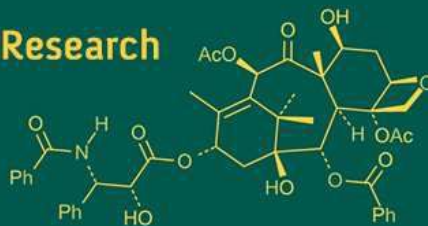
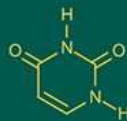


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Interaction studies between *Beauveria bassiana* (Balsamo) Vuillemin and commonly used insecticides

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Abstract

Among the insecticides evaluated, the combination of novaluron and indoxacarb had the least impact on *B. bassiana* and was considered safe at both the recommended and lower concentrations. Spinetoram, spirotetramat + imidacloprid, tolfenpyrad, and beta-cyfluthrin + imidacloprid caused mild to moderate inhibition depending on the dosage. In contrast, sulfoxaflor, cyantraniliprole, pyriproxyfen + bifenthrin, and afidopyropen resulted in strong inhibitory effects, particularly at higher concentrations, with afidopyropen completely suppressing fungal growth.

Keywords: *Beauveria bassiana* (Balsamo) Vuillemin, compatibility, entomopathogenic fungus, insecticides

Introduction

Entomopathogenic fungi constitute a major component of microbial communities, encompassing an estimated 1.5 to 5 million species distributed across more than 100 genera (St. Leger and Wang, 2010) [7]. Among the most widely used and commercially significant species are *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmermann) Zare and Gams. These fungi have evolved intricate parasitic mechanisms, making them valuable biological control agents against a variety of insect pests and diseases. Representing one of the most diverse microbial groups, entomopathogenic fungi include nearly 1,000 species known to cause natural mortality in invertebrates such as insects, ticks, and mites (Boomsma *et al.*, 2014) [2]. Typically, these fungi infect arthropod hosts by directly penetrating the outer cuticle, functioning primarily as contact pathogens.

Among them, *Beauveria bassiana* (Balsamo) Vuillemin, a member of the class Deuteromycetes, is one of the most extensively studied and globally distributed species due to its high potential as a biocontrol agent against numerous agricultural insect pests (Quesada *et al.*, 2007; Akello *et al.*, 2009) [6, 1]. The *Beauveria* genus holds both ecological and economic importance worldwide (Chen *et al.*, 2018) [3]. *B. bassiana* is commonly used as a mycoinsecticide to manage various pest species, while *B. brongniartii* (Sacc.) Petch, also distributed globally, is employed particularly for controlling soil-dwelling beetles. Since the 1960s, over 80 companies have developed approximately 171 mycoinsecticidal and mycoacaricidal formulations, of which *B. bassiana* accounts for 33.9% and *B. brongniartii* for 4.1%. Other *Beauveria* species likewise show strong potential for the biological control of numerous insect pests.

Understanding the compatibility between entomopathogenic fungi and commonly applied pesticides is essential in modern crop protection. Studying these interactions helps to optimize pest control strategies, lower production costs, and minimize toxic residues in food and the environment. In particular, assessing the compatibility of *Beauveria bassiana* with various insecticides and fungicides is crucial for identifying suitable chemical partners and scheduling treatments that preserve its biocontrol effectiveness. Such evaluations support the successful incorporation of *B. bassiana* into groundnut Integrated Pest Management (IPM) programs. Overall, these findings highlight the opportunity to integrate entomopathogenic fungi with less harmful insecticides, promoting safer and more sustainable pest management practices.

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Compatibility studies of *B. bassiana* with different insecticides

The current laboratory study on the compatibility of a local strain of *Beauveria bassiana* with different insecticides,

fungicides, and sprayable fertilizers was carried out using the poison food technique at the Biocontrol Research Laboratory, Department of Entomology, Junagadh Agricultural University, Junagadh.

Table 1: Compatibility studies of *B. bassiana* with different insecticides

Sr. No.	Insecticides	Concentration (%)		
		Lower	Recommended	Higher
1	Tolfenpyrad 15 EC	0.015	0.030	0.045
2	Sulfoxaflor 21.8 SC	0.008	0.016	0.025
3	Spinetoram 11.70 SC	0.005	0.010	0.015
4	Cyantraniliprole 10.26 OD	0.006	0.012	0.018
5	Afidopyropen 50 g/L DC	0.050	0.100	0.150
6	Novaluron 5.25 + Indoxacarb 4.50 SC	0.008	0.017	0.025
7	Beta-cyfluthrin 8.49+ Imidacloprid 19.81 OD	0.006	0.012	0.017
8	Pyriproxyfen 10 + Bifenthrin 10 EC	0.012	0.024	0.045
9	Spirotetramat 11.01+ Imidacloprid 11.01 SC	0.011	0.023	0.033
10	Control	-	-	-

