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RESEARCH ARTICLE

Bio-efficacy of *Beauveria bassiana* RB PTB against rice bug (*Leptocorisa acuta*, Thunberg) and brown plant hopper (*Nilaparvata lugens*, Stål) and its safety to natural enemies

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Abstract

Beauveria bassiana (Balsamo) Vuillemin is an entomopathogenic fungus, ubiquitous in distribution, with a remarkably broad and diverse host range. A new native isolate of *B. bassiana* RB PTB was obtained from an epizootic observed on rice bugs at the Regional Agricultural Research Station (RARS), Pattambi, Kerala, India. The study aimed to evaluate the bio-efficacy of the new isolate on major sucking pests of rice *viz.,* rice bug, *Leptocorisa acuta*, brown planthopper (BPH), *Nilaparvata lugens and* its safety on beneficial insects in the rice ecosystem under laboratory conditions. The isolate was characterized by its high speed of kill, causing 100 % mortality in adult rice bugs and brown planthopper at 120 hr after treatment with a spore concentration of 10^8 spores mL^{-1}. Probit analysis revealed a time-dependent increase in virulence in rice bugs, with an LC_{50} of 1.4×10^8 spores mL^{-1} at 24 hr after treatment (HAT) and LT_{50} was 1.63 days at 10^8 spores mL^{-1}. Additionally, *B. bassiana* RB PTB (10^8 spores mL^{-1}) was safe to key natural enemies, including the coccinellid predator *Micraspis discolor*, long-jawed orb-weaver spider *Tetragnatha mandibulata* and parasitoids, *viz*., *Goniozus nephantidis*, *Trichogramma chilonis* and *T. japonicum*. These findings highlight the potential of *B. bassiana* RB PTB as a promising biocontrol agent against rice bugs and BPH, major sucking pests of rice, while ensuring the safety to natural enemies in the rice ecosystem.

Keywords: bio-efficacy; *Beauveria bassiana* RB PTB; entomopathogenic fungus; *Leptocorisa acuta; Nilaparvata lugens*

Introduction

Rice (*Oryza sativa*) is a vital crop, ranking third in global production after corn and wheat and a staple part of the daily diet for almost half of the world's population. Its importance is underscored by the current global production of 538.9 million tonnes (1). In India, rice plays a vital role in the national economy and serves as a key pillar of food security. However, its production is threatened by major biotic factors, including insect pests, disease and weeds. Rice earhead bug, *Leptocorisa* spp. and brown planthopper, *Nilaparvata lugens* are the most serious sucking pests of paddy and are prevalent throughout all rice growing tracts in India (2). Currently, pest management primarily relies on the application of chemical pesticides. Excessive use of synthetic insecticides during the flowering and milky stages of rice can leave harmful residues in grains, posing risks to human health. Rising concerns over the residues and the demand for residue-free food emphasize the need for focused research on insect microbial pathogens (3, 4).

Entomopathogenic fungi (EPF) have been receiving significant attention as potential biocontrol agents for the management of rice pests. One of the bottlenecks in the use of entomopathogens for rice pest management is the nonavailability of indigenous isolates. An indigenous isolate was obtained from an epizootic observed on rice bugs. It was subsequently identified as a new isolate of *B. bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) based on molecular and morphological criteria (5). The sequence has been deposited in NCBI (Accession no: OP023314). Indigenous isolates are more virulent and highly pathogenic compared to exotic strains and are well adapted to the prevailing environmental conditions. Extensive research worldwide has documented variations in the virulence of *B. bassiana* isolates from different regions against various insect species. Therefore, an investigation was initiated at the College of Agriculture, Vellanikkara, Kerala Agricultural University, Kerala, to evaluate the bio-efficacy of *B. bassiana* RB PTB against rice bug, *L. acuta* and brown planthopper, *N. lugens.* Additionally, its safety to natural enemies in the rice ecosystem was assessed.

Materials and Methods

Fungal isolate

Pure culture of *B. bassiana* RB PTB was maintained on Sabouraud Maltose Agar and Yeast (SMAY) slants and stored at 4 °C. Fourteen-day-old cultures in SMY broth were blended and sieved through double-layered muslin cloth and the spore count was estimated using a Neubauer haemocytometer. Different concentrations of spore suspension were prepared with 0.01 % Tween-80 and vortexed thoroughly.

