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Short communications

In-vitro compatibility of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin RB PTB with selected fungicides and insecticides

M. Aany[1], P. Sreeja[1*], C.F. Gleena Mary[2] and Mani Chellappan[1]

[1]*Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur-680 656, Kerala, India*

[2]*Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur 680 656, Kerala, India*

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Abstract

The *in vitro* compatibility of a potent indigenous isolate of *Beauveria bassiana* RB PTB with selected pesticides recommended for pest management in rice was evaluated using the poisoned food technique. Among insecticides, flubendiamide 20 WG (0.005%) and thiamethoxam 25 WG (0.005%) were compatible with the lowest growth inhibition of 10.58 and 12.02 per cent, respectively. However, acephate 75 SP (0.15%) and malathion 50 EC (0.1%) were incompatible with 66.35 and 59.61 per cent inhibition. Among fungicides, copper hydroxide 77 WP (0.1%) was compatible, while carbendazim 50 WP (0.1%), mancozeb 75 WP (0.2%), hexaconazole 5 EC (0.2%) and tebuconazole 50% + trifloxystrobin 25% 75 WG (0.04) were incompatible with 100 per cent growth inhibition. Results of the present study suggest that flubendiamide 20 WG (0.005%), thiamethoxam 25 WG (0.005%) and copper hydroxide 77 WP (0.1%) can be safely integrated with the entomopathogenic fungus *B. bassiana* RB PTB for rice pest management.

Key words: *Beauveria bassiana* RB PTB, Compatibility, Entomopathogenic fungus, Fungicides, Growth inhibition, Insecticides

Plant protection in the current scenario is focused on new strategies and good agricultural practice sowing to the deleterious effects of chemical insecticides. Microbial control is a safer option than chemicals and a promising approach for sustainable pest management. Entomopathogenic fungi (EPF) play a pivotal role in regulating insect pests due to their broad host range and capacity to suppress pest populations without posing any health or environmental risks to humans (Pathak et al., 2022). Among different fungi, *Beauveria bassiana* is aubiquitous species witha broad host spectrum (Kidanu and Hagos, 2020). A major limitation in using entomopathogens is the limited availability of virulent, indigenous isolates adapted to local conditions. Indigenous isolates are more virulent and highly pathogenic than exotic strains, adapted to the prevailing climatic conditions and are environmentally stable. *Beauveria bassiana* RB PTB is an indigenous fungal isolate obtained from an epizootic observed on rice bugs from the Regional Agricultural Research Station (RARS), Pattambi, Palakkad, Kerala. It was subsequently identified as a new isolate of *B. bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) on molecular and morphological criteria. The sequence has been deposited in NCBI (Accession no.: OP023314) (Sreeja et al., 2025). The isolate was characterized by its high speed of kill, causing 100 per cent mortality in adult rice bugs and BPH at 120 h after treatment with a spore concentration of 10^8 spores mL^{-1} (Aany, 2025).

Insecticides and fungicides, among other abiotic factors, may impair conidial germination and growth of microbial pathogens (Mathew and Louis, 2019). Information on the compatibility of pesticides with entomopathogenic fungi (EPN) is crucial for selecting appropriate pesticides for integrated pest management programme (IPM) (Deka et al., 2021). Hence, a study was conducted to assess the compatibility of a novel isolate of *B. bassiana* RB PTB with selected fungicides and insecticides recommended for pest management in rice cultivation using the poisoned food technique.

The compatibility of different insecticides and fungicides with *B. bassiana* RB PTB was conducted using the poisoned food technique as suggested by Sain et al. (2019). Compatibility of four insecticides *viz*., malathion 50% EC, acephate 75% SP, flubendiamide 20% WG, thiamethoxam 25% WG and five fungicides such as carbendazim 50% WP, mancozeb 75% WP, hexaconazole 5% EC, copper hydroxide 77% WP, and tebuconazole 50% + trifloxystrobin 25%, 75%

* Author for Correspondences: Phone : 9446343611; Email: sreeja.p@kau.in; Orcid ID : 0000-0002-1768-6331

Table 1: Insecticides tested against *Beauveria bassiana* RB PTB

No.	Insecticides	Trade name	Concentrations (%)
1.	Malathion 50% EC	Koshathion	0.1
2.	Acephate 75% SP	Asataf	0.15
3.	Flubendiamide 20% WG	Takumi	0.005
4.	Thiamethoxam 25% WG	Actara	0.005

SP, soluble powder; EC, emulsifiable concentrate; WG, wettable granule

Table 2: Fungicides tested against *Beauveria bassiana* RB PTB

No.	Fungicides	Trade name	Concentrations (%)
1.	Carbendazim 50% WP	Bavistin	0.1
2.	Mancozeb 75% WP	Indofil M-45	0.2
3.	Hexaconazole 5% EC	Contaf	0.2
4.	Copper hydroxide 77% WP	Kocide 2000	0.2
5.	Tebuconazole 50% + Trifloxystrobin 25% 75% WG	NATIVO	0.04

WP, wettable powder; EC, emulsifiable concentrate; WG, wettable granule

WG were studied (Table 1 and 2). Compatibility of the isolate was evaluated by exposing it to the recommended field dose as per the package of practices, KAU (2024).

