ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Compatibility Analysis of Entomopathogenic Fungi *Beauveria bassiana* (NCIM No-1300) With Several Pesticides.

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ABSTRACT

Entomopathogenic fungi *Beauveria bassiana* has the potential to infect a large number of arthropods. In this experiment compatibility of mentioned fungi with Imidacloprid, Endosulfane, Acetamiprid, Neemarin and Profenofos and effect of these pesticides on sporulation and biomass production of the fungus was studied. Results suggested that Acetamaprid and Profenofos require further investigation. Also field evaluation of the interactions between *B.bassiana* and these pesticides should be undertaken to evaluate their effect on pest and beneficial insects.

Keywords: *Beauveria bassiana,* Entomopathogenic, Fungi, Chemical Pesticide, Pests

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INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* is an imperfect fungus in the subdivision Deuteromycotonia. The genus *Beauveria* is a parasite of a great number of arthropods, occurring in more than 200 species of insects and acaridae. Conidial survival can be effected by interaction with agrochemicals, environmental factor or by bio-pesticide and/or chemical product used to protect plants [1, 2, 3, 4, 5]. The pesticides and herbicides may antagonize or synergize efficacy and potential insecticidal activity of *B. bassiana* and may disrupt natural epizootics of this pathogen [6]. De Olivera and Neves (2004) evaluated compatibility of *B. bassiana* whit 12 acaricides formulation and showed that the formulations more compatible with *B. bassiana* were Avermectin and the pyrethroids [7]. Alizadeh et al., (2007) studied the compatibility of *B.bassiana* with imidacloprid, flufenoxuron, teflubenzuron+ phuzalon, endosulfan and amitraz and effect of these pesticides on conidial germination, vegetative growth and sporulation of the fungus [8]. The formulations of pesticides were tested in three concentrations (mean concentration-MC, half MC & twice the MC). The results indicated that flufenoxuron is not compatible with *B. bassiana* and it caused complete or strong inhibition in its development. The compatible formulation with *B. bassiana* (isolate DEBI008) was imidacloprid. This formulation could be used simultaneously with this entomopathogenic in integrated pest management. Dhar and Kaur (2009) tested the compatibility of the entomopathogenic fungi, *B. bassiana* and Metarhizium anisopliae with neonicotinoid insecticide, Acetamiprid at three concentrations- 1X (field recommended), 0.1X and 10X [9]. No significant difference in growth compared to the control was observed for all ten isolates. Most of the fungal strains showed slightly increased radial growth at 0.1X and 1X concentrations. *B. bassiana* isolates showed late germination than Metarhizium anisopliae isolates. Gatarayiha et al., (2010) tested the compatibility of the fungicides azoxystrobin (a strobilurin) and flutriafol (a triazole) in vitro for their effects on germination of conidia and mycelial growth of *Beauveria bassiana* (Bals.) Vuill. and in bioassay for their effect on fungal activity against Tetranychus urticae Koch, at three different concentrations [10]. They find azoxystrobin as most compatible with *B. bassiana*, while flutriafol as the most harmful fungicide that inhibit the growth of mycelia and germination of the fungal conidia at all concentrations tested in vitro, and also reduced the efficacy of *B. bassiana* in bioassays against mites.

MATERIALS AND METHODS

Fungus:

Beauveria bassiana NCIM No.1300 used in this study was kindly supplied by National Collection of Industrial Microorganism, Pune, India.

Inoculum:

B. bassiana spores were produced in Erlenmeyer flasks (250 mL) containing 50 mL of Potato Dextrose Agar, incubated at 26º C for 10 days under static condition. The spore suspension was prepared by the addition of 40 mL sterile distilled water, 15 g of glass beads and

Tween 80 (0.1%) and stirred for 30 minutes on a magnetic stirrer. The spores were counted in Neubauer chamber [11,12,13].

Chemical Pesticides:

Table1: List of Chemical Pesticides used in this study

S. No	Active Ingredient	Trade name	Formulation	MC	Source
1	Endosulphan	Kamdhenu	35 EC	35 EC	Swastic Pesticide Ltd., Muzaffarnagar,UP
2	Imidacloprid	Dharbar	17.8 SP	17.8 SP	Sree Ramcids Chemicals Pvt. Ltd., Chennai
3	Acetamaprid	TATA Manek	20SP	20SP	Rallis India Ltd., Mumbai
4	Profenofos	Celeron	50EC	50EC	Excel Crop Core Ltd., Maharashtra
5	Neem Kernel Extract	Neemarin	0.15EC	0.15EC	Biotech International Ltd., New Delhi

Medium used for compatibility analysis of entomopathogenic fungi with agrochemicals:

The pesticides selected for these experiments are shown on Table 2. For compatibility tests, the pesticides used in three different concentrations, mean concentration (MC), half MC and twice the MC (De Olivera & Neves, 2004) [7].

