

Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin, an entomopathogenic fungi, with selective insecticides for enhanced pest management

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ABSTRACT: Six insecticides, viz. Imidacloprid 17.8 SL, Chlorpyrifos 20 EC, Quinalphos 25 EC, Dimethoate 30 EC, Dichlorvos 76 EC and Fenvalerate 20 EC, at three concentrations [recommended dose - RD, and 0.5 per cent each below RD (BRD) and above RD (ARD)] were used to treat on the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin. Impact on the conidial germination, vegetative growth, and spore production was studied by grading as harmless (<50%), slightly harmful (50-79%), moderately harmful (80-90%), and harmful (>90%). Among all these treatments, imidacloprid and fenvalerate were rated as harmless to *B. bassiana*, whereas chlorpyrifos, was slightly harmful in all three concentrations. In the case of treatment with dimethoate, the toxicity was slightly high in the RD and BRD but moderate at ARD. Quinalphos was moderately toxic in all three concentrations; however, dichlorvos was highly toxic, and there was no mycelial growth in all the three concentrations. Imidacloprid and fenvalerate were found highly compatible with *B. bassiana*. © 2025 Association for Advancement of Entomology

KEY WORDS: Toxicity, conidial germination, vegetative growth, spore production

INTRODUCTION

Synthetic pesticides are the most commonly used crop protection chemicals, particularly in conventional cultivation, but their injudicious use causes uncountable environmental and health hazards. About 99.9 per cent of the pesticide used reaches nontarget organisms and only 0.1 per cent reaches the target organism (Pimentel 1995; Carriger *et al.*, 2006). The biological and biochemical activity of soil microorganisms can influence the effectiveness of even reduced use of synthetic insecticides (Cycon *et al.*, 2006; Vig *et al.*, 2008; Cycon *et al.*, 2010). Entomopathogenic

fungi play an important role in integrated pest management strategies (Amutha *et al.*, 2010). Relying on entomopathogenic fungus as biocontrol agents facilitates the reduction of insecticidal usage, and thereby minimizes the environmental contamination and the incidence of pest resistance to insecticide (Ambethgar, 2009). *Beauveria bassiana*, which can easily be cultured on potato dextrose agar in the laboratory, is a potential biocontrol agent against certain insect pests (Wu *et al.*, 2014). Fungus infected insects develop hyphae that cause mortality of the pests by damaging their internal organs (Bidochka and Khachatourians 1994). Detailed compatibility studies

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are required for the integration of microbial pesticides with chemicals in pest management strategies. Many experiments were carried out, aiming to detect pesticide side effects on entomopathogenic fungi (Clark *et al.*, 1982; Gardner and Storey 1985; Neves *et al.*, 2001).

Despite their potential, microbial agents face limitations such as slow pest mortality, environmental susceptibility, and high production costs, which hinder their widespread adoption (Sharma *et al.* 2024). Combining entomopathogenic fungi with low concentrations of chemical insecticides have shown synergistic effects, improved pest control efficacy and reducing pesticide dependency (El-Ashry and Ramadan 2021; Robertson *et al.*, 2017; Thakur, 2022). This integrated approach offers a cost-effective and environmentally sustainable solution, addressing challenges in pest management while maintaining the essential role of synthetic insecticides (Singh, 2016). The objective of this investigation studied the impact of commonly used insecticides at different doses on *B. bassiana*.

MATERIALS AND METHODS

Fungus culture: Isolates of the entomopathogenic fungus, *B. bassiana*, obtained from the ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru were sub-cultured on PDA ($26 \pm 1^\circ\text{C}$; RH 70%), and the conidia produced were used for further investigation. The study was carried out at the ICAR- Central Tuber Crops Research Institute, Thiruvananthapuram.

Insecticides: All the insecticides selected for the current investigation (Table 1) were classified into recommended dose (RD), below recommended dose (BRD) and above recommended dose (ARD). Except for imidacloprid, the concentrations (in %) for other insecticides were 0.10 (BRD), 0.20 (RD), and 0.30 (ARD); whereas it was 0.02 (BRD), 0.03 (RD) and 0.04 (ARD) in the case of imidacloprid.

Conidial germination: An appropriate concentration of each pesticide was added to 50 mL of Potato-Dextrose-Broth (PDB) at 45°C to

reach the required concentration (Table 1). Conidial suspension of *B. bassiana* (1mL) at 10^6 spore/mL, prepared in sterile distilled water amended with 0.02 per cent Tween 20 was added to the above medium; and an aliquot without pesticides was used as control. These were transferred into an incubator ($26 \pm 1^\circ\text{C}$; 12 h photophase), and after 24 h, the germinated spores in 100 conidia per quadrant were observed, both in treatments and control, by using stereomicroscope (Carl Zeiss, Stemi 2000C) under 40X.

