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Efficacy of oil infused rice-based formulations of *Beauveria bassiana* (Balsamo) Vuillemi isolates (BPA/B7 and BKN-20) against *Helopeltis theivora* Waterhouse infesting tea

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Abstract

The *Beauveria bassiana,* a soil-borne, filamentous fungus known for its entomopathogenic nature, and commonly used as a myco-insecticide to control pests. This white muscardine fungus produces a number of cyclodepsipeptides such as beauvericin which is considered for its insect toxicity. The insecticidal effectiveness of two native isolates of *Beauveria bassiana* (BPA/B7 and BKN-20) from tea ecosystem was evaluated against *Helopeltis theivora*, a major pest of tea plantations, and studied the potential of oil infused rice media to enhance fungal performance. These indigenous isolates were cultured on rice-based substrates supplemented with different organic oils, and the parameters like fungal growth, sporulation, spore viability, and pathogenicity were assessed under laboratory conditions besides its effect on tea mosquito bugs at varying concentrations. Results showed that, coconut oil-infused rice media produced the highest mycelia growth (3.78cm) and spore germination (86.1%). The isolate BPA/B7 grown on 1% coconut oil infused rice media caused over 80% mortality of *H. theivora*, outperforming commercial formulations. The findings indicated that, rice powder supplemented with 1% coconut oil is a cost effective and efficient substrate for large scale production of *B. bassiana* under laboratory condition.

***Keywords**: Beauveria bassiana*, entomopathogen, biocontrol, tea mosquito, *Helopeltis theivora*,

Introduction

Tea (*Camellia sinensis* L. Kuntze) is one of the most economically significant plantation crops in India, contributing substantially to employment, export earnings, and rural livelihood, especially in the north-eastern states such as Assam and West Bengal. The tea production is continuously challenged by a variety of insect pests, among which the tea mosquito (*Helopeltis theivora* Waterhouse) is considered as a major sucking pest. This mirid bug infests the tender shoots and young leaves, sucking with their needle like rostrum after injecting toxic saliva containing several digesting enzymes into the tissues. The characteristic reddish brown necrotic lesions at feeding sites lead to curling, drying, and dieback of the shoots, causing direct loss in both yield and quality (Hazarika *et al.* 2009; Roy *et al.* 2021). The management of *H. theivora* has been traditionally relied on chemical insecticides, which initially are effective, however this approach gradually causes several ecological and agronomic problems, including pest resurgence, development of insecticide resistance, and contamination of tea leaves with pesticide residues (Sarmah *et al.* 2022). Since the manufactured tea is consumed as an infusion; the presence of chemical residues not only violates safety standards but also jeopardizes market acceptability and export potential. These concerns have driven the search for alternative pest management strategies that are environmentally safe, economically viable, and well-suited for sustainable tea cultivation. Among various biological agents, the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin has gain popularity due to its broad host range, natural occurrence, and safety to non-target organisms. It

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has been successfully used against numerous insect pests of different agroecosystems worldwide (Maniania, 1993; Feng *et al.*, 1994; Mascarin and Jaronski, 2016; Feng, 2004 and Shi and Landa *et al.* 2020). The fungal spores (conidia) attach to insect cuticle, germinate, and penetrate the host eventually leading to insect mortality. Importantly, *B. bassiana* can be mass produced and formulated into myco-insecticides suitable for integration into pest management system. Low-cost spore production methods can benefit any crop-based industry (Ferron 1978; Majumdar *et al.* 1995; Hazarika and Puzari 1997; Arcas *et al.* 1999). Despite its promise, widespread use of *B. bassiana* is constrained by challenges in mass production, limited shelf life, and inconsistent field performance. These limitations are often linked to the selection of sub optimal fungal strains and unsuitable culture media for conidial production (Feng *et al.* 1994; Mohammed *et al.* 2021). Strains isolated from the crop specific environment, such as tea soils or pests, are generally more effective under field conditions due to better adaptability and virulence (Majumder *et al.* 1995; Nelson *et al.* 1996). Therefore, it is essential to isolate, characterize, and optimize native strains of *B. bassiana* for each agro ecosystem.

