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

Joshi M, Gaur N and Pandey R

Abstract
The present investigation was conducted to test the toxicological impact of chemical pesticides on entomopathogenic fungus. This study reports the in-vitro toxicity of six insecticides (Profenophos 50% EC, Chlorantraniliprole 18.5%SC, Lambda cyhalothrin 4.9% CS, Novaluron 10% EC, Emamectin benzoate 5% WG, Indoxacarb 15.8% EC), four fungicides (Mancozeb 75% WP, Carbendazim 50% WP, Propiconazole 25% EC and Hexaconazole 5% EC) at different concentration for their effect on growth, inhibitory or synergistic effects and spore germination of *Beauveria bassiana*, *Metarhizium anisopliae* by growing them on insecticides and fungicides treated media. Profenophos 50% EC was the most toxic insecticide to the linear growth, percent inhibition and spore germination. Some lower concentration (0.5-0.25) were found to be safe for *B. bassiana* only. Chlorantraniliprole 18.5% SC was found compatible with *B. bassiana.* Where as in *M. anisopliae* at different concentration (0.6, 0.3, 0.15, 0.075%) showed detrimental effect on spore germination whereas only at lowest conc. (0.037%), 76.00 percent spore germination was observed. Whereas other Insecticides like Lambda cyhalothrin 4.9% CS, Novaluron 10% EC, Emamectin benzoate 5% WG, Indoxacarb 15.8% EC were found to be compatible with *B. bassiana* and *M. anisopliae*. Among all fungicides tested only Mancozeb75% WP proved safe up to some extent at lower concentrations (0.5 and 0.25%) to test fungi with average amount of spore germination, whereas Carbendazim 50% WP, Hexaconazole 5% EC and Propiconazole 25% EC were completely inhibitory in its action at all the concentrations.

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, Compatibility, entomopathogenic fungi, insecticide, fungicide

Introduction
Among the several micro-organisms viz. bacteria, fungi, virus, protozoans and nematode, entomopathogenic fungi fill an extremely important niche for control of insect pests. It is one of the important groups of bio-agents that associate with the insects living in diverse habitats, including fresh water, soil surface and aerial location. Entomopathogenic fungi are often reported as causing high level of epizootics in nature and are the most versatile biological control agent and are environmentally safe [1]. There are more than 700 species of fungi from 9 genera that infect insects [2]. Many entomopathogenic fungi especially *Beauveria bassiana* and *Metarhizium anisopliae* are used as biological control agents of insects including gregarious insect pests. Integration of selected strain of entomopathogenic fungi with selective insecticides can improve the control efficiency, besides decreased amount of insecticides required it also minimize the risks of environmental contamination and delay the expression of insecticide resistance in insect pests [3]. Application of insecticide in combination with entomogenous fungi or separately may affect effectiveness of later through their effect on growth, sporulation and germination [4]. Thus it is important to test the compatibility of insecticides with entomogenous fungi. Conidial survival can be effected by interaction with agrochemicals, environmental factors or by biopesticides and or chemical product used to protect plants [5]. The present investigation was conducted to test the toxicological impact of selected chemical pesticides on entomopathogenic fungus; *Beauveria bassiana* and *Metarhizium anisopliae*

Materials and Methods
Six different insecticides (Table 1) and four fungicides (Table 1.1) with different concentration were selected to test their influence on mycelial growth and sporulation.

The poison food technique method was used for observation of mycelia inhibition due to insecticides and fungicides solutions were preferred. Simultaneously, double strength of PDA media was also prepared. The media was autoclaved for 15 minutes at 15 lb psi. After cooling media to about 40^0C, a previously prepared double strength insecticide and fungicide solution was mixed in media under aseptic condition in laminar flow. About 2 ml poisoned media was poured in petri-dishes and kept for solidification.

The solidified media was inoculated with 5 mm disc cut from a 15 days old actively growing culture. The PDA media without insecticides and fungicides were also poured and seeded with fungus as a control for comparison. The petri plates were incubated at 27 ± 2^0C. Each treatment was replicated thrice. Observations were recorded by measuring the radial growth of fungus up to 15 days from the date of inoculation. The mean diameter was calculated for each concentration and compared with control. Percent inhibition was recorded by comparing out with control.

