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Compatibility of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin isolated from Pulney hills, Western Ghats of Tamil Nadu with insecticides and fungicides

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Introduction

Coffee berry borer, Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae) is serious pest of coffee. The pest native of South Africa, is now available in all coffee growing countries (Le Pelley, 1968; Dufour et al., 1999; Soto-Pinto et al., 2002) including India (Kumar et al., 1990). Initially the pest was effectively controlled by the application of endosulfan (Decazy, 1985), but it was found to be harmful to humankind and environment. Repeated application of synthetic chemicals to control this pest also led to development of resistance to commercially available insecticides (Brun, 1989). Alternative to chemical insecticides, the entomopathogenic fungus Bassiana bassiana (Balsamo) Vuillemin was found to be promising biological control agent against several insect pest populations (De La Rosa et al., 1997; Bustillo, 2005). Many authers have reported effective control of the coffee berry borer in coffee plantations using B. bassiana (Varela and Morales, 1996; Samuels et al., 2002; Posada and Vega, 2005; Monzón et al., 2008). It is also noted that the success of using *B. bassiana* in integrated pest control strategies, depends on its conidial survival in environment (Benz, 1987). The conidial survival can be affected by the environmental factors (Furlong and Pell, 1997) or application of bio pesticides/chemicals which are commonly used to control the pest (Anderson and Roberts, 1983; Loria et al., 1983; Alves and Lecuona, 1998). The conservation of entomopathogens is necessary, whether they are present naturally or applied to control the insects. Hence, studies on compatibility of insecticides with entomopathogenic fungus will be useful for farmers at the time of application to select

ABSTRACT

The entomopathogenic fungus *Beauveria bassiana* is a promising biocontrol agent against coffee berry borer, *Hypothenemus hampei* in coffee. The fungus conidial survival was influenced by both environment and agrochemicals normally used to protect crop plants. The present study evaluates the fungitoxic effect of commonly used chemical insecticides (endosulfan, chlorpyriphos, dimethoate and quinalphos) and fungicides (bordeaux, hexaconazole and triadimefon) on germination, vegetative growth and sporulation of *B. bassiana*. The insecticides and fungicides were tested at three concentrations (Field Recommendation (FR), half FR, and twice FR). All the tested concentrations inhibited the germination (9.0-81.19% and 19.3-100%), vegetative growth (0.5-62.9% and 37.1-100%) and sporulation (7.0-99.9% and 99-100%) of *B. bassiana* by the insecticides and fungicides respectively, but dimethoate exhibited minimum inhibitory effect. Dimethoate showed better compatibility to *B. bassiana* in all the three concentrations. As dimethoate is safer to biological control agent, could be used as an integrated pest management in coffee.

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suitable insecticides, chemicals and entomopathogens in integrated pest management (Butt et al., 2001; Inglis et al., 2001; Lacey et al., 2001). This alternative strategy, lead to decrease in the use of non-compatible insecticides to control pests and also increase the control efficacy of fungus and minimize the environmental contamination (Rosin et al., 1996; Moino and Alves, 1998; Quintela and McCoy, 1998; Goettel et al., 2000). The objective of the present study is to assess the compatibility of commonly used insecticides and fungicides in coffee plantation on *B. bassiana* especially in conidial germination, vegetative growth and spore production.

Material and Methods

Isolation and inoculum preparation of Beauveria bassiana

The fungus *Beauveria bassiana* (BbNK007) was isolated from infested coffee berry borer from Pulney Hills, Tamil Nadu, India and the spores were stored on anhydrous sterile silica gel crystals at -20°C (Windels et al., 1993). The isolate BbNK007 showed highest virulence against *H. hampei* among 16 isolate screened at *in vitro* condition (Authors unpublished data). The spores were retrieved on Sabourad Dextrose Agar supplemented with 0.2% yeast extract medium (SDYA) (Varela and Morales, 1996) and incubated at $25\pm2^{\circ}$ C until dense sporulation was observed. The spores were harvested using a sterile spatula into sterile water containing 0.02% Tween 20. The conidial concentration in the suspension was calculated using Neubauer Chamber under the microscope. The suspensions were diluted until the desired concentration.

Insecticides and fungicides

The insecticides and fungicides used in this study were commonly used in coffee plantation in Pulney Hills to control

insect pests and diseases (Table 1). The concentrations used were the field recommendation (FR) dose (1X - 200 l/ha), half the concentration (0.5X - FR-50%) and double the concentration (2X - $2 \times$ FR).

Germination assessment

The insecticides with pre-established concentrations were mixed in sterile water containing Tween 20 (0.02%) and *B. bassiana* spores which were kept aside for one hour. Later 0.5 ml of aliquot from each suspension was spread on petri dishes containing sterile water agar medium. The dishes were incubated at $25\pm2^{\circ}$ C with 12:12 photo period for 24 hours. The germinated spores were quantified by counting 100 spores in three different random zones on the petri plates. Five replicates per concentration were maintained and the whole experiments were repeated three times a week.