Recent studies have shown that supplementing solid state fermentation substrates with natural plant-based oils such as coconut, sesame, and karanj oil can enhance fungal biomass, conidial production, and spore viability (Verma *et al.* 2020; Oliveira *et al.* 2023). Coconut oil improves the sporulation efficiency and spore viability, thereby increasing pest mortality upon application. Utilization of the agro-wastes such as rice husk, and saw dust seems to be an economically feasible alternative to synthetic media for fungal mass production (Arcas *et al.* 1999; Hazarika and Puzari, 1997). In the tea growing regions of Assam and West Bengal, rice-based substrates and organic oils are locally available, making them ideal candidate for developing region specific myco-insecticide formulations. However, there is limited literature evaluating the comparative performance of native *B. bassiana* isolates cultured on oil infused rice media for their bio-efficacy against *H. theivora* under laboratory conditions.

The present study was undertaken to (i) evaluate the compatibility of selected oil with native *Beauveria bassiana* isolates (BPA/B7 and BKN-20), (ii) to assess the influence of oil-infused rice-based substrates on fungal growth, viability, conidial production, viability and sporulation; (iii) identify economically viable substances for mass multiplication of the isolates and (iv) to evaluate the pathogenicity of the produced conidia against *H. theivora* under laboratory conditions.

Materials and methods

Fungal isolates

The entomopathogenic fungal isolates of *Beauveria bassiana*, BPA/B7 and BKN-20 were isolated from soils collected from tea plantations in Assam and West Bengal, respectively. Pure cultures were obtained through single-spore isolation and the isolates were identified by Dr. T. Pameela Devi, Indian Type Culture Collection (ITCC), IARI, New Delhi. The isolates were maintained on Sabouraud Dextrose Agar (SDA) with streptomycin sulphate (20µg/l) and incubated at 25±2ºC. Observations on mycelia growth and sporulation were recorded 10 days after incubation (DAI).

Compatibility with organic oils

Coconut, sesame, sunflower, castor, *karanj*, and neem oils were procured from local commercial suppliers. The compatibility of the fungal isolates with various organic oils was assessed using poisoned food technique (Nene and Thapliyal, 1993). The oils were mixed with molten PDA at 1%, and 5 mm fungal discs were placed centrally on solidified plats. Plates were incubated at 28±2ºC and observations were taken for 3, 6, 9, 10 and 14 days. Control plates contained PDA without oil. The percentage of growth inhibition was calculated using the formula provide by Hokkanen and Kotiluoto (1992).

$$Growth\ inhibition\ (\%) = \frac{Rcontrol\ Rtreatment}{Rcontrol}\ X100$$

$R_{control}$= radial growth in control; $R_{treatment}$= radial growth in treatment

Mass production

Solid substrates (rice powder, rice husk, or thatch; 100 g each) were soaked overnight in 100 ml distilled water, autoclaved at 121 º C and mycelial biomass was harvested on days 7, 14, and 21.

Determination of spore viability

One-gram of dried fungal biomass was mixed in 10 ml of distilled water with 0.1%tritonX100. The suspension was mixed well in vortex, filtered and adjusted to1X 10^7 conidia/ml using haemocytometer. The moisture content of fungal biomass was analyzed using the given formula

$$Moisture\ content\ (\%) = \frac{MLod\ Mlad}{Mlod} X100$$

Where, M_{Lod}= moisture content loss with oven drying; M_{Lad}= moisture content loss with air drying

Spore concentration

One gram of dried fungal culture was suspended in 10ml of sterilized distilled water with0.1% tritonX 100.The mixture was shaken, filtered, and conidia were counted using a haemocytometer. The conidial density was expressed as the number of conidia per gram of fungal biomass.

