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Research Article

Exploration of three native strains of entomopathogenic fungi against rice yellow hairy caterpillar, *Psalis pennatula* (Lepidoptera: Lymantriidae) in Assam

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ABSTRACT: Rice yellow hairy caterpillar *Psalis pennatula* Fabricius (Lepidoptera: Lymantriidae) is an emerging pest of rice and an increased incidence is expected in different rice-growing areas of Assam in the coming years. The caterpillar is a voracious leaf feeder and can consume the whole leaves within a day. Keeping this in view, an attempt was made to evaluate the bio-efficacy of three important native isolates of entomopathogenic fungal strains *viz. Beauveria bassiana* (KR855715), *Cordyceps javanica* (OM321438) and *Isaria fumosorosea* (MH414514) against *P. pennatula* larvae under laboratory conditions during the *Sali* season of rice. All three EPF strains at different conidial dilutions ($1x10^3$, $1x10^5$,$1x10^7$, $1x10^8$ and$1x10^9$ conidia/ml) were found to be pathogenic to the 3rd instar larvae of *P. pennatula*. Among the tested fungal strains, the strain *Isaria fumosorosea* (MH414514) showed the highest mortality (100%) at $1x10^7$ conidia/ml and *Cordyceps javanica* (OM321438) showed the lowest mortality (51%) at $1x10^7$conidia/ml at 10th day of post-treatment. The tested fungal isolates could play a vital role as microbial biopesticides in suppressing the *P. pennatula* population in Assam and could be incorporated into IPM strategies.

KEYWORDS: Entomopathogens, pathogenicity, rice crop, rice yellow hairy caterpillar

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INTRODUCTION

Rice is one of the most important cereal crops grown around the world and is considered an important staple food for half of the world's population. In terms of area India stands first (43.78 million hectares) and the world's second-largest producer (118.43 million tonnes) of rice, next to China, contributing to 43 per cent of food grain production and 46 per cent of cereal production in the country (Anonymous, 2020). In the regional context, rice is the single most important Crop of Assam, occupying an area of 2.35 million hectares with production of 43.82 million tonnes with productivity of 1886 kg/ha (Directorate of Economics and Statistics, Government of India, 2023). Assam has unique climatic and physiographic features, which renders it favorable for rice cultivation in three distinct seasons *viz*. Ahu (Feb/Mar-June/July), Sali (Jun/Jul-Nov/Dec) and Boro (Nov/Dec-May/Jun). The state produces 43.82 million tonnes of total rice, of which, the highest production was recorded during Sali (33.69 million tonnes), followed by Boro (8.44 million tonnes) and Ahu (1.68 million tonnes) (DES 2023).

Among all cereal crops, rice harbors many insect species at different growing stages right from seedling to harvesting of the crop. A total of 300 insect pest species attack the rice crop at different growing stages, with 23 of them posing an especially significant threat (Pasalu & Katti, 2006). In India, insect pests pose the biggest threat to rice production, resulting in a 25% loss (Mondal *et al*., 2017) and the most prevalent insect pests of rice belong to the order lepidoptera. Among lepidopteran pests of rice, *Sciropophaga incertulas* (Walker) and leaf folder, *Cnaphalocrocis medinalis* (Guenee), are considered the major economically important pest but in the present day scenario, another lepidopteran pest, i.e., Rice yellow hairy caterpillar *P. pennatula* Fabricius (Lepidoptera: Lymantriidae) is creating a havoc in some rice growing pockets of Assam which now can be considered as the emerging pest of paddy. The caterpillar is an acquisitive feeder, devouring whole tiller leaves in a single day. Management of rice pests has been a major problem for the farming community. Over the last decades, indiscriminate use of pesticides has hurt the environment as well as human

health. Moreover, several pests had developed resistance against the insecticides. However, the Government of India has also banned some insecticides and major emphases have been focused on to use of organic pesticides against the pest.