*Figures in parenthesis are original values, while outside are arcsine transformed values. DAT -Days after Treatment

Methodology

This study evaluated the effect of selected insecticides on the growth inhibition of *Beauveria bassiana*. Three concentrations—lower, recommended, and higher—were established for each insecticide based on their respective field application rates. A total of five insecticides, each tested at three dose levels, were examined using the poisoned food technique described by Dhingra and Sinclair (1986) on Potato Dextrose Agar (PDA) medium. For the assay, 20 ml of PDA was sterilized in individual boiling tubes, and the insecticide emulsions at the desired concentrations were aseptically mixed into the molten sterile PDA. The mixture was then poured into 9 cm diameter sterile Petri dishes and allowed to solidify under a laminar airflow cabinet. Mycelial discs of *B. bassiana* (5 mm in diameter) were excised from the actively growing margin of a 10-day-old culture using a cork borer and placed at the center of each prepared PDA plate. PDA plates inoculated with the fungal disc but containing no insecticide served as untreated controls.

Observations to be recorded

The degree of fungal growth inhibition observed in each treatment was recorded and subsequently classified using a 1-4 scoring scale for in vitro toxicity evaluation, following the classification system proposed by Hassan.

Grades	Per cent growth
0	0%
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

The grades given after the observed growth was imparted for the calculation of the growth inhibition percentage of *B. bassiana* by using the following formula.

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

Where,

X- Per cent growth inhibition

Y- Grade obtained to the growth in the control plate

Z- Grade obtained to the growth in treated plate

The growth inhibition of the fungus was obtained from each treatment and it will further be classified in evaluation categories of the following 1- 4 scoring index in toxicity tests *in vitro* according to following Hassan's classification scheme (Hassan, 1989) ^[5].

Scoring Index	Toxicity
1	Harmless (<50% reduction in beneficial capacity)
2	Slightly harmful (50-79%)
3	Moderately harmful (80-90%)
4	Harmful (>90%)

The data was obtained based on the above indexes in each treatment and was subjected to arcsine transformation and analysed statistically.

Compatibility Studies of *B. bassiana* with different insecticides

The compatibility between entomopathogenic fungi and chemical insecticides must be evaluated individually under laboratory conditions. Achieving the optimal integration of fungal and chemical agents for pest management may involve their concurrent application. Therefore, the compatibility of *B. bassiana* with commonly used insecticides was assessed under controlled laboratory conditions, and the findings are presented below.

Compatibility at lower dose

The data on percent growth inhibition presented in Table 2 revealed that novaluron 5.25 + indoxacarb 4.50 SC at 0.008% (46.67%) caused minimal inhibition of *B. bassiana* growth at the lower concentration. Treatments with tolfenpyrad 15 EC (0.015%), spinetoram 11.70 SC (0.005%), spirotetramat 11.01 + imidacloprid 11.01 SC (0.011%), and beta-cyfluthrin 8.49 + imidacloprid 19.81 OD (0.006%) moderately inhibited fungal growth, showing inhibition levels of 51.85%, 60.00%, 61.11%, and 61.48%, respectively. Furthermore, the remaining insecticides.

Table 2: Effect of different insecticides on the growth of *B. bassiana* at a lower dose

Sr. No.	Treatment	Conc. (%)	Dose /Liter	Growth Inhibition (%)	Grades	Scoring index
1	Tolfenpyrad 15 EC	0.015	1.00 ml	46.06 (51.85)	3	2
2	Sulfoxaflor 21.8 SC	0.008	1.00 ml	66.49 (84.07)	5	3
3	Spinetoram 11.70 SC	0.005	0.60 ml	50.77 (60.00)	3	2
4	Cyantraniliprole 10.26 OD	0.006	0.43 ml	66.50 (84.07)	5	3
5	Afidopyropen 50 g/L DC	0.050	0.60 ml	68.92 (87.04)	5	3
6	Novaluron 5.25 + Imidacloprid 4.50 SC	0.008	0.20 ml	43.09 (46.67)	3	1
7	Beta-cyfluthrin 8.49 + Imidacloprid 19.81 OD	0.006	0.38 ml	51.64 (61.48)	4	2
8	Pyriproxyfen 10 + Bifenthrin 10 EC	0.012	0.85 ml	68.02 (85.93)	5	3
9	Spirotetramat 11.01 + Imidacloprid 11.01 SC	0.011	0.50 ml	51.42 (61.11)	4	2
10	Control	-	-	8.3 (0.00)	-	-
	S.E.m. $\pm$			0.70		
	C.D. at 5%			2.08		
	C.V. %			2.33		

Figures in parenthesis are original values, while outside values are angular transformed. A local strain of *B. bassiana* @ 2×10^9 cfu/g was used.