Bioassay

The third instar nymphs of *Helopeltis theivora* were used for the bioassay. Three replication per treatment were used and ten number of insects were used in each replication. The conidial suspension of isolates was applied using glass atomizer. Treated insects were kept at 25±2ºC and 80±5% RH. Percent mortality were recorded up to 7 days after treatment. Dead insects were surface sterilized and incubated to confirm mycosis.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) with the help of SPSS 16.0. Treatments means were compared using the Least Significant Difference (LSD) test at p<0.05. Values are expressed as Mean ± SE. All experiments followed completely randomized design with three replications per treatments. Percent mortality data were arcsine transformed prior to statistical analysis to normalize variance distribution. Toxicological classification of organic oils (very toxic, toxic, moderately toxic, and compatible) was based on T values from spore germination percentages.

Results

Results of compatibility study highlighted the impact of different oil infused rice media on the growth, conidial production, spore size, and germination of *B. bassiana* isolate. Table 1 shows that coconut oil infused media provide maximum growth of fungal mycelia (37.8 cm), conidial production (98.2X10^6 conidia/ml), and spore germination of 86.12%. Coconut oil is significantly outperforming the efficacy of other oils including control, indicating it provides optimal conditions for fungal growth and proliferation (**Fig. 1**). Sunflower, sesame, castor, and *karanj* oil also support the fungal growth and conidial production. Neem oil caused severe suppression of fungal growth and sporulation, reducing mycelia growth by 69.35%, although slight growth was recorded after prolonged incubation.

Table 1. Compatibility test of fungal isolate (BPA/B7) with the organic oils

Treatments	9 Days	Conidia number (10^6 per ml)	Spore Size (µm)*	Spore Germination*	T value	Classification
	Mycelial Growth (mm)*	% Reduction	10 DAI	10 DAI	10 DAI	
Karanj oil	29.3 ± 0.2	13.05	32.7± 3.7	64.2 ±0.5	57.2	C
Neem oil	11.2 ± 0.1	69.35	0	0	2.3	VT
Sesame oil	33.4 ± 0.7	0.89	28.1 ± 1.8	81.6 ± 21.0	71.9	C
Castor oil	30.0 ± 0.1	10.98	30.9 ± 1.8	74.1 ± 1.5	65.3	C
Sunflower oil	32.5 ± 0.2	3.5	30.2 ± 3.5	78.2 ± 2.0	69.1	C
Coconut oil	37.8 ± 0.2	-12.17	29.1±0.2	98.2 ± 0.9	86.1	C
Control	33.7 ± 0.2	0.00	34.1 ± 1.2	96.2 ± 0.9	-	-
SEm (+)	3.26 ± 0.3	2.07	4.5 ± 0.5	12.6 ± 2.9	-	-
F	12.92	15.10	2.221	9.86	-	-
CD(P=0.05)	0.73	18.01	2.160	4.55	-	-
CV%	18.51	73.86	4.672	3.70	-	-

* Values represent Mean ± SE of three replications (0-30= Very Toxic (VT), 31-46=Toxic, 46-60=moderately Toxic (MT) and ≥60= Compatible)

Table 2. Day-wise effect of different organic oil on the growth and sporulation of *Beauveria bassiana* (BPA/B7)

Treatments	Colony area						Number of Conidia
	3 Days		6 Days		9 Days		9 Days
	Mycelial Growth (cm)	% Reduction over control	Mycelial growth (cm)	% Reduction over control	Mycelial growth (cm)	% Reduction over control	(10^6 ml /L)
Control	1.9 ± 0.1	-	2.8 ± 0.10	-	3.7 ± 0.2	-	10.3 ± 0.8
Karanj oil	1.5 ± 0.2	27.1	2.2±0.16	4.39	2.9 ± 0.2	21.24	11.4 ± 0.2
Neem oil	0	100	0	100	0.4 ± 0.2	90.05	0
Sesame oil	1.7 ± 0.1	17.1	2.1±0.49	15.1	3.3 ± 0.7	10.22	8.1 ± 1.1
Castor oil	1.3 ± 0.2	33.2	1.9±0.10	20.82	3.0 ± 0.1	19.35	8.3 ± 0.1
Sunflower oil	1.8 ± 0.2	10.1	2.4±0.19	4.08	3.3 ± 0.2	12.63	16.0 ± 0.9
Coconut oil	1.8 ± 0.2	5.33	2.5±0.22	-1.22	3.78 ± 0.5	-1.61	2.1 ± 0.3
SEM	0.38	-	0.36	-	0.41	-	-
F	0.909	-	0.805	-	0.41	-	-
CD (P=0.05)	0.423	-	0.352	-	0.717	-	-
CV %	23.397	-	14.357	-	19.287	-	-

