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Influence of systemic and non-systemic fungicides on growth of *Beauveria bassiana*

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Abstract
In Integrated Pest Management, entomopathogenic fungi are a promising biocontrol agent for managing insect populations. Understanding the detrimental impacts of agrochemicals and testing their compatibility are crucial to effective integrated pest management. The present *in vitro* study was carried out at the Department of Plant Pathology, College of Agriculture, Parbhani. Seven systemic (500, 1000 and 1500 ppm) and non-systemic fungicides (1500, 2000 and 2500 ppm) were evaluated by Poisoned Food Technique. The compatibility was inversely proportional with the concentrations of these fungicides. Among the systemic fungicides only Pyraclostrobin was found compatible at 500 and 1000 ppm and incompatible at 1500 ppm (38.50, 21.66 and 13.83 mm, respectively). Whereas Metalaxyl (08.00, 06.83 and 05.16 mm) and Thiophanate methyl (09.16, 07.66 and 07.00 mm) was incompatible at all concentrations followed by Triazole group and Carbendazim. All the non-systemic fungicides tested were found moderately compatible at 1500 ppm and sulphur and Chlorothalonil were slightly compatible at 2000 and 2500 ppm. At higher concentrations except Sulphur and Chlorothalonil other fungicides were incompatible.

Keywords: Entomopathogenic fungi, *Beauveria, in-vitro,* Fungicides, Inhibition

Introduction

In agriculture use of agrochemicals is a common practice because of their immediate effects. It suppresses rapidly expanding insect populations and disease infection. Traditionally the agrochemicals are used to decrease the economic losses, but their indiscriminate use can cause serious problems (Golshan *et al*., 2013) [16].

Biological control agents such as entomopathogenic fungi (EPF) (*Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Verticillium lecanii*, *Acremonium* spp. and *Fusarium* spp.) can be used as a component of Integrated Pest Management (IPM) of many insect pests. Under natural conditions, these pathogens are a frequent and often cause of natural mortalities of insect populations. (Saranraj and Jayaprakash, 2017) [13]. The white muscardine disease caused by *Beauveria bassiana* in major insect pests like lepidopteron, coleopteron and arthopteron etc. (Sandhu *et al*., 2017) [12].

Selective insecticides can significantly increase control efficiency in Entomopathogenic fungi, decreasing resistance and minimizing environmental contamination hazards. These methods also increases susceptibility to insecticides by suppressing enzyme activities (Ambethgar, 2009) [1]. The agrochemicals may interfere with these fungi's normal epizootic process and have unfavorable impacts on their activity. Understanding how pesticides affect the beneficial organisms utilized in integrated pest management is crucial. Pesticides have been reported to affect entomopathogenic fungus adversely in terms of growth, sporulation, and pathogenicity (Faraji *et al*., 2016) [3].

Therefore, the knowledge of compatibility between *Beauveria bassiana* and fungicides (systemic and non-systemic fungicides) may facilitate the choice of proper products for Integrated Pest Management (IPM) program considering the fungus as an important pest control agent, the present study was undertaken.

Materials and Methods

The fungicides were evaluated for compatibility with entomopathogenic fungi in *in vitro* by using Poisoned Food Technique given by (Nene and Thapliyal, 1993) [10]. Three Petri plates / treatment along with untreated control plates were maintained.

Details of the experiment

- **Design:** CRD
- **Treatments:** Eight
- **Replications:** Three

Systemic fungicides

Tr. No.	Treatment details	Trade Name
T_1	Propiconazole 25% EC	Tilt
T_2	Tebuconazole 5.4% FS	Folicur
T_3	Difenoconazole 25% EC	Score
T_4	Metalaxyl 75% WP	Rampart
T_5	Thiophanate methyl 70% WP	Roko
T_6	Pyraclostrobin 20% WG	Headline
T_7	Carbendazim 50% WP	Bavistin
T_0	Untreated Control	

Non-Systemic fungicides

Tr. No.	Treatment details	Trade Neme
T_1	Copper Oxychloride 50% WP	Blitox
T_2	Copper Hydroxide 77% WP	Dupont Kocide
T_3	Sulphur 80% WP	Sulphaboost
T_4	Chlorothalonil 75% WP	Kavach
T_5	Propineb 70% WP	Antracol
T_6	Zineb 75% WP	Indofil Z-78
T_7	Mancozeb 75% WP	Dithane M-45
T_0	Untreated Control	

Observations on radial mycelial growth / colony diameter of the *B. bassiana* was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the test fungicides over the untreated control were calculated by using the formula (Vincent, 1927) [14].