Preparation of amount of pesticides required to be used:

The amount of toxicant (i.e. actual ingredient) in required quantity of water was calculated with the help of following formula

$$\text{Amount of Pesticides required} = \frac{\text{Quantity of solution required X per cent of solution desired}}{\text{Strength of formulation available}}$$

Table 2: List of Pesticides used with their active ingredient, formulations and dilutions in water (in Litres)

Chemical Pesticides used	Active Ingredient	Formulation	Dilution in water (In litres)
Endosulphan	35 EC	1.5 Litres	1000
Imidacloprid	17.8 SL	0.35 litres	1875
Acetamiprid	20 SP	50 g	500-600
Profenofos	50 EC	859.11 ml	1000
Neemarin	0.15 EC	6.67 litres	1000

Conidia Yield:

The appropriate concentration of each pesticide was added to 50 ml of cooled (45°C) PDA. This treated directly when solidified was inoculated with 1 ml of a conidial suspension of *B.*

bassiana containing 10^6 spores/ml that diluted in sterile distilled water amended with 0.01% Tween 80. PDA inoculated with same concentration of conidial suspension without pesticides, was used as control. The treatments were transferred to an incubator 26°C for ten days. After incubation, by hemocytometer conidia were count as number of spores per g of dextrose.

Mycelia Growth:

Mycelial growth in response to the three concentrations of the five pesticides was evaluated in PDB. Samples (100 ml) of the broth containing the appropriate amount of pesticides were placed in 250ml Erlenmeyer flasks and inoculated with a 0.1ml of conidial suspension (3.7 x 10^5 spores/ml). Each pesticide concentration was replicated two times with a sixth flask serving containing no pesticide as control. Flasks were incubated at 26°C for 6 days on a rotary shake table (200 rpm). After incubation, contents of each flask were centrifuge, oven dried, and weighed to yield mycelial dry weights.

Method for conidia (spore) count:

Conidia production was monitored by placing beads and premeasured volume of distilled water containing 0.1% Tween 80 in Erlenmeyer flask containing uniform surface of fungus. The Erlenmeyer flasks were then placed on magnetic stirrer for 30 minutes, to disperse the conidia as conidia are hydrophobic in nature.

The suspension containing conidia were passed through muslin cloth to obtain the suspension of spores only. Conidia in the suspension were diluted as appropriate and were counted in a hemocytometer at 400X magnification in phase contrast microscope.

RESULT AND DISCUSSION

In this experiment compatibility of mentioned fungi with Imidacloprid, Endosulfane, Acetamiprid, Neemarin and Profenofos and effect of these pesticides on sporulation and biomass production of the fungus was studied. The formulations of pesticides were tested in three concentrations (mean concentration-MC, half of MC & twice of MC).

Comparison of conidia yield of entomopathogenic fungi grown on PDA in presence of different chemical pesticides:

The effect of pesticides in different concentration on sporulation of *B.bassiana* is shown in Table 3.

This study shows that endosulphan causes almost 100% reduction of conidial germination. Profenofos shows a better compatibility at 0.5MC, only 45% reduction as compared to control, mean concentration and twice of mean concentration shows 86% and 99.78% reduction in spore production respectively.

According to Alizadeh (2007) [8] imidacloprid is compatible with *B.bassiana*. Our study also shows that imidacloprid has better compatibility with *Beauveria bassiana*, it shows only 36% reduction at 0.5MC and 48% reduction at mean concentration, but shows quite noticeable reduction at twice of MC that is 78%.

Acetamiprid has very good compatibility with the fungi, even at 2MC only 44.8% reduction in spore yield was observed. Other concentrations of acetamiprid have very less toxic effect on *Beauveria bassiana* and show only 20% - 30% reduction in spore yield.

Thus the inference we can have by this study is that acetamiprid is compatible with the fungi even at twice of MC, while imidacloprid also have better compatibility with the fungi at mean concentration. Profenofos can be used in formulation with *Beauveria bassiana* only at half of the MC while endosulphan is not compatible with *Beauveria bassiana* at the tested concentrations.

Table 3: Effect of pesticides in three different concentrations on sporulation of the entomopathogenic fungus *B. bassiana* in studies conducted on formulation-amended PDA media at 26°C. (Reduction % - up to 40% =Compatible, 41% -60% = Moderately Toxic, above 60% = Toxic). Spore per gram of dextrose in Control = 1.95 X 10^9