* Values represent Mean ± SE of three replications

Time course studies on compatibility between organic oils and *Beauveria bassiana* (BPA/B7)

BPA/B7 (*Beauveria bassiana*) isolate placed on media infused with neem oil showed complete suppression, followed by castor oil with a reduction percentage of 33.17 % after the third day of inoculation (Table 2). Maximum mycelia growth was recorded in the media infused with coconut, followed by control and sunflower oil, with a growth of 1.99 cm, 1.83, and 1.79 cm, respectively. Similarly, after 6th day, the neem oil maintained growth suppression, while castor oil caused 20.82% reduction in growth. The maximum growth was observed in coconut oil-infused media, followed by control and sunflower oil, with a growth of 3.78 cm, 3.72 cm, and 2.35 cm respectively. By the 9th day, *Beauveria bassiana* on neem oil infused media showed a partial suppression, with a mycelial diameter of 0.37 cm, indicating a reduction due to the inhibitory effect in neem oil over time. Notably all the organic oils tested were compatible with the growth of BPA/B7 (*Beauveria bassiana*), and only neem oil, which demonstrated anti-fungal properties throughout the study. On the7th day of incubation, the mean radial growth was found to be the highest on media infused with 1% *Karanj* oil (2.2cm), followed by media infused with 1% sesame oil (1.9 cm). Minimum mean radial growth was observed by media infused with 1% coconut oil (1.4 cm), followed by media infused with 1 % yeast. On the 14th day of incubation, the mean radial growth of coconut increased by double recording a maximum mean radial growth of 2.3 cm, which is equal to the mean radial growth of the fungi on media infused with 1% *karanj* oil (2.3cm). Minimum mean radial growth was observed in media infused with 1% yeast (1.9cm). The observations of growth on coconut oil suggest that the fungi require time to acclimatize to it, and once adjusted, they can grow rapidly.

Comparative percent mortality caused by BPA/B7 culture infused with 1% coconut oil and commercial formulation (CS)

The sporulation percentage is highest in the control followed by coconut oil and sesame oil. The sporulation percentage was recorded as the highest in the control followed by coconut oil and sesame oil. The Sporulation percentage in sunflower oil and castor oil were more than 70%, and *Karanj* oil-infused media supported *B. bassiana* sporulation with 64%. The percent mortality of BPA/B7 grown on media infused with 1% coconut oil and commercial formulation at a concentration of $1X10^6$ was tested against the pest and found that BPA/B7 gave a better result compared to the commercial formulation (**Fig.** 2). At a concentration of 25ml, the commercial sample gave a maximum mortality rate of 60%, whereas the BPA/B7 grown on infused media gave more than 80% mortality of the pest. However, the mortality caused by BPA/B7 (25ml) grown on infused media is lower than that of the crude extract of BPA/B7 (25ml) (Fig. 3)

Fig 1. Differential growth of BPA/B7on media infused with different organic oil and control (1% yeast)