The pure culture of *B. bassiana* RB PTB was maintained in Sabouraud Maltose Agar and Yeast (SMAY) slants. The SMAY medium was sterilized in 100 mL conical flasks, each with 50 mL of medium. The appropriate quantities of insecticides and fungicides were aseptically incorporated and homogenized under a laminar airflow cabinet. Fifteen millilitres of each of the poisoned medium was dispensed into 90 mm Petri dishes. A fungal culture disc (1 mm thick and 8 mm diameter) of non sporulating mycelium from a 4-day-old culture of *B. bassiana* RB PTB was collected by a cork borer and kept at the middle of a Petri dish with the solidified SMAY poisoned with each fungicide and insecticide. Unpoisoned SMAY medium inoculated with fungus served as control. The Petriplates were kept under incubation at 27°C and RH 80%. The experiment was laid out in a Completely Randomized Design where each treatment was replicated four times. Observations on the radial growth of fungus at 2, 4, 6, 8, 10, 12, and 14 DAI (Days after inoculation) were recorded.

Growth inhibition of *B. bassiana* RB PTB in the poisoned media was worked out using the following formula suggested by Sain et al. (2019).

I = (C-T) / C X100

Where,

I = Percentage growth inhibition, C = diameter of the mycelial growth in control Petri plates, T = diameter of mycelial growth in treated Petri plates.

The data were subjected to analysis of variance (ANOVA) through the WASP 2 software.

The effect of insecticides and fungicides on the radial growth of *B. bassiana* RB PTB at recommended doses is depicted in Tables 3 and 4. Among the tested insecticides, flubendiamide 20 WG (0.005%) and thiamethoxam 25 WG (0.005%) were compatible, with lowest per cent inhibition of 10.58 and 12.02, and colony diameter of 4.65 cm and 4.57 cm at 14 DAI respectively as against 5.2 mm in control (Fig.1). In contrast, Malathion 50 EC and acephate 75 SP were slightly harmful, with inhibition of 59.61 and 66.35 per cent, respectively. The highest radial growth observed on 14th day of incubation was 2.10 and 1.75 cm for malathion 50 EC and acephate 75 SP, respectively.

Table 3. Radial growth of *Beauveria bassiana* RB PTB on SMAY poisoned with insecticides

Insecticides	Colony diameter (cm)*DAI						
	2	4	6	8	10	12	14
Malathion 50% EC	0.00^{b}	0.00^{c}	0.17^{d}	0.60c	1.00^{c}	1.50^{d}	2.10^{d}
Acephate 75% SP	0.00^{b}	0.00^{c}	0.12^{d}	0.55^{c}	0.90^{d}	1.17^{e}	1.75^{e}
Flubendiamide 20% WG	0.00^{b}	0.45^{b}	1.00^{c}	2.15^{ab}	2.85^{b}	4.05^{b}	4.65^{b}
Thiamethoxam 25% WG	0.00^{b}	0.45^{b}	1.22^{b}	2.12^{b}	2.85^{b}	3.95^{c}	4.57^{c}
Control	0.15^{a}	0.70^{a}	1.50^{a}	2.20^{a}	3.10^{a}	4.40^{a}	5.20^{a}
CD (0.05)	0.039	0.055	0.058	0.182	0.055	0.121	0.065
SE (m)	0.013	11.411	0.019	0.021	0.018	0.021	0.021

*Mean of four replications.; DAI- Days after inoculation; In each column figures followed by same letter do not differ significantly

Table 4. Radial growth of *Beauveria bassiana* RB PTB on SMAY poisoned with fungicides

Fungicides	Colony diameter (cm)*DAI						
	2	4	6	8	10	12	14
Carbendazim 50 WP	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}
Mancozeb 75 WP	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}
Hexaconazole 5 EC	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}
Copper hydroxide 77 WP	0.00^{b}	0.65^{b}	1.47^{a}	2.17^{a}	3.07^{b}	4.37^{a}	5.15^{b}
(Tebuconazole 50% + Trifloxystrobin 25%) 75 WG	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}
Control	0.15^{a}	0.70^{a}	1.50^{a}	2.20^{a}	3.10^{a}	4.40^{a}	5.20^{a}
CD (0.05)	0.035	0.035	0.030	0.030	0.030	0.030	0.035
SE (m)	0.012	0.016	0.010	0.010	0.010	0.010	0.012

