



ISOLATION AND IDENTIFICATION OF INDIGENOUS ENTOMOPATHOGENIC FUNGI FROM BROWN PLANTHOPPER IN RICE

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ABSTRACT

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A promising alternative for insect pest control in agriculture is the use of entomopathogenic fungi. The objectives of this work were to isolate and identify strains of entomopathogenic fungi from brown plant hopper, *Nilaparvatha lugens* in rice ecosystems of Nellore and West Godavari districts of Andhra Pradesh. Two native strains of entomopathogenic fungi were isolated from the infected BPH cadavers. According to their macroscopic and microscopic traits, one isolate was identified as *Beauveria bassiana* species (coded as Bb-NLR) and another isolate was identified as *Metarhizium anisopliae* species (coded as Ma-NLR). Bb-NLR was isolated from a BPH cadaver collected from the Agricultural Research Station (ARS), Nellore, while Ma-NLR was isolated from a BPH cadaver collected from Chinnacherukuru village in T.P. Gudur mandal of Nellore district. No entomopathogenic fungi were recovered from soil samples despite multiple isolations. These native strains offer potential for development of biocontrol agents targeting major rice insect pests.

KEYWORDS: Entomopathogenic fungi, brown planthopper, *nilaparvathalugens*, morphological identification.

INTRODUCTION

Rice (*Oryzasativa* L.) is a vital food crop globally, serving as the staple diet for over half of the world's population. It plays a crucial role in ensuring food security and provides a major source of livelihood and employment, particularly in developing nations such as India. Approximately 90 per cent of global rice cultivation and consumption occurs in Asia. However, sustaining productivity amidst increasing pest pressure remains a major challenge for farmers and agricultural scientists. Despite technological advancements, rice production continues to face significant challenges from both abiotic and biotic stresses. Insect pests remain a major biotic constraint, causing substantial yield losses and threatening crop sustainability. Among the insect pests, the brown planthopper (*Nilaparvatalugens*, Hemiptera: Delphacidae) has economic importance in rice ecosystems across Asia. The brown planthopper is a monophagous herbivore is recognized as one of the most destructive insect pests of rice in Asia, capable of causing 20–80 per cent yield losses through both direct and indirect damage (Balachiranjeevi *et al.*, 2019). *N. lugens* causes damage by feeding on phloem sap and transmitting viral pathogens, including rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV), resulting in significant physiological stress and hopperburn symptoms (Helina *et al.*, 2019). However, indiscriminate chemical use for

the control of *N. lugens* has led to adverse consequences such as pest resurgence, resistance development, non-target effects and environmental contamination. These concerns have prompted the adoption of Integrated Pest Management (IPM), with an emphasis on ecologically sustainable approaches. Among these, biological control utilizing microbial agents has gained attention as an environmentally benign and effective alternative. Entomopathogenic fungi (EPF) are promising candidates for biological control due to their ability to infect and kill a wide range of insect pests. More than 700 fungal species, mainly from Deuteromycetes and Entomophthorales, are known to be entomopathogenic (Wang *et al.*, 2020). Rice ecosystems, characterized by high humidity and a favorable microclimate, support the natural occurrence and activity of EPF. Species such as *Beauveria bassiana* and *Metarhiziumanisopliae* have demonstrated high virulence against key rice pests (Malekan *et al.*, 2015). The successful application of EPF in field conditions often depends on the selection of locally adapted and highly virulent strains. Indigenous isolates tend to perform better under native environmental conditions (Jackson *et al.*, 2000). In this context, the present study aims to isolate and identify indigenous entomopathogenic fungi associated with brown plant hopper in the rice ecosystem.