Scoring Index*: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (>90%) sulfoxaflor 21.8 SC 0.008% (84.07%), cyantraniliprole 10.26 OD 0.006% (84.07%), pyriproxyfen 10 + bifenthrin 10 EC 0.012% (85.93%) and afidopyropen 50 g/L DC 0.050% (87.04) were also significantly reduced the growth of the fungus.

The results indicated that among the insecticides tested, novaluron + indoxacarb was classified as *relatively harmless* (Scoring Index 1) to *B. bassiana*, showing less than 50% growth inhibition at the lower concentration. Four insecticides—tolfenpyrad, spinetoram, beta-cyfluthrin + imidacloprid, and spirotetramat + imidacloprid—were found to be *slightly harmful* (Scoring Index 2). In contrast, sulfoxaflor, cyantraniliprole, pyriproxyfen 10% + bifenthrin, and afidopyropen were categorized as *moderately harmful* (Scoring Index 3) to *B. bassiana*.

Compatibility at recommended dose

Table 3: Effect of different insecticides on the growth of *B. bassiana* at recommended dose

Sr. No.	Treatment	Conc. (%)	Dose/liter	Growth Inhibition (%)	Grades	Scoring index
1	Tolfenpyrad 15 EC	0.030	2.0 ml	84.44 (66.80)	4	2
2	Sulfoxaflor 21.8 SC	0.016	2.0 ml	86.67 (68.62)	4	2
3	Spinetoram 11.70 SC	0.010	1.2 ml	83.70 (66.21)	4	2
4	Cyantraniliprole 10.26 OD	0.012	0.85 ml	97.78 (82.99)	5	3
5	Afidopyropen 50 g/L DC	0.100	1.2 ml	89.63 (71.22)	4	2
6	Novaluron 5.25 + Imidacloprid 4.50 SC	0.017	0.4 ml	74.81 (59.90)	3	2
7	Beta-cyfluthrin 8.49+ Imidacloprid 19.81 OD	0.012	0.75 ml	72.59 (58.49)	4	2
8	Pyriproxyfen 10 + Bifenthrin 10 EC	0.024	1.7 ml	88.52 (70.20)	4	2
9	Spirotetramat 11.01+ Imidacloprid 11.01 SC	0.023	1.0 ml	82.22 (65.12)	4	2
10	Control	-	-	8.3 (0.00)	-	-
	S.E.m. $\pm$			1.46		
	C.D. at 5%			4.33		
	C.V. %			4.08		

Figures in parenthesis are original values, while outside values are angular transformed. Local strain of *B. bassiana* @ 2×10^9 cfu/g was used.

Scoring Index*: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (>90%)

The study on the compatibility of *B. bassiana* with various insecticides at their recommended doses revealed that beta-cyfluthrin + imidacloprid, novaluron + indoxacarb, spirotetramat + imidacloprid, spinetoram, tolfenpyrad, sulfoxaflor, pyriproxyfen 10% + bifenthrin, and afidopyropen were classified as *slightly harmful* (Scoring Index 2) to the fungal growth. In contrast, the insecticide

The results presented in Table 3 demonstrate the effects of various insecticides on the growth of *B. bassiana* at their recommended doses. The lowest growth inhibition (58.49%) was recorded with beta-cyfluthrin 8.49 + imidacloprid 19.81 OD (0.012%), followed closely by novaluron 5.25 + indoxacarb 4.50 SC (0.017%) with 59.90% inhibition. The insecticides spirotetramat 11.01 + imidacloprid 11.01 SC (0.023%), spinetoram 11.70 SC (0.010%), tolfenpyrad 15 EC (0.030%), and sulfoxaflor 21.8 SC (0.016%) exhibited moderate inhibition of fungal growth, with inhibition levels of 65.12%, 66.21%, 66.80%, and 68.62%, respectively. Further, pyriproxyfen 10 + bifenthrin 10 EC (0.024%), afidopyropen 50 g/L DC (0.100%), and cyantraniliprole 10.26 OD (0.012%) caused higher growth inhibition of 70.20%, 71.22%, and 82.99%, respectively. Among all treatments, cyantraniliprole 10.26 OD (0.012%) resulted in the greatest reduction in mycelial growth of *B. bassiana*, with an inhibition rate of 82.99%.

cyantraniliprole exhibited a *moderately harmful* effect (Scoring Index 3) on *B. bassiana* growth.