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where,
C = Growth of the test fungus in untreated control plates.
T = Growth of the test fungus in treated plates

Evaluation scale for the direct application of insecticides on the insect pest through glass plate, leaf or sand (soil) was given by Hassan (1989) [4]. To elucidate the precise results, sub categorization of Hassan's scale was done as follow.

Table 1: Scale of evaluation for compatibility of agrochemicals

Per cent inhibition (%)	Compatibility	Radial mycelial growth (mm)
81-100	Incompatible	0-15
61-80	Slightly compatible	16-30
41-60	Moderately compatible	31-50
21-40	Compatible	51-70
0-20	Highly compatible	71-90

Result and Discussion

Systemic fungicides

Radial mycelial growth

The results (Table 2) revealed that, at 500, 1000 and 1500 ppm concentration, the fungicide Pyraclostrobin (T_6) showed highest (38.50, 21.66 and 13.83 mm, respectively) mycelial growth of *B. bassiana* and found moderately compatible (500 ppm), slightly compatible (1000 ppm) and incompatible (1500 ppm). The other fungicides were found incompatible with *B. bassiana* at all the concentrations tested. The mycelial growth of *B. bassiana* recorded in treatment T_5 (Thiophanate methyl) was 09.16, 07.66 and 07.00 mm, respectively followed by treatment T_4 (Metalaxyl), which recorded 08.00, 06.83 and 05.16 mm mycelial growth, respectively. The other fungicides like Propiconazole (T_1), Tebuconazole (T_2) and Difenoconazole (T_3) and Carbendazim (T_7) were found highly toxic to the fungus *B. bassiana* and not allowed mycelial growth (00.00 mm) and statistically at par with each other.

Percent inhibition: The results (Table 2 and Figure 1) revealed that, treatments (T_6) inhibited 57.21, 75.92 and 89.96% mycelial growth of *B. bassiana* at 500, 1000 and 1500 ppm concentrations, respectively. These fungicides were moderately compatible (500 ppm), slightly compatible (1000 ppm) and incompatible (1500 ppm) with *B. bassiana*. The other treatments were found incompatible and inhibited mycelial growth. The treatment T_5 inhibited mycelial growth up to 89.81, 91.47 and 92.26%, respectively and followed by T_4 which recorded 91.10, 91.45 and 94.25%, respectively. At 1000 ppm, T_4 and T_5 were statistically at par with each other. The treatments *viz*. T_1, T_2, T_3 and T_7 were found completely incompatible and inhibited cent per cent (100%) mycelial growth of *B. bassiana* and were found statistically at par with each other.

The similar results were earlier reported by, Faraji *et al*. (2016) [3] studied compatibility of two systemic fungicides *viz*., Cabendazim 52.5% WP and Benomyl 50% WP against

Beauveria and they reported that, both Cabendazim and Benomyl showed complete inhibition on the conidial germination of *Beauveria bassiana* and incompatible to the fungus. Joshi *et al*. (2018) [7] studied three systemic fungicides *viz*., Carbendazim 50% WP, Propiconazole 25% EC and Hexaconazole 5% EC at different concentration for their effect on growth of *Beauveria bassiana* and *M. anisopliae* and they reported that, among all fungicides tested Carbendazim 50% WP, Hexaconazole 5% EC and Propiconazole 25% EC were completely inhibitory in its action at all the concentrations tested. Some other researchers also reported similar findings such as Reddy *et al*. (2018) [11] and Khun *et al*. (2020) [8].

