

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(3): 04-08
www.biochemjournal.com
Received: 08-12-2025
Accepted: 11-01-2026

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In vitro compatibility of entomopathogenic fungi, *Beauveria bassiana* (Balsam) with insecticides and fungicides

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3Sa.7783>

Abstract

Compatibility of *Beauveria bassiana* with six commonly used insecticides (buprofezin, acetamiprid, imidacloprid, flonicamid, thiamethoxam and fipronil) and three fungicides (carbendazim, copper oxychloride and propiconazole) were tested to identify and incorporate the in Integrated pest management (IPM). Among the treatments, flonicamid 50 WG showed maximum growth (46.50 mm) and it was found the most compatible insecticide and it was followed by copper oxychloride and buprofezin which recorded growth of 45.20 and 44.50 mm, respectively. The vegetative growth of *B. bassiana* in the other assessed treatments viz., imidacloprid, fipronil, acetamiprid, and thiamethoxam was measured at 37.89, 35.73, 34.25, and 34.25 mm, respectively. However, the media treated with propiconazole (7.93 mm) and carbendazim (8.20 mm) remained comparable to one another with significantly lower vegetative growth. Based on compatibility categorization analysis, all investigated treatments were found to be compatible with *B. bassiana* with the exception of carbendazim and propiconazole.

Keywords: Compatibility, *Beauveria bassiana*, IPM, treatment, insecticides

Introduction

In many parts of the world, agricultural development has a lengthy history. Around 10,000 years ago, agricultural activities started in the Fertile Crescent of Mesopotamia, which roughly corresponds to the majority of modern-day Iraq, Turkey, Syria, and Jordan. Individuals who lived in these regions and gathered edible seeds using techniques like forest gardening and firestick farming. Large quantities of wheat, barley, peas, lentils, chickpeas, bitter vetch, and flax were grown as the people grew more sedentary and lived on farms. Around 7500 years ago, sorghum and rice were first cultivated in the Sahel region of Africa (Davies, 1968) [1]. Furthermore, according to Davies (1968) [1], several indigenous crops were independently domesticated approximately 7500 years ago in Ethiopia, New Guinea, and West Africa. China was the country that domesticated millet and rice. Sunflowers, potatoes, corn, and squash were all domesticated by Americans on their own. Pests, weeds, and illnesses frequently plague farmed crops, which can significantly reduce crop productivity. Without the use of pesticides, diseases and pests would cause up to 78%, 54%, and 32% of the loss of fruits, vegetables, and cereals, respectively (Zhang *et al.*, 2011) [2]. Thus, it is imperative that researchers and the general public find solutions to the issues brought on by illnesses and pests. In many areas, high-yielding cultivars, better soil and water management, fertilization, and other farming practices can boost crop productivity. However, larger crop output potential is frequently linked to increased susceptibility to pest attack, which raises absolute losses and loss rates. Pre-harvest pests cost farmers around the world an average of 35% of their potential crop output. Conventional pesticides are thousands of years behind the evolutionary relationships between farmers and pests. Different levels of loss can be distinguished, such as direct and indirect losses or primary and secondary losses, demonstrating that pests can have an impact on the supply of food and feed, the economies of rural areas, and even entire nations, in addition to threatening crop productivity and