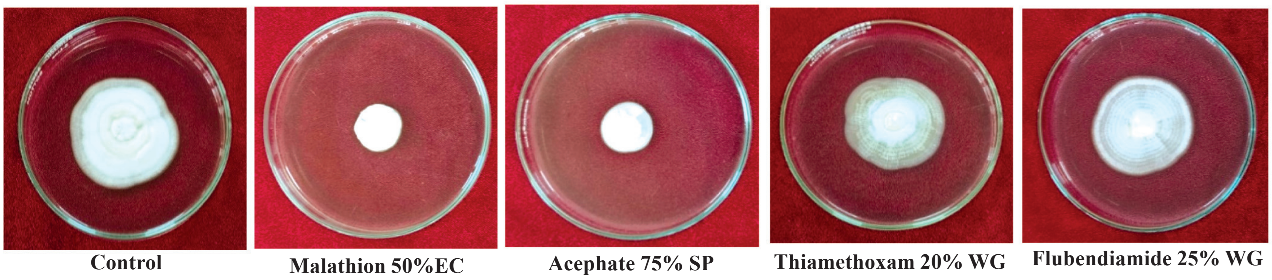

Figure 1. Radial growth of *Beauveria bassiana* RB PTB with insecticides on 14 DAI

Among the five fungicides tested, copper hydroxide 77 WP (0.2%) was compatible, which exhibited a significant radial growthwith colony diameters of 5.15 cm at 14 DAI (Fig. 2) and per cent inhibition was only 0.96. However, carbendazim 50 WP (0.1%), mancozeb 75 WP (0.2%), hexaconazole 5 EC (0.2%) and tebuconazole 50% + trifloxystrobin 25% 75 WG (0.04%) completely inhibited the growth of *B. bassiana* RB PTB as no fungal growth was observed at 14 DAI with 100 per cent inhibition.

In the study, flubendiamide 20 WG and thiamethoxam 25 WG were found to be compatiblewith *B. bassiana* (Fig. 3).

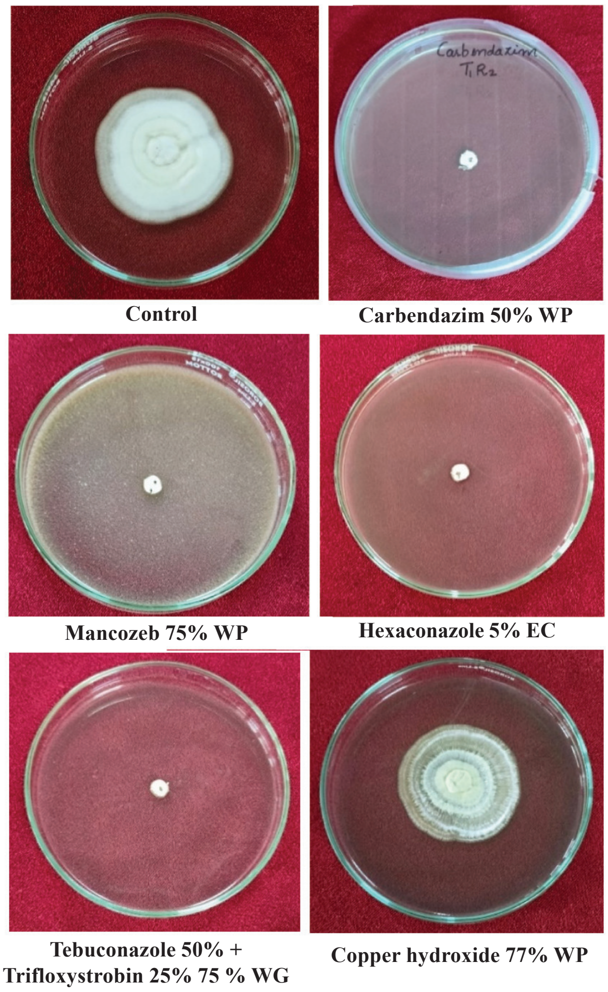

Figure 2. Radial growth of *Beauveria bassiana* RB PTB with fungicides on 14 DAI

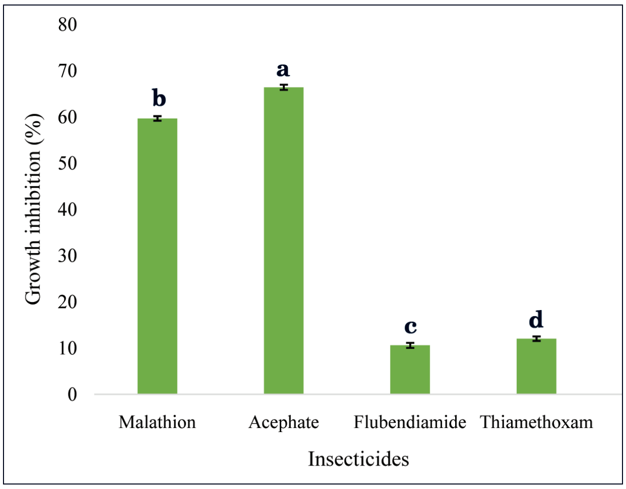

Figure 3. Effect of insecticides on the growth inhibition of *Beauveria bassiana* RB PTB