Compatibility at higher dose

All the insecticides tested exhibited varying levels of compatibility with *B. bassiana* at their higher concentrations (Table 4). Analysis of the growth inhibition data revealed that spirotetramat 11.01 + imidacloprid 11.01 SC (0.033%) and spinetoram 11.70 SC (0.015%) caused 83.70% and 87.78% inhibition, respectively. The treatments tolfenpyrad

15 EC (0.045%), sulfoxaflor 21.8 SC (0.025%), novaluron 5.25 + indoxacarb 4.50 SC (0.025%), beta-cyfluthrin 8.49 + imidacloprid 19.81 OD (0.017%), and cyantraniliprole 10.26 OD (0.018%) followed, with growth inhibition values of 91.11%, 91.48%, 95.93%, 95.93%, and 97.78%, respectively. Complete growth suppression was recorded in afidopyropen 50 g/L DC (0.150%), where no fungal growth was observed.

At higher concentrations, all tested insecticides demonstrated a stronger inhibitory effect on the mycelial growth of *B. bassiana* compared to their lower and recommended doses. The findings revealed that the insecticides differed in their toxicity levels and impact on fungal development. Spirotetramat + imidacloprid and spinetoram were classified as *moderately harmful* (Scoring Index 3), while tolfenpyrad, sulfoxaflor, novaluron + indoxacarb, beta-cyfluthrin + imidacloprid, cyantraniliprole, and afidopyropen were categorized as *harmful* (Scoring Index 4), exhibiting more than 90% growth inhibition. Overall, the study demonstrated that all insecticide

formulations suppressed the mycelial growth of *B. bassiana* on Potato Dextrose Agar (PDA) medium to varying degrees, ranging from partial to complete inhibition, depending on the concentration. Significant differences were observed among the insecticides in their inhibitory effects on fungal growth.

The current findings are in agreement with those reported by Joshi *et al.* (2018) [8], who found that insecticides such as lambda-cyhalothrin, novaluron, indoxacarb, and emamectin benzoate were compatible with *B. bassiana* and *M. anisopliae*. Similarly, Kim *et al.* (2020) [9] evaluated *B. bassiana* with trichlorfon, acephate, indoxacarb, sulfoxaflor, and spinetoram, and concluded that trichlorfon, acephate, and indoxacarb were compatible with *B. bassiana*, while formulated sulfoxaflor had an adverse effect. The present results also align with Kiruthiga *et al.* (2022), who observed that spinetoram exhibited moderate inhibition of fungal growth (76.60-89.00 mm radial growth), confirming its relatively lower toxicity compared to other insecticides.

Table 4: Effect of different insecticides on the growth of *B. bassiana* at higher dose

Sr. No.	Treatment	Conc. (%)	Dose/litre	Growth Inhibition (%)	Grades	Scoring index
1	Tolfenpyrad 15 EC	0.045	3.00 ml	73.37 (91.11)	5	4
2	Sulfoxaflor 21.8 SC	0.025	3.00 ml	73.47 (91.48)	5	4
3	Spinetoram 11.70 SC	0.015	1.80 ml	69.70 (87.78)	5	3
4	Cyantraniliprole 10.26 OD	0.018	1.28 gm	81.43 (97.78)	5	4
5	Afidopyropen 50 g/L DC	0.150	1.80 ml	91.70 (100.00)	5	4
6	Novaluron 5.25 + Indoxacarb 4.50 SC	0.025	0.60 ml	78.54 (95.93)	5	4
7	Beta-cyfluthrin 8.49+ Imidacloprid 19.81 OD	0.017	1.13 ml	78.44 (95.93)	5	4
8	Pyriproxyfen 10 + Bifenthrin 10 EC	0.045	2.55 ml	78.38 (95.93)	5	4
9	Spirotetramat 11.01+ Imidacloprid 11.01 SC	0.033	1.50 ml	66.21 (83.70)	5	3
10	Control	-	-	8.3 (0.00)	-	-
	S.Em. ±			1.50		
	C.D. at 5%			4.44		
	C.V. %			3.70		

Figures in parenthesis are original values, while outside values are angular transformed. A local strain of *B. bassiana* @ 2×10^9 cfu/g was used.

Scoring Index*: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (>90%)

The present study revealed that the insecticides tolfenpyrad, novaluron + indoxacarb, and spirotetramat + imidacloprid, categorized as *slightly harmful* at lower and recommended doses (50-79% inhibition), could potentially be used in combination with the tested *B. bassiana* strain to a certain extent. The slight discrepancies between these findings and those of previous studies may be due to variations in the *B. bassiana* strains employed by different researchers.

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