Vegetative growth and conidia production assessment

The sterile SDYA medium was cooled to 40±5°C and preestablished concentrations of the insecticides were added with the antibiotic streptomycin (0.5/l). Approximately 20 ml of each concentration was poured into 9 cm petridish. Medium without insecticide but, containing streptomycin was used as control. After solidification the medium was inoculated with *B. bassiana* in three points per dish (Alves et al., 1998a). The dishes were incubated at 25±2°C and 12:12 photo period for 8 days (five dishes/concentration). After 8 days, the formulation effects were determined by measuring the radial growth of the colony using a Vernier caliper. Ten central colony discs (6mm) were randomly selected for conidial production assessment. Each disc was placed in a screw cap tube containing 10 ml of sterile water with (0.02%) Tween 20. The tubes were agitated until the conidia was completely released and the condial concentration was counted using Neubauer chamber. A Completely Randomized Design (CRD) was used in all experiments.

Compatibility study

Toxicity of chemicals against entomopathogenic fungi was calculated using the formula of Alves et al. (1998b);

т	20 (VG)	+	80 (ESP)
1	100		

In this formula, values for vegetative growth (VG) and sporulation (ESP) were given in relation to control (100%). Where T = 0 to 30 (very toxic); 31 to 45 (toxic); 46 to 60 (moderately toxic); >60 (compatible).

Statistical analysis

Data were analyzed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range tests ($P \le 0.05$) using SPSS v.16.

Results

Conidial germination

A significant reduction in conidial germination of *B.* bassiana was observed in all concentrations of tested insecticides and their effects were concentration dependent (Table 2). The maximum inhibition of germination was observed in treatment of endosulfan (81.1%) followed by quinalphos (66%) and chlorpyriphos (54.2%) at 2×FR concentration, whereas dimethoate had little effect on conidial germination with reduction of 9.0% at lowest concentration ($0.5 \times FR$). The fungicides *viz.* hexaconazole, bordeaux and triadimefon adversely affected the growth of the conidia. The hexaconazole inhibited 100% spore germination at all the concentrations. Least inhibition of conidial germination (19.3%) was noticed in triadimefon at lowest concentration ($0.5 \times FR$).

Vegetative growth

The insecticides endosulfan, quinalphos and chlorpyriphos inhibited the fungus vegetative growth in all the treated concentrations (Table 2). The quinalphos exhibited maximum inhibition (62.9%) on vegetative growth of *B. bassaina* at 2xFR concentration, followed by chlorpyriphos (59.8%) and endosulfan (57.2%), while dimethoate showed the least reduction on vegetative growth (-1, 0.5 and 12.4%) with increasing concentrations over control. The fungicide, bordeaux showed 100% inhibition at all the tested concentrations on vegetative growth of the fungus. Hexaconazole exhibited more than 80% inhibition in vegetative growth of the fungus in all the concentrations, whereas triadimefon showed less than 40% reduction in vegetative growth of B. bassiana at various test concentrations. Bordeaux and triadimefan did not show significant difference within concentrations (Table 3). Spore production

In the present study dimethoate showed minimum reduction (7, 34.1 and 46%) in spore production at half FR, FR and $2\times$ FR concentrations, respectively. Other insecticides showed 90 to 99.9% reduction in spore production of *B. bassiana*. Fungicidal bordeaux and hexaconazole showed 100% inhibition in spore production of *B. bassiana*. Triadimefon showed 99-99.8% reduction in spore production.

Compatibility study

Insecticides (endosulfan, quinalphos, and chlorpyriphos) and fungicides (bordeaux, hexaconazole, and triadimefon) were more toxic to *B. bassiana*. It was denoted by the average 'T' (toxic) value of 14.54, 12.90, 75.97, 11.96, 1.78 and 12.95 for endosulfan, quinalphos, chlorpyriphos, bordeaux, hexaconazole and triadimefon respectively (Table 4). However, dimethoate showed excellent compatibility with the entomopathogen with a "T" value of 75.97.

Discussion

The chemical pesticide, dimethoate in the present study showed less than 10% inhibition in spore germination, vegetative growth and spore production compared to other insecticides and fungicides at half FR concentration. These results are in agreement with the findings of Alizadeh et al. (2007) where it was reported that imidacloprid exhibited less than 10% inhibition of spore germination, vegetative growth and spore production at 0.5×FR concentration, when compared to other insecticides. It was also revealed in the report that endosulfan recorded 90% inhibition in germination, 89% inhibition in vegetative growth and 87% reduction in spore production. Similarly, in the present study endosulfan inhibited the germination by 81.19%, reduction in vegetative growth by 57.2% and spore production by 99.9%. The percentage inhibition by various insecticides and fungicides on germination, vegetative growth and spore production of *B. bassiana* in the study were significantly varied. Similar such findings were observed by Li and Holdom (1994) on the effect of insecticides and fungicides on vegetative growth and sporulation of Metarhizium anisopliae. Vanninen and Hokkanen (1988) and Anderson et al. (1989) have also reported the fungitoxic effect of insecticides on germination and vegetative growth of various strains of entomopathogenic fungi.

