

Full Length Research Paper

Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides

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A study was designed to assess the toxicity of insecticides and fungicides on mycelial growth and spore production of *Metarhizium anisopliae*. All insecticides significantly inhibited mycelial growth and spore production of the fungus. Chlorpyrifos, match, profenofos and metalaxyl+mancozeb were the most toxic chemicals to mycelial growth and conidial germination followed by emamectin, cypermethrin, acetameprid, imidacloprid and sinophos which were relatively less toxic to mycelial growth and spore production ($P = 0.05$) of the fungal pathogen. On the contrary, spinosad and indoxacarb were significantly compatible and were found safe to conidial germination and growth of the fungi. Further studies related to their field evaluation are needed to confirm the findings.

Key words: *Metarhizium*, mycelial growth, compatibility, insecticides, fungicides.

INTRODUCTION

Among entomopathogens, fungi are the most wide spread group of microorganisms closely associated with agriculture. Many entomopathogenic fungi especially *Metarhizium anisopliae* are used as biological control agents of insects including gregarious insect pests (Moorhouse et al., 1992, 1993a, b; Booth and Shanks, 1998; Bruck, 2005; Bruck and Donahue, 2007; Freed et al., 2012). But field application of fungi cannot give satisfactory results as pesticides due to many abiotic and biotic factors (Ferron, 1978; Villani et al., 1992; Anderson and Roberts, 1983; Loria et al., 1983; Alves and Lecuona, 1998). The use of fungi in integrated pest management (IPM) cannot be ignored. A lot of examples exist where application of different selective chemical insecticides and fungi when used in combination provide satisfactory control against many agricultural insect pests (Quintela and McCoy, 1998; Dayakar et al., 2000; Serebrove et al., 2005; Purwar and Sachen, 2006). On

the other hand, the use of non selective or incompatible chemical pesticides may possibly have the potential to hinder the vegetative growth and development of fungi adversely affecting the IPM (Anderson and Roberts, 1983; Duarte et al., 1992; Malo, 1993). For this reason, an understanding about the adverse effects of different insecticides on entomopathogenic fungi is very necessary. A number of experiments have been done to evaluate the deleterious effects of chemical insecticides on different developmental stages of fungi (Er and Gokce, 2004; Rachapa et al., 2007; Aliazadeh et al., 2007). The effect of these products may vary in different species and strains of fungi (Vänninen and Hokkanen 1988; Anderson et al., 1989). The results from such experimental work would direct the farmers to choose a more compatible pesticides and the adverse effects of the injudicious use of insecticides can be minimized (Butt et al., 2001; Inglis et al., 2001).

The aim of present study was to manipulate the inhibitory effects of different insecticides and fungicides on the mycelial growth and sporulation of four isolates of *M. anisopliae*, as well as, to check the compatibility of these chemicals with *M. anisopliae*.

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Table 1. The isolates of *M. anisopliae* isolated from different soils.

S/N	Isolate	Source	Location
1	M 11.1	Cotton field	Makhdoom Rasheed, Multan
2	M2	Barseen field	Bund Bosan, Multan
3	M2.2	Cotton field	Tawakal Town, Multan
4	M70	Cotton field	Shujaabad, Multan

Table 2: Insecticides and fungicides used in the study.

S/N	Common name	Active ingredient	Dose/acre in g or ml	Dose/90 ml media in μ L or μ g
1.	Acetameprid	Acetameprid	125	112.5
2.	Imidacloprid	Imidacloprid	250	225
3.	Tracer	Spinosad	40	36
4.	Profenophos	Profenofos	800	720
5.	Emamectin benzoate	Emamectin Benzoate	200	180
6.	Match	Lufenoron	200	180
7.	Steward	Indoxacarb	175	157
8.	Cypermethrin	Cypermethrin	330	297
9.	Chlorpyrifos	Chlorpyrifos	750	675
10.	Sinophos	Fosetyl-aluminium	250	225
11.	Metalaxyl + mencozeb	Metalaxyl + mencozeb	200-250	180

MATERIALS AND METHODS

Entomopathogenic fungus

The isolates of *M. anisopliae* used in this study were isolated from the soil samples collected from different agricultural fields of Southern Punjab, Pakistan (Table 1). After the isolation and identification, these isolates were cultured on potato dextrose agar (PDA) medium autoclaved at 121°C for 20 min. For this purpose 10 ml of PDA was spread onto the sterilized petri plates. After the solidification of the media, these petri dishes were inoculated with the respective isolates of *M. anisopliae* (Table 1) and were incubated in dark at 28±1°C and at a relative humidity of 85±5%. After 10 to 12 days, the spores of the fungi were harvested by scraping the upper surface with sterilized inoculation needle. The collected spores were suspended in sterilized Tween solution 0.05%. The mixture was shaken by using a magnetic stirrer for 10 to 15 min. The hyphal debris and mycelial clumps were removed by muslin cloth sieve and the required concentration for compatibility with insecticides were made by serial dilution.