Chemical pesticide used	Concentration of pesticide applied	Conidial yield per gram of dextrose	%Reduction in spore production	Toxicity level
ENDOSULPHAN	0.5 X MC	2.24 X 10^7	98.85	TOXIC
	1 X MC	2.774 X 10^7	98.58	TOXIC
	2 X MC	2.73 X 10^7	98.60	TOXIC
PROFENOFOS	0.5 X MC	1.066 X 10^9	45.00	MODERATELY TOXIC
	1 X MC	2.67 X 10^8	86.00	TOXIC
	2 X MC	4.3 X 10^6	99.78	TOXIC
IMIDACLOPRID	0.5 X MC	1.248 X 10^9	36.00	COMPATIBLE
	1 X MC	1.0004 X 10^9	48.00	MODERATELY TOXIC
	2 X MC	4.2 X 10^8	78.46	TOXIC
ACETAMIPRID	0.5 X MC	1.56 X 10^9	20.00	COMPATIBLE
	1 X MC	1.3635 X 10^9	30.00	COMPATIBLE
	2 X MC	1.0764 X 10^9	44.80	MODERATELY TOXIC

Comparision of biomass production of *Beauveria bassiana* in presence of chemical pesticides:

Biomass production of *B.bassiana* in PDB in presence of Endosulphan, Profenofos, Imidacloprid, Aceatamiprid and Neemarin was done and results are shown in Table 4.

Biomass production is observed at the 6th day of the incubation and the results shows that Endosulphan as observed in SSF gives quite noticeable reduction and it is not very useful to apply Endosulphan with the fungi to the field showing at average 70%- 80% of reduction.

Only half of mean concentration of Profenofos is compatible with the fungi showing only 45% reduction while rest two concentrations, 1MC and 2MC shows 70%-80% of reduction in biomass production.

As it is already observed that neem seed extract and neem leaves extract is compatible with *Beauveria bassiana* [14], but neemarin, neem kernel extract based pesticide at all three concentrations shows 44%-66%, that means it is toxic to fungi but concentrations lower than half of MC of neemarin can give some positive results.

The observations for the pre evaluated and enormously used chemical pesticide Imidacloprid with *Beauveria bassiana* shows quite better biomass production which shows only 25%, 26.7% and 30% reduction at 0.5MC , 1MC and 2MC respectively. This is in agreement with the literature that imidacloprid is compatible with *Beauveria bassiana.*

Acetamiprid when applied in half of mean concentration causes only 23% reduction of the mycelia production and even at higher concentrations it causes 26% and 32% reduction at mean concentration and twice of mean concentration respectively.

Thus this study suggest that imidacloprid, acetamiprid are compatibility with the *Beauveria bassiana. S*ome further investigation on the acetamiprid and profenofos compatibility with the fungus should be done. However, filed evaluation of the interactions between *B.bassiana* and these pesticides should be under taken to evaluate their effect on pest and beneficial insects.

Biomass produced in control = 1.67 g

Table 4: Percentage Reduction of biomass of *B.bassiana* in presence of Chemical Pesticides.

Chemical pesticide used	Concentration applied	Biomass Produced (grams)	% Reduction in Biomass Production
Endosulphan	0.5 x MC	0.5012	69.98
	1 x MC	0.2707	83.79
	2x MC	0.3650	78.14
Profenofos	0.5 x MC	0.9175	45.05
	1 x MC	0.3798	77.25
	2 x MC	0.2228	86.65
Neemarin	0.5 x MC	0.9342	44.06
	1 x MC	0.7634	54.29
	2 x MC	0.5565	66.67
Imidacloprid	0.5 x MC	1.2521	25.02
	1 x MC	1.2241	26.70
	2 x MC	1.1559	30.78
Acetamiprid	0.5 x MC	1.2804	23.33
	1 x MC	1.2300	26.35
	2 x MC	1.1204	32.91

CONCLUSION

Two way compatibility analysis of *B.bassiana* with chemical pesticides was carried out, one in terms of spore yield and another in terms of mycelium mass. Reduction in spore yield was lowest in presence of Acetamiprid which shows compatibility with 0.5MC and MC as only 20% and 30% reduction in spore yield respectively was observed. Imidacloprid shows compatibility at 0.5MC and MC as only 36% and 48% reduction in spore count was observed. Profenofos can be used in formulation with the fungus only at 0.5MC while Endosulphan is not compatible at any tested concentrations.

Compatibility in terms of biomass production shows that only imidacloprid and acetamaprid are compatible with *B.bassiana*. Since imidacloprid shows only 25%, 26.7% and 30% reduction in biomass at 0.5MC, MC and 2MC, similarly acetamaprid shows only 23%, 26% and 32% reduction in biomass at 0.5MC, MC and 2MC respectively. Profenofos is compatible only at 0.5MC, while endosulphan and neemarin is not compatible at all concentration tested.

Future Prospect:

To decrease the dose of chemical pesticide used in agriculture, combination of chemical pesticides with biopesticides can be done so as to minimize the negative effect of former on the environment. Acetamaprid and Profenofos require further investigation. Also field evaluation of the interactions between *B.bassiana* and these pesticides should be undertaken to evaluate their effect on pest and beneficial insects.

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