For conidial viability, tests were performed using microscope slides disinfected with 70% alcohol. After demarcation of three area on the bottom surface, the slides were placed on petri plates, with a high relative humidity (100%) being maintained by two cotton pads moistened with distilled water. Two matches were placed in the horizontal position under each slide to prevent it to touch the plate's bottom. Each slide's surface was covered with 4 ml of culture medium and one drop (approximately 0.05ml) of the conidial suspension (10^7conidia per ml) was placed in each area. One-hundred conidia, both germinated and non-germinated ones, were observed in the specified area. Four slides, each representing a replication were made for each treatment and the percentage of viable conidia was calculated. A spore was considered to be viable if the germ tube was twice the length of the spore.

Table 1: Insecticides used with their concentrations

S. No.	Name of insecticides	(a. i. %)	Concentrations (Percent)
1.	Profenophos	50 EC	4.0, 2.0*, 1.0, 0.5, 0.25
2.	Chlorantraniliprole	18.5 SC	0.6, 0.3*, 0.15, 0.075, 0.037
3.	Lambda cyhalothrin	4.9 CS	1.2, 0.6*, 0.3, 0.15, 0.025
4.	Novaluron	10 EC	3.0, 1.5*, 0.75, 0.375, 0.187
5.	Emamectin benzoate	5 WG	0.50, 0.25*, 0.125, 0.062, 0.031
6.	Indoxacarb	15.8 EC	1.32, 0.66*, 0.33, 0.165, 0.082

*Recommended dose

Table 1.1: Fungicides used with their concentrations

S. No.	Name of fungicides	(a.i %)	Concentrations (Percent)
1.	Mencozeb	75WP	4, 2*,1, 0.50,0.25
2.	Carbendazim	50WP	2, 1*,0.5,0.25,0.125
3.	Propiconazole	25EC	1,0.5*, 0.25, 0.125,0.062
4.	Hexaconazole	5EC	0.5, 0.25*, 0.125, 0.062, 0.031

*Recommended dose

Results and Discussion

Six insecticides Profenophos 50% EC, Chlorantraniliprole 18.5%SC, Lambda cyhalothrin 4.9% CS, Novaluron 10% EC, Emamectin benzoate 5% WG, Indoxacarb 15.8% EC (Table 1) and four fungicides Mancozeb 75% WP, Carbendazim 50% WP, Propiconazole 25% EC and Hexaconazole 5% EC (Table 1.1) at different concentration were tested for their effect on growth, inhibitory or synergistic effects and spore germination of *B. bassiana* and *M. anisopliae* by growing them on insecticides and fungicides treated media. With the comparison of *B. bassiana* and *M. anisopliae* it was concluded that in case of *M. anisopliae* spores were not viable even at lower concentration of test insecticides however some viability was recorded in lower concentrations in *B. bassiana.*

Chlorantraniliprole 18.5% SC was found compatible with *B. bassiana* (Table 3). Where as in *M. anisoplaie* at different concentration (0.6, 0.3, 0.15, 0.075%) showed detrimental effect on spore germination whereas only at lowest conc. (0.037%), 76.00 percent spore germination was observed (Table 3.1). Relatively higher spore viability (83.33-91.66%) was recorded in *B. bassiana* as compared to *M. anisopliae* (0.00-76.00%).

Compatibility data from two entomogenous fungi with Lambda cyhalothrin 4.9% CS revealed that *B. bassiana* (Table 4) was more susceptible than *M. anisopliae* (Table 4.1), to the same doses of same insecticide.

Novaluron 10% EC was found safe for the growth and viability of *B. bassiana* (Table 5) followed by *M. anisopliae* (Table 5.1). No spore germination was recorded at three higher conc. (3.0, 1.5 and 0.75) whereas 89.33 percent spore germination was observed at two lower conc. (0.375, 0.187%) over control.

In case of Emamectin benzoate 5% WG compatibility with *B. bassiana* and *M. anisopliae*, it was observed that *B. bassiana* and *M. anisopliae* were found compatible with this insecticide (Table 6) (Table 6.1).

Indoxacarb 15.8% EC was found compatible with *M. anisopliae* (Table 7.1) and *B. bassiana* (Table 7) with 27.38% and 46.78 percent inhibition at highest concentration.