Insecticides

Insecticides and fungicides commonly used in the field for the control of insect pests and diseases were used at their recommended field doses to check their compatibility with the entomopathogenic fungi (Table 2).

Growth inhibition assay

Insecticides with recommended field doses were added in PDA (90 ml) in Erlenmeyer flask before the solidification and then mixed thoroughly by gentle shaking. The medium containing insecticides were poured into sterilized petri plates. After the complete solidification of poisoned medium, 1 to 2 μ l of conidial suspension was added in the centre of each petri plate with the help of

micropipette. The conidia were allowed to settle on the PDA for 10 min, petri dishes were sealed and were incubated at 28±1°C, 85±5 % relative humidity. There were twelve treatments including control and each treatment was replicated four times. Standard control without poison (Tween 80, 0.05%) was also kept for comparison under same conditions. The radial growth of pathogenic fungal colony starts to measure after two days of inoculation with a caliper rule for the next consecutive ten days. The data taken were compared with the control to check the extent of toxicity of insecticides used in the study.

Spore yield

To assess the effect of insecticides on the spore production, the spores of individual plates were harvested after 10 days of inoculation in sterile conical flasks (50 ml) with 20 ml 0.05% Tween 80 solution and were quantified using a Neubauer chamber. The data collected were compared with that of control to find the effect on spore yield.

Statistical analysis

The data collected was analyzed by using SAS (SAS, 2002) under completely randomized design (CRD) and the treatments means were compared by Duncan's multiple range test (DMRT) at 0.05 probability levels.

RESULTS

Compatibility of different insecticides and fungicides with *M. anisopliae* (M11.2)

Compatibility effects of insecticides and fungicides

Table 3. Compatibility of different insecticides and fungicides with *M. anisopliae* (M11.2).

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Acetameprid	4.23 ± 0.13d	6.45 ± 0.35d	7.9±0.44d	9.06±0.52d	10.00±0.53d	10.72±0.43d	11.44±0.34d	12.31±0.49d	13.25±0.48e	14.25±0.60d
Imidacloprid	5.04±0.25c	6.7±0.17d	9.1±0.19c	10.93±0.24c	12.44±0.33c	13.69±0.62c	15.06±0.75c	16.13±0.77c	17.38±0.55c	18.25±0.25c
Spinosad	5.84±0.06b	8.69±0.17b	11.24±0.31b	13.81±0.36b	16.09±0.45b	18.50±0.29b	22.25±1.20b	24.38±0.24b	26.00±0.29b	27.25±0.52b
Eammetcin	0±0.00g	0±0.00g	2.75±0.25f	3.41±0.25g	3.94±0.20f	4.59±0.16f	4.83±0.16e	5.34±0.16e	5.75±0.20f	6.34±0.21e
Indoxacarb	5.84±0.14b	7.75±0.32c	10.59±0.44b	13.83±0.69b	16.00±1.02b	18.38±0.94b	22.06±0.78b	24.13±0.58b	25.31±0.57b	26.63±0.55b
Cypermethrin	2.97±0.16f	4.03±0.44f	5.5±0.46e	6.31±0.47f	7.50±0.65e	7.75±0.85e	11.00±1.08d	13.06±1.08d	14.19±1.04de	14.69±1.04d
Sinophos	3.84±0.05e	4.88±0.07e	6.25±0.35e	8.01±0.37e	10.00±0.31d	11.63±0.22d	13.88±0.26c	16.54±0.20c	15.31±0.21d	17.44±0.19c
Control	8.38±0.30a	11.38±0.30a	14.5±0.23a	17.5±0.23a	20.31±0.16a	23.38±0.22a	26.19±0.41a	29.63±0.46a	32.88±0.74a	36.13±0.82a

*The means sharing same letters are not significantly different (DMRT, P= 0.05%).

