

**DETECTION OF COMPATIBILITY OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* (BALS.) VUILL. WITH PESTICIDES, FUNGICIDES AND BOTANICALS.**J. Usha¹, M. Naren Babu^{2*} and V. Padmaja³^{1,2,3}Department of Botany, Andhra University, Visakhapatnam- 530 003. India.

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ABSTRACT: Knowledge about compatibility of biocontrol agents with an array of chemicals in the form of pesticides, fungicides and botanicals in the agro environments is a prerequisite for deployment of the biocontrol agent. Combined utilization of selective insecticides in association with fungal pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance. By using isolates of *Beauveria bassiana* compatibility assessment was made with insecticides, fungicides and botanical, at three concentrations (0.1X, 0.5X and 1X) in the laboratory based on the recommended dose for field application by food poison technique and their effect on conidial germination, vegetative growth and sporulation. Chlorpyrifos is highly detrimental to all the isolates even at low concentrations. Isolate B55 was compatible with imidacloprid even at higher concentration (1X). Sulphur displayed high compatibility to all isolates at higher concentration except for B57 and Copper oxy-chloride was compatible to all the isolates at lower concentration but showed toxicity at higher levels. All the botanicals were compatible to the isolates. Data obtained in this study may guide future recommendations of these active ingredients in IPM programs where *B. bassiana* is intended to be used as a biocontrol agent.

Key words: Compatibility, *Beauveria bassiana*, Toxicity, Pesticides.

INTRODUCTION

Biological control, particularly by entomopathogenic fungi, is important for reducing the population density of pests in Integrated Pest Management (IPM) programs. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) is a registered biopesticide with a broad host range of approximately 700 insect species used for management of several crop insect pests. The integration of microbial pesticides with chemical pest management practices requires detailed compatibility studies. Data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments so that benefits from compatible sets can be accrued and, with noncompatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized [15,22,31]. The knowledge of the compatibility between entomopathogenic fungi and pesticides may facilitate the choice of proper products for integrated pest management (IPM) program considering the fungus as an important pest control agent [40]. Combined utilization of selective insecticides in association with fungus pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance [34,42]. Conidial survival can be effected by interaction with agrochemicals, environmental factor (Benz; 1987) or by bio-pesticide and/or chemical product used to protect plants [4, 9,33]. Fungal biocontrol agents and selective insecticide may act synergistically increasing the efficacy of control, allowing the lower doses of insecticides, preservation of natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent [6]. De Olivera and Neves [16] evaluated compatibility of *B. bassiana* with 12 acaricides and showed that Avermectin and the Pyrethroids were more compatible with *B. bassiana* than the others. Amutha et al. [8] suggested use of Quinolphos and chloripyrifos in combination with *B. bassiana* which were proved to be less toxic to the fungal pathogen in his studies on compatibility. Monocrotophos, endosulfan and deltamethrin were the most harmful insecticides to *B. bassiana* development.

Thiamethoxam, diafenthiuron and acephate were compatible with *B. bassiana*, with no effect on reproductive or vegetative growth. carbosulfan was classified as incompatible, significantly affecting conidial production, and imidacloprid was moderately compatible. Shafa khan [46] concluded that imidacloprid, monocrotophos and quinalphos were highly safe and most compatible to *B. bassiana* and *M. anisopliae* whereas chlorpyrifos is found to be highly deleterious. Alizadeh et al [2] reported that imidacloprid is compatible with *B. bassiana* and can be used simultaneously in IPM programmes. Monocrotophos was reported to be compatible to *B. bassiana* by Umadevi et al. [49]. Durán et al. [17] mention that benomyl, dimethomorph-mancozeb, chlorothalonil, propineb, mancozeb, and mancozeb-cymoxanil mixture fungicides significantly affect germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride do not. Mancozeb and copper oxychloride were not compatible and caused complete or strong inhibition of vegetative growth as well as sporulation [46].

In recent years there has been an attempt to replace the synthetic insecticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals [23]. Hirose et al. [21] observed 45% reduction in spore germination of *B. bassiana* when mixed with neem oil at 2%. *B. bassiana* activity was enhanced by agroneem (a commercial neem insecticide) [3]. When an IPM strategy is devised, it is important to take into account the compatibility of products sprayed on the crop, avoiding the use of the most toxic, or using them during seasons when the effect over a natural control agent is minimized. Therefore, the toxic effect impact on the control agent will be smaller, contributing indirectly to control the host pest-insect and, consequently, to reduce damage in the cultivated field [24]. The objective of this study was to evaluate the in vitro effect of commercial pesticides, fungicides and botanicals on conidial germination, vegetative growth and sporulation of selected isolates of entomopathogenic fungus *B. bassiana*, an important bio control agent used in integrated pest management programmes.