Bioassay on rice ear head bug, *Leptocorisa acuta*

As the new isolate was obtained from rice bugs and its pathogenicity was already established (5), a bioassay with different concentrations of *B. bassiana* RB PTB was conducted. The field-collected rice bugs were reared on rice plants at the milky grain stage under polyhouse conditions. Adult insects of uniform age and physiological conditions collected from the cage were used for conducting the bioassay. Panicles at the milky grain stage were clipped off, the cut ends were covered with moist cotton to prevent drying up and kept in plastic jars. Adult rice bugs were released at the rate of ten per plastic jar and six replications were maintained. Spore suspensions of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 spores mL^{-1} were sprayed uniformly over rice bugs using an atomizer. Sterile water spray served as a control. Mortality was recorded every 24 hr up to 240 hr. Dead insects were kept in a moist chamber (Petri dish lined with a moistened filter paper) and observed for mycosis to confirm fungal infection as the cause of death.

Bioefficacy on brown planthopper, *Nilaparvata lugens*

Brown planthopper, *N. lugens* was reared on one-month-old rice plants established in pots under rearing cages. For carrying out host range studies, two-week-old rice seedlings were grown in paper cups and each paper cup was covered using cylindrical cages made up of a mylar sheet. Adults were collected from the rearing cages using an aspirator. Ten insects were released per plant, with three replications. The insects were subjected to a topical spray of *B. bassiana* RB PTB 10^8 spores mL^{-1} and observations on mortality were recorded at 24 hr intervals up to 120 hr. Dead insects in the treated plants were transferred singly to humid chambers prepared with Petri dishes lined with moist tissue paper to facilitate mycosis.

Re-isolation of fungus from cadaver

After the appearance of fungal outgrowth on insect cadavers, they were surface sterilized using sodium hypochlorite Hi-AR™/ACS 4 % (NaOCl) for 3 min. The cadavers were then passed through sterile water thrice to remove the traces of sodium hypochlorite and were kept over sterile tissue paper to ensure the removal of water. The cadavers were then placed over SMAY plates and incubated at 27 °C in darkness in a BOD incubator (Rotek Plus, India). The Petri plates were regularly observed for fungal growth from cadavers (6). Fungal growth observed on the cadavers was transferred aseptically into SMAY tubes. Slides were prepared and the colonies were characterized based on morphological keys (7). Fungal structures were observed under a trinocular microscope (DM 200LED, Leica) for morphological confirmation.

Safety of *Beauveria bassiana* RB PTB to natural enemies

Predators

***Micraspis discolour* Fabricius**: The safety of the effective dose of *B. bassiana* RB PTB was tested on predators and parasitoids commonly found in the rice ecosystem. Adults of *M. discolor* were collected from rice fields using an aerial net and transferred to plastic bottles (17 cm × 10 cm) at 10 insects per bottle. Treatment was replicated thrice and treatment with sterile water served as control. Spore suspensions of 10^8 spores mL^{-1} were directly sprayed over test insects using an atomizer. After an hour, treated insects were provided with 10 % honey solution (8). Mortality was recorded at 24 hr intervals up to 120 hr.

***Tetragnatha mandibulata* Walckenaer**: Long-jawed orb-weaver spiders, *T. mandibulata* were collected from the rice field using an aerial net and kept in plastic bottles of 17 cm × 10 cm dimensions with five spiders per bottle. Safety test was conducted with direct conidial application of 10^8 spores mL^{-1} with five replications. After an hour of treatment, the treated group was provided with aphid colonies as a food source (8). Mortality was recorded at 24 hr intervals up to 120 hr.

Parasitoids

***Goniozus nephantidis* Muesebeck:** Ten adults of *G. nephantidis* were released to plastic bottles of 17 cm × 10 cm dimensions and provided with honey solution as a food source. The walls of the bottles were given a fine spray of spore suspensions of 10^8 spores mL^{-1} prior to the release of parasitoids. Treatment was replicated thrice and treatment with sterile water served as control (8).

***Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead:** Egg cards of *T. japonicum* and *T. chilonis* were cut into six bits of 4 cm × 2 cm from cards of each species. The trichobits of *T. chilonis* and *T. japonicum* were directly sprayed with a spore suspension of 10^8 spores mL^{-1}, while the control was sprayed with sterile distilled water (9). Three replications were maintained and observations were recorded at 24 hr intervals for 120 hr.

Data analysis

The data of bioefficacy studies were subjected to Analysis of Variance (ANOVA) using WASP 2.0 software and probit analysis was carried out using PoloPlus software.

Results

Bioassay on rice earhead bug, *Leptocorisa acuta*

Beauveria bassiana RB PTB, with 10^8 spores mL^{-1}, was pathogenic to adult rice bugs and a dose-dependent mortality was recorded at varying spore concentrations. The highest mean cumulative mortality of 41.67 % was observed at 24 HAT, which was further increased to 58.33, 91.67, 96.67 and 100 % at 48, 72, 96 and 120 HAT (Table 1). The corresponding mean mortality elicited with 10^7 spores mL^{-1} was 30.00, 46.67, 73.33, 83.33 and 90.00 % and for the spore suspension of 10^6 spores mL^{-1}, the mean cumulative mortality was 16.67, 31.67, 65.00, 73.33 and 81.67 respectively.

The LC_{50} value of *B. bassiana* RB PTB (Table 2) at 24 hr after treatment on *L. acuta* was $1.4×10^8$ spores mL^{-1}. While the LC_{50} value was reduced to $2.3×10^7$ spores mL^{-1} after 48 hr. Similarly, *B. bassiana* RB PTB caused 50 % mortality at $3.1×10^4$, $2.9×10^3$, $2.6×10^2$ and $1.2×10^2$ spores mL^{-1} at 72, 96, 120 and 144 hr, respectively. At 168 hr of treatment, the lowest LC_{50} of $3.4×10^1$ spores mL^{-1} was recorded. The LT_{50} value computed for *B. bassiana* RB PTB against *L. acuta* is presented in Table 3. At the highest concentration of $1×10^8$ spores mL^{-1}, *B. bassiana* RB PTB showed the lowest LT_{50} of 1.63 days. As the spore concentration decreased, the LT_{50} value increased from $1×10^7$ spores mL^{-1} (2.28 days) to $1×10^6$ spores mL^{-1} (2.94 days), $1×10^5$ spores mL^{-1} (3.80 days), $1×10^4$ spores mL^{-1} (4.14 days) and $1×10^3$ spores mL^{-1} (4.67 days).

Bioefficacy on brown planthopper, *Nilaparvata lugens*

Strong virulence of *B. bassiana* RB PTB against the *N. lugens* were observed at spore suspension of 10^8 spores mL^{-1}. After 24 hr of treatment, 16.67 % mortality was recorded. The mortality increased to 40 % and 53.33 % at 48 and 72 HAT respectively. Mortality further increased to 76.66 % and 100 % at 96 and 120 HAT respectively (Fig. 1).

Table 1. Bioefficacy of *Beauveria bassiana* RB PTB against rice bug *Leptocorisa acuta*