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MATERIAL AND METHODS

The study was conducted during the *rabi* season of 2024 - 2025 at the Agricultural Research Station, Nellore (14.4303°N, 79.9987°E), with the objective of Isolation, identification of indigenous entomopathogenic fungi (EPF) associated with brown plant hopper in the rice. A systematic roving survey was carried out in rice fields of Nellore and West Godavari districts, Andhra Pradesh during October 2024 – February 2025. In total, 18 villages were selected across six mandals (three mandals per district, three villages per mandal). Naturally infected insect cadavers showing external mycosis symptoms were collected using sterile fine brushes into sterile glass vials by labelling them. Rhizospheric soil samples (top 5–10 cm) were also collected near cadaver sites using a sterile spatula, placed in sterile polyethylene bags and stored at room temperature ($25 \pm 2^\circ\text{C}$) until processing.

media and incubated for 7–14 days under sterile conditions to allow colony development. To obtain pure cultures, hyphal tips were subcultured repeatedly onto fresh agar media. The purified fungal isolates were maintained on agar slants and preserved in glycerol at -19°C for long-term storage and future experimentation.

Isolation of Entomopathogenic fungi from soil

Ten soil samples were collected from surveyed fields and 1 gram from each sample was used for serial dilution. The soil was initially suspended in 10 ml of sterile distilled water (autoclaved at 121°C , 15 psi for 15 minutes) to prepare a 10^{-1} dilution, followed by successive 1 ml transfers into 9 ml sterile water to achieve dilutions up to 10^{-11} . From the 10^{-11} dilution, 1 ml was aseptically transferred into sterile Petri plates inside a Laminar Air Flow chamber. Approximately 10–20 ml of molten and cooled Sabouraud's Maltose Agar with Yeast Extract was



Fig. 1. Survey map showing collection of insect cadavers of BPH and soils from Nellore & West godavari districts of Andhrapradesh, India

Isolation of entomopathogenic fungi from insect cadavers

The insect cadavers collected during the field survey were initially surface-sterilized by immersing them in 1 per cent sodium hypochlorite solution for 1 minute. This was followed by three successive rinses in sterile distilled water to remove any residual disinfectant. Following sterilization, the cadavers were transferred aseptically onto sterile Petri plates lined with moistened filter paper to maintain humidity. The plates were incubated at $25 \pm 1^\circ\text{C}$ for 3–5 days to facilitate the outgrowth of fungal mycelia from the cadaver surface.

Emerging fungal growth was carefully transferred to Sabouraud Dextrose Agar with Yeast extract (SDAY)

poured into the plates and gently swirled for even mixing before allowing it to solidify. The inoculated plates were incubated at $25^\circ\text{C} \pm 2^\circ\text{C}$ for a duration of 10 days to facilitate fungal growth and isolation.

Morphological characterization of entomopathogenic fungi

Fungal morphology was examined based on colony texture, mycelial structure, conidiophores, phialides, and conidia. Fungal mounts were prepared using lactophenol cotton blue stain on sterile slides and observed under a Phase Contrast Microscope (OLYMPUS BX43) at 40X & 100X magnification. Identification was based on standard taxonomic keys and morphological traits.



Fig. 2. Collection of insect cadavers of BPH from rice field in Nellore and west godavari districts of Andhra Pradesh

RESULTS AND DISCUSSION

Survey efforts were focused in three mandals per district; Nellore Rural, Kodavaluru, and T.P. Gudur in Nellore district; and Penumantra, Achanta, and Palakollu in West Godavari district—covering different rice varieties and crop stages to enhance the possibility of capturing natural epizootics of entomopathogenic fungi in the field. In Nellore district brown planthopper cadavers were collected across all locations, mainly from rice varieties BPT 5204, KNM 1638, and MTU 1010 during tillering to grain filling stages. In West Godavari district, brown planthopper cadavers were predominantly found in MTU 7029 fields at the flowering stage. During the course of the survey, a total of 10 BPH cadavers suspected to be infected with entomopathogenic fungi were collected. The cadavers were collected based on visible signs of fungal infection under field conditions and brought to the laboratory for fungal isolation. Additionally, ten rhizospheric soil samples were also collected from the surveyed sites. However, none of the soil samples yielded entomopathogenic fungi despite repeated isolations using standard culturing techniques.

From the BPH cadavers collected from rice ecosystems, two fungal isolates showing typical characteristics of entomopathogenic fungi were isolated. Of these fungi, one isolate corresponded to *Beauveria bassiana* (coded as Bb-NLR) and one isolate corresponded to *Metarhizium anisopliae* (coded as Ma-NLR). Bb-NLR was isolated from a BPH cadaver collected from the Agricultural Research Station (ARS), Nellore, while Ma-NLR was identified from a BPH cadaver collected

from Chinnacherukuru village in T.P. Gudur mandal of Nellore district. Remaining eight isolates were identified as plant pathogens morphologically under microscope.

