Effect of Different Fungicides on Growth, Sporulation and Germination of *Beauveria bassiana* (Balsamo) Vuillemin

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SUMMARY

An experiment was carried out to study the effect of different fungicides *i.e.* wanis, propiconazole, kitazin, validamycin, triadimefan and bitertenol on sporulation, germination of *Beauveria bassiana* (Balsamo) Vuillemin. There were eight concentrations *i.e.* 5000, 2500, 1000, 500, 300, 200, 150 and 100 ppm of each test fungicide which were replicated thrice. It was found that all the test fungicides reduced the growth, sporulation and germination compared to control. Among the test fungicides, wanis (botanical extract) was found to be compatible with *B. bassiana* on the basis of spore germination while higher linear growth and sporulation was recorded in validamycine treated media. Fungicides propiconazole was proved to be highly detrimental for *Beauveria bassiana*.

Key words : *Beauveria*, Fungicide, Sporulation, Germination.

Beauveria and *Metarhizium* are important entomopathogenic fungi which have been proved as a potential substitute of chemical insecticides with other control measures and use of such entomopthogenci fungi has greatly increased due to broad spectrum of pathogenic activity (Martin *et al.*, 2000). This has also proved helpful in avoiding the adverse effects of widespread use of pesticides (Strasser *et al.*, 2000). The disease causing organisms are more or less persistent in every soil type like clay, sand, etc. (Vanninen *et al.*, 2000). However, besides microbial agents, fungicides are also regularly used to control insect pests, plant diseases and weeds which reduce the effectiveness of these entomogenous fungi by affecting their growth, sporulation and germination (Beevi and Jacob, 1987). Therefore, it is necessary to identify those fungicides which affect the growth, sporulation and germination of entomopathogenic fungi in order to maintain their effectiveness. In light of above fact, a laboratory experiment was carried out to test the effect of different fungicides on growth, sporulation and germination of *Beauveria bassiana.*

MATERIALS AND METHODS

An experiment was carried out in Department of Entomology at G.B. Pant University of Agriculture and Technology, Pantnagar to study the effect of different pesticides on growth, sporulation and germination of *Beauveria bassiana*. The fungicides used for the study were wanis, propiconazole, kitazin, validamycin, triadimefan and bitertenol. There were eight concentrations out of which three (*i.e.* 5000 (0.5%), 2500 (0.25%) and 1000 (0.1%) ppm) were higher than recommended dose (500 ppm (0.05%) while four (*i.e.* 300 (0.03%), 200 (0.02%), 150 (0.015%) and 100 (0.001%) ppm) and each concentration were replicated thrice for each experiment.

The fungus, *Beauveria bassiana*, was grown on SDA medium at 25 ± 2°C and 95 ± 5 per cent relative humidity for experimental use. The poison food technique (method) was used to observe linear growth and conidial germination. Double strength of different pesticides solutions was prepared for further study. Simultaneously, double strength of SDA medium was also prepared for each concentration of fungicides. The media were autoclaved for 15 minutes at 15 lb psi separately to sterilize the media. After cooling of medium to about 30-40°C, previously prepared double strength pesticide solutions were mixed in medium, separately, under aseptic condition under laminar flow. About 25 ml of poisoned medium was poured in Petridishes and kept for solidification. The solidified medium was inoculated with five mm disc of 10 days old actively growing culture of *Beauveria bassiana*. The Petridishes were incubated at 25±2°C and 95 ± 5 per cent RH for growth of inoculated fungus. The SDA medium without pesticides was also poured in Petridishes and

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seeded with 10 day old actively growing culture of *Beauveria bassiana* as control for comparison. Observations of each concentration of pesticides were recorded by measuring the radial growth of fungus up to 18 days from the date of inoculation. The mean diameter was calculated of each concentration of pesticides and compared with control to find out per cent inhibition.