MATERIALS AND METHODS

Fungal culture and maintenance

Four isolates of *B. bassiana* were studied were obtained from ARSEF (Agricultural Research Service Collection of Entomopathogenic Fungi), Ithaca-type culture collections as listed.

Isolate	ARSEF Acc. No	Host [^]	Taxonomic order
B 44	ARSEF- 1514	<i>Musca autmnalis</i>	Diptera
B 55	ARESF - 654	<i>Nilaparvata lugens</i>	Homoptera
B 56	ARSEF - 679	<i>Nilaparvata lugens</i>	Homoptera
B 57	ARSEF- 736	<i>Chalcodermus aeneus</i>	Coleoptera

The microscopic cultures were grown on SDAY medium (Sabouraud dextrose Agar with yeast extract medium) – 4% dextrose, 1% peptone, 1% Yeast extract, 2% Agar, pH 7.0, incubation of slants at 25 +1° C. The sporulated cultures seen with cream colored powdery coating on the white mycelial mat were stored at 4°C. Viability of the isolates was maintained through strain passage by infecting the natural insect host (*Spodoptera litura*). All the solvents used as medium components of the culture media were from Merck (India) Ltd.

Preparation of conidial suspensions

Conidia of *Beauveria* were obtained from 15 day old sporulating cultures grown at 25°C on agar slants of SDAY medium. Conidia were harvested from the surface of these cultures directly by scraping and suspending in 5 ml of sterile 0.02% Tween 80 solution. The suspension was then vortexed to decimate conidial clumps in order to obtain homogenous mixtures which were then adjusted to defined concentrations based on haemocytometer counts.

Germination assay

For evaluating the germination rates of conidia of the fungal isolates, the method followed by Bugeme et al, [14] was adopted with slight modifications. Conidial suspension (100µl) with 1×10^8 conidia ml⁻¹ concentration was spread on SDA plates suspended with media mixed with pesticides, fungicides, botanicals at 0.1X, 0.5X and 1X of the recommended doses. A sterile cover slip was randomly placed before sealing the plates with Para film and incubated at 25 ± 1°C in complete darkness. At 24 hr post-inoculation, 1 ml formaldehyde (0.5%) was added onto the plates to halt germination and the germination counts were made from 300 spores from each plate at 400X magnification. Triplicates were maintained for each treatment.

Evaluating the compatibility with fungicides, pesticides and botanicals

Pesticides, fungicides and botanicals used in the experiment and their active ingredients were detailed in T-1.

The culture medium SDAY was autoclaved at 15 lbs for 20 min. and the requisite quantity of pesticides, fungicides and botanicals were added to the medium before solidification (medium temperature 45°C) at 0.1X, 0.5X and 1X doses recommended by the manufacturer (T-1) and mixed thoroughly before pouring into Petri dishes measuring 9 cm in diameter. After medium solidification, a well of 05mm diameter was made using a sterile cork borer, into which 40µl of conidial suspension of *B.bassiana* at $1 \times 10^8 \text{ ml}^{-1}$ concentration was transferred using a micropipette. For each treatment three triplicates were maintained. Controls without the toxin (pesticide, fungicide and botanical) were kept for comparison under the same condition. The dishes were maintained in an incubator at $25 \pm 1^\circ\text{C}$ for 10 days.

For evaluating compatibility with the fungi, the colony size and spore output were taken in to consideration. Diameter of the colonies was measured on the 10th day with the common ruler by measuring in two directions and the mean for the two values was tabulated. Inhibition of colony growth over untreated check was worked out for the respective chemicals. For estimating spore output, 5 ml of 75 per cent ethanol was added to 10 day old culture to arrest growth and washed 10 times with 9.5 ml of 0.01 per cent Tween 80 and aliquot was collected in vials. Number of conidia of each culture was determined using haemocytometer and the average number of conidia per colony in each plate was calculated to measure the conidia output [32]. Mean colony size and mean number of conidia in each treatment was submitted to analysis of variance followed by the F test and Tukey test at 5% level of significance.

Compatibility assessment was done as per Alves et al, (1998) using the formula:

$$T = \frac{20 [VG] + 80 [SP]}{100}$$

Where *T* is the corrected value of vegetative and reproductive growth for product classification, *VG* is percent vegetative growth and *SP* is percent sporulation compared to control. The *T* values for classification of the effect of chemical products on the fungi are as: 0 to 30 (highly toxic), 31 to 45 (toxic), 46 to 60 (moderately toxic) and >60 (compatible).