The variation in inhibitory effects of insecticides due to their types or classes or nature of chemicals and characteristic interaction with microbial species were observed by several workers (Inglis et al., 2001; Antonio et al., 2001; Kumar et al., 2008). At 2×FR concentration, fungal spore germination was reduced in the treatment of endosulfan and least in the treatment of dimethoate (46.9%). Significant reduction in vegetative growth (62.9%) was observed in quinalphos treatment. Maximum reduction in spore production was noticed in chlorpyriphos (99.9%) and minimum in dimethoate (46%). Loureiro et al. (2002) noticed that the insecticides thiamethoxam, imidacloprid and cyromazine did not show any inhibitory effect on vegetative growth and sporulation of B. bassiana. Similarly, Oliveira et al. (2003) stated that thiamethoxam and cyfluthrin showed least inhibition of germination, vegetative growth and spore production of B. bassiana, whereas endosulfan, chlorpriphos and triazophos completely inhibited the germination, vegetative growth and spore production. On the contrary, dimethoate increased the vegetative growth (-0.1%) of B. bassiana at 0.5×FR concentration. Neves et al. (2001) were also reported that germination, vegetative growth and sporulation of fungi increased when treated with several insecticides. The possible reasons suggested for the difference in the response of fungi to synthetic insecticides were due to the fungal physiological mechanism to insecticides and their metabolism that liberated compounds used as secondary nutrients. The chemicals present in the insecticide formulations which could also be used directly as nutrients to increase the vegetative growth and spore production (Moino and Alves, 1998).

The fungicides, bordeaux and hexaconazole are highly toxic to *B. bassiana* that completely inhibited germination, vegetative growth and spore production. It was also found that triadimefon inhibited 99-99.8% spore production, while the vegetative growth inhibition was less than 40%. Similar results were found in the earlier reports of Rachappa et al. (2007) that fungicides hexaconazole, carbendazim, propiconazole and chlorothalonil were highly toxic (100%). Loureiro et al. (2002) revealed that the fungicides *viz.* thiophanate methyl cartap, tebuconazole, metalaxyl and mancozeb inhibited the germination, vegetative growth and sporulation of *B. bassiana*. It was reported that fungicides were found to be more toxic to entomopathogenic fungi (Tamai et al., 2002) and that was once again proved in the present investigation.

Conclusion

In the present study dimithoate confirmed 9 to 46% spore germination, -1 to 12.4% vegetative growth, 7 to 46% spore production of *B. bassiana* with a "T" value of 75.97 when compared to other insecticides and fungicides. Therefore formulations with dimethoate are more compatible with *B. bassiana* for the control of insect pests in coffee plantations. It is also recommended that appropriate time interval should be maintained during application of other insecticides or fungicides that are toxic to *B. bassiana* and so they did not influence the efficiency of the entomopathogenic fungus, *B. bassiana*.

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Table 1. Insecticides and fungicides used in the compatibility study with Beauveria bassiana

Trade Name	Active ingredient	Formulation	Recommended	Category
			Dose g or ml l ⁻¹	
Endocel	Entosulfan	EC 35	1.7	Insecticide
Classic 20	Chlorpyriphos	EC 20	3.0	Insecticide
Rogor	Dimethoate	EC 30	0.85	Insecticide
Ekalux	Quinalphos	EC 25	1.5	Insecticide
Bordeaux mixture	Copper sulphate and Calcium carbonate (1:1)	WP	5.0	Fungicide
Contaf	Hexaconazole	EC5	2.0	Fungicide
Bayleton	Triadimefon	WP 25	0.8	Fungicide

Table	2. Effect	of insecticides	on germination,	vegetative	growth	and sporulation	of entomopathogenic
			£	Dansa Daar			