The poison food technique (method) was again used to observe the effect of fungicides on sporulation. Conidia of *Beauveria bassiana* grown on different fungicides treated medium were harvested separately after eighteen days from the date of inoculation. The upper surface of media bearing mycelial growth was washed in laminar flow under aseptic condition from the conical flask using 100 ml of sterilized distilled water having 0.02 per cent Tween 80 as wetting agent. The surface was gently scrubbed with a sterilized infection-loop. The conidial counts were made with the help of Improved Nuebauer Weber, England Haemocytometer (Jones, 1962).

For observations of conidial germination, the fungal suspension of 0.1 ml was poured in Petridishes having very thin layer of fungicides treated medium, under aseptic condition. The Petridishes were incubated at 25±2°C and 95 ± 5 per cent RH. Observations were taken after 24 hours as suggested by Walstad *et al*. (1970).

RESULTS AND DISCUSSION

On the perusal of data, it was found that different pesticides significantly affected the growth, sporulation and germination of *Beauveria bassiana* (Tables 1, 2 and 3). It was revealed that different pesticides affected the growth, sporulation and germination of *Beauveria bassiana* on the basis on chemical concentration.

Effect fungicides on Beauveria bassiana:

Six fungicides, namely, wains (botanical extract), propiconazole, kitazin, validamycin, triadimefan and bitertenol were tested to observe their effect on linear growth, sporulation and germination of conidia of *Beauveria bassiana* (MTCC 984). It was found that different fungicides influenced the linear growth, germination and sporulation significantly.

On linear growth:

The data presented in Table 1 reveal that all the test fungicides influenced the fungal growth depending on concentrations. It was noticed that all the test fungicides caused hundred per cent inhibition of fungal growth at

Table 1: Linear growth of *Beauveria bassiana* (MTCC 984) on SDA medium treated with fungicides

Treatments	Liner growth (in mm) at concentration (in ppm)								Mean
	5000	2500	1000	700	500	250	150	100	
Wanis	0.00	0.00	6.16	8.67	10.00	13.00	15.00	17.67	11.75
Propiconazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kitazin	0.00	0.00	6.33	7.33	7.67	9.33	10.33	11.00	8.67
Validamycin	0.00	0.00	6.67	7.00	10.33	14.33	18.33	19.33	12.67
Triadimefan	0.00	0.00	0.00	0.00	7.33	9.00	12.00	14.67	7.16
Bitertenol	0.00	0.00	0.00	0.00	0.00	8.66	10.00	13.00	5.27
Control	43.33	43.33	43.33	43.33	43.33	43.33	43.33	43.33	43.33
C.D. (P=0.05)	-	-	1.44	1.57	1.62	1.75	1.83	1.98	-
S.E.±	-	-	0.47	0.51	0.53	0.57	0.60	0.65	-

Table 2: Sporulation of *Beauveria bassiana* (MTCC 984) on SDA medium treated with fungicides

Treatments	Spore production ($x10^5$) at concentration (ppm)								Mean
	5000	2500	1000	700	500	250	150	100	
Wanis	0.00	0.00	0.00	0.00	0.00	5.00	6.33	11.67	5.75
Propiconazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kitazin	0.00	0.00	0.00	0.00	0.00	0.00	7.67	9.00	4.16
Validamycin	0.00	0.00	0.00	0.00	0.00	6.67	11.0	12.67	7.58
Triadimefan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	1.67
Bitertenol	0.00	0.00	0.00	0.00	3.00	5.66	7.33	12.00	7.00
Control	667.0	667.0	667.0	667.0	667.0	667.0	667.0	667.0	667.0
C.D. (P=0.05)	-	-	-		1.25	2.13	2.51	2.99	-
S.E.±	-	-	-	-	0.41	0.69	0.82	0.97	-

Table 3: Spore germination of *Beauveria bassiana* (MTCC 984) on SDA medium treated with fungicides