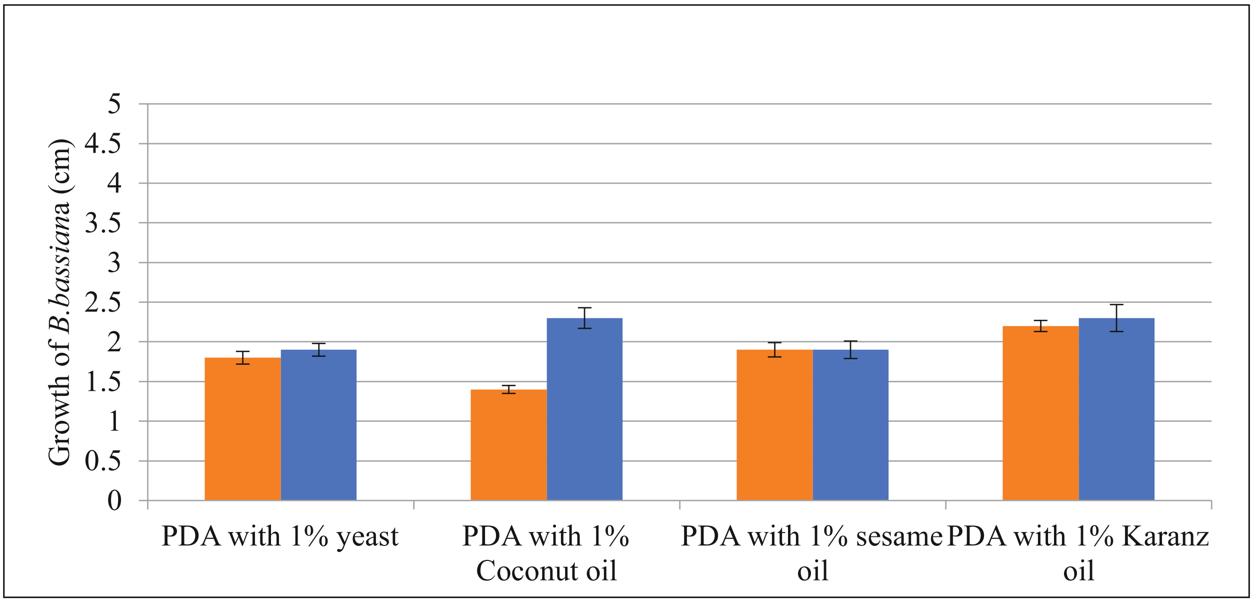
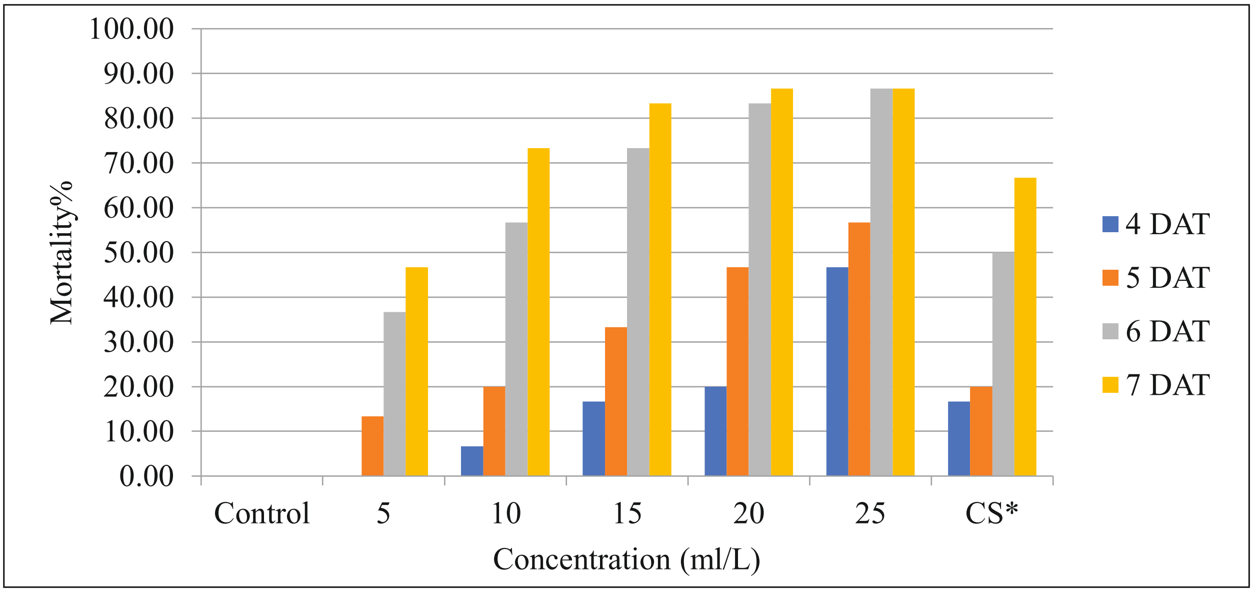