Table 4. Compatibility of different insecticides and fungicides with *M. anisopliae* (M2.2).

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Acetameprid	4.25±0.14cd	4.88±0.13c	5.82±0.25d	6.38±0.22e	7.63±0.39f	9.25±0.67d	11.00±0.35d	11.44±0.33e	12.13±0.24e	12.63±0.24f
Imidacloprid	3.94±0.19d	5.94±0.19b	7.94±0.19b	9.94±0.19b	12.13±0.16c	14.38±0.16b	15.94±0.21b	17.19±0.12c	18.63±0.13c	19.75±0.23d
Spinosad	6.00±0.34a	8.06±0.41a	10.69±0.12a	12.44±0.36a	14.44±0.48a	16.75±0.42a	8.38±0.52a	20.75±0.78a	23.38±0.63a	24.75±0.48b
Eammetcin	3.75±0.14d	5.50±0.20bc	7.00±0.20c	8.00±0.20d	9.88±0.31e	11.25±0.43c	12.50±0.50c	13.53±0.54d	14.63±0.55d	16.88±0.63e
Indoxacarb	5.06±0.33b	7.88±0.81a	10.38±0.63a	11.94±0.70a	13.38±0.63b	14.94±0.82b	16.38±0.81b	18.38±0.85b	20.13±0.75b	22.25±0.83c
Cypermethrin	4.63±0.24bc	6.13±0.24b	7.63±0.24bc	9.13±0.24c	10.75±0.14d	11.88±0.24c	13.38±0.24c	14.38±0.24d	15.63±0.24d	17.81±0.37e
Sinophos	2.50±0.00e	3.00±0.00d	4.25±0.14e	5.25±0.14f	5.75±0.14g	6.75±0.32e	7.25±0.32e	7.69±0.31f	8.25±0.32f	8.75±0.32g
Control	6.06±0.36a	8.31±0.37a	10.69±0.33a	12.69±0.33a	14.69±0.33a	16.88±0.22a	18.94±0.41a	20.88±0.47a	23.88±0.47a	26.69±0.43a

*The means sharing same letters are not significantly different (DMRT, P= 0.05%).

with *M. anisopliae* (M11.2) showed significant results. The maximum radial growth of *M. anisopliae* was observed in spinosad on the 10th day of treatment with diameter of 27.25±0.5b ($f = 72.34$, $df = 14$, $p = < 0.0001$). On the other hand, compared to the control (36.13±0.82a), indoxacarb ($r = 26.63±0.55b$), imidacloprid ($r = 18.25±0.25c$), sinophos ($r = 17.44±0.19c$), cypermethrin ($r = 14.69±1.04d$), acetamiprid (14.25±0.60d), and emamectin ($r = 6.34±0.21e$) showed moderate conidial germination, when measured radially (Table 3). Cypermethrin and

acetamiprid almost showed equal result. In contrast to this, profenofos, match, chlorpyrifos and metalaxyl+mancozeb showed complete inhibition of conidial germination of *M. anisopliae* (M11.2), with no apparent germination.

Compatibility of different insecticides and fungicides with *M. anisopliae* (M2.2)

The same chemicals were tested against *M. anisopliae* (M2.2) in which spinosad showed the

maximum fungal radial growth with a diameter of 24.75±0.48b ($f = 114.95$, $df = 14$, $p = < 0.0001$). When compared to the control (26.69 ± 0.43a), indoxacarb ($r = 22.25±0.83c$), imidacloprid ($r = 19.75±0.23d$), cypermethrin ($r = 17.81±0.37e$), emamectin ($r = 16.88±0.63e$), acetamiprid ($r = 12.63±0.24f$), sinophos ($r = 8.75±0.32g$) also showed growth of *M. anisopliae* in the decreasing order respectively (Table 4). For the chemicals, profenofos, match, chlorpyrifos and metalaxyl+mancozeb showed complete inhibition of conidia germination of *M. anisopliae* (M2.2) in

Table 5. Compatibility of different insecticides and fungicides with *M. anisopliae* (M2).

