and imidacloprid, at lower concentration, could be used with entomopathogenic fungi in integrated pest management programs. Combination of sub-lethal concentrations of chemical insecticides and entomopathogenic fungi can cause increased stress, immunocompromise, and alteration in insect physiology and behavior leading to improved performance in insect-control programs that include a biological component (Pelizza *et al.* 2015)

The results of this research suggest that most pesticides were not compatible with *B. bassiana* and *M. anisopliae* at higher concentration and caused complete or inhibition in their developments. Only spinosad was compatible at three concentrations for both *B. bassiana* and *M. anisopliae* and all fungicides tested were incompatible at all the concentrations.

These *in vitro* studies expose the entomopathogen to the maximum action of the pesticides that usually do not happen under field conditions. Experiments under field conditions for evaluating the efficacy of these entomopathogenic fungi in combination with chemical pesticides are recommended.

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