lowering farmers' net income. To meet the rising food demand, herbicides, insecticides, fungicides, and nematicides are frequently used to lower the pest pressure (Sarkar *et al.*, 2021) [3]. Pesticides are thought to be crucial for both crop protection and the treatment of human diseases. Pesticides are categorized as either organism targets, repellents, or destroyers. Many insects and pests cannot be controlled by store-bought insecticides. Large quantities of commercial insecticides are also utilized to manage pests. Depending on the type of pest and the methods used for control, pests are treated both preventively and remedially. Insecticides, herbicides, fungicides, molluscicides, nematicides, plant growth regulators, and rodenticides are among the pesticides categorized by the species they target. The environment is negatively impacted by the widespread usage of pesticides. Research indicates that pesticides have a negative impact on biodiversity and human health. Usually stable and soluble in water, pesticides have an impact on aquatic life, wildlife, and native plants. Pesticides are harmful in agricultural applications and expose workers to occupational hazards during the manufacturing process. Because chemical pesticides are persistent in the environment and can infiltrate the food chain, they have a negative impact on human health. Chemicals from pesticides can enter the human food chain through a variety of sources, including contaminated water, polluted air, and vegetables and fruits. Fish, birds, mammals, and beneficial insects are all harmed by a number of pesticides. The toxicity of pesticides, their quantity, timing, frequency, application technique, weather, and soil type all affect wildlife poisoning. The use of carbamate and organophosphate has significantly expanded over the last forty years. Endosulfan and other organochlorines are quite persistent in the environment in the south. The bulk of the population has decreased as a result of pesticide poisoning as a result of habitat alteration. Entomopathogenic fungi show great promise in controlling a range of arthropod pests. However, their field application may result in inconsistent control because biotic factors, such as interactions with antagonistic bacteria, and abiotic factors, such as temperature and humidity, can easily affect infections of hosts with insect pathogenic fungus. Entomopathogenic fungi have been used in conjunction with botanicals and sublethal chemical dosages to boost efficacy and accelerate insect mortality. Pesticides. In many instances, the effectiveness of entomopathogenic fungi in combating insect pests has been enhanced by the application of certain insecticides. The effects of insecticides and fungicides on mycelial development and sporulation of fungi have been the focus of most fungus-insecticide compatibility studies. While the effects of insecticides on conidial germination, which are commonly overlooked, are the most significant factor to consider when evaluating insecticide compatibility since it is the first stage of the infection. *Beauveria bassiana* (Balsam) Vuillemin is a necrotrophic fungus that lives in soil and is commonly referred to as white muscardine. This fungus is used for its entomopathogenic qualities as well as its other advantageous qualities as plant endophytes, plant disease antagonists, helpful rhizosphere colonizers, and plant growth boosters (Vega *et al.*, 2009) [4]. Based on above mentioned problem, research is required to examine which insecticides and fungicides compatible with entomopathogenic fungus *B. bassiana*. Thus, the purpose of

this study was to determine the compatibility of various insecticides and fungicides at particular doses to entomopathogenic fungus *B. bassiana*.

Materials and Methods

The present investigation was conducted at plant pathology laboratory, R. A. K. College of Agriculture, RVSKVV, Sehore, Madhya Pradesh. The impact of these chemical insecticides and fungicides on the vegetative growth and spore germination of *B. bassiana* was assessed using poisoned food technique (Grover and Moore, 1962) [5]. The list of various insecticides and fungicides are used which showed in Table-1.

Maintenance of pure culture of *B. bassiana*

For the current investigations, a pure fungal culture of *B. bassiana* was obtained from ICAR-NBAIR. A loopful of the inoculum from the mother culture slants of *B. bassiana* was moved to PDA slants and Petri plates in order to preserve the pure culture. The PDA medium was autoclaved for 30 minutes at 121 °C and 15 psi of pressure. The autoclaved PDA medium was then transferred to sanitized Petri plates, chilled, and aseptically injected with a pure culture of *B. bassiana*. After that, the plates were cultured for fourteen days at room temperature (25±2 °C). After 12-15 days of incubation, fungal mat was blended and filtered through muslin cloth and diluted with sterile distilled water. The required concentration of conidia for compatibility studies were made by using hemocytometer.

Assessment of vegetative growth (VG)

Required concentrations of each test insecticides and fungicides were incorporated into 200 ml of melted sterile PDA media under aseptic condition, mixed thoroughly, poured into 9 cm diameter sterile petri plates and allowed to solidify under laminar air flow cabinet. Using a 5 mm diameter cork borer, the mycelium mat and agar disc of *B. bassiana* were cored from the edges of the fourteen-day-old colony and positioned in the middle of the PDA plate. PDA media treated as an untreated control that was infected with mycelial disc but did not include pesticides. In order to allow for radical growth, Parafilm sealed Petri dishes were incubated for two days at 25±1 °C, 60±5% relative humidity, and 12:12 hours of photo:dark phase. Each treatment was replicated four times. The radial growth (mm) of entomopathogenic fungus *B. bassiana* was measured using caliper rule when control plates attained full growth. Per cent inhibition of *B. bassiana* growth in the poisoned media was calculated using the formula suggested by Vincent (1927).