Fig 2. Percent mortality tea mosquito bug caused by BPA/B7 culture reared on PDA infused with 1% coconut oil compared with Commercial Formulation sample (CS)



Mass production on cheap alternate material

The mean mycelial weight was maximum at PDB (5.09gm) followed by broth infused with 1% Coconut oil (5.13gm) and minimum was recorded at broth infused with saw dust (27gm) followed by broth infused with rice husk (2.9gm) (Fig.3). Observations were made up to 21th days in incubation, and mean mycelial weight was found to increase with each week starting from 2.71gm to5.80gm at 7th day to 21 day of incubation respectively. The rice husk gave result similar to oil infused broth so the different form of rice *viz* rice husk, thatch and rice powder were studied.

The rice powder media supported the maximal production of conidia for both isolates of *B. bassiana* (BPA/B7 and BKN20). Rice media infused with coconut oil yielded highest conidial production, followed by rice media with *karanj* oil (Fig. 3). It was also recorded that BKN-20 produces more conidia on rice forms than BPA/B7. Both the isolates showed the highest conidial production on rice media, with 1.47 g for BPA/B7 and 1.55 g for BKN-20. The lowest was recorded on rice husk and thatch, with BPA/B7 (0.55 g). BKN-20 was also found to retained higher moisture content compared to BPA/B7. The highest percent moisture was observed in BKN-20 grown on rice powder (6.98%), followed by BKN-20 grown on rice thatch (6.29%), while the minimum moisture content was noted in BPA/B7 grown on rice thatch (2.92%).

The fungal isolates grown on rice powder had higher sporulation and greater conidial virulence, leading to higher mortality rates compared to those grown on other rice substrates. The addition of nutritional supplements to the growth media enhanced sporulation conidial production, sporulation and insecticidal activity regardless of the substrate used (Table 3). Among the treatments, rice media supplemented with 1% coconut oil recorded the highest conidial production, sporulation and mortality for both isolates. BPA/B7 cultured on rice media infused with 1% coconut oil recorded highest conidial production, sporulation and mortality (78.33%), followed by rice media supplemented with 1% *karanj* oil (73.33%) (Table 3). The lowest mortality rate, around 35%, was observed for both isolates grown on rice thatch.

Fig 3. Mycelial growth of BPA/B7 on (A) PDB+1% Coconut Oil, (B) PDB+1% Sesame Oil, © PDB+1% Karanj Oil, (D) PDB+1% Rice Husk, (E) PDB+1% Saw Dust and (F) Potato Dextrose Broth