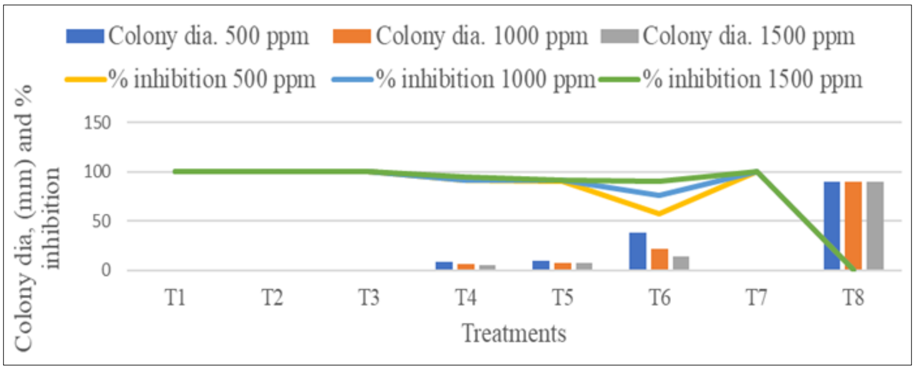

Fig 1: Compatibility of systemic fungicides with *Beauveria bassiana*

Table 2: Effect of systemic fungicides on radial mycelial growth of entomopathogenic fungi

Tr. No.	Treatments	Radial mycelial growth (mm)			Per cent inhibition (%)		
		Conc. (ppm)					
		500	1000	1500	500	1000	1500
T_1	Propiconazole	00.00	00.00	00.00	100.00 (90.00)*	100.00 (90.00)*	100.00 (90.00)*
T_2	Tebuconazole	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	Difenoconazole	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	Metalaxyl	08.00	06.83	05.16	91.10 (72.62)	91.45 (73.00)	94.25 (76.10)
T_5	Thiophanate methyl	09.16	07.66	07.00	89.81 (71.36)	91.47 (73.00)	92.26 (72.80)
T_6	Pyraclostrobin	38.50	21.66	13.83	57.21 (49.13)	75.92 (60.59)	89.96 (66.88)
T_7	Carbendazim	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_8	Control (Untreated)	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
S.E. (m) ±		0.186	0.177	0.132	0.207	0.329	0.273
C.D. at 1%		0.563	0.535	0.398	0.626	0.994	0.827

Non-systemic fungicides

Radial mycelial growth

The results (Table 3) revealed that, the fungicide Sulphur (T_3) at 1500, 2000 and 2500 ppm showed 36.16, 27.66 and 24.66 mm mycelial growth of *B. bassiana*, respectively and found moderately compatible at 1500 ppm and slightly compatible at 2000 and 2500 ppm. Chlorothalonil (T_4) was found slightly compatible at all three concentrations and recorded 23.33, 22.83 and 21.33 mm radial mycelial growth, respectively of *B. bassiana*. Copper hydroxide (T_2), Zineb (T_6) and Mancozeb (T_7) were found slightly compatible at 1500 and 2000 ppm and incompatible at 2500 ppm with *B. bassiana*. Copper hydroxide (T_2) recorded 27.16, 20.33 and 14.83 mm radial mycelial growth of *B. bassiana*, respectively followed by Zineb (T_6) (24.00, 18.50 and 14.00 mm) and Mancozeb (T_7) (22.66, 19.00 and 12.66 mm). Zineb (T_6) was found statistically at par with Mancozeb (T_7) 1500 ppm and 2000 ppm and with Chlorothalonil (T_4) (1500 ppm), Copper hydroxide (T_2) (2000 ppm) and Propineb (T_5) (2500 ppm). Propineb (T_5) and Copper oxychloride (T_1) were found slightly compatible with *B. bassiana* at 1500 ppm (21.50 and 19.50 mm, respectively) and incompatible at higher concentrations *i.e*., 2000 ppm (15.50 and 16.66 mm, respectively) and 2500 ppm (14.16 and 15.33 mm, respectively). Copper hydroxide (T_2) was statistically at par with Copper oxychloride (T_1).

Percent inhibition: The result (Table 3 and Figure 2) revealed that, at 1500, 2000 and 2500 ppm, the treatment T_3 was found moderately compatible at 1500 ppm and slightly compatible at 2000 and 2500 ppm with *B. bassiana* which showed 59.81, 69.25 and 72.29% inhibition of mycelial growth, respectively. Treatment T_4 was found slightly compatible at all three concentrations which showed 74.07, 74.62 and 76.93% inhibition of radial mycelial growth, respectively. Treatments T_2, T_6 and T_7 were found slightly compatible at 1500 and 2000 ppm and incompatible at 2500 ppm. Treatment T_2 recorded 69.81, 77.40 and 83.33% inhibition of radial mycelial growth of *B. bassiana*, respectively followed by T_6 (73.33, 77.77 and 84.43%) and T_7 (74.81, 78.88 and 85.92%, respectively). Treatment T_6 was found statically at par with T_7 1500 and 2000 ppm concentrations and with T_4 at 1500 ppm whereas with T_2 at 2000 ppm and with T_5 at 2500 ppm. Treatments T_5 and T_1 were found slightly compatible at 1500 ppm (76.61 and 78.33%, respectively) and incompatible at higher concentrations *i.e*., 2000 ppm (82.77 and 81.47%, respectively) and 2500 ppm (84.25 and 82.95%, respectively) with *B. bassiana*. Treatment T_1 and T_2 were statistically at par at 2500 ppm.