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

Where,

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

Spore (conidia) production

After vegetative growth measurement, the central 5 colony discs from each test were drawn for conidia production analysis. Each disc was placed in test tube containing 10ml of sterile distilled water with Tween 80 (0.02 per cent). The tubes were vortexed for 30 seconds to extract the conidia from the disc. Number of conidia per ml were counted using haemocytometer.

Statistical analysis

The compatibility data were analyzed using Completely Randomised Design (CRD) with Analysis of Variance (ANOVA). Toxicity of each insecticide was calculated using formula proposed by Alves *et al.*, (1998). In this

formula calculation of vegetative growth (VG) and sporulation (SP) is made in relation to control (100%): $T = [20 (VG) + 80 (SP)]/100$. Where T = 0 to 30 (very toxic); 31 to 45 (toxic); 46 to 60 (moderately toxic); >60 (compatible).

Table 1: List of insecticides and fungicides used for compatibility studies with *B. bassiana*

S. No.	Insecticides/fungicides	Dosage g or ml/l	Trade name	Manufacturing company
1	Buprofezin 25% SC	1.00 ml	Applaud	Tata Rallis
2	Acetamiprid 20 % SP	0.25 gm	Dhanpreet	Dhanuka Agritech Limited
3	Imidacloprid 17.8 % SL	0.50 ml	Victor	Insecticides (India) Limited
4	Flonicamid 50 WG	0.30 g	Ulala	UPL Limited
5	Thiamethoxam 25 WG	0.40 g	Actara	Syngenta India Pvt. Ltd.
6	Fipronil 5 % SC	1.5 ml	Regent	Bayer Crop Science Pvt. Ltd.
7	Carbendazim 50 WP	1.0 g	Bavistin	Crystal Crop Protection
8	Copper oxychloride 50 WP	1.5 g	Blue Copper	Crystal Crop Protection
9	Propiconazole	0.6 ml	Tilt	Syngenta India Pvt. Ltd.
10	Control	-	-	-

Results and Discussion

Vegetative growth (mm)

When implementing an IPDM approach, it's vital to consider the compatibility of the chemicals sprayed on the crop, avoiding the use of the most hazardous ones or applying them during seasons when the effect on a natural control agent is minimized. The integration of microbial pesticides with chemical pest management practices requires detail compatibility studies. Keeping the fact in background, the present investigation on compatibility of *Beauveria bassiana* with various insecticides and fungicides was carried out at plant pathology laboratory, R. A. K. College of Agriculture, RVSKVV, Sehore, Madhya Pradesh during the year 2025. Data on growth performance of *B. bassiana* in different chemical treatments are presented in Table-2 and Fig. 1

At ten days after inoculation (control plates attained full growth), there were noticeable variations between the vegetative growth of *B. bassiana* on various chemical treated media. Among the treatments, flonicamid 50 WG showed maximum growth (46.50 mm) and it was found the most compatible insecticide and it was followed by copper oxychloride 50 WP and buprofezin 25 SC, which recorded growth of 45.20 and 44.50 mm, respectively. The other evaluated treatments viz., imidacloprid 17.8 SL, fipronil 5 SC, acetamiprid 20 SP, thiamethoxam 25 WG recorded 37.89, 35.73, 34.25, and 34.25 mm vegetative growth of *B. bassiana*, respectively. However, treatment carbendazim 50 WP (8.20 mm) and propiconazole 25 EC (7.93 mm) treated media remained at par with each other with significantly least vegetative growth. Significantly highest vegetative growth was observed in control (54.78 mm). The results of our experiments were in agreement with those previously reported and showed strong or complete inhibition of *B. bassiana* in laboratory tests.