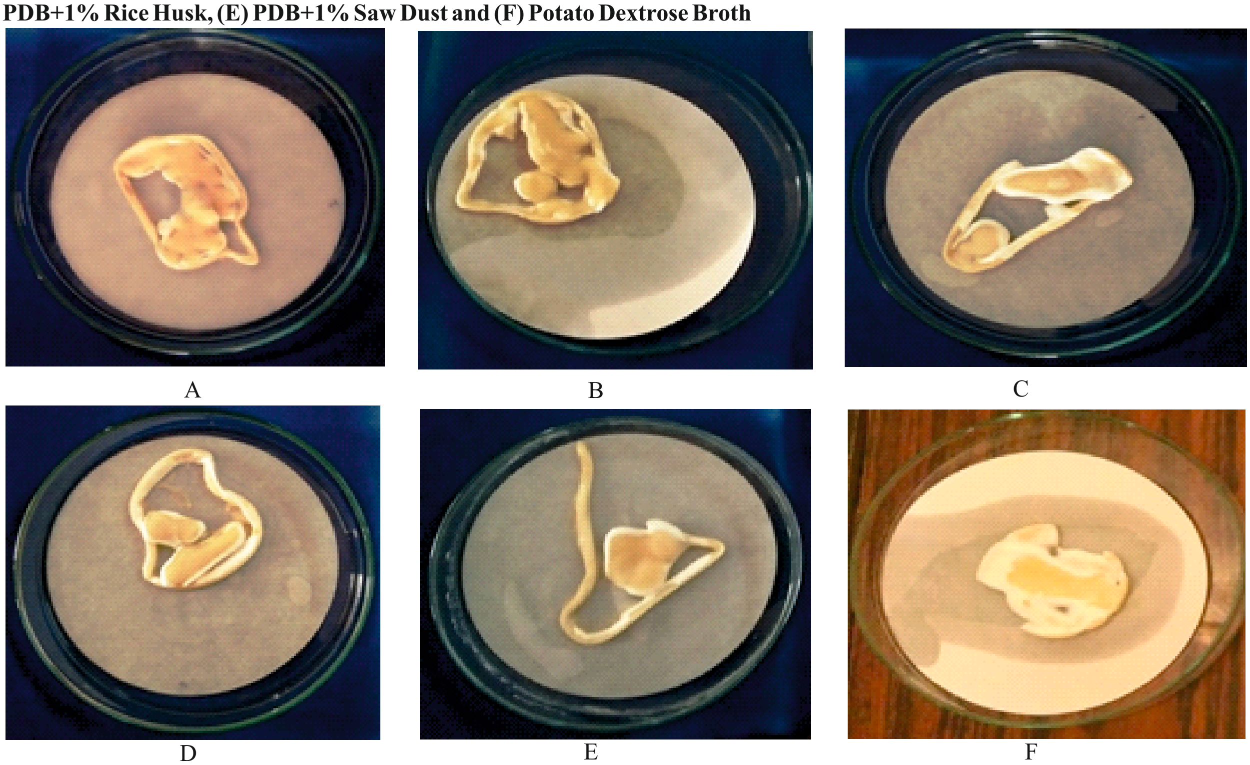


Table 3. Effect of rice-based substrate on conidial production, sporulation and percent mortality of *Beauveria bassiana* isolates (BPA/B7 and BKN 20)

Substrate	Conidial production (gm/100 gm of substrate)		Sporulation		Percent mortality	
	BPA/B7	BKN-20	BPA/B7	BKN-20	BPA/B7	BKN-20
Rice husk	0.55^{a}	0.88^{a}	4.8^{e}	6.0^{d}	55.00^{h}	46.67^{h}
Rice thatch	0.55^{a}	0.90^{ab}	3.0^{d}	6.3^{d}	35.00^{g}	35.00^{g}
Rice powder	1.57^{b}	1.55^{b}	4.9^{e}	6.9^{de}	66.67^{i}	56.67^{i}
Rice media infused with coconut oil	1.91^{c}	2.00^{c}	5.6^{f}	7.2^{de}	78.33^{j}	73.33^{j}
Rice infused with *karanj* oil	1.75^{bc}	1.80^{bc}	5.2^{ef}	7.0^{e}	73.33^{j}	60.00^{ij}

Values in columns followed by the different letter are significantly different with each other according to LSD ($p<0.01$)

Economics of the media

Among the different media used, the rice media with 1% coconut oil is found to be cost effective followed by rice media infused with 1% *karanj* oil and rice media with a production cost of Rs. 0.32, 0.33 and 0.37 respectively (Table 4). The production cost of fungi on commercial media is comparatively higher than the rice media.