Fungal isolate	Dose (spores mL^{-1})	Mean cumulative mortality of rice bugs (%)									
		24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	240 h
B. bassiana RB PTB	1×10^{3}	0.00^{d}	0.00^{e}	48.33^{d}	51.67^{d}	65.00^{d}	68.33^{c}	73.33^{d}	73.33^{d}	78.33^{c}	83.33^{b}
		(0.906)	(0.906)	(44.04)	(45.96)	(53.88)	(55.99)	(59.00)	(59.00)	(62.57)	(66.14)
	1×10^{4}	0.00^{d}	15.00^{d}	50.00^{d}	56.67^{d}	66.67^{d}	71.67^{c}	76.67^{d}	81.67^{c}	83.33^{c}	85.00^{b}
		(0.906)	(22.20)	(45.00)	(48.93)	(54.78)	(57.89)	(61.22)	(65.03)	(66.14)	(67.50)
	1×10^{5}	3.33^{d}	18.33^{d}	50.00^{d}	58.33^{d}	68.33^{d}	73.33^{c}	88.33^{c}	90.00^{b}	91.67^{b}	95.00^{a}
		(5.182)	(24.96)	(45.00)	(49.85)	(55.89)	(59.00)	(71.77)	(73.13)	(76.05)	(80.33)
	1×10^{6}	16.67^{c}	31.67^{c}	65.00^{c}	73.33^{c}	81.67^{c}	90.00^{b}	93.33^{bc}	98.33^{a}	98.33^{a}	98.33^{a}
		(23.85)	(34.07)	(53.88)	(59.10)	(65.04)	(73.13)	(77.40)	(86.17)	(86.17)	(86.17)
	1×10^{7}	30.00^{b}	46.67^{b}	73.33^{b}	83.33^{b}	90.00^{b}	93.33^{b}	96.67^{ab}	98.33^{a}	100.00^{a}	100.00^{a}
		(33.10)	(43.08)	(59.10)	(67.96)	(74.94)	(77.40)	(83.25)	(86.17)	(89.09)	(89.09)
	1×10^{8}	41.67^{a}	58.33^{a}	91.67^{a}	96.67^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
		(40.10)	(49.93)	(77.62)	(84.82)	(89.09)	(89.09)	(89.09)	(89.09)	(89.09)	(89.09)
	Control	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{d}	13.33^{c}
		(0.906)	(0.906)	(0.906)	(0.906)	(0.906)	(0.906)	(0.906)	(0.906)	(0.906)	(21.14)
	CD (0.05)	6.718	8.711	8.047	9.905	8.047	5.661	5.545	4.575	5.545	5.051
	CV	43.773	30.603	12.704	14.086	10.190	7.658	6.400	6.114	6.003	5.246

Figures in the parenthesis are arc sin transformed values. Mean values in each column followed by a common letter not significantly different.

Table 2. Dose mortality response of *Beauveria bassiana* RB PTB on adult *Leptocorisa acuta*

Exposure time (h)	LC_{50} (spores mL^{-1})	95 % fiducial limit (spores mL^{-1})	Slope ± SE	Chi square	df
24	1.4×10^{8}	5.2×10^{7}- 6.6×10^{8}	0.545 ± 0.078	3.19	4
48	2.3×10^{7}	7.3×10^{6}- 1.4×10^{8}	0.335 ± 0.057	0.62	3
72	3.1×10^{4}	3.9×10^{2}- 1.5×10^{5}	0.237 ± 0.075	0.82	2
96	2.9×10^{3}	1.1×10^{1}- 2.6×10^{4}	0.292 ± 0.045	6.49	4
120	2.6×10^{2}	0.00 – 2.6×10^{2}	0.290 ± 0.050	8.38	4
144	1.2×10^{2}	0.0003×10^{1}- 2.2×10^{3}	0.316 ± 0.055	6.46	4
168	3.4×10^{1}	0.05×10^{1}- 2.9×10^{2}	0.348 ± 0.065	1.80	4

LC_{50}= Concentration (spores mL^{-1}) to inflict 50 % mortality

Table 3. Exposure time response of *Beauveria bassiana* RB PTB on adult *Leptocorisa acuta*

Dose (spores mL^{-1})	LT_{50} (days)	95 % Fiducial limit (days)	Chi square	df	Slope
1 x 10^{3}	4.667	3.464 to 5.890	35.749	8	3.234
1 x 10^{4}	4.141	3.298 to 4.951	18.156	8	3.086
1 x 10^{5}	3.803	3.248 to 4.306	9.404	8	3.716
1 x 10^{6}	2.939	2.475 to 3.336	2.886	8	4.017
1x 10^{7}	2.286	1.742 to 2.734	3.645	8	3.661
1x 10^{8}	1.626	1.030 to 2.052	9.900	8	4.446