RESULTS

Compatibility of *Beauveria* isolates with commercial pesticides

Germination rates of *B.bassiana* isolates observed at 24 hours post inoculation containing pesticides amended in the medium at the three concentrations 0.1X, 0.5X and 1X varied from 20 – 90%. A dose dependant decrease with increasing concentration of the pesticides was observed for all the pesticides in the study. Isolate B55 showed only 7.5% reduction at 1x concentration with imidacloprid whereas at 0.5x and 0.1x concentrations there was no reduction in conidial germination (T-2a).

The results of the present study revealed that all the four pesticides tested have different compatible levels with all the isolates at different concentrations. Among the pesticides tested, Chlorpyrifos was proved to be highly detrimental to all the isolates at 1x and 0.5x concentrations. Similarly, the other pesticides monocrotophos and quinalphos were also categorized as either highly toxic or moderately toxic to all the isolates at all concentrations but Conversely, B55 displayed its compatibility with all pesticides except Chlorpyrifos where it showed moderate toxicity. Moreover, B55 was highly compatible with imidacloprid even at higher concentrations showing least inhibition of sporulation less than 3% at lower concentration. quinalphos has drastically inhibited sporulation of all the isolates in spite of showing vegetative growth and the percent reduction of sporulation varied among isolates but has followed a similar decreasing tendency on the concentration of the pesticide amended in the medium (T-2b,c).

Compatibility of *Beauveria* isolates with commercial fungicides

All the fungal isolates showed differential sensitivity to the fungicides tested at the different concentrations used. The conidial germination of all the isolates was completely inhibited at 1x concentration of the bavistin amended in the SDA medium where as the germination rates were drastically reduced at 0.5x and 0.1x concentration ranging from 86% to 24%. Isolate B57 showed complete inhibition of germination with all concentrations of mancozeb and bavistin tested. Isolate B56 displayed 40% inhibition at higher concentration, but only 9% has been recorded at lower concentration of copper oxychloride (T-3a). Sulphur showed no inhibition at 0.1x concentration. Copper oxy chloride was compatible to all the isolates at lower concentration but showed toxicity at higher levels, nevertheless sulphur displayed high compatibility to all isolates at higher concentration except B57 which is moderately toxic even at low concentrations.

Table 1: Pesticides, fungicides and botanicals with their active ingredients and chemical group.

	Active ingredient (Commercial name)	IUPAC name	Chemical group (Formula)	RD* (per litre)
Pesticides	Chlorpyrifos (Hilban ®)	<i>O,O</i> -diethyl <i>O</i> -3,5,6-trichloropyridin-2-yl phosphorothioate	Organophosphate (C ₉ H ₁₁ C ₁₃ NO ₃ PS)	2 ml
	Imidacloprid (Media®)	<i>N</i> -[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide	Chloronicotine (C ₉ H ₁₀ ClN ₅ O ₂)	2 ml
	Monocrotophos (Monodhan 36)	Dimethyl (<i>E</i>)-1-methyl-2-(methylcarbamoyl)vinyl phosphate	Etylene (C ₇ H ₁₄ N ₃ O ₂)	1.5 ml
	Quinalphos (Ekalux ®)	<i>O,O</i> -Diethyl <i>O</i> -2-quinoxalanyl phosphorothioate	Bisdithiocarbamate (C ₁₂ H ₁₅ N ₂ O ₃ PS)	3 ml
Fungicides	Mancozeb (Indofil® M- 45)	-	Carbon disulfide	3 gm
	Sulphur (Sulfex®)	-	Sulphur compounds	3 gm
	Copper oxychloride (Cuprocarb 500)	-	Copper compounds	2 gm
	Carbendazim (Bavistin)	Methyl <i>1H</i> -benzimidazol-2-ylcarbamate	Benzimidazole carbamate (C ₉ H ₉ N ₃ O ₂)	3 gm
Botanicals	- (Herbastim)	-	-	2 ml
	- (Exodos)	-	-	3 ml
	- (Biospark)	-	-	3 gm
	- (Neem gold)	-	-	2 ml

*Recommended dose

Table 2: Effect of pesticides in three different concentrations on germination (a), vegetative growth (b) and spore output (c) of the entomopathogenic fungal isolates of *B. bassiana* compared to control in studies conducted on compatibility.