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Acetameprid	3.50±0.32b	5.00±0.20bc	6.00±0.20de	7.13±0.13d	7.63±0.13d	8.38±0.24d	11.00±0.14d	12.13±0.24c	13.00±0.10d	14.13±0.22d
Imidacloprid	4.25±0.18b	5.19±0.12b	7.25±0.14c	9.25±0.14c	12.25±0.25b	14.25±0.25b	18.69±1.21c	21.13±1.01b	22.13±1.01c	23.25±0.75c
Spinosad	6.44±0.12a	7.69±0.21a	10.50±0.20a	12.69±0.19a	14.81±0.12a	16.94±0.52	21.13±0.55b	23.00±0.27a	24.69±0.28b	26.25±0.34b
Emamectin	3.94±0.54b	4.94±0.54bc	6.69±0.47cd	7.25±0.83d	8.50±1.13cd	9.75±1.20c	11.63±1.13d	13.06±1.19c	13.56±1.36d	14.63±1.42d
Indoxacarb	6.31±0.62a	8.38±0.58a	9.63±0.55b	11.06±0.52b	12.13±0.66b	14.63±0.24b	19.19±0.73c	20.50±0.68b	21.81±0.64c	23.25±0.57c
Cypermethrin	4.00±0.18b	5.00±0.18bc	6.13±0.26de	7.69±0.06d	9.06±0.16c	10.39±0.32c	12.00±0.46d	13.38±0.43c	14.50±0.54d	15.50±0.54d
Sinophos	3.50±0.18b	4.25±0.10c	5.75±0.10e	7.00±0.18d	8.75±0.10c	9.81±0.31c	11.25±0.43d	12.75±0.25c	13.50±0.41d	14.38±0.52d
Control	5.94±0.06a	8.38±0.07a	10.88±0.07a	13.38±0.07a	15.63±0.07a	17.73±0.10a	23.63±0.52a	23.75±0.59a	26.28±0.49a	28.75±0.48a

The means sharing same letters are not significantly different (DMRT, $P = 0.05\%$).

this experiment.

Compatibility of different insecticides and fungicides with *M. anisopliae* (M2)

The test of the same compounds with the isolate (M2) revealed that spinosad showed a higher fungal growth with the diameter of $26.25 \pm 0.34b$ ($f = 285.44$, $df = 14$, $p < 0.0001$) compared to the control ($28.75 \pm 0.48a$). Also, indoxacarb ($r = 23.25 \pm 0.57c$), imidacloprid ($r = 23.25 \pm 0.75c$), cypermethrin ($r = 15.50 \pm 0.54d$), emamectin ($r = 14.63 \pm 1.42d$), sinophos ($r = 14.38 \pm 0.52d$), and acetamiprid ($r = 14.13 \pm 0.22d$) showed conidia germination of *M. anisopliae* when measured radially (Table 5). But profenofos, match, chlorpyrifos and metalaxyl+mancozeb showed complete inhibition of conidia germination of *M. anisopliae* (M2) in this experiment.

Compatibility of different insecticides and fungicides with *M. anisopliae* (M70)

The results of compatibility of the chemicals with the isolate (M70) showed that, spinosad and

imidacloprid resulted in maximum growth of *M. anisopliae* with the values of $r = 25.81 \pm 0.21b$ and $r = 24.81 \pm 0.61b$ respectively, when compared with control ($r = 29.50 \pm 0.59a$) ($f = 198.57$, $df = 14$, $p < 0.0001$); while sinophos ($r = 21.31 \pm 0.51c$), indoxacarb ($20.81 \pm 0.81cd$), emamectin ($r = 19.63 \pm 1.03de$), cypermethrin ($r = 18.56 \pm 0.99e$), acetamiprid ($r = 18.50 \pm 0.40e$), showed moderate conidial germination of *Metarhizium*. M70 showed the same extent of compatibility with profenofos with a radial growth ($r = 7.69 \pm 0.62f$), but showed no conidia germination when tested with other isolates of *M. anisopliae* (Table 6).

On the other hand, match, chlorpyrifos and metalaxyl+mancozeb showed complete inhibition of conidia germination of (M70), as recorded for the other isolates.

Spore yield

The spore yield of different isolates of *M. anisopliae* when mixed with insecticides showed that spinosad yielded a higher number of spores ($3.41 \times 10^7 \pm 1.36 \times 10^6b$) compared to control ($4.35 \times 10^7 \pm 1.23 \times 10^6a$) (Table 7); sinophos yielded the lowest number of spores

($4.62 \times 10^6 \pm 2.53 \times 10^5ef$). Profenofos, match, chlorpyrifos and metalaxyl+mancozeb yielded no spores ($f = 67.59$, $df = 14$, $p < 0.0001$).