Table 4. Production economics of different media

Media	Media code	Cost ₹/ kg (A)	Media quantity required/ litre (B)	Cost ₹ /litre media (C=A/ 1000XB)	Quantity of media used (ml or g per treatment) (D)	Cost ₹ / treatment (E=C/1000 X D)	Gram Biomass produced (F)	Production cost₹ /g (G=E/F)
PDA	MH096-500G	4210	39	164.19	25	4.10475	1.78	2.31
SDA	M063-500G	4300	65	279.5	25	6.9875	1.56	4.48
Rice husk	-	15	1000	15.00	25	0.375	0.55	0.68
Rice thatch	-	12	1000	12.00	25	0.3	0.56	0.54
Rice media	-	22	1000	22.00	25	0.55	1.48	0.37
Rice media with 1% KO	-	23.2	1000	23.20	25	0.58	1.75	0.33
Rice media with 1% CO	-	24.7	1000	24.70	25	0.62	1.91	0.32

Discussion

The current study investigated the compatibility, conidial productivity, and bio-efficacy of two native *Beauveria bassiana* isolates (BPA/B7 and BKN-20) cultured on rice-based media infused with different organic oils, targeting *Helopeltis theivora.* Among the tested oils, coconut oil was the most compatible and stimulatory, significantly enhancing fungal growth, conidial yield, and pest mortality. Organic oils have been previously reported to influence the growth and sporulation of entomopathogenic fungi. Our results corroborate the findings of Oliveira *et al.* (2023), who reported that coconut oil significantly increased conidial germination and virulence in *B. bassiana* and *M. anisopliae* isolates. Similarly, Verma *et al*. (2020) found that the addition of 1% coconut oil to rice media improved the yield and viability of *B. bassiana* conidia, supporting the hypothesis that certain fatty acids present in coconut oil (e.g. lauric acid) act as growth enhancers for fungal metabolism.

In contrast, we found the neem oil completely suppressed the growth and sporulation of both isolates of *B. bassiana.* This is consistent with the inhibitory effects observed by Maniania (1993) and Hokkanen and Kotiluoto (1992), who reported that neem-based formulations exert fungistatic activity against *B. bassiana* due to the presence of azadirachtin and related limonoids. While neem oil has insecticidal properties, its integration with entomopathogenic fungi may be antagonistic unless carefully optimized. We also found that BPA/B7 isolate cultured on coconut oil infused rice media caused over 80% mortality of *H. theivora*, outperforming a commercial formulation which is similar to BPA/B7 liquid formulation (76.7%) prepared in collaboration with Varsha Bio-science and Technology India Pvt. Ltd. (Ekka *et al.* 2023). This aligns with results from Mascarin and Jaronski (2016), who emphasized the role of substrate and strain compatibility in maximizing fungal virulence. Similarly, Sang Myeong *et al.* (1996) reported that native strains of *B. bassiana* grown on rice on rice and millet-based media showed enhanced sporulation and higher insecticidal activity when compared to non-native commercial isolates.

Further, our evaluation on alternative substrates including rice husk, rice thatch, and rice powder revealed that rice powder supported the highest biomass and conidial yield. These findings align with Nelson *et al.* (1996) findings who demonstrated the superior performance of finely milled rice derivatives. Different *B. bassiana* isolates exhibit variability in growth and sporulation across culture media. For instance, isolate GY1-17 showed maximal sporulation in rice bran and saw dust mix, while isolates Bb 9207and Bb 9301 achieved optimal mycelial growth and sporulation in composite media containing rice and millets (Sang Myeong *et al.* 1996). The advantage of rice-based substrate lies in their high carbohydrates content, moisture retention, and regional availability, which make them well suited for low-cost fungal cultivation (Arcas*et al.* 1999; Majumdar *et al.* 1995). Among the two isolates studied, BKN-20 consistently showed higher conidial output and moisture retention than BPA/B7 across all substrates. This inter-strain variability is in agreement with Mohammed *et al.* (2021), who emphasized that the ecological origin of *B. bassiana* isolates strongly influences their adaptability and performance on various culture media.

Conclusion

The different parts of rice can be effectively utilized as substrates for the cost-efficient mass production of *B. bassiana* under laboratory condition. Among them, rice powder infused with coconut oil is most supportive media, promoting optimal growth and sporulation of both isolates, BPA/B7and BKN-20. Further field evaluation and shelf-life studies can be done for large scale application in the field.

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Authorship contribution statement: PE: Methodology, Investigation, Formal analysis; **LRS & AB**: Funding acquisition, Formal analysis, Supervision and critical reviewing of manuscript.

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