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Biological compatibility of *Beauveria bassiana* (Balsamo) Vuillemin isolate with different insecticides and neem formulations commonly used in rice pest management

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ABSTRACT: Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin (isolate BbCm KKL 1100) with twelve insecticides and three neem formulations was examined on agar plate to develop suitable combinations for the management of insect pests in rice fields. All chemical and botanical insecticides inhibited mycelial growth of *B. bassiana* either partially or completely depending on their concentrations (10X, 1X and 0.1X, where X = treated concentration). Chemical insecticides completely inhibited the mycelial growth of *B. bassiana*, while the neem formulations inhibited 70-86% biomass production of the fungus at 10X concentration. At 1X concentration, carbofuran caused total inhibition, but all other insecticides caused 47.4–75.5% inhibition. However, at 0.1X concentration, only neem seed kernel extract, chlorpyriphos and dimethoate exhibited 22.2%, 27.3% and 32.6% mycelial inhibition, respectively, and these could be used with *B. bassiana* in the field condition.

KEY WORDS: *Beauveria bassiana*, compatibility, insecticides, neem products, rice pests.

INTRODUCTION

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has attracted significant interest as a biological control agent since it infects a wide range of insect pests in diverse agroecosystems. In peninsular India, *B. bassiana* often causes natural epizootics on different insect pests in rice ecosystem (Rao, 1975; Nayak and Srivastava, 1979; Hazarika and Puzari, 1990; Ambethgar, 1996). Although not commercial, it is used for the management of rice hispa, *Dicladispa armigera* (Olivier), rice brown planthopper, *Nilaparvata lugens* Stål, white backed planthopper, *Sogatella furcifera* (Horvath), green leafhopper, *Nephotettix virescens* Distant, rice leaf folder *Cnaphalocrocis medinalis* (Guenée) (Hazarika and Puzari, 1990; Rama Mohan Rao, 1989), and several other crop pests across the country. Chemical pesticides are also frequently used to manage rice pests which may impede natural epizootics of the fungus (Aguda *et al.,* 1986).

Information on the compatibility of entomopathogenic fungi with conventional pesticides would not only reduce the cost of crop protection, but also mitigate the accumulation of toxic residues in food and environment. Knowledge of compatibility between *B. bassiana* and insecticides is crucial to select the appropriate compound for scheduling treatments in order to minimize any deleterious effects on biocontrol efficiency and to incorporate *B. bassiana* into the integrated pest management programme of rice. Therefore, the present study was aimed to examine the compatibility of a virulent *B. bassiana* isolate (BbCm KKL 1100) from rice leaf folder (*C. medinalis*) larva with some selected insecticides and neem formulations on agar plates to predict compatible combinations to exploit for management of insect pests in the field.

MATERIALS AND METHODS

Fungal isolate

The isolate of *Beauveria bassiana* (BbCm KKL 1100) was isolated from rice leaf folder, *C. medinalis* cadavers from Karaikal, Puducherry Union Territory, India (Ambethgar, 1996). The fungus was isolated on Sabouraud dextrose agar slants fortified with 1% yeast extract (SDAY) and selected based on virulence against *C. medinalis* larvae assayed by two-way screening using an initial single-dose assay with a standard concentration of $1x10^8$ conidia ml^{-1} in 0.02% Tween 80®, followed by multiple-dose mortality assay with six different conidial concentrations containing $1x10^4$, 10^5, 1^6, 10^7, 10^8 and 10^9 viable conidia ml^{-1} in 0.02% Tween 80® as surfactant.

Chemical insecticides and neem formulations

The chemical insecticides and neem formulations used for rice pest management were selected for this study. The common name, chemical name, trade name, active ingredient, formulation and field doses of these insecticides are mentioned in Table 1. The effects of these insecticides on the radial growth of *B. bassiana* were evaluated in the laboratory. Fifteen chemicals including twelve insecticides and three neem formulations were tested on SDAY each at three concentrations, *viz.,* the normal field application rate (I.0X) (based on 500L ha^{-1} spray), 10-fold lower rate (0.1X) and 10-fold higher rate (10.0X) for inhibition of radial growth of the fungus (Moorhouse *et al.,* 1992).

***In vitro* inoculation procedure**

Twenty ml of SDAY medium was sterilized separately, cooled to 55^{o}C and the required concentration of each insecticide was incorporated separately and plated into 9cm sterile Petri dishes. An agar-mycelial mat of *B. bassiana* (10 mm dia., 10-day-old) was inverted on the center of a SDAY plate amended with different concentrations of insecticides. SDAY plates without insecticide inoculated with a mycelial disc served as control. The plates were sealed and incubated at 26 ± 2^{o}C in a BOD incubator for 14d when radial growth on control covered the Petri dish. The diameter of radial growth of the culture in excess of the plugs on 14d after inoculation was measured. The effect of the insecticides was scored as fungicidal if growth dropped totally, otherwise it was taken as fungistatic. The experiments were replicated three times.