Spinosad and Indoxacarb were compatible with two *M. anisopliae* isolates in all tested concentrations [6]. Indoxacarb showed no significant inhibition of radial growth of *B. bassiana* strains, but caused significant inhibition of sporulation and spore viability in some strains [7].

Table 2: Biological characters of *B. bassiana* on PDB media treated with Profenophos 50 EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
4	0.0(0.71)*	100(90.00)**	0.00(0.00)**
2	0.0(0.71)	100(90.00)	0.00(0.00)
1	0.0(0.71)	100(90.00)	0.00(0.00)
0.5	2.20(1.64)	51.46(45.83)	83.00(67.50)
0.25	2.96(1.86)	45.99(42.67)	85.33(67.50)
Control	4.56(2.25)	0.00(0.00)	91.00(75.56)
SEM±	0.09(0.02)	4.76(2.77)	0.76(0.60)
Cd (5%)	0.29(0.07)	14.66(8.53)	2.33(1.86)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 2.1: Biological characters of *M. anisopliae* on PDB media treated with Profenophos 50 EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
4	0.0(0.71)*	100(90.00)**	0.00(0.00)**
2	0.0(0.71)	100(90.00)	0.00(0.00)
1	0.0(0.71)	100(90.00)	0.00(0.00)
0.5	2.20(1.64)	56.00(48.45)	0.00(0.00)
0.25	4.00(2.12)	19.93(26.48)	0.00(0.00)
Control	5.00(2.34)	0.00(0.00)	92.33(73.98)
SEM±	0.07(0.01)	1.06(0.68)	0.36(0.39)
Cd (5%)	0.21(0.05)	3.28(2.10)	1.10(1.21)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 3: Biological characters of *B. bassiana* on PDB media treated with Chlorantraniliprole 18.5 SC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
0.6	4.46(2.23)*	38.03(38.05)**	83.33(65.95)**
0.3	5.00(2.34)	30.49(33.48)	87.67(69.50)
0.15	5.16(2.38)	28.23(32.09)	91.00(72.56)
0.075	5.66(2.48)	21.13(27.16)	90.00(72.56)
0.0375	7.10(2.76)	1.36(5.40)	91.33(75.88)
Control	7.19(2.77)	0.00(0.00)	91.66(73.22)
SEM±	0.15(0.03)	2.15(1.83)	0.92(0.76)
Cd (5%)	0.49(0.10)	6.63(5.64)	2.84(2.34)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 3.1: Biological characters of *M. anisopliae* on PDB media treated with Chlorantraniliprole 18.5 SC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
0.6	3.06(1.89)*	50.23(45.13)**	0.00(0.00)**
0.3	4.00(2.12)	35.09(36.32)	0.00(0.00)
0.15	4.26(2.18)	30.82(33.68)	0.00(0.00)
0.075	4.30(2.19)	30.22(33.31)	0.00(0.00)
0.0375	5.03(2.35)	18.36(25.34)	76.00(60.68)
Control	6.16(2.58)	0.00(0.00)	92.33(73.98)
SEM±	0.10(0.02)	1.69(1.06)	0.54(0.48)
Cd (5%)	0.31(0.07)	5.22(3.29)	1.67(1.48)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 4: Biological characters of *B. bassiana* on PDB media treated with Lambda cyhalothrin 4.9CS

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
1.2	5.56(2.46)*	19.33(26.06)**	71.00(57.42)**
0.6	5.30(2.41)	23.18 (28.77)	78.00(62.05)
0.3	6.00(2.55)	13.01(21.07)	86.33(68.35)
0.15	5.93(2.54)	13.99(21.94)	88.00(69.76)
0.075	6.10(2.57)	11.59(19.90)	89.33(70.94)
Control	6.90(2.72)	0.00(0.00)	91.00(72.55)
SEM±	0.06(0.01)	0.91(0.73)	1.04(0.82)
Cd (5%)	0.21(0.04)	2.80(2.25)	3.22(2.52)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 4.1: Biological characters of *M. anisopliae* on PDB media treated with Lambda cyhalothrin 4.9 CS