Treatments	Per cent spore germination at concentration (ppm)								Mean
	5000	2500	1000	700	500	250	150	100	
Wanis	0.00	0.00	0.00	0.00	0.00	11.67	19.0	26.33	19.00
Propiconazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kitazin	0.00	0.00	0.00	0.00	0.00	0.00	14.00	17.67	10.56
Validamycin	0.00	0.00	0.00	0.00	0.00	10.33	12.00	16.33	12.88
Triadimefan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bitertenol	0.00	0.00	0.00	0.00	0.00	11.67	15.00	19.67	15.44
Control	96.33	96.33	96.33	96.33	96.33	96.33	96.33	96.33	96.33
C.D. (P=0.05)	-	-	-	-		1.79	2.02	2.26	-
S.E.±	-	-	-	-	-	0.59	0.66	0.74	-

higher concentrations *i.e.* 5000 and 2500 ppm. Among the test fungicides, propiconazole caused hundred per cent inhibition of growth at all the test concentrations (from lower 100 to higher 5000 ppm). At 1000 ppm, highest fungal growth was 6.67 mm in validamycin treated medium while the growth of fungus on wanis and kitazin treated medium was non-significantly lower than validamycin. No growth was recorded in triadimefan and bitertenol treated medium at and above 700 and 500 ppm, respectively. Similar trend of growth was recorded in rest of the test concentrations *i.e.* higher growth was recorded in validamycin treated medium. The growth increased with reduction in concentration of test fungicides. At 100 ppm, highest fungal growth was again recorded in validamycin (19.33 mm) treated medium followed by wanis (17.67 mm). Lowest (55.3%) inhibition of fungal growth was recorded in validamycin treated medium compared to untreated control having linear growth of 43.33 mm.

Effect on sporulation:

All the fungicides were detrimental for the sporulation of *Beauveria bassiana* and produced less spore as compared to control (Table 1 and 2) which produced 6.67 x 10^7 conidia ml^{-1}. No sporulation was recorded above 700 ppm concentration in all the fungicides treated medium whereas propiconazole completely inhibited sporulation at all the test concentrations (from 5000 to 100 ppm). At 500 ppm concentration, only bitertenol treated medium produced spore *i.e.* 3.0 x 10^5 conidia ml^{-1} while rest of the fungicides at this concentration inhibited the sporulation. However, the sporulation of test fungus increased gradually as the concentrations decreased. Among all the fungicides, validamycin proved relatively safe compared to all test fungicides by producing higher spore (Table 2). At lowest concentration (100 ppm), highest sporulation was recorded in validamycin treated medium (12.6 x 10^5 conidia ml^{-1}) which was non-significantly higher than bitertenol (12.0 x 10^5 conidia ml^{-1}) and wanis (11.6 x 10^5 conidia ml^{-1}). Lowest spore production was recorded in triadimefan treated medium *i.e.* 6.6 x 10^5 conidia ml^{-1}.

On spore germination:

Like spore production, all the test fungicides inhibited the germination of *Beauveria bassiana* spore above 500 ppm concentration (Table 3). However, in control 96.33 per cent germination of spore was recorded which was highest among the treatments. Among the test fungicides at 250 ppm, highest spore germination (11.67%) was recorded in wains treated medium while at 100 ppm concentration germination was 26.33 per cent which was significantly higher than rest of the treatments. Lowest conidial germination was recorded in validamycin treated medium *i.e.* 16.33 per cent. However, minimum inhibitory concentration of validamycin was found below 400 ppm for the growth of *Beauveria bassiana* (Itoh *et al.*, 1994). No conidial germination was noticed in propiconazole and triadimefan treated medium at all the test concentrations. Result of Vainio and Hokkanen (1990) supports this finding. According to them propiconazole inhibited germination of *Beauveria bassiana*.

On the perusal of data, it may be concluded that among the fungicides, wanis (botanical extract) was found to be compatible with *Beauveria bassiana* on the basis of spore germination while higher linear growth and sporulation was recorded in validamycine treated media. Fungicides propiconazole was proved to be highly detrimental for *Beauveria bassiana*.

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