In the same way, when the same chemicals were tested with *M. anisopliae* (M2.2), a significant spore production was observed in spinosad ($5.46 \times 10^7 \pm 4.85 \times 10^5a$), followed by indoxacarb ($3.8 \times 10^7 \pm 4.7 \times 10^6b$) and imidacloprid ($f = 179.76$, $df = 14$, $p < 0.0001$) (Table 7).

The test of insecticides and fungicides with *M. anisopliae* (M70) showed that spinosad ($1.26 \times 10^8 \pm 3.21 \times 10^6ab$) was significant in spore yield with control ($2.09 \times 10^8 \pm 9.79 \times 10^7a$) while the minimum yield was observed in sinophos ($4.05 \times 10^6 \pm 2.18 \times 10^5c$) followed by match, chlorpyrifos and metalaxyl+mancozeb with no spore production ($f = 38.57$, $df = 14$, $p < 0.0001$) (Table 7).

DISCUSSION

The current study was planned to evaluate the compatibility of different insecticides and fungicides being used in the field with different isolates of *M. anisopliae*. The results revealed that spinosad and indoxacarb were the most

Table 6. Compatibility of different insecticides and fungicides with *M. anisopliae* (M70).

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Acetameprid	4.94±0.21c	7.00±0.29c	9.25±0.32b	11.38±0.31de	13.25±0.32c	14.75±0.32cd	16.50±0.46c	17.69±0.57d	18.31±0.43e	18.50±0.40e
Imidacloprid	6.75±0.18b	10.00±0.62b	12.75±0.72a	14.06±0.93bc	18.50±0.23a	20.13±0.44b	21.56±0.36b	24.38±0.24b	24.63±0.22b	24.81±0.61b
Spinosad	6.81±0.12b	11.19±0.73b	14.50±0.66a	15.63±1.01b	18.69±0.62a	21.19±0.77ab	23.31±0.47a	25.94±0.79ab	25.63±0.24b	25.81±0.21b
Emamectin	4.50±0.35c	6.13±0.80cd	8.13±1.49b	9.63±1.14e	14.50±0.87bc	15.38±0.90cd	16.63±0.77c	18.13±0.83d	19.25±0.97de	19.63±1.03de
Profenofos	0±0.00e	1.38±0.07e	2.25±0.10c	3.19±0.28f	4.69±0.28d	5.19±0.28e	5.69±0.28d	7.06±0.49e	7.56±0.49f	7.69±0.62f
Indoxacarb	6.25±0.27b	11.00±0.71b	12.75±0.63a	13.00±0.64cd	15.31±0.34b	16.19±0.40cd	17.69±0.43c	20.25±0.66c	20.69±0.70cd	20.81±0.81cd
Cypermethrin	4.88±0.13c	6.88±0.31c	9.13±0.24b	11.25±0.32de	13.00±0.20c	14.44±0.48d	16.13±0.66c	18.13±1.09d	18.38±1.06e	18.56±0.99e
Sinophos	2.88±0.24d	5.25±0.32d	7.75±0.95b	12.25±0.43cd	13.75±0.52bc	16.50±0.65c	17.50±0.46c	20.88±0.72c	21.13±0.62c	21.31±0.51c
Control	10.00±0.41a	12.88±0.46a	14.44±0.66a	18.50±1.51a	20.06±1.38a	22.63±1.26a	24.63±1.34a	27.63±0.68a	29.38±0.63a	29.50±0.59a

*The means sharing same letters are not significantly different (DMRT, P= 0.05%).

Table 7. Spore yield of different isolates of *M. anisopliae* after insecticides and fungicides treatment.