Data computation and analysis

The fungus-insecticide compatibility data were analyzed according to Hassan's classification scheme (Hassan, 1989). The mean fungus radial growth data were expressed as percentage of growth inhibition in comparison to corresponding control following Hokkanen and Kotiluoto (1992) as given below:

$$X = \frac{Y - Z}{Y} \times 100$$

where X, Y and Z stand for percentage of growth inhibition, radial growth of fungus in control and radial growth of fungus in poisoned medium, respectively. The pesticides were further classified on a 1-4 scoring: 1 = harmless (<20% reduction), 2 = slightly harmful (20-35%), 3 = moderately harmful (35-50%), harmful (>50%) in toxicity tests (Hassan, 1989). All data were analyzed using the Statistical Analysis System (SAS Institute, Inc., 1982) programme.

RESULTS AND DISCUSSION

All the insecticides and neem formulations hampered the mycelial development of B. bassiana in SDAY medium either partially or completely at all the three concentrations, viz., the normal field rate (1.0X), 10 times subnormal rate (0.1X) and 10 times higher rate (10.0X) (Table 2). However, there were significant differences in the inhibition rate of fungus growth by the insecticides (Table 2). All the concentrations of carbofuran and carbaryl and

Table 2. Effect of different insecticides on growth of *B. bassiana*

Insecticides	Subnormal dose (0.1X)		Normal dose (1X)		Higher dose (10X)		Scoring at 0.1X
	MCD	PIC	MCD	PIC	MCD	PIC	
Acephate	31.33	65.18	22.00	75.55	0.0	100.00	4
Carbaryl	0.0	100.00	0.0	100.00	0.0	100.00	4
Carbofuran	0.0	100.00	0.0	100.00	0.0	100.00	4
Chlorpyriphos	69.63	27.33	46.50	48.33	0.0	100.00	2
Dichlorvos	50.66	43.71	35.33	60.74	0.0	100.00	3
Dimethoate	60.66	32.60	41.00	54.44	0.0	100.00	2
Endosulfan	51.33	42.96	36.66	59.66	0.0	100.00	3
Fenthion	40.66	54.82	26.66	70.33	0.0	100.00	4
Monocrotophos	59.33	34.07	47.33	47.41	0.0	100.00	2
Phosalone	54.00	40.00	36.66	59.66	0.0	100.00	3
Phosphamidon	56.00	37.77	46.00	48.88	0.0	100.00	3
Quinalphos	39.00	56.66	52.66	41.48	0.0	100.00	4
Azadirachtin	57.66	35.93	44.00	51.11	18.3	80.00	3
Neem Oil	57.33	36.30	40.33	55.18	12.33	86.30	3
NSKE	70.00	22.22	46.33	48.52	20.66	70.04	2
Control	90.00	0.00	90.00	0.00	90.00	0.00	-

MCD = Mean colony diameter (mm); PIC = Per cent inhibition over control; 1 = harmless (<20% inhibition); 2 = Slightly harmful (20-35% inhibition); 3 = Moderately harmful (36-50% inhibition); 4 = Harmful (>50% inhibition).

Table 1. Insecticides used to study compatibility with *Beauveria bassiana*

Insecticides	Chemical Name	Brand Name	Active Ingredient (%)	Field dose (ml/g L^{-1})
Acephate	O,S-dimethyl acetylphosphorami dothioate	Ortain	75 SP	1.0
Carbaryl	1-Naphthyl N-methylcarbamate	Sevin	50 WP	2.0
Carbofuran	2,3-dihydro-2,2-dimethyl 1,7 benzofuranyl methylcarbamate	Furadan	3 G	1.0
Chlorpyriphos	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Durmet	20 EC	2.5
Dichlorvos	O,O-Dimethyl 2, 2-Dichlorovinyl phosphate	Nuvan	76 WSC	1.5
Dimethoate	O,O-dimethyl –S- [2-(methylamino)-2 oxoethyl] phosphorodithioate	Rogar	30 EC	2.0
Endosulfan	6, 7, 8, 9, 10, 10-hexachloro- 1, 5, 5a, 6, 9, 9a-hexahydro- 6,9-methano-2,4,3-benzodioxathiepin-3-oxide	Thiodan	35 EC	2.0
Fenthion	O,O-dimethyl O-[3- methyl-4-(methylthiophenyl phosphorothioate)]	Lebaycid	100 EC	1.0
Monocrotophos	Dimethyl (E)-1-methyl-P methyl carbomoylvinyl phosphate	Nuvacron	36 WSC	2.0
Phosalone	O,O-diethyl S-(6-chloro-2-oxo-benzoxazoilin-3-yl) methyl phosphorodithioate	Zolone	35 EC	2.0
Phosphamidon	Dimethyl phosphate, Ester with 2-chloro-N,N-Diethyl-3- Hydroxycrotonamide	Dimecron	85 WSC	1.0
Quinalphos	O,O-diethyl-O-quinoxaxalinyl-(2) thionophosphate	Ekalux	25 EC	2.0
Azadirachtin	Azadirachtin	Achook	0.03 EC	3.0
Neem Oil	Azadirachtin	–	3	30.0
Neem Seed Kernel Extract	Azadirachtin	–	5	50.0