This could be attributed to the characteristics of *B. bassiana,* in which the fungus can use the active ingredient (a.i.) and/or components for growth as nutrient sources, thereby promoting vegetative growth and conidiogenesis. The results are in agreement with Oliveria et al. (2003), who stated that there was no inhibition on the growth and sporulation of *B. bassiana* with thiamethoxam 25WG.It is further supported by Nirgude et al. (2023) who reported that the per cent inhibition of *B. bassiana* with thiamethoxam 25 WG was only 7.12 per cent and 15.59 per cent with flubendiamide 39.5 SC with mean mycelial growth of 30.33 mm and 26.67 mm, respectively, at 12 DAI.

Beegum and Subramanian(2019) reported that malathion 50 EC registered a higher per cent inhibition of 70.29 per cent, followed by acephate with per cent inhibition of 31.29. The inhibition of growth can be attributed to the direct disruption of cell wall formation by organophosphate compounds, as Ghini and Kimati (2000) opined, which inhibits the enzyme responsible for converting phosphatidylethanolamine into chitin. The inhibition in sporulation and colony growth mayalso be due to the action of active components of insecticides, which directly disrupt cell membrane permeability, enzyme synthesis, and the metabolic processes of EPF that facilitate conidial germination (Fauziah and Rohdiana, 2016).

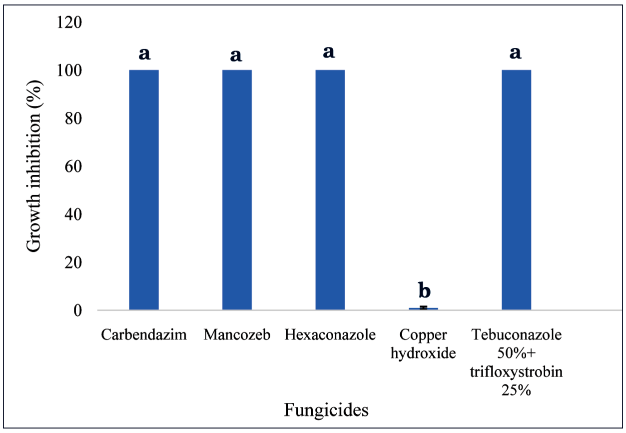

Figure 4. Effect of fungicides on the growth inhibition of *Beauveria bassiana* RB PTB

The study indicated that *B. bassiana* RB PTB is compatible withcopper hydroxide 77 WP. These results confirm the findings of Erdoðan and Saðlan (2023), who reported that in *B. bassiana*, copper hydroxide exhibited the highest mycelial growth of 22.40 mm and the lowest inhibition rate of 26.80 per cent compared to the control. Fungicides *viz*., carbendazim 50 WP, mancozeb 75 WP, hexaconazole 5 EC, and tebuconazole 50% + trifloxystrobin 25% 75 WG, completely prevented the growth of *B. bassiana* RB PTB (Fig. 4). Bartlett et al. (2002) reported thattriazole fungicides, such as tebuconazole, inhibit ergosterol biosynthesis, thereby hindering the formation of the fungal cell membrane.

Similarly, Joshi et al. (2018), documented that *B. bassiana* was inhibited at all the tested concentrations of carbendazim 50 WP, hexaconazole 5 EC, and propiconazole 25 EC. Similarly, Nirgude etal., (2023) reported that all three tested fungicides *viz*., carbendazim 50 WP, copper oxychloride 50 WP, and propiconazole 25 EC resulted in the highest growth suppression of 84.69 per cent, indicating their incompatibility with *B. bassiana*

Conclusion

The study revealed that the indigenous isolate *of B. bassiana* RBPTB is compatible with selected insecticides and fungicides used in rice pest management under *in vitro* conditions. Further field studies are to be undertaken to validate the results observed in the laboratory conditions. The integrated use of *B. Bassiana* with compatible chemical pesticides is a promising strategy for IPM programs. The findings of this study may facilitate to develop recommendations in the future for the use of chemical pesticides in IPM programs where *B. bassiana* is utilized as a control strategy against rice bugs.

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