Parameters	M2	M2.2	M11.2	M70
Acetameprid	8.35x10 ⁶ ±1.66 x10 ⁶ de	2.06x10 ⁷ ±8.12x10 ⁵ c	9.53x10 ⁶ ±7.22x10 ⁵ c	4.01x10 ⁷ ±4.65x10 ⁶ bc
Imidacloprid	2.23x10 ⁷ ±4.11x10 ⁶ c	3.57x10 ⁷ ±1.99x10 ⁶ b	7.26x10 ⁶ ±3.57x10 ⁵ cd	7.63x10 ⁷ ±1.77x10 ⁷ bc
Spinosad	3.41x10 ⁷ ±1.36x10 ⁶ b	5.46x10 ⁷ ±4.85x10 ⁵ a	1.47x10 ⁷ ±2.29x10 ⁶ b	1.26x10 ⁸ ±3.21x10 ⁶ ab
Emamectin	1.26x10 ⁷ ±1.00x10 ⁶ d	1.98x10 ⁷ ±8.77x10 ⁵ c	3.14x10 ⁶ ±7.54x10 ⁴ e	6.53x10 ⁷ ±6.07x10 ⁶ bc
Profenofos	0±0.00f	0±0.00e	0±0.00f	4.98x10 ⁶ ±2.29x10 ⁶ bc
Indoxacarb	2.27x10 ⁷ ±8.75x10 ⁵ c	3.8x10 ⁷ ±4.7x10 ⁶ b	1.65x10 ⁷ ±2.14x10 ⁶ b	9.27x10 ⁷ ±6.27x10 ⁶ bc
Cypermethrin	1.24x10 ⁷ ±8.75x10 ⁵ c	2.21x10 ⁷ ±1.88x10 ⁶ c	7.37x10 ⁶ ±1.60x10 ⁶ cd	3.49x10 ⁷ ±8.70x10 ⁵ bc
Sinophos	4.62x10 ⁶ ±2.53x10 ⁵ ef	5.63x10 ⁶ ±6.04x10 ⁵ d	5.67x10 ⁶ ±1.23x10 ⁵ de	4.05x10 ⁶ ±2.18x10 ⁵ c
Control	4.35x10 ⁷ ±1.23x10 ⁶ a	5.21x10 ⁷ ±7.79x10 ⁵ a	5.21x10 ⁷ ±7.79x10 ⁵ a	2.09x10 ⁸ ±9.79x10 ⁷ a

compatible insecticides when used in combination with all isolates (Tables 3, 4, 5 and 6) which show that the results of the current studies are in accordance with the results of Mohammad et al. (1987) and Rachappa et al. (2007). The utilization of incompatible insecticides may lead to suppression of development and reproduction of pathogens such as *M. anisopliae* and confine their appliance in IPM program (Anderson and Roberts, 1983; Duarte et al., 1992; Malo, 1993). In contrast to this, all of the four isolates were badly

affected by chlorpyrifos, match, profenofos and metalaxyl (Tables 3, 4, 5 and 6). Our results confirm the findings of Asi et al. (2010) and Li and Holdon (1996), who reported that these insecticides were toxic to *M. anisopliae* with the exception of one isolate (M.70) which showed a bit compatibility with profenofos (P < 0.05). Our findings confirms earlier results of Mietkiewski and Gorski (1995) and Gupta et al. (1999) who observed the changes in toxicity of entomopathogenic fungi from synergistic,

antagonistic or neutral to insecticides. All the four isolates were affected with (chlorpyrifos, lufenoron and metalaxyl+mancozeb) in the present investigation. The same results have been reported by Asi et al. (2010). Profenophos was less detrimental in case of isolate M70 but it showed complete inhibition for other three isolates (M11.2, M2, and M2.2), same results were reported by Rachapa et al. (2007).

The variation in prospective of pesticides to restrain the growth and sporulation of the insect

pathogenic fungi can be due to their inherent changes as reported earlier (Freed et al., 2011a, b). This outcome of insecticides on the growth of fungi can be different due to the chemical nature of products and the fungal species that are interacting with it (Antonio et al., 2001; Kumar et al., 2000). The lethal effects of insecticides are different at various stages of fungus (Li and Holdom, 1994). Wettable powder insecticides show synergism with *M. anisopliae* (Duarte et al., 1992; Moino and Alves, 1998), but the lethal effect of active ingredient on the growth of fungi cannot be disregarded. Conidial germination is the most significant characteristic in initiating biological control, because it is the primary step for the instigation of infection procedure (Oliveria et al., 2001; Hirose et al., 2001). Our results disclose that insecticides were more deleterious to mycelial growth.