Isolate	Conc (X)	%germination in Control	%germination* with Pesticides			
			Chlorpyrifos	Imidacloprid	Monocrotophos	Quinolphos
B44	0.1	84.2 ± 0.95	80.00 ± 0.00	75.00 ± 0.56	72.00 ± 0.45	54.00 ± 0.00
	0.5		73.54 ± 0.40	69.45 ± 1.10	66.50 ± 0.90	48.90 ± 0.23
	1		68.20 ± 0.25	60.20 ± 0.25	58.20 ± 0.18	40.45 ± 0.80
B55	0.1	100 ± 0.00	63.42 ± 0.12	100 ± 0.00	85.20 ± 0.32	78.90 ± 1.10
	0.5		54.40 ± 1.20	100 ± 0.00	80.00 ± 0.10	70.00 ± 1.00
	1		48.90 ± 0.84	92.45 ± 0.25	49.43 ± 0.45	48.44 ± 1.20
B56	0.1	100 ± 0.00	68.20 ± 0.45	85.48 ± 0.90	88.60 ± 0.82	63.50 ± 0.68
	0.5		62.45 ± 1.20	76.66 ± 0.39	65.40 ± 1.10	60.00 ± 0.50
	1		54.25 ± 0.90	63.42 ± 0.36	55.45 ± 0.88	45.50 ± 0.38
B57	0.1	89.2 ± 0.40	80.00 ± 0.00	80.00 ± 0.00	85.40 ± 0.29	62.45 ± 0.45
	0.5		74.56 ± 0.72	78.45 ± 0.10	78.40 ± 1.10	48.90 ± 1.23
	1		70.34 ± 0.50	64.20 ± 0.62	50.42 ± 1.00	10.00 ± 0.00

*%germination at 24th hour post inoculation

(a)

Isolate	Conc (X)	Vegetative growth* with pesticides			
		Chloropyrifos	Imidacloprid	Monocrotophos	Quinolphos
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	13.77 ± 0.02	9.09 ± 0.05	16.52 ± 0.05	25.61 ± 0.01
	0.5	23.96 ± 0.06	8.26 ± 0.07	14.6 ± 0.04	26.72 ± 0.02
	1	58.67 ± 0.02	11.01 ± 0.04	23.96 ± 0.01	43.25 ± 0.00
B55	0.1	28.93 ± 0.03	2.25 ± 0.01	16.85 ± 0.03	14.88 ± 0.03
	0.5	30.89 ± 0.08	3.26 ± 8.42	17.69 ± 0.08	18.53 ± 0.05
	1	33.7 ± 0.02	3.2 ± 0.06	47.75 ± 0.07	30.89 ± 0.06
B56	0.1	28.12 ± 0.08	9.89 ± 0.04	15.88 ± 0.08	14.06 ± 0.01
	0.5	28.9 ± 0.01	16.66 ± 0.01	32.29 ± 0.01	33.33 ± 0.01
	1	49.73 ± 0.08	28.12 ± 0.08	45.31 ± 0.02	43.75 ± 0.02
B57	0.1	6.9 ± 0.01	9.00 ± 0.03	3.00 ± 0.03	23.12 ± 0.02
	0.5	32.13 ± 0.05	17.12 ± 27.02	15.91 ± 0.05	44.14 ± 0.01
	1	57.05 ± 0.03	27.02 ± 0.07	45.04 ± 0.01	84.13 ± 0.03

*vegetative growth in centimeters (cm)

(b)

Isolate	Conc (X)	Spore output with pesticides			
		Chloropyrifos	Imidacloprid	Monocrotophos	Quinolphos
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	82.94 ± 0.14	24.35 ± 1.16	24.6 ± 1.00	43.92 ± 2.14
	0.5	87.24 ± 1.1	52.49 ± 1.13	58.01 ± 3.03	56.86 ± 1.10
	1	92.83 ± 0.09	72.65 ± 1.08	78.8 ± 0.18	66.26 ± 1.23
B55	0.1	56.86 ± 2.58	2.82 ± 2.43	13.68 ± 6.42	28.24 ± 7.13
	0.5	66.13 ± 1.2	5.77 ± 1.18	16.67 ± 2.14	35.21 ± 3.82
	1	80.06 ± 1.36	7.33 ± 1.12	23.59 ± 1.34	41.87 ± 1.23
B56	0.1	35.62 ± 1.35	15.13 ± 3.12	16.35 ± 1.21	77.91 ± 0.62
	0.5	63.22 ± 0.46	35.90 ± 2.06	40.80 ± 4.10	86.54 ± 0.04
	1	72.64 ± 0.18	49.52 ± 4.01	59.87 ± 0.16	87.33 ± 0.04
B57	0.1	73.29 ± 0.05	13.01 ± 2.20	70.94 ± 3.09	67.38 ± 1.86
	0.5	91.77 ± 0.12	28.22 ± 3.09	82.51 ± 1.11	79.28 ± 3.68
	1	98.85 ± 0.01	37.17 ± 2.14	94.14 ± 0.10	100

(c)

Table 3: Effect of fungicides in three different concentrations on germination (a), vegetative growth (b) and spore output (c) of the entomopathogenic fungal isolates of *B. bassiana* compared to control in studies conducted on compatibility.