Assam is one of God's gifted hubs of many macro and micro-fauna, therefore emphasizing organic farming and natural farming are some of the important aspects and need of the hour to manage the insect pests by using such biological agents in this region. Some potent Entomopathogenic Fungi (EPF) are prevalent in the rice ecosystem and these Entomopathogenic fungi can also be utilized for the management of insect pests in rice. Among all the species of EPF, *B. bassiana* and *Metarhizium anisopliae* are considered important (Puzari & Hazarika 1992; Puzari *et al*. 1994; Mudoi *et al*. 2017) as they have great potential in controlling 700 insect species under the order Lepidoptera, Hemiptera, Orthoptera, Coleoptera and Diptera (Puzari & Hazarika 1992; Puzari *et al*. 1994). *Metarhizium anisopliae* and *B. bassiana* have also been widely used for controlling rice Brown plant hoppers, rice bugs, coconut beetles, grasshoppers, termites, etc. All microbial agents are safe, environmentally friendly, less threat to human health and generally harmless to non-target organisms. In India, though many commercial biopesticides have been recommended for the control of insect pests in different crops, their application has shown very limited efficiency because of the unavailability of target-specific bioformulation and non-adaptability to the climatic conditions of that region. Therefore to increase their efficiency in the field, knowledge of the biology of entomopathogenic fungi and its mode of action on the target pest is necessary. On the other hand, EPF can be applied in the organic rice pest management programme and could also be integrated as a key component of IPM strategies for *P. pennatula* control. In addition to these entomopathogenic fungi, there are some other potent entomopathogenic fungi which are effective against other pests of crops but their efficacy on lepidopteran pests of rice has not yet been ascertained. One such EPF is *I. fumosorosea* which is yet to be evaluated against the insect pest of rice other than *P. pennatula*. Hence, the objective of this work was to find out the effective fungal isolates that can kill the immature stage of *P. pennatula* and to develop microbial-based biopesticides against this notorious lepidopteran pest in Assam and abroad.

MATERIALS AND METHODS

The present study was carried out in the Physiology Laboratory, Department of Entomology, Assam Agricultural University, Jorhat during 2020-2022.

Sources of fungal strain

Three strains of entomopathogenic fungi namely *B. bassiana* (KR855715), *Cordyceps javanica* (OM321438) and *I. fumosorosea* (MH414514) were collected from the Department of Entomology, AAU, Jorhat.

Culturing of the fungal isolate

Potato Dextrose Agar (PDA, Himedia) was used as a medium for culturing fungal isolate. All the fungal strains were initially passed through larvae of *P. pennatula* and re-isolated from the infected cadavers showing typical fungal fluffy growth and then it was purely cultured and kept in a BOD incubator at 25 ± 1°C for 10 days for further study.

Field collection of *P. pennatula*

The mass rearing of *P. pennatula* in laboratory conditions was carried out in a rearing case providing rice seedlings. The adults as well as egg mass were collected from the field during the active tillering stage of the crop. The freshly emerged larvae were transferred into another set of tubs having fresh rice seedlings and the third-stage larvae were used for the pathogenicity study.

Pathogenicity test

The pathogenicity test of three strains was evaluated against the third instars larvae of *P. pennatula* following Das(2021)at room temperature (30±1°C, RH 85–90%). Five different conidial concentrations, namely $1x10^3$, $1x10^5$, $1x10^7$, $1x10^8$ and $1x10^9$ conidia/ml, were prepared by serial dilution technique. The fungal strain was diluted in water and combined with tween-80 @ 0.023%. Seedlings of 40 days old were placed in a conical flask (50ml) and then placed in a plastic tub. Third instar larvae of *P. pennatula* were discharged with the aid of a thin camel brush into a conical flask (50 ml) containing rice seedlings and caged in paired chimneys, one kept on top of the other and muslin cloth was put over the top chimney's open end and similar set of tubs were used for control (Figure 1).The larvae were then sprayed with the respective concentrations using a hand glass atomizer @ 25ml (Figure 2). Control tubs were treated with a sterile distilled water solution containing tween-80 (0.023%, v/v). Data on mortality were collected starting 24 hrs after treatment and continuing up to the 10^{th} day of treatment.



Figure 1. Overview of pathogenicity test under laboratory conditions.