The similar results were earlier reported by, Jaros-Su *et al*. (1999) [6] studied effects of selected fungicides on *Beauveria bassiana*. They reported that, fungicides Copper hydroxide was deleterious to the fungus than Mancozeb and Chlorothalonil at their field recommended dose in both laboratory and field conditions. Challa and Sanivada (2014) [2] studied the effect of two non systemic fungicides *viz*., Copper oxychloride and

Mancozeb on germination and mycelial growth of 30 isolates of *Beauveria bassiana* and they reported that, Copper oxychloride showed no significant inhibition of conidial germination except one isolate and Mancozeb completely inhibited the growth of the isolates. Some other researchers also reported similar findings such as Wari *et al*. (2020) [15] and Hirapara *et al*. (2020) [5].

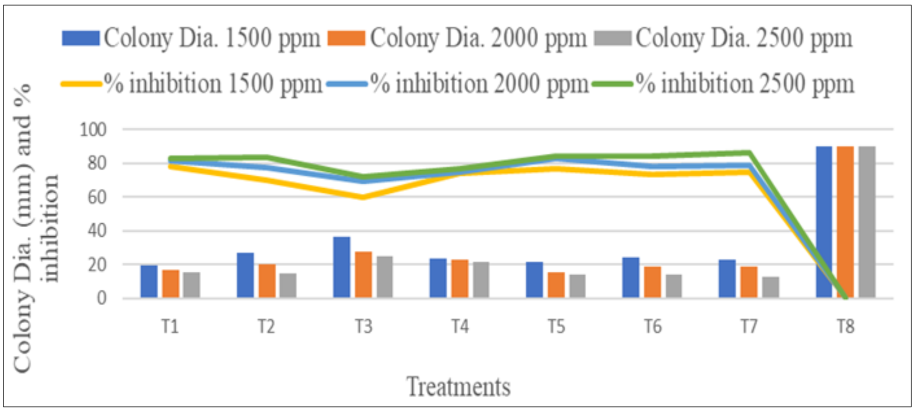

Fig 2: Compatibility of non-systemic fungicides with *Beauveria bassiana*

Table 3: Effect of non-systemic fungicides on radial mycelial growth of entomopathogenic fungi

Tr. No.	Treatments	Radial mycelial growth (mm)			Per cent inhibition (%)		
		Conc. (ppm)					
		1500	2000	2500	1500	2000	2500
T_1	Copper oxychloride	19.50	16.66	15.33	78.33 (62.33)*	81.47 (64.48)*	82.95 (65.59)*
T_2	Copper hydroxide	27.16	20.33	14.83	69.81 (56.65)	77.40 (61.59)	83.33 (66.01)
T_3	Sulphur	36.16	27.66	24.66	59.81 (50.64)	69.25 (56.30)	72.29(58.04)
T_4	Chlorothalonil	23.33	22.83	21.33	74.07 (59.36)	74.62 (59.72)	76.93 (60.84)
T_5	Propineb	21.50	15.50	14.16	76.61 (60.71)	82.77 (65.45)	84.25 (66.59)
T_6	Zineb	24.00	18.50	14.00	73.33 (58.88)	77.77 (61.88)	84.43 (66.74)
T_7	Mancozeb	22.66	19.00	12.66	74.81 (59.85)	78.88 (62.61)	85.92 (66.93)
T_8	Control (Untreated)	90.00	90.00	90.00	00.00(00.00)	00.00 (00.00)	00.00 (00.00)
S.E. (m) ±		0.471	0.276	0.306	0.524	0.739	0.315
C.D. at 1%		1.425	0.836	0.926	1.583	2.234	0.915

Conclusion

Beauveria bassiana showed more compatibility with non-systemic fungicides than the systemic fungicides.

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