Isolate	Conc (X)	% germination in Control	%germination* with fungicides			
			Copper oxychloride	Sulphur	Bavistin	Mancozeb
B44	0.1	84.2 ± 0.95	70.00 ± 0.00	75.45 ± 0.08	64.4 ± 1.10	38.00 ± 0.00
	0.5		62.45 ± 0.66	68.20 ± 0.45	42.40 ± 0.95	20.00 ± 0.00
	1		38.45 ± 0.90	42.10 ± 0.18	-	-
B55	0.1	100 ± 0.00	78.40 ± 0.20	82.34 ± 0.20	58.90 ± 0.02	40.00 ± 0.00
	0.5		70.22 ± 1.10	80.00 ± 0.12	40.00 ± 0.00	16.60 ± 0.96
	1		60.00 ± 0.00	76.40 ± 0.38	20.00 ± 0.00	-
B56	0.1	100 ± 0.00	90.45 ± 0.20	84.56 ± 0.90	42.44 ± 1.00	45.20 ± 0.82
	0.5		73.90 ± 0.08	79.48 ± 1.28	26.45 ± 2.00	14.48 ± 0.40
	1		60.20 ± 0.54	64.49 ± 0.18	-	-
B57	0.1	89.2 ± 0.40	68.90 ± 0.38	74.50 ± 0.68	61.39 ± 1.40	-
	0.5		60.66 ± 0.14	61.36 ± 0.86	53.82 ± 0.68	-
	1		52.60 ± 0.45	60.00 ± 0.00	-	-

*%germination at 24th hour post inoculation (a)

Isolate	Conc (X)	Vegetative growth* with fungicides			
		Copper oxychloride	Sulphur	Bavistin	Mancozeb
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	13.49 ± 0.02	19.56 ± 0.01	29.48 ± 0.01	44.91 ± 0.00
	0.5	22.86 ± 0.01	28.93 ± 0.04	47.94 ± 0.00	59.23 ± 0.00
	1	27.00 ± 0.90	42.15 ± 0.00	100	100
B55	0.1	18.82 ± 0.00	10.20 ± 0.05	34.84 ± 0.01	57.31 ± 0.01
	0.5	29.77 ± 0.04	17.61 ± 0.01	46.92 ± 0.02	78.66 ± 0.02
	1	34.83 ± 0.10	19.67 ± 0.07	65.17 ± 0.00	100
B56	0.1	7.29 ± 0.00	21.88 ± 0.01	50.79 ± 0.01	48.96 ± 0.01
	0.5	14.84 ± 0.05	32.82 ± 0.01	67.71 ± 0.00	77.87 ± 0.01
	1	24.47 ± 0.09	39.59 ± 0.01	100	100
B57	0.1	13.21 ± 0.03	22.53 ± 0.01	25.55 ± 0.02	100
	0.5	23.12 ± 0.03	29.13 ± 0.04	42.95 ± 0.00	100
	1	27.32 ± 0.01	39.94 ± 0.00	100	100

*vegetative growth in centimeters (cm) (b)

Isolate	Conc (X)	Spore output with fungicides			
		Copper oxychloride	Sulphur	Bavistin	Mancozeb
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	21.88 ± 2.48	14.07 ± 1.29	77.00 ± 0.4	100
	0.5	44.15 ± 1.87	32.63 ± 1.82	92.80 ± 0.08	100
	1	57.82 ± 0.90	45.32 ± 2.00	100	100
B55	0.1	11.91 ± 4.32	10.80 ± 2.31	57.02 ± 0.9	79.86 ± 0.32
	0.5	34.52 ± 2.86	25.00 ± 6.54	65.57 ± 1.72	92.37 ± 1.45
	1	65.21 ± 1.89	29.24 ± 2.85	100	100
B56	0.1	20.00 ± 3.62	28.05 ± 4.12	54.27 ± 0.70	69.77 ± 0.64
	0.5	31.46 ± 1.48	36.50 ± 2.42	78.27 ± 0.4	87.45 ± 0.20
	1	50.68 ± 1.00	51.13 ± 2.00	100	100
B57	0.1	33.97 ± 4.12	53.01 ± 1.18	80.18 ± 1.45	100
	0.5	41.45 ± 1.48	62.29 ± 3.00	93.47 ± 0.63	100
	1	49.41 ± 1.10	73.91 ± 1.48	100	100