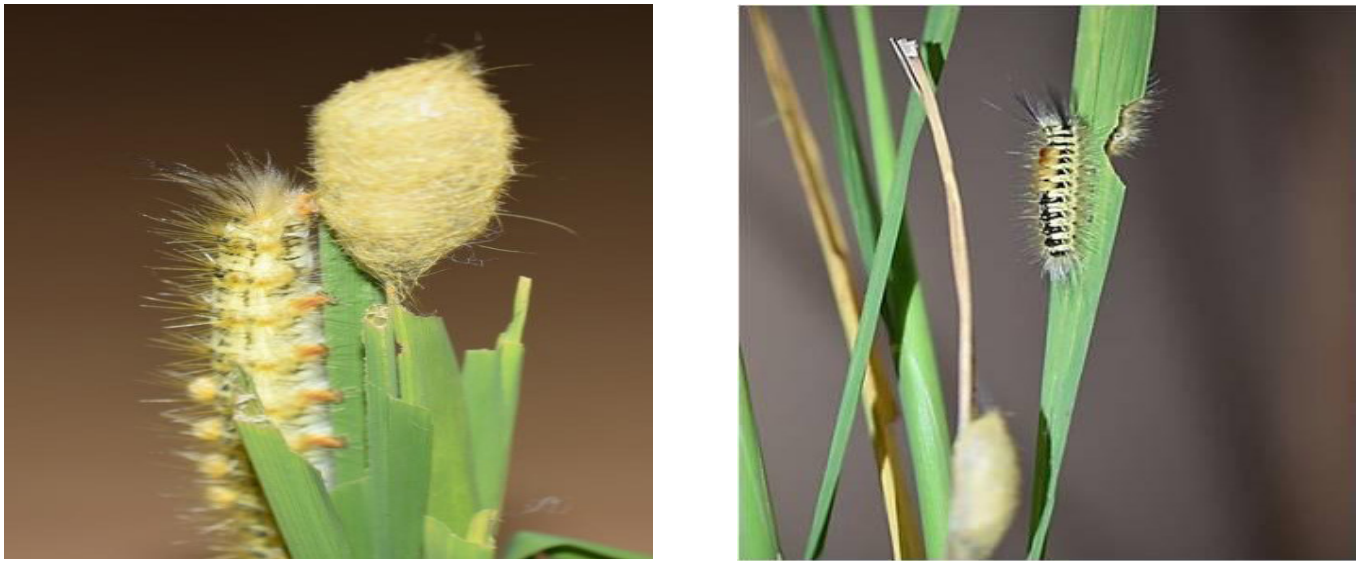
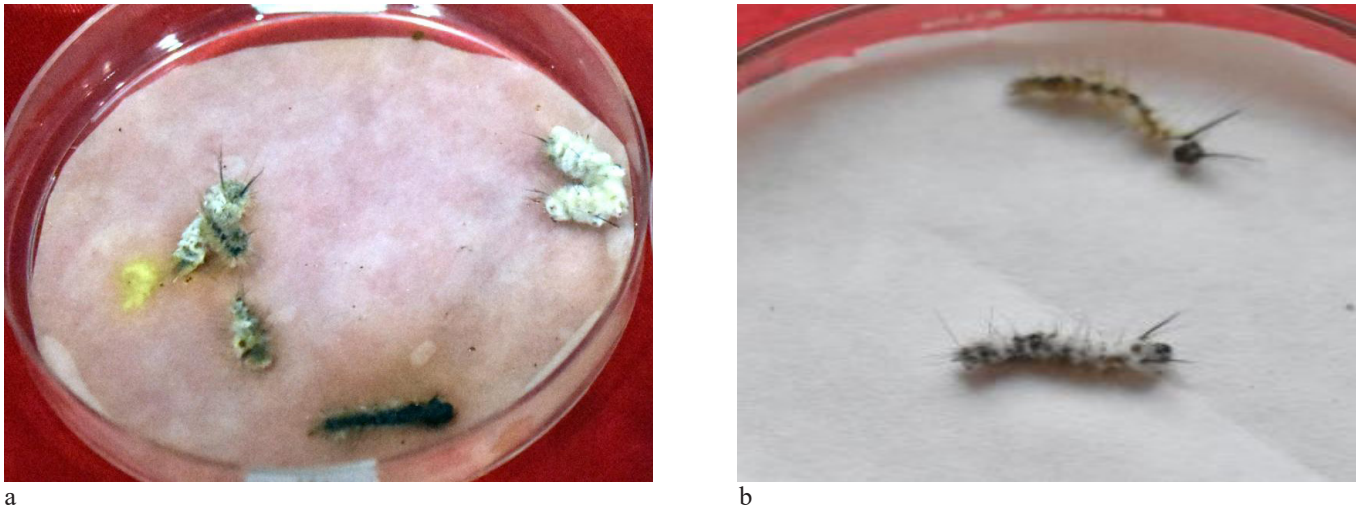