According to our suggestions, all tested chemicals except chlorpyrifos, match, profenophos and metalaxyl+mancozeb, were compatible with *M. anisopliae*. Spinosad, indoxacarb, imidacloprid and acetameprid were more compatible with insect pathogenic fungi compared to other insecticides tested in the experiment. Alternatively, field application can give dissimilar results due to low doses of insecticides or due to the revitalization of fungi after the breakdown of insecticides. Therefore, once an insecticide has been established to be compatible in the laboratory, it must be selective under field conditions. On the other hand, high *In vitro* toxicity of a product will not always be same in the field (Butt and Brownbridge, 1997; Alves et al., 1998). The present study showed multifaceted and changing outcomes of insecticides and fungicides on the insect pathogenic fungi. In connection to that, the effect of insecticides on fungi in this field needs further exploration and research for the collective application of insecticides and fungi for the control of insect pests.

REFERENCES

- Alialzadeh A, Samih MA, Khezri M, Riseh RS (2007). Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. Intl. J. Agric. Biol., 9(1): 31-34.
- Alves SB, Lecuona RE (1998). Epizootologia aplicada ao controle microbiano de insetos. p. 97-170. In: S.B. Alves, (ed.). "Controle Microbiano de Insetos" São Paulo, Fealq, pp. 1163.
- Alves SB, Moino A Jr, Almeida JEM (1998). Produtos fitossanitários e entomopatogênicos. In: (Ed.): S.B. Alves. *Controle microbiano de insetos. Piracicaba*. FEALQ, pp. 217-238.
- Anderson TE, Hajek AE, Roberts DW, Preisler K, Robertson JL (1989). Colorado potato beetle (Coleoptera: Chrysomelidae): Effects of combinations of *Beauveria bassiana* with insecticides. J. Econ. Entomol., 82(1): 83-89.
- Anderson TE, Roberts DW (1983). Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado Potato Beetle (Coleoptera: Chrysomelidae) control. J. Econ. Entomol., 76: 1437-1441.
- Anderson TE, Roberts DW (1983). Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. J. Econ. Entomol., 76:1437-1441
- Antonio BF, Almeida JEM, Clovis L (2001). Effect of Thiamethoxam on entomopathogenic microorganisms. Neotrop. Entomol., 30(3): 437-447.
- Asi MR, Bashir MH, Afzal M, Ashfaq M, Sahi ST (2010). Compatibility of entomopathogenic fungi, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* with selective insecticides. Pak. J. Bot., 42(6): 4207-4214.
- Booth SR, Shanks CH Jr (1998). Potential of a dried rice mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. Biocontrol Sci. Technol., 8:197-206.
- Bruck DJ (2005) Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biol. Control., 32:155-163.
- Bruck DJ, Donahue KM (2007) Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals. J. Invertebr. Pathol., 95:146-150.
- Butt TM, Brownbridge M (1997). Fungal pathogens of thrips. In: *Thrips as crop pests*. (Ed.): T. Lewis. CAB international, Wallingford, UK. pp. 399-433.
- Butt TM, Jackson CW, Magan N (2001). Fungal biological control agents: Progress, Problems and Potential. CABI International, Wallingford, Oxon, UK, pp. 377-384
- Dayakar S, Kanaujia KR, Rathore RRS (2000). Compatibility of entomogenous fungi with commonly used insecticides for management of *Spodoptera litura* (Fab.). In S. Ignacimuthu and A. Sen (Eds.). *Microbials in Insect Pest Management*. Oxford and IBH Publishing Co. Pvt. Ltd, M. Delhi, Kolkata, pp. 47-52.
- Duarte A, Menendez JM, Trigueiro N (1992) Estudio preliminar sobre la compatibilidad de *Metarhizium anisopliae* con algunos plaguicidas químicos. *Revista Baracoa*, 22: 31-39.
- Ferron P (1978). Biological control of insect pests by entomogenous fungi. Ann. Rev. Entomol., 23: 409-442.
- Freed S, Jin FL, Ren SX (2011a). Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. World J. Microbiol. Biotechnol., 27 (2): 359-370.
- Freed S, Jin FL, Ren SX (2011b). Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. Pak. J. Zool., 43 (3): 417-425.
- Freed S, Jin FL, Naeem M, Ren SX Hussian M (2012). Toxicity of proteins secreted by entomopathogenic fungi against *Plutella xylostella* (Lepidoptera: Plutellidae). Int. J. Agric. Biol., 14: 291-295.
- Gupta P, Paul MS, Sharma SN (1999). Studies on compatibility of white muscardine fungus *Beauveria bassiana* with neem products. Indian Phytopathol., 52(3): 278-280.
- Hirose EP, Neves MOJ, Zequi JAC, Martins LH, Peralta CH, Moino A Jr (2001). Effect of biofertilizers and neem oil on the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch) Sorok. Brazilian Arch. Biol. Technol., 44(4): 419-423
- Inglis GD, Goettel MS, Butt TM, Strasser H (2001). Use of hyphomycetous fungi for managing insect pests. T.M., C. Jackson and N. Magan (eds.) *In Butt, Fungi as biocontrol agents progress, problems and potential*. CABI International, UK, pp. 23-70
- Kumar A, Mukherjee P, Guha A, Adyantaya SD, Mandale AB, Kumar R, Sastry M (2000). Amphoterization of colloidal gold particles by capping with valine molecules and their phase transfer from water to toluene by electrostatic complexation with fatty amine molecules. Langmuir, 16: 9775- 9783.
- Li DP, Holdom DG (1994). Effects of pesticides on growth and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). J. Invertebr. Pathol., 63: 209-211.
- Loria R, Galaini S, Roberts DW (1983) Survival of inoculum of the entomopathogenic fungus *Beauveria bassiana* as influenced by fungicides. Environ. Entomol., 12:1724-1726.
- Malo AR (1993). Estudio sobre la compatibilidad del hongo *Beauveria bassiana* (Bals.) Vuill. con formulaciones comerciales de fungicidas e insecticidas. *Revista Colombiana de Entomología*, 19: 151-158.
- Mietkiewski R, Gorski R (1995). Growth of selected entomopathogenic fungi species and isolates on media containing insecticides. Acta. Mycol., 30(1): 27-33.
- Mohammad AKA, Joanin PP, Nelson FRS (1987). Compatibility of *Metarhizium anisopliae* var. *anisopliae* with chemical pesticides. Mycopathologia, 99(2): 99-105.