(c)

The spore output was highly inhibited in all the isolates regardless of the concentration used, conversely B55 displayed less than 5% and 11% reduction respectively at lower concentrations. As far as vegetative growth is concerned except isolate B55 all others showed cent percent inhibition at 1x concentration and the percent reduction ranged from 30% to 74% at other concentrations with bavistin. The mycelia growth was highly reduced even at lower concentrations (more than 43%) for all isolates. The fungicides bavistin and mancozeb were highly deleterious totally inhibiting the reproductive growth of the fungal isolates irrespective of the concentration of the active ingredient present in the culture medium. Fungicides which retarded fungal germination resulted in relatively slower growth i.e., mycelia mat was observed 4-6 days post inoculation, whereas in plates where fungicides had no significant effect on germination, plates were fully covered with mycelia mat within 2 days post inoculation (T-3b,c).

Compatibility of *Beauveria* isolates with commercial botanicals

Exodon and biospark permitted the conidial germination of all the fungal isolates tested in the study at all the concentrations with percent inhibition ranging from 52.5% to 4.4%. The germinating efficiency of the *beauveria* isolates used in the present study was not negatively affected by neemgold. Isolate B57 displayed 0.2% reduction at low concentration (T-4a). Herbastimm was found to be incompatible to all the fungal isolates at 1x concentration where as at lower concentrations they displayed compatibility. However, exodon and biospark were compatible to all the isolates at lower concentration whereas isolate B44 showed toxicity at higher concentration. Moreover, neemgold was found to be highly compatible to all the isolates at all the concentrations. All other isolates were also found to be less affected showing a percent reduction less than 26% even at high concentrations. Neemgold displayed least effect on sporulation when compared to other botanicals used in the present study (T-4b,c). Table 5 indicates the T-values that represent the toxicity levels of the fungal isolates with the pesticides, fungicides and botanicals.

Table 4: Effect of botanicals in three different concentrations on germination (a), vegetative growth (b) and spore output (c) of the entomopathogenic fungal isolates of *B. bassiana* compared to control in studies conducted on compatibility.

Isolate	Conc (X)	%germination in Control	%germination* with botanicals			
			Herbastim	Exodon	Biospark	Neemgold
B44	0.1	84.2 ± 0.95	64.4 ± 0.85	70.50 ± 0.45	80.45 ± 0.45	79.20 ± 0.78
	0.5		52.00 ± 0.00	64.40 ± 0.22	72.64 ± 0.62	72.45 ± 0.80
	1		46.55 ± 0.87	58.90 ± 0.90	62.00 ± 0.00	66.20 ± 0.14
B55	0.1	100 ± 0.00	86.90 ± 1.00	89.39 ± 0.36	86.32 ± 0.98	90.20 ± 0.20
	0.5		80.45 ± 0.38	80.00 ± 0.00	82.40 ± 0.62	84.32 ± 0.33
	1		76.49 ± 0.77	75.66 ± 0.89	78.45 ± 0.20	80.45 ± 0.22
B56	0.1	100 ± 0.00	90.20 ± 1.22	90.10 ± 0.75	84.56 ± 0.45	80.66 ± 0.11
	0.5		82.36 ± 0.89	82.44 ± 0.80	72.85 ± 0.98	80.00 ± 0.00
	1		76.80 ± 0.35	70.45 ± 1.10	66.66 ± 0.00	78.66 ± 0.10
B57	0.1	89.2 ± 0.40	76.66 ± 0.62	52.40 ± 0.50	70.00 ± 0.00	89.00 ± 0.55
	0.5		70.00 ± 0.00	40.62 ± 0.66	58.45 ± 1.00	72.45 ± 0.42
	1		58.45 ± 0.84	32.83 ± 0.98	42.42 ± 0.40	65.00 ± 0.22

*%germination at 24th hour post inoculation (a)