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
1.2	3.20(1.92)*	55.50(48.16)**	70.67(52.21)**
0.6	5.36(2.42)	23.10(28.67)	75.33(60.24)
0.3	5.46(2.44)	24.04(29.35)	81.33(64.45)
0.15	6.40(2.62)	11.07(19.37)	84.00(66.56)
0.075	6.76(2.69)	5.97(13.25)	88.33(70.04)
Control	7.20(2.77)	0.00(0.00)	90.00(71.57)
SEM±	0.10(0.02)	1.79(1.76)	1.53(1.17)
Cd (5%)	0.32(0.06)	5.53(3.43)	4.72(3.62)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 5: Biological characters of *B. bassiana* on PDB media treated with Novaluron 10EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
3	4.53(2.24)*	36.25(36.99)**	87.00(68.90)**
1.5	5.43(2.43)	23.45(28.93)	88.33(70.04)
0.75	5.43(2.43)	23.45(28.93)	88.67(70.35)
0.375	5.20(2.39)	40.16(39.02)	89.00(70.63)
0.1875	6.50(2.65)	8.41(16.51)	90.66(72.23)
Control	7.10(2.76)	0.00(0.00)	91.00(72.55)
SEM±	0.11(0.02)	6.51(3.99)	0.75(0.67)
Cd (5%)	0.34(0.07)	20.06(12.29)	2.29(2.07)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 5.1: Biological characters of *M. anisopliae* on PDB media treated with Novaluron 10EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
3	4.00(2.12)*	45.12(42.19)**	78.00(62.05)**
1.5	5.56(2.46)	24.32(29.46)	80.00(63.43)
0.75	5.90(2.53)	19.90(26.43)	82.67(65.42)
0.375	6.63(2.61)	14.15(22.06)	88.67(70.35)
0.1875	6.43(2.63)	12.81(20.64)	88.33(70.04)
Control	7.53(2.83)	0.00(0.00)	92.00(73.72)
SEM±	0.07(0.01)	2.03(1.60)	1.10(0.98)
Cd (5%)	0.22(0.04)	6.24(4.95)	3.40(3.02)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 6: Biological characters of *B. bassiana* on PDB media treated with Emamectin benzoate 5WG

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
0.50	0.00(0.71)*	100.00(90.00)**	0.00(0.00)**
0.25	2.33(1.68)	64.68(53.56)	73.33(61.59)
0.125	3.40(1.97)	46.30(42.88)	79.00(62.72)
0.0625	5.40(2.43)	11.51(19.68)	83.33(65.95)
0.0312	5.66(2.48)	10.45(18.64)	85.00(67.21)
Control	6.33(2.61)	0.00(0.00)	91.00(72.55)
SEM±	0.09(0.02)	1.71(1.36)	0.09(0.69)
Cd (5%)	0.28(0.06)	5.29(4.19)	2.87(2.15)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 6.1: Biological characters of *M. anisopliae* on PDB media treated with Emamectin benzoate 5WG

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
0.50	4.30(2.19)*	43.66(41.35)**	68.00(55.56)**
0.25	4.40(2.21)	42.36(40.60)	71.00(57.41)
0.125	4.60(2.26)	39.73(39.07)	74.33(59.56)
0.0625	6.16(2.58)	19.10(25.98)	77.67(61.81)
0.0312	7.30(2.79)	4.34(11.15)	82.33(61.18)
Control	7.63(2.85)	0.00(0.00)	92.00(73.72)
SEM±	0.09(0.01)	1.26(1.43)	1.25(1.00)
Cd (5%)	0.28(0.05)	3.89(1.41)	3.84(3.11)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 7: Biological characters of *B. bassiana* on PDB media treated with Indoxacarb 15.8 EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
1.32	3.06(1.89)*	46.78(43.15)**	72.66(58.49)**
0.66	3.16(1.91)	43.73(41.37)	78.00(62.05)
0.33	3.90(2.09)	32.33(34.64)	79.00(62.72)
0.165	4.30(2.19)	25.42(30.24)	84.00(66.44)
0.0825	5.00(2.35)	13.23(28.28)	89.33(70.94)
Control	5.76(2.50)	0.00(0.00)	90.33(71.89)
SEM±	0.10(0.02)	1.92(1.18)	0.97(0.68)
Cd (5%)	0.30(0.06)	5.94(3.65)	2.99(2.09)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 7.1: Biological characters of *M. anisopliae* on PDB media treated with Indoxacarb 15.8 EC