Vegetative growth and spore production: The required concentration of the test insecticides was added in to the autoclaved PDA at 40°C , and from this 20 mL was transferred into a Petri plate (90 mm). Medium without insecticides served as the control. After solidification, each plate was inoculated with a PDA plug (4mm dia) with *B. bassiana*, as described by Todorova *et al.* (1998). The plates were incubated at $26 \pm 1^\circ\text{C}$ and growth of the culture was measured on alternative days until 14 days after the treatment (DAT). The experiment was replicated thrice. After 14 d, the conidia were harvested by scraping and suspended in 1 mL of 0.01 per cent Tween 80. The concentration of the conidia was estimated using Neubauer counting chamber, and the percentage growth inhibition of *B. bassiana* by insecticide-treated PDA was estimated by Hokkanen and Kotiluoto (1991).

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

Where: X = Growth inhibition (%); Y = Growth diameter (cm) in control; Z = Growth diameter (cm) in treatment.

The insecticides were further categorised into harmless (<50%), slightly harmful (50-79%), moderately harmful (80-90%), and harmful (>90%) and given score from 01 to 04 as classified by Hassan (1989). The data were statistically analysed.

RESULTS AND DISCUSSION

The tested insecticides exhibited varying degrees of inhibition on the germination, vegetative growth and sporulation of *B. bassiana* (Table 1).

Table 1. Effect of insecticides in different doses on sporulation of the entomopathogenic fungus *B. bassiana*

Treatments	Germination		Colony		Number of conidia	
	Total	Reduction (%)	Total area (mm)	Reduction (%)	X *10 ⁶	Reduction (%)
1.1 Effect of insecticides in below recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	74.67±4.53b	13.57	76.33±4.73b	15.19	92.13±9.91b	34.44
Chlorpyrifos	43.13±2.53c	50.08	30.67±3.51c	67.40	52.67 ± 5.27 c	62.52
Quinalphos	16.20±2.58e	81.25	15.67±1.53d	82.60	34.13 5.68d	75.71
Dimethoate	26.27±2.00d	69.59	28.00±2.00c	68.89	30.47±4.76d	78.31
Dichlorvos	4.67±1.15f	94.60	8.67±0.58e	90.37	11.53±1.15e	91.80
Fenvalerate	72.73±5.78b	15.82	78.33±2.52b	12.96	93.33±12.34b	33.58
1.2 Effect of insecticides in recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	65.33±3.50b	24.38	74.33±2.52b	17.77	72.70± 9.82b	48.26
Chlorpyrifos	32.87±2.76c	61.96	28.33±1.53d	68.62	40.00±6.34c	71.54
Quinalphos	12.53±1.75e	85.49	11.67±1.15f	87.04	22.53±6.12d	83.97
Dimethoate	20.00±2.05d	76.85	20.00±2.65e	77.78	21.67 ±6.78d	84.57
Dichlorvos	0.00±0.58f	100	0.00±0.00g	100	0.00 ± 0.00e	100
Fenvalerate	65.07±4.34b	24.69	70.67±1.53c	21.48	73.07±12.71b	48.00
1.3 Effect of insecticides in above recommended dose						
Control	86.40±5.20a	-	90.00±0.00a	-	140.53±11.78a	-
Imidacloprid	54.67±4.67b	36.72	58.33±3.51b	35.17	61.47 ±8.23b	56.25
Chlorpyrifos	24.00±1.78c	72.22	24.67±1.53d	72.59	30.00±5.00c	78.65
Quinalphos	5.33±1.53e	93.83	8.67±0.58f	90.37	14.73 ±5.96d	89.51
Dimethoate	15.40±1.65d	82.17	17.00±2.65e	81.11	14.33± 4.00d	89.80
Dichlorvos	0.00±0.58f	100	0.00±0.00f	100	0.00 ± 0.00e	100
Fenvalerate	55.73±3.88b	35.36	52.00±2.65c	42.22	58.47±10.45b	58.39

X= Conidia in number

Imidacloprid and fenvalerate showed the least inhibition on conidial germination at all three tested concentrations compared with other insecticides. Chlorpyrifos, quinalphos, and dimethoate at BRD, RD and ARD showed >50.00 per cent germination inhibition. The effect of dichlorvos on *Beauveria* germination was statistically on par. However, 100 per cent reduction was observed in RD and ARD where as it was 94.60 per cent reduction in the BRD

In the case of radial growth, imidacloprid in all concentrations and fenvalerate at BRD showed the least inhibition. The vegetative growth inhibition

induced by fenvalerate at RD and ARD was significantly different from BRD. On the other hand, in chlorpyrifos, quinalphos and dimethoate fungal growth inhibition was higher (>65%) in all concentrations. Dichlorvos significantly reduced radial growth at all three concentrations compared to the control, with percentage reductions ranging from 90.37 at BRD to 100 inhibition at RD and ARD.