Isolate	Conc (X)	Vegetative growth* with botanicals			
		Herbastim	Exodon	Biospark	Neemgold
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	11.3 ± 0.04	17.36 ± 0.00	4.14 ± 0.04	4.96 ± 0.03
	0.5	14.61 ± 0.01	24.25 ± 0.05	11.30 ± 0.00	11.85 ± 0.01
	1	20.39 ± 0.00	31.13 ± 0.01	20.39 ± 0.01	17.36 ± 0.00
B55	0.1	11.80 ± 0.10	12.36 ± 0.01	8.99 ± 0.00	4.50 ± 0.00
	0.5	19.67 ± 0.04	21.92 ± 0.02	12.93 ± 0.02	7.89 ± 0.03
	1	29.22 ± 0.01	28.66 ± 0.01	18.54 ± 0.01	12.65 ± 0.01
B56	0.1	10.15 ± 0.02	6.25 ± 0.00	9.35 ± 0.03	15.11 ± 0.05
	0.5	21.88 ± 0.00	14.36 ± 0.23	11.72 ± 0.02	19.55 ± 0.02
	1	24.74 ± 0.03	21.88 ± 0.00	21.88 ± 0.00	25.79 ± 0.04
B57	0.1	17.12 ± 0.02	24.33 ± 0.01	12.92 ± 0.04	6.91 ± 0.00
	0.5	24.33 ± 0.03	32.14 ± 0.01	20.73 ± 0.02	14.42 ± 0.01
	1	57.06 ± 0.02	45.95 ± 0.00	33.94 ± 0.01	22.53 ± 0.02

*vegetative growth in centimeters (cm) (b)

Isolate	Conc (X)	Spore output with botanicals			
		Herbastim	Exodon	Biospark	Neemgold
		% increase/decrease	% increase/decrease	% increase/decrease	% increase/decrease
B44	0.1	17.8 ± 1.36	29.52 ± 1.43	17.80 ± 2.00	9.25 ± 3.00
	0.5	27.20 ± 1.48	5.40 ± 3.00	38.33 ± 1.46	17.80 ± 3.60
	1	67.19 ± 0.96	66.29 ± 2.45	57.64 ± 3.60	30.00 ± 1.45
B55	0.1	55.21 ± 4.42	28.61 ± 4.65	10.17 ± 3.48	7.16 ± 3.00
	0.5	63.22 ± 2.12	37.95 ± 2.86	17.70 ± 2.00	12.69 ± 1.98
	1	66.07 ± 1.48	40.23 ± 1.45	25.43 ± 1.50	20.36 ± 2.80
B56	0.1	9.96 ± 3.82	11.01 ± 2.00	12.02 ± 1.45	5.63 ± 1.00
	0.5	29.86 ± 2.86	28.17 ± 2.36	26.71 ± 3.20	17.63 ± 1.80
	1	44.20 ± 4.00	42.43 ± 1.80	52.71 ± 0.90	24.67 ± 2.00
B57	0.1	42.33 ± 3.86	23.07 ± 1.90	18.08 ± 3.85	14.95 ± 2.45
	0.5	70.36 ± 3.58	34.37 ± 2.00	29.83 ± 2.40	23.50 ± 3.96
	1	79.68 ± 2.00	37.04 ± 1.45	37.21 ± 1.40	32.66 ± 2.48

(c)

Table 5: The 'toxicity levels' for classification of the effect of pesticides, fungicides and botanicals on the entomopathogenic fungal isolates of *B. bassiana*.

		Isolate	Concentration		
			0.1X	0.5X	1X
PESTICIDES	Chlorpyrifos	B44	HT	HT	HT
		B55	MT	T	HT
		B56	C	T	HT
		B57	T	HT	HT
	Imidacloprid	B44	C	MT	T
		B55	C	C	C
		B56	C	C	MT
		B57	C	C	C
	Monocrotophos	B44	C	MT	T
		B55	C	C	C
		B56	C	C	T
		B57	T	T	HT
	Quinolphos	B44	MT	MT	T
		B55	C	C	C
		B56	T	HT	HT
		B57	T	HT	HT
FUNGICIDES	Copper Oxychloride	B44	C	C	T
		B55	C	C	T
		B56	C	C	MT
		B57	C	C	MT
	Sulphur	B44	C	C	MT
		B55	C	C	C
		B56	C	C	MT
		B57	MT	T	T
	Bavistin	B44	T	HT	HT
		B55	MT	T	HT
		B56	MT	T	HT
		B57	HT	HT	HT
	Mancozeb	B44	HT	HT	HT
		B55	HT	HT	HT
		B56	T	HT	HT
		B57	HT	HT	HT
BOTANICALS	Herbastim	B44	C	C	MT
		B55	MT	T	T
		B56	C	C	MT
		B57	C	T	HT
	Exodon	B44	C	MT	T
		B55	C	C	C
		B56	C	C	C
		B57	C	C	C
	Biospark	B44	C	C	MT
		B55	C	C	C
		B56	C	C	MT
		B57	C	C	C
	Neemgold	B44	C	C	C
		B55	C	C	C
		B56	C	C	C
		B57	C	C	C