Figure 2. Larvae and cocoons of *P. pennatula.*



a b

Figure 3. The infected cadaver of *P. pennatula* with white fluffy growth of (a) *B. bassiana* and (b) *I. fumosorosea.*

Each of the plastic tubs represented as a replication, five replications were kept for each treatment having ten insects. Hence for each replication, a total of 50 insects were used for each concentration.

RESULTS

The mortality of *P. pennatula* larvae was found to be significantly different among the different concentrations on different Days Of Treatment (DOT). All three strains, *i.e.*, *Isaria, Beauveria* and *Cordyceps* have shown significant efficacy against the tested insect. The larva was severely affected by *Isaria* as compared to *Beauveria* and *Cordyceps* and the larval mortality increased as the days after treatment increased. At $1x10^7$spore/ml concentration, strains *B. bassiana* (KR855715) and *I. fumosorosea* (MH414514) exhibited the highest mortality percentage of 76% and 100% on the 10^{th} day of treatment. This was followed by the mortality of 74% and 78% in strains *B. bassiana* (KR855715) and *I. fumosorosea* (MH414514), respectively at $1x10^9$spore/ml concentration. *Isaria* was found to have a mortality factor of 100% after 9 days of treatment whereas, *Beauveria* showed 76.56% after 10 days of treatment and *Cordyceps* proved to be the least effective as compared to the other two strains used and recorded a mortality of 52% after 10 days of treatment (Figure 4). The appearance of white cottony growth on tested larvae proved the virulence of *Beauveria* and *Isaria* (Figure 3). LC_{50} and LC_{90}values of entomopathogenic fungi at 10 days after treatment and LT_{50} and LT_{90} values of different strains of entomopathogenic fungi at 1×10^7 condia/ml were presented in Table 1 and Table 2.

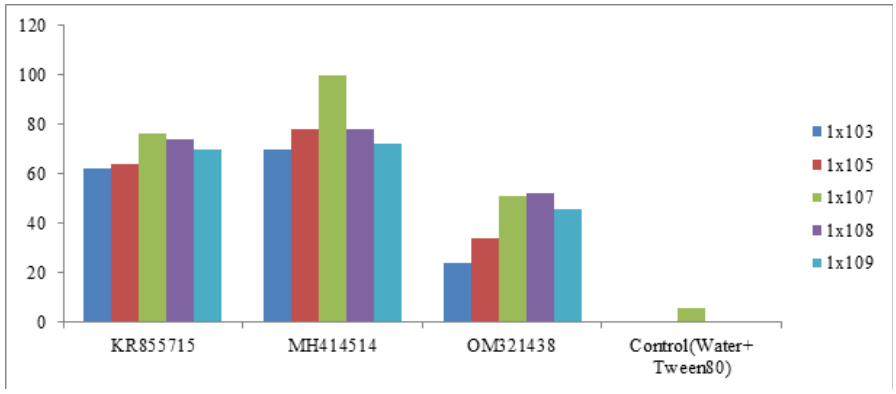


Figure 4. Efficacy of different strains of entomopathogenic fungi on larval mortality of *P. pennatula* at different spore concentration upto 10 Days of Treatment (DOT).