LT_{50}= Time (days) to inflict 50 % mortality

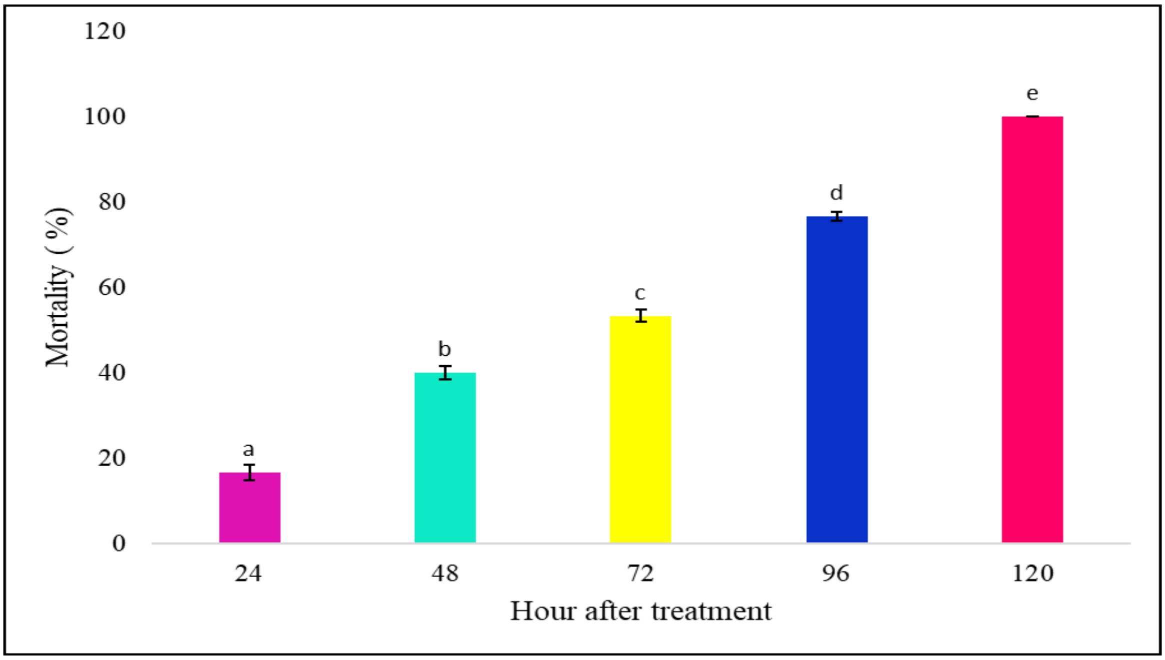


Fig. 1. Mean cumulative mortality (%) of *Nilaparvata lugens* caused by *Beauveria bassiana* RB PTB at 1×10^{8} spores mL^{-1}.

Mycosis on treated insects

Pathogenesis was observed in both rice bug and BPH. Mycosis was evident on the treated insects five days after their death. Dead insects exhibited growth of white mycelia, initially emerging from the legs, followed by antennal bases, inter-segmental membranes of the abdominal region and around the compound eyes gradually spreading over the entire body (Fig. 2 & 3). Re-isolation of the fungus from each mycosed insects confirmed the identity of *B. bassiana* RB PTB, which showed a radial growth of 7 cm at 21 DAI (Fig. 4).

Safety of *B. bassiana* RB PTB to natural enemies

Predators

The results of the safety studies revealed that the new isolate was not pathogenic to the coccinellid beetle, *M. discolour*. No death was observed till the end of the experimental period. However, in case of *T. mandibulata,* a mortality rate of 33.33 % was recorded at 120 HAT, but it did not develop any symptoms of mycosis after death.

Parasitoids

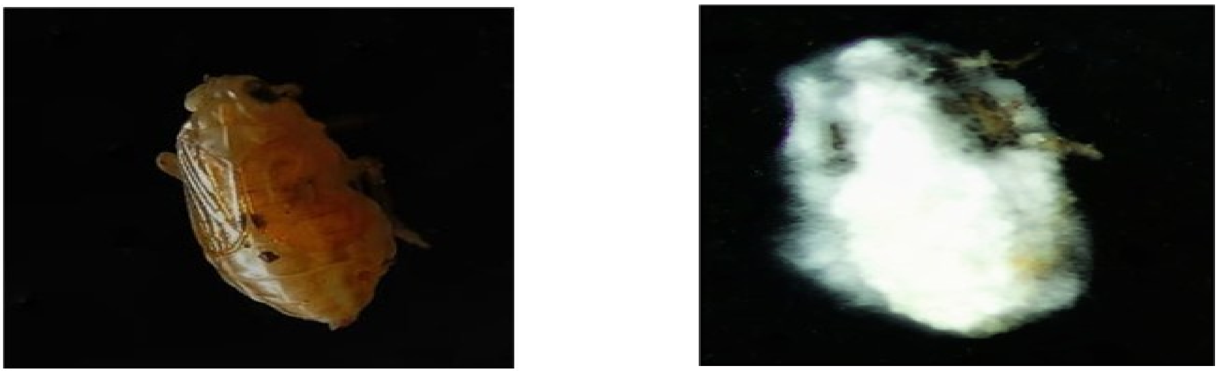
Beauveria bassiana RB PTB was safe for *G. nephantidis, T. chilonis* and *T. japonicum*, as no mortality and adverse effects were observed throughout the experimental period.