- Moino Jr, AR, Alves SB (1998). Efeito de Imidacloprid e Fipronil sobre *Beauveria bassiana* (Bals.) Vuill. E *Metarhizium anisopliae* (Metsch.) Sorok. e no comportamento de limpeza de *Heterotermes tenuis* (Hagem). Anais da Sociedade Entomológica do Brasil, 27: 611-619.
- Moorhouse ER, Charnley AK, Gillespie AT (1992). Review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Ann. Appl. Biol., 121:431-454
- Moorhouse ER, Easterbrook MA, Gillespie AT, Charnley AK (1993a). Control of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larvae on a range of hardy ornamental nursery stock species using the entomogenous fungus. Biocontrol Sci. Technol., 1:63-72.
- Moorhouse ER, Gillespie AT, Charnley AK (1993b). Application of *Metarhizium anisopliae* (Metsch.) Sor. conidia to control *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larvae on glasshouse pot plants. Ann. Appl. Biol., 122:623-636.
- Oliveria GN, Neves PMOJ, Kawazoe LS (2003). Compatibility between the entomopathogenic fungus *Beauveria bassiana* and insecticides used in coffee plantations. Scientia Agricola., 60(4): 663-667.
- Purwar JP, Sachan GC (2006). Synergistic effect of entomogenous fungi on some insecticide against Bihar hairy caterpillar *Spilarctia oblique* (Lepidoptera: Arctiidae) Microbiol. Res., 161(1): 38-42.
- Quintela ED, McCoy CW (1998). Synergistic effect of imidacloprid and two entomogenous fungi on behaviour and survival of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. J. Econ. Entomol., 91(1): 110-122.
- Rachappa V, Lingappa S, Patil RK (2007). Effect of agrochemicals on growth and sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. Karnataka J. Agri. Sci., 20(2): 410-413.
- Serebrov VV, Khodyrev VP, Gerber ON, Tsvetkova VP (2005). Perspectives of combined use of entomopathogenic fungi and chemical insecticides against Colorado beetle (*Leptinotarsa decemlineata*). Mikologiya I Fitopatologiya, 39(3): 89-98.
- Vänninen I, Hokkanen H (1988). Effects of pesticides on four species of entomopathogenic fungi. Ann. Agric. Fenn., 27: 345-353.
- Villani MG, Krueger SR, Nyrop JP (1992). A case study of the impact of the soil environment on insect/pathogen interactions: Scarabs in turfgrass. In: use of pathogens in Scarab pest management. (Eds.): T.R. Glare and T.A. Jackson. Intercept, Hampshire, pp. 111-126.