Table 1. LC_{50} and LC_{90} values of different strains of entomopathogenic fungi at 10 days after treatment

STRAIN	Regression equation (y= a + bx)	Chi-square (X^2)	df	Intercept ± S.Ed.	LC_{50} (Nos. of spores/ ml)	95% confidence limit		LC_{90} (Nos. of spores/ ml)	95% confidence limit	
						Lower	upper		Lower	upper
KR855715 (B. *bassiana*)	Y=0.045+0.544X	22.171	23	0.544±0.272	0.000	-	-	2.830×10^{16}	-	-
MH414514 (I. *fumosorosea*)	Y=0.062+0.121X	11.835	23	0.121±0.256	0.011	-	-	5.516×10^{18}	-	-
OM321438 (C. *javanica*)	Y=0.132-1.045X	5.165	23	-1.045±0.264	8.49×10^{7}	5242348.539	32220700576	4.525×10^{17}	3.098×10^{13}	4.474×10^{32}
Control (Water+Tween80)	Y=0.010-2.207X	33.853	23	-2.207±0.666	1.646×10^{227}	-	-	-	-	-

Table 2. LT_{50} and LT_{90} values of different strains of entomopathogenic fungi at 1×107 condia/ml

STRAIN	Regression equation (y= a + bx)	Chi-square (X^2)	df	Intercept ± S.Ed.	LT_{50} (DAYS)	95% confidence limit		LT_{90} (DAYS)	95% confidence limit	
						Lower	upper		Lower	upper
KR855715 (B. *bassiana*)	Y=3.505-19.951X	61.589	48	-1.951±0.203	3.603	3.191	3.99	8.362	7.302	10.020
MH414514 (I. *fumosorosea*)	Y=6.113-5.094X	16.805	48	-5.094±0.477	6.813	6.448	7.201	11.040	10.083	12.500
OM321438 (C. *javanica*)	Y=4.501-4.124X	20.080	48	-4.124±0.434	8.245	7.650	9.055	15.883	13.465	20.436
Control (Water+Tween80)	Y=4.061-5.845X	19.312	48	-5.845±2.229	27.489	-	-	56.847	-	-

DISCUSSION

The ability of Entomopathogenic fungi to control the *P. pennatula,* an emerging lepidopteran pest of rice is in close conformity with the findings of Ibrahim *et al.* (2016) who studied the effects of *B. bassiana* and *Paecilomyces lilacinus* strains on the lepidopteran larva of *Galleria mellonella* a pest of Honey bee. Dhar *et al.* (2019) evaluated the efficacy of *B. bassiana* against *Spodoptera litura*. Das *et al*. (2019) and Das *et al*. (2011) investigated the efficacy of *I. fumosorosea* and *B. bassiana* against the larvae and pupae of *E. amabilis*, a lepidopteran pest affecting lac cultivation under laboratory conditions. Their studies demonstrated the infectivity of these pathogens against the target pest, whereas the highest mortality recorded in the present study was observed against *P. pennatula.* Similarly, Opisa *et al.* (2018) tested the efficacy of 24 EPF isolates from three genera (14 *Metarhizium anisopliae*, 9 *B. bassiana*, and 1 *I. fumosorosea*) against second instar larvae of *S. recurvalis*. Likewise, Fergani *et al.* (2020) examined the efficacy of *B. bassiana* (Y-F_ ITS1) against 4th instar larvae of *Galleria mellonella*. The highest mortality recorded at $1x10^{7}$conidia/ml in the current experiment was similar to the results of Permana *et al*. (2022) who performed a laboratory bioassay of *Cordyceps* sp. and *B. bassiana* against 3rd instar larvae of *Crocidolomia pavonana* and at $1x10^{7}$ conidia/ml concentrations both the EPF showed 100% and 98.33% mortality.

Biological control using entomopathogenic fungi plays a significant part in the organic rice pest management program by altering the dynamics of the insect population. This is the first report of using EPF against the emerging pest of rice, *P. pennatula.* All three entomopathogenic strains, *I. fumosorosea* (MH414514), *B. bassiana* (KR755815) and *C. javanica* (OM321438) could be used in the management of *P. pennatula*. However, the data indicated that among all the evaluated strains of EPF, *I. fumosorosea* (MH414514) can prove as the most potent strain against larvae of *Psalis*. Further studies are also necessary to evaluate the biopesticidal efficacy of these potent EPF isolates under field conditions before their integration into sustainable management of *P. pennatula.*

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