Discussion

The laboratory bioassays were conducted to evaluate the dose response to arrive at an accurate estimate of the virulence of an isolate. The effective dose of *B. bassiana* RB PTB against *L. acuta* was determined as 10^8 spores mL^{-1} with 100 % mortality at 120 HAT. The dose-dependent mortality observed in this study conforms with the findings of a previous study, where adult rice bugs treated *B. bassiana* VKA 01 showed similar dose-dependent mortality. They recorded 90.74 and 98.15 % mortality at 168 HAT in adults, when treated with 10^7 and 10^8 spores mL^{-1}, respectively (10).

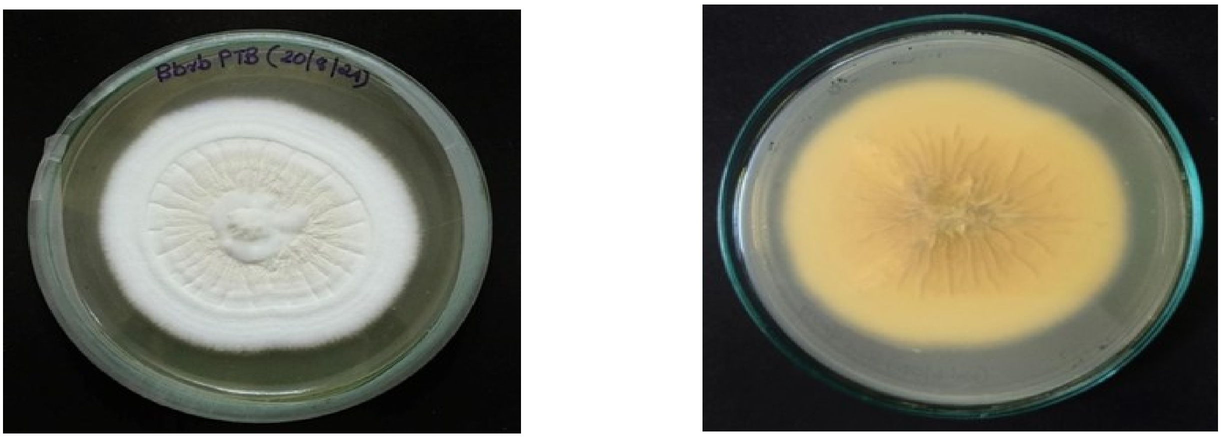
(a) **(b)**

Fig. 2. Symptom of mycosis on rice bug (a) Control, (b) Mycosis on treated rice bug, *Leptocorisa acuta*.



(a) **(b)**

Fig. 3. Symptom of mycosis on brown planthopper (a) Control, (b) Mycosis on treated BPH, *Nilaparvata lugens*.



(a) **(b)**

Fig. 4. *Beauveria bassiana* RB PTB re-isolated from mycosed insect (a) front view, (b) rear view.

The LC_{50} value of *B. bassiana* RB PTB decreased over time, indicating increased effectiveness with prolonged exposure. According to a previous study, *B. bassiana* HaBa at 1 ×10^8 conidia mL^{-1} caused a mortality of 75.39 % against *Aphis craccivora* at 7 DAT and recorded an LC_{50} value of 1.2×10^8 spores mL^{-1} on 72 HAT (11). The pathogenicity of *B. bassiana* (202) to *Mysus persicae,* which resulted in a lowest LC_{50} of 6.70 × 10^4 conidia mL^{-1} at 168 HAT (12). The *L. saksenae* spores were highly infective to rice bugs, where in concentrations of 10^8 and 10^7 spores mL^{-1} resulted in 100 % mortality within 72 HAT. The LC_{50} value for the spore suspension of *L. saksenae* was determined to be 2.99×10^4 spores mL^{-1} at 6 DAT for adult *L. acuta* (13).