This study proves that *B. bassiana* is affected by the various fungicides and insecticides and also they inhibit the development and reproduction of the entomopathogen *B. bassiana* that would be a limiting factor in their future applications in the IPM programs (Anderson & Roberts, 1983) [6]. The degree to which the insecticides inhibited *B. bassiana* germination, vegetative growth, and sporulation varied and was mostly influenced by the chemical characteristics and concentrations of each compound.

Because the ingredients in the insecticide formulations can be employed directly as nutrients, the greatest vegetative growth and spore yield seen with flonicamid 50 WG treatment boosting the pathogen's conidia generation and vegetative development.

The current finding that buprofezin, a chitin inhibitor, was relatively safe at higher concentrations is supported by Anderson *et al.* (1989) [8], who also reported that insecticides of microbial origin and chitin inhibitors like abamectin, thuringiensin, and triflumuron were compatible with *B. bassiana* even at higher doses. Additionally, Andrew *et al.* (2005) verified that buprofezin, the active ingredient, offered a satisfactory degree of *L. muscarium* spore germination.

According to Deb *et al.* (2017) [9], the isolate of *Beauveria* BP1.1 exhibited the highest growth in copper oxychloride (80.50 mm), followed by neem leaf extract (74.75 mm) and neem seed kernel extract (70.75 mm). In contrast, the fungicide carbendazim showed the highest inhibition of fungal growth.

Due to its multisite activity, copper oxychloride prevents fungal growth by disrupting the spore and mycelium's enzyme systems. As the copper binding protein (CuBp) of *B. bassiana* is a member of metallothioneins, a cysteine-rich protein devoid of aromatic residues and composed of copper (I)-thiolate cluster, Challa and Savinda (2014) reported that *B. bassiana* was found to be highly compatible with copper oxychloride. *B. bassiana* has a higher affinity for copper because of this. Similarly, in the present study, copper oxychloride 50WP was found as the most compatible fungicide with 45.20 mm radial growth of *B. bassiana*.

In our study carbendazim showed significant inhibition of entomopathogenic fungi and found very toxic to *B. bassiana* according to toxicity classification, which might be due to carbendazim inhibits the development of fungus by interfering with spindle formation at mitosis during cell division, thus, inhibiting germ tube development, formation of appressorium and growth of mycelia. Butters *et al.* (2003) [11] has also reported the abnormal bent appearance of the germination peg of *B. bassiana* with bursting at the tip in the presence of bavistin. Similarly, in the present study, the total inhibition of mycelial growth was observed in carbendazim 50WP at all three concentrations.

Spore yield

The data from Table-2 indicated that the total quantity of conidial spore produced by various pesticides varied significantly. With 5.63×10^4 conidial spores/ml, the control treatment had the highest spore yield. This was followed by flonicamid 50 WG and copper oxychloride 50 WP, which had 5.15×10^4 and 4.82×10^4 conidial spores/ml, respectively. Nevertheless, a spore yield of 4.78, 4.52, 4.38, 4.29, 3.69 and 1.23×10^4 conidial spores/ml was recorded in the treatment of buprofezin 25 SC, imidacloprid 17.8 SL, fipronil 5 SC, acetamiprid 20 SP, thiamethoxam 25 WG and carbendazim 50 WP, respectively.

Compatibility classification

Table-3 revealed the pesticides classification according to their toxicity and compatibility with *B. bassiana*. In agreement with the proposed formula by Alves *et al.* (1998) the insecticides/fungicides buprofezin 25 SC, acetamiprid 20 SP, imidacloprid 17.8 SL, flonicamid 50 WG, thiamethoxam 25 WG, fipronil 5 SC and copper oxychloride

50 WP were compatible with *B. bassiana*. For this entomopathogenic fungi, the average 'T' values, were 84.15, 73.43, 78.10, 90.29, 62.76, 75.25 and 84.97, respectively. Whereas, carbendazim 50 WP and propiconazole 25 EC found very toxic to *B. bassiana* with 'T' values of 20.36 and 17.70, respectively. The only insecticide that was found to be incompatible with *B. bassiana* and to restrict growth was diafenthiuron, according to Hiremath *et al.* (2020) [13]. However, on days 14 and 30 following inoculation, imidacloprid, thiamethoxam, acetamiprid, clothianidin, acephate, fipronil, buprofezin, flonicamid, and azadirachtin were shown to be compatible. According to Oliveira *et al.* (2003) [14], the fungus was compatible with formulations of thiamethoxam at all three concentrations and cyfluthrin at the two lowest values. The Alpha-Cypermethrin formulation, the Fenpropathrin and Deltamethrin formulations at the lowest concentrations, and the Thiamethoxam and Cyfluthrin formulations can all be regarded as compatible when germination/viability is taken into account as a compatibility factor.