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
1.32	3.50(1.99)*	27.38(31.52)**	68.00(55.56)**
0.66	4.20(2.17)	19.84(26.42)	76.66(61.13)
0.33	6.36(2.62)	10.97(19.33)	81.00(64.16)
0.165	7.03(2.74)	9.26(17.60)	83.00(65.67)
0.0825	7.16(2.77)	4.62(12.22)	86.66(68.62)
Control	7.63(2.85)	0.00(0.00)	92.66(74.43)
SEM±	0.11(0.02)	1.20(1.09)	1.22(1.00)
Cd (5%)	0.35(0.07)	3.71(3.36)	3.77(3.10)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Among all fungicides tested Carbendazim 50% WP, Hexaconazole 5% EC and Propiconazole 25% EC were completely inhibitory in its action at all the concentrations. While Mencozeb 75% WP proved safe upto some extent at lower concentrations to test fungi with average amount of spore germination.

Almost negligible mycelia growth (1.13 and 1.46 cm) and 74.00, 66.34 percent growth inhibition was found at two lower conc. 0.5 and 0.25% respectively. Relatively higher spore viability (63.33 and 72.33%) at lower concentration (0.5 and 0.25%) respectively was recorded in *B. bassiana* (Table 8) as compared to *M. anisopliae* (Table 8.1).

Table 8: Biological characters of *B. bassiana* on PDB media treated with Mencozeb 75 WP

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
4	0.00(0.71)*	100.00(90.00)**	0.00(0.00)**
2	0.00(0.71)	100.00(90.00)	0.00(0.00)
1	0.00(0.71)	100.00(90.00)	0.00(0.00)
0.5	3.10(1.89)	49.16(44.51)	63.33(52.75)
0.25	3.36(1.97)	44.80(42.01)	72.33(58.28)
Control	6.10(2.57)	0.00(0.00)	90.33(71.57)
SEM±	0.03(0.08)	0.53(0.30)	1.17(0.73)
Cd (5%)	0.11(0.02)	1.63(0.93)	3.60(2.26)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Table 8.1: Biological characters of *M. anisopliae* on PDB media treated with Mencozeb 75 WP

Concentration (percent)	Linear Growth	Percent Inhibition	Spore Germination %
4	1.36(1.36)*	77.80(61.92)**	0.00(0.00)**
2	1.96(1.57)	68.09(55.61)	0.00(0.00)
1	2.20(1.64)	64.27(53.30)	0.00(0.00)
0.5	2.63(1.77)	57.24(49.17)	0.00(0.00)
0.25	4.26(2.18)	30.76(33.67)	0.00(0.00)
Control	6.16(2.58)	0.00(0.00)	91.33(72.92)
SEM±	0.07(0.02)	1.46(0.90)	0.36(0.37)
Cd (5%)	0.23(0.07)	4.49(2.78)	1.10(1.14)

*Parenthesis values are square root transformed $\sqrt{x+0.5}$

**Parenthesis values are angular transformed

Complete growth inhibition of *M. anisopliae* in propiconazole, carbendazim and flusilazole at field recommended dose [8]. High toxicity of carbendazim and mancozeb to *N. rileyi* has also reported [9, 11] which indicates that carbandazim, propiconazole, chlorothalonil and hexaconazole were found highly detrimental to fungus by retarding the growth totally and Captan and triadimefan were comparatively safe to *M. anisopliae.*

Summary & Conclusion

Profenophos 50% EC was the most toxic insecticide to the linear growth, percent inhibition and spore germination. Some lower concentration (0.5-0.25) were found to be safe for *B. bassiana* only. Chlorantraniliprole 18.5% SC was found compatible with *B. bassiana*. Where as in *M. anisoplaie* at different concentration (0.6, 0.3, 0.15, 0.075%) showed detrimental effect on spore germination whereas only at lowest conc. (0.037%), 76.00 percent spore germination was observed. Whereas other Insecticides like Lambda cyhalothrin 4.9% CS, Novaluron 10% EC, Emamectin benzoate 5% WG, Indoxacarb 15.8% EC were found to be compatible with *B. bassiana* and *M. anisopliae*.

Among all fungicides tested Carbendazim 50% WP, Hexaconazole 5% EC and Propiconazole 25% EC were completely inhibitory in its action at all the concentrations. While Mencozeb 75% WP proved safe upto some extent at lower concentrations to test fungi with average amount of spore germination.

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