			Tungus	s Deauveria da	ssiana		
Treatments	Concentration	Germination	% reduction	Colony	% reduction	Conidia	% reduction
		(%)	over control	Diameter (%)	over control	Number	over control
						$(X \times 10^7)$	
Entosulfan	$0.5 \times FR^{1}$	64.6±0.38 ^g	28.9	13.1±1.23 ^e	32.5	4.24±0.3 ^t	92.6
	FR	$41.1\pm0.61^{\circ}$	54.8	11.2 ± 0.39^{d}	42.3	2.59±0.93 ^e	95.5
	$2 \times FR$	17.2 ± 0.79^{a}	81.19	8.3±0.33 ^{ab}	57.2	0.35±0.96 ^{bc}	99.4
Chlorpyriphos	$0.5 \times FR$	67.7 ± 0.88^{g}	25.5	15.7 ± 0.32^{f}	19.1	0.98 ± 1.97^{d}	98.3
	FR	57.8±1.01 ^e	36.4	12.5±0.76 ^{de}	35.6	0.13 ± 0.33^{ab}	99.8
	$2 \times FR$	41.6±1.3°	54.2	7.8 ± 0.34^{a}	59.8	0.03 ± 0.29^{a}	99.9
Dimethoate	$0.5 \times FR$	82.7±0.73 ⁱ	9.0	19.6±0.6 ^g	-1.0	53.15 ± 0.62^{j}	7.0
	FR	67.3 ± 0.76^{h}	26.0	19.3±0.71 ^g	0.5	37.64 ± 1.78^{i}	34.1
	$2 \times FR$	48.3 ± 1.29^{d}	46.9	17.0 ± 1.55^{f}	12.4	30.84 ± 2.18^{h}	46.0
Quinalphos	$0.5 \times FR$	60.2 ± 0.85^{f}	33.8	11.0±0.71 ^{cd}	43.3	4.51±0.53 ^g	92.1
	FR	49.3±1.03 ^d	45.8	9.6 ± 0.52^{bc}	50.5	0.50±0.71 ^c	99.1
	$2 \times FR$	30.9±0.77 ^b	66.0	7.2 ± 0.48^{a}	62.9	0.13 ± 0.13^{ab}	99.8
Control		90.9 ± 0.87^{j}		$19.4{\pm}0.87^{g}$		57.14 ± 1.81^{k}	

Mean followed by same letter within the column are not significantly different (P≤0.05) by Tukey's test.

 1 FR = Field recommendation.

Table	3. Fungai	toxic effe	ct o	n germ	ination,	vegetative Beauveria	growth bassian	and a	sporula	tion	of	ento	mop	athogenic	fungus	
-	ã		a				a .					a				_

Treatments	Concentration	Germination (%)	% reduction over control	Colony Diameter (%)	% reduction over control	Conidia Number $(X \times 10^7)$	% reduction over control
	$0.5 \times FR$	16.0±0.57 ^c	82.4		100		
Bordeaux	FR	1.9±0.42 ^b	97.9		100		
	$2 \times FR$	0.3 ± 0.26^{a}	99.6		100		
	$0.5 \times FR$	0.0^{a}	100	3.6±0.97°	81.4		
Hexaconazole	FR	0.0^{a}	100	1.6±1.2 ^b	91.8		
	$2 \times FR$	0.0^{a}	100		100		
	$0.5 \times FR$	73.4 ± 1.28^{t}	19.3	12.1±0.38 ^d	37.6	0.56±1.63 ^b	99.0
Triadimefon	FR	38.3±0.25 ^e	57.9	11.7±0.75 ^d	39.7	0.56±1.04 ^b	99.0
	$2 \times FR$	24.8±0.5 ^d	72.8	12.2±0.78 ^d	37.1	0.12±0.13°	99.8
Control		90.9±0.87 ¹		19.4±0.87 ^g		57.14±1.81 ^k	

 $Mean followed by same letter within the column are not significantly different (P \! \leq \! 0.05) by Tukey's test.$

 1 FR = Field recommendation

Table 4. Toxic factor and compatibility classification of different insecticides and fungicides to their fungitoxic effect against B. bassiana

Destinidas	Concentrations	B. bassiana						
resticides	Concentiations	'T' value	Average of 'T' Value	Classification				
	0.5× FR≠	19.44		Τ*				
Entosulfan	FR	15.13	14.54	Т				
	$2 \times FR$	9.05		Т				
	$0.5 \times FR$	17.56		Т				
Chlorpyriphos	FR	13.07	12.90	Т				
	$2 \times FR$	8.08		Т				
	$0.5 \times FR$	94.62		C#				
Dimethoate	FR	72.60	75.97	С				
	$2 \times FR$	60.70		С				
	$0.5 \times FR$	17.65		Т				
Quinalphos	FR	10.61	11.96	Т				
-	$2 \times FR$	7.62		Т				
			Fungicides					
	$0.5 \times FR$	3.71		Т				
Hexaconazole	FR	1.65	1.78	Т				
	$2 \times FR$	0.0		Т				
	$0.5 \times FR$	13.26		Т				
Triadimefon	FR	12.85	12.95	Т				
	$2 \times FR$	12.75		Т				
m.t.m. :		1						

T * Toxic; C# compatible; $FR \neq$ Field recommended concentration