Compatible; T: Toxic; MT: Moderately toxic; HT: Highly toxic

DISCUSSION

Insecticides have potential to affect the various developmental stages of entomopathogenic fungi. In the present study all tested insecticides displayed varying degree of potential to inhibit growth and conidial germination. Fungal germination is an important factor of pesticides compatibility evaluation with entomopathogenic fungi considering the pest management, because the beginning of epizootics is conditioned by the capacity of conidia to germinate on the host [2, 9]. The entomopathogenic fungus success, however, depends on conidial viability [16]. Our research showed that, in general the pesticides tested (except imidacloprid) significantly affected *B. bassiana* germination, vegetative growth and sporulation *in vitro* at higher concentration. Isolate B55 was compatible with imidacloprid even at higher concentrations. According to James and Elzen [26], imidacloprid had no negative effect on *B. bassiana*. Synergistic interaction of imidacloprid with fungal agents in insect control have been reported previously [28, 42, 30, 43, 19, 50] classified imidacloprid, deltamethrin and trichlorfon as compatible with *B. bassiana*. In earlier reports, chlorpyrifos and monocrotophos were found to be slightly harmful to *B. bassiana* at normal field dose [7]. Chlorpyrifos had been reported to strongly inhibit the growth and sporulation of *B. bassiana* [11] in a dose-dependent manner even at concentrations lower than recommended rates of field use [44, 35] reported that chlorpyrifos and endosulfan strongly inhibit the growth of *B. bassiana*. Oliveira et al, [41] reported triazophos, chlorpyrifos and endosulfan formulations inhibited 100% of the germination of *B. bassiana*. These reports support our present study in which chlorpyrifos and monocrotophos at higher concentrations inhibited the growth of all fungal isolates. Quinolphos was found to be toxic to all the fungal isolates tested. Among the fungicides tested, sulphur was found to be compatible with isolate B55 at all concentrations. Tamai et al, [48] classified sulphur as compatible and tebuconazol, mancozeb and copper oxychloride as highly toxic to this mycopathogen. Mani et al, [36] found that exposure to copper oxychloride reduced the longevity and fecundity of the citrus mealybug parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae). A glasshouse pot trial by McLean et al. [37] confirmed that *Trichoderma harzianum*, an effective biocontrol agent of the onion white rot pathogen *Sclerotium cepivorum*, was sensitive to mancozeb. Durán et al, [17] mentioned that benomyl, dimethomorph-mancozeb, chlorothalonil, propineb, mancozeb, and mancozeb-cymoxanil mixture of fungicides significantly affect germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride do not. Kouassi et al, [29] found that simultaneous application of the fungicides metalaxyl, mancozeb and copper oxide with *B. bassiana* reduced insect infection, suggesting that the fungicides inhibited germination on the cuticle. Background information about the different degrees of entomopathogenic fungi showing fungicide tolerance was reported by Maribel et al, [38]. Alam et al. [1] reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found Bavistin, Dithane M-45 tilt to be the most effective fungicides. With respect to botanicals, neem gold, biospark and exodon showed compatibility to all the isolates in the study and neemgold displayed synergism with B55 which was manifested by enhanced vegetative growth of the isolate when grown in combination. Sahayaraj et al., [47] also observed that the commercial plant based pesticides were well tolerated by *B. bassiana*. Neemgold and biospark were relatively safe for combined use. Jayaraj [27] hinted the possibility of combining botanicals with microbial for enhanced efficacy against insect pests. The compatibility of isolates of *B. bassiana* with azadirachtin formulations has been investigated previously [45, 12, 20, 18]. However, a few isolates were tested and contradictory results have been reported. For example, neem oil was found compatible with *B. bassiana* by Rodríguez et al. [45] but was reported to be inhibitory by Bajan et al, [12] and Depieri et al, [18]. The observed difference could be due to inherent variability of chemicals to biological creatures. Mohan et al, [39] studied the compatibility of AZA and neem oil extract (0.15 % AZA) with 30 different isolates of *B. bassiana*. Of those studied 23 combinations were found to be compatible. *B. bassiana* activity was enhanced by agroneem (a commercial neem insecticide) as observed by Al-Mazraaw et al., [3]. The germination percentage of *B. bassiana* is slightly affected (reduction percentage is not more than 12%) by various neem concentrations [25]. It is evident that the action of all insecticides was mainly dependent on the chemical nature of the compounds as well as concentrations used and different fungal isolates utilized in the experiment.

CONCLUSION

B55 isolate proved to be more promising for development as mycopesticides and for application along with the pesticides like imidacloprid and fungicide, sulphur respectively in the IPM programme. However, field evaluation of the interactions between *B. bassiana* and these pesticides should be under taken to evaluate their effect on pest and beneficial insects.

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