At a highest concentration of 1×10^8 spores mL^{-1}, *B. bassiana* RB PTB recorded the lowest LT_{50} of 1.63 days. The results of the exposure time-response bioassay showed that LT_{50} values decreased with an increase in the concentration of fungal spore suspension. These findings are in close agreement with a previous study in which the LT_{50} values decreased with increasing concentration of *B. bassiana* HaBa. At the highest concentration of 1×10^{10} spores mL^{-1}, LT_{50} of 73.24 hr was recorded and at the lowest concentration of 1×10^4 spores mL^{-1}, LT_{50} of 157.39 hr was reported (11). The *L. saksenae* against rice bugs recorded LT_{50} values of 18.58 hr for 10^8 spores mL^{-1} and 19.97 hr for 10^7 spores mL^{-1} respectively, indicating effectiveness at both concentrations (13).

High virulence of *B. bassiana* RB PTB against the *N. lugens* was observed at spore suspension of 10^8spores mL^{-1} (Fig. 4). Conidial suspension of *B. bassiana* KN801 1×10^8 conidia mL^{-1} exhibited a significant lethal effect on brown planthopper adults (p = 0.0495, Tukey's test). Similarly, for *B. bassiana* KN802, at 1 × 10^8 conidia mL^{-1} also showed a lethal effect on adults (p < 0.0001, Tukey's test). These results highlight the potential of *B. bassiana* strains KN801 and KN802 as effective agents for controlling *N. lugens* populations (14). *Beauveria bassiana* at 1×10^8 spores mL^{-1} was pathogenic to *N. lugens* where mortality was 79.1 % after 10 days of treatment (15). The rapid mortality observed in *B. bassiana* RB PTB, as evidenced by 41.67 % mortality after 24 hr of treatment, is attributed to the insecticidal metabolites present in the spore suspensions apart from conidia. Pathogenicity through conidia alone is a slow process, typically requiring three to four days to cause mortality. This is supported by a previous study that the role of some toxic metabolites, in addition to the role of conidia to cause pathogenesis in *L. saksenae* to bring 100 % mortality within 96 hr of application (12).

Beauveria bassiana RB PTB was safe to natural enemies *viz*., *M. discolor, G. nephantidis, T. chilonis and T. japonicum* as no mortality was observed throughout the experimental period. However, in the case of *T. mandibulata,* a mortality rate of 33.33 % was recorded at 120 HAT, but it did not develop any symptoms of mycosis after death. These results are in close agreement with the findings of a previous study that the application of *B. bassiana* isolates (Bb-5a, Bb-9, Bb -36 and Bb-68) at a dose of 10^8 spores mL^{-1} had no adverse effect on *Cheilominus sexmaculata* at 7 DAT. In the case of *Micromus timidis*, the isolates tested showed very low larval mortality of 0.33- 2.90 % at the same time interval (16). The population of coccinellids and spiders before the treatment of *B. bassiana* ranged from 1.33 to 1.67 $plant^{-1}$ which was increased to 2.00 to 2.33 $plant^{-1}$ after 14 days of treatment. Therefore, *B. bassiana* was reported to be safe to natural enemies like spiders and coccinellids in the rice ecosystem as evidenced by their increased population (17).

Conclusion

A native isolate adapted to the prevailing local environmental conditions is preferable to an exotic one and the notable efficacy of *B. bassiana* RB PTB against rice bugs and brown planthopper highlights its potential as an alternative to chemical management of major rice pests. Safety of the bioagent revealed in this study on coccinellid predators, parasitoids *viz*., *G. nephantidis*, *T. chilonis* and *T. japonicum* also supports its integrated use with beneficial insects for sustainable pest management. Further studies are warranted for the field-level evaluation of *B. bassiana* RB PTB against rice bugs and BPH.

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Authors' contributions

AM contributed to investigation, visualization, and writing original draft. SP contributed to conceptualization, methodology, writing review and editing, and supervision. KK contributed resources. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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