Table 2: Effect of different insecticides and fungicides on vegetative growth and spore yield of *B. bassiana*

Tr. No.	Treatments	Vegetative growth (mm)	Spore yield (spores/ml)
1	Buprofezin 25 SC	44.50	4.78×10^4
2	Acetamiprid 20 SP	34.25	4.29×10^4
3	Imidacloprid 17.8 SL	37.89	4.52×10^4
4	Flonicamid 50 WG	46.50	5.15×10^4
5	Thiamethoxam 25 WG	28.40	3.69×10^4
6	Fipronil 5 SC	35.73	4.38×10^4
7	Carbendazim 50 WP	8.20	1.23×10^4
8	Copper oxychloride 50 WP	45.20	4.82×10^4
9	Propiconazole 25 EC	7.93	1.05×10^4
10	Untreated control	54.78	5.63×10^4
	S.E.m.±	1.06	0.12
	F test	Sig.	Sig.
	C.V. %	6.19	5.93

Table 3: Classification of insecticides based on compatibility and toxicity values towards *B. bassiana*

Tr. No.	Treatments	Growth inhibition (%)	Sporulation inhibition (%)	Toxicity values (T)	Classification
1	Buprofezin 25 SC	18.76	15.12	84.15	Compatible
2	Acetamiprid 20 SP	37.47	23.84	73.43	Compatible
3	Imidacloprid 17.8 SL	30.85	19.66	78.10	Compatible
4	Flonicamid 50 WG	15.11	8.36	90.29	Compatible
5	Thiamethoxam 25 WG	48.15	34.51	62.76	Compatible
6	Fipronil 5 SC	34.79	22.24	75.25	Compatible
7	Carbendazim 50 WP	85.03	78.29	20.36	Very Toxic
8	Copper oxychloride 50 WP	17.48	14.41	84.97	Compatible
9	Propiconazole 25 EC	85.54	81.49	17.70	Very Toxic
10	Untreated control	0.00	0.00	0.00	-

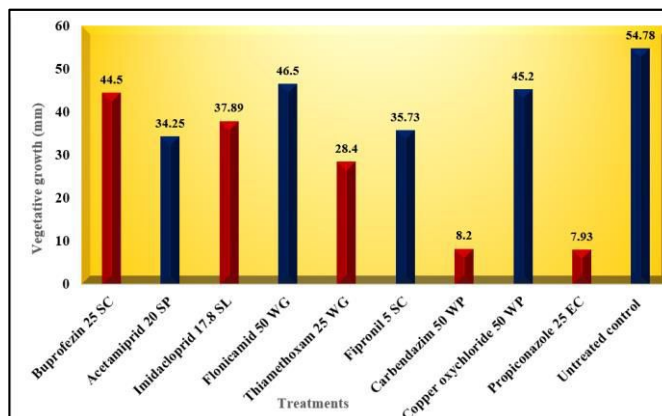


Fig 1: Vegetative growth of *Beauveria bassiana* influenced by different treatments

Conclusion

Since the onset of epizootics depends on these structures' ability to germinate on the host, conidial germination is a crucial stage in fungal pest management. However, conidial vigor is necessary for the entomopathogenic fungus to succeed. With the exception of carbendazim and propiconazole, our investigation revealed that the majority of pesticides were compatible with *B. bassiana*. However, the highest spore production and vegetative growth have been observed with flonicamid and copper oxychloride treatment. However, field investigation of the interactions between *B. bassiana* and insecticides/fungicides should be undertaken to examine their impact on pests and beneficial insects.

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