Concerning spore production, the imidacloprid and fenvalerate formulations showed the best results at the lowest concentrations, relative to the control. Dichlorvos at the three concentrations was poorer

than the other treatments concerning spore production. Dimethoate and quinalphos at the highest concentrations (ARD) also showed significant reduction relative to the control. Considering Hassan's classification scheme (Hassan 1989), imidacloprid and fenvalerate formulations at the three concentrations were compatible with the fungus (Table 2).

Several studies, (Mohamed *et al.*, 1987; Anderson *et al.*, 1989; Castineiras *et al.*, 1991; Oliveira and Neves, 2004; Alizadeh *et al.*, 2007; Abidin *et al.*, 2017) have examined the compatibility of chemical insecticides with the entomopathogens. Hassan and Charnley (1989) identified inconsistent interactions between fungi and insecticides, resulting in varying degrees of inhibition on the conidial germination, vegetative growth and sporulation of *B. bassiana*. The importance of conidial germination in pest management using fungi was emphasized by Batista Filho *et al.* (1998) and De Olivera and Neves (2004). The effect of insecticides on conidia germination is considered a critical aspect of product compatibility in the field, as entomopathogenic fungi primarily infect insects through conidial germination (Anderson and Roberts, 1983; Neves *et al.*, 2001).

Dichlorvos exhibited strong inhibition of *B. bassiana*, while imidacloprid and fenvalerate demonstrated relatively minor fungal inhibition. Boucias *et al.* (1988) demonstrated that the treatment of entomopathogenic fungi conidia with chemical products can neutralize the electrostatic charge of the surface or remove the mucous layer covering conidia, impacting substrate recognition and the transduction of the signal that initiates germination. The role of coat removal in substrate recognition and the transduction of the signal-triggering germ tube formation in *Metarhizium anisopliae* was demonstrated by Leger and Cooper (1987) and Leger *et al.* (1991).

According to James and Elzen (2001) and Alizadeh *et al.* (2007), imidacloprid and fenvalerate exhibited lower toxicity to *B. bassiana*, with imidacloprid showing no adverse effects on the fungus. In the current study, chlorpyrifos demonstrated slight toxicity to *B. bassiana*, although previous reports indicated slight harm to the fungus normal field doses

Table 2. Toxicity scoring index of insecticides according to Hassan's (1989) classification scheme

Treatments	Conc	Germi	Colony	Spore
Imidacloprid	BRD	1	1	1
	RD	1	1	1
	ARD	1	1	2
Chlorpyrifos	BRD	2	2	2
	RD	2	2	2
	ARD	2	2	2
Quinalphos	BRD	3	3	3
	RD	3	3	3
	ARD	4	4	3
Dimethoate	BRD	2	2	2
	RD	2	2	3
	ARD	3	3	3
Dichlorvo	BRD	4	4	4
	sRD	4	4	4
	ARD	4	4	4
Fenvalerate	BRD	1	1	1
	RD	1	1	1
	ARD	1	1	2

Grades: 1: harmless (<50%), 2: slightly harmful (50-79%), 3: moderately harmful (80-90%), 4: harmful (>90%)

BRD- Below recommended dose; RD- Recommended dose; ARD-- above recommended dose

Conc- Concentration; Germi- Germination; Colony- Colony area; Spore- Sporulation

(Ambethgar, 2003). Other studies suggested that chlorpyrifos strongly inhibits the growth and sporulation of *B. bassiana*, even at concentrations lower than the recommended field usage rates (Barbosa and Moreira, 1982; Rao, 1989).

The findings highlighted the incompatibility of dichlorvos with *B. bassiana*, suggesting the potential of combined applications of *B. bassiana* with compatible chemical pesticides as a promising strategy for integrated pest management programs. Insecticides such as imidacloprid and fenvalerate were recommended in pest management programmes due to their compatibility with the entomopathogenic fungus *B. bassiana*, commonly utilized as a biocontrol agent.

The study elucidates the differential impact of tested

insecticides on *B. bassiana*, crucial for biocontrol. Imidacloprid and fenvalerate demonstrate consistent compatibility, showing minimal inhibition on conidial germination, radial growth, and spore production. Conversely, chlorpyrifos, quinalphos and dimethoate exhibits substantial inhibitory effects, posing risks to *B. bassiana*. The findings underscore the needful insecticide selection in integrated pest management. Imidacloprid and fenvalerate emerge as favorable choices, while caution is advised with chlorpyrifos, quinalphos, dimethoate and dichlorvos to preserve beneficial fungi efficacy.

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