G= Granules; SP= Soluble powder; WP= Wettable powder; EC= Emulsifiable concentrate; WSC= Water soluble concentrate

10X concentration of other insecticides totally inhibited fungus growth. Clark *et al.* (1982) also found complete inhibition of *B. bassiana* in broth cultures containing carbofuran and azinphos methyl at higher concentrations. Three insecticides, chlorpyriphos, monocrotophos and phosphamidon, caused 47.4%, 48.3% and 48.8% inhibition of the mycelial growth, respectively, at 1X concentration, while all other insecticides caused more than 50% inhibition (Table 2). At 0.1X concentration, only chlorpyriphos and dimethoate were slightly harmful, causing 27.3% and 32.6% mycelial inhibition but all other insecticides were highly inhibitory (Table 2). This observation suggests that chlorpyriphos at sublethal concentration can be combined with *B. bassiana* formulation. Similarly, *B. bassiana* with half dose of chlorpyriphos to control *Ostrinia nubilalis* Hub. (Ribba *et al.,* 1983) and low doses of chlorpyriphos (0.9 kg ha^{-1}) to increase mortality of *O. nubilalis* in fields (Foschi and Grassi, 1985) were recommended.

The enhanced effects of *B. bassiana* on the processes of germination, radial growth and sporulation vary depending on the fungal isolates and the nature and concentrations of the insecticide (Olmert and Kenneth, 1974; Anderson *et al.,* 1989). Subnormal doses of fenitrothion, chlorfenvinphos and permethrin were reported to be innocuous to *B. bassiana* (Cadatal and Gabriel, 1970; Clark *et al.,* 1982). Similarly, insecticides of microbial origin and chitin inhibitors such as abamectin, thuringiensin and triflumuron were compatible with *B. bassiana* even at higher rates (Anderson *et al.,* 1989).

Out of twelve insecticides, only chlorpyriphos and dimethoate at subnormal concentration (0.1X) were found to be slightly harmful to *B. bassiana* (Table 2). Contrary to this finding, chlorpyriphos had been reported to strongly inhibit the growth and sporulation of *B. bassiana* at lower than recommended field rates (Rama Mohan Rao, 1989). Although different insecticides inhibited the growth of *B. bassiana*, the combined use of entomopathogenic fungi and insecticides need not be totally ruled out as enhanced control of insect pests was occasionally observed in some crops. For instance, increased mortality of *Melolontha melolontha* by *Beauveria tenella* with reduced doses of some insecticides (Ferron, 1978) and Colorado potato beetle (Anderson *et al.,*1989), and suppression of the second generation outbreak of *Cydia pomonella* by *B. bassiana* formulation 'Boverin' with reduced doses of trichlorophon were observed.

Neem products such as azadirachtin, crude neem oil and neem seed kernel extract (NSKE) at three concentrations caused varying levels of colony inhibition of *B. bassiana* similar to chemical insecticides (Table 2). However, only NSKE at 0.1X concentration exhibited minimum (22.2%) inhibition as against 35.9% and 36.3% by azadirachtin and neem oil (Table 2). Aguda *et al.* (1986) found that neem oil at 5% had strong inhibitory effect on conidial germination and sporulation of *Metarhizium anisopliae,* but Gupta *et al.* (2002) reported that higher concentrations of neem formulations like Achook, Field marshal, Margocide and Nimbecidine in 10,000, 1000, and 100 ppm were also comparable to *B. bassiana* under *in vitro* conditions. Such variations of inhibitory action may be due to variations of the composition of different formulations. The results indicate that *B. bassiana* can be combined with NSKE, chlorpyriphos and dimethoate at 0.1X concentration to control leaf folder in the field.

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