The morphological identification (employing macroscopic and microscopic information) allowed the identification of the strains to the genera and species levels. In the present work, the fungal isolations of *B. bassiana* and *M. anisopliae* presented similar shapes, sizes and dispositions of conidia with the descriptions made by Samson *et al.* (1988), Humber (2005, 2012) and Rehner *et al.* (2005). All the fungal strains obtained corresponded to microorganisms of the Phylum Ascomycota, Sordariomycetes class, and the families Cordycipitaceae for *Beauveria*, and Clavicipitaceae in the case of *Metarhizium* (Humber, 2005, 2012).

The isolate Bb-NLR exhibited white, powdery colonies with cottony to velvety aerial mycelium and a uniformly circular growth pattern. After 10–12 days of incubation at $25 \pm 1^\circ\text{C}$, colonies attained a diameter of approximately 6.5 cm. Microscopic examination showed globose, hyaline conidia borne singly on a denticulated, zig-zag rachis, a characteristic feature of *Beauveria bassiana*. The conidia were smooth-walled, hyaline and measured $2.3\text{--}3.0\ \mu\text{m}$ in length and $2.0\text{--}2.5\ \mu\text{m}$ in width. Kirubhadharsini *et al.* (2017) also recorded the macroscopic characters of *Beauveria* isolates, which exhibited a white colony colour, powdery to cottony texture and a round shape and microscopic examination of *Beauveria* isolates revealed globose to sub-globose conidia with hyaline hyphae.

Table 1. Survey Locations and details of Entomopathogenic Fungi-Infected BPH Cadavers and soil collected from Rice Ecosystems of Nellore and West Godavari Districts (rabi 2024–2025)

District	Mandal	Village	Variety	Crop stage	latitude	longitude	Pest cadaver collected	Entomopathogenic fungi isolated from soil
Nellore	Nellore rural	Nellore(ARS)	BPT5204	Tillering stage	14.430195°N	79.99867°E	BPH	Not determined
		Mullamudi	KNM 1638	Tillering stage	14.45651°N	79.98019°E	BPH	Not determined
		Magallapalem	KNM1638	Tillering stage	14.44259°N	79.98645°E		Not determined
		Northrajupalem	MTU1010	Grain filling stage	14.5531°N	79.9887°E	BPH	Not determined
	Kodavaluru	Ramannapalem	KNM733	Grain filling stage	14.54139°N	79.99723°E	--	Not determined
		Kodavaluru	KNM1638	Grain filling stage	14.5669°N	80.0034°E	--	Not determined
	T P gudur	Chinnacherukuru	MTU1010	Tillering stage	14.3833°N	80.1000°E	BPH	Not determined
		Koduru	KNM1638	Tillering stage	16.0086°N	81.03441°E	BPH	Not determined
		Varigonda	KNM1638	Flowering stage	16.60999°N	80.72144°E	BPH	Not determined
		Maruteru	MTU7029	Flowering stage	16.631162°N	81.7389°E	BPH	Not determined
West Godavari	Penumantra	Neggipudi	MTU1318	Flowering stage	16.6366°N	81.7308°E	BPH	Not determined
		Alamuru	MTU1318	Flowering stage	16.7833°N	81.9000°E	BPH	Not determined
		Koderu	MTU1061	Grain filling stage	16.60386°N	81.85659°E	---	Not determined
		Kodamanchili	MTU1064	Grain filling stage	16.60516°N	81.83343°E	---	Not determined
	Achanta	Penumanchili	MTU1061	Grain filling stage	16.58581°N	81.82376°E	---	Not determined
		Palakollu rural	MTU1318	Grain filling stage	16.5333°N	81.7333°E	BPH	Not determined
		Palamuru	MTU1282	Grain filling stage	16.5487°N	81.6515°E	---	Not determined
		Pulapalli	BPT 5204	Grain filling stage	16.5187°N	81.7062°E	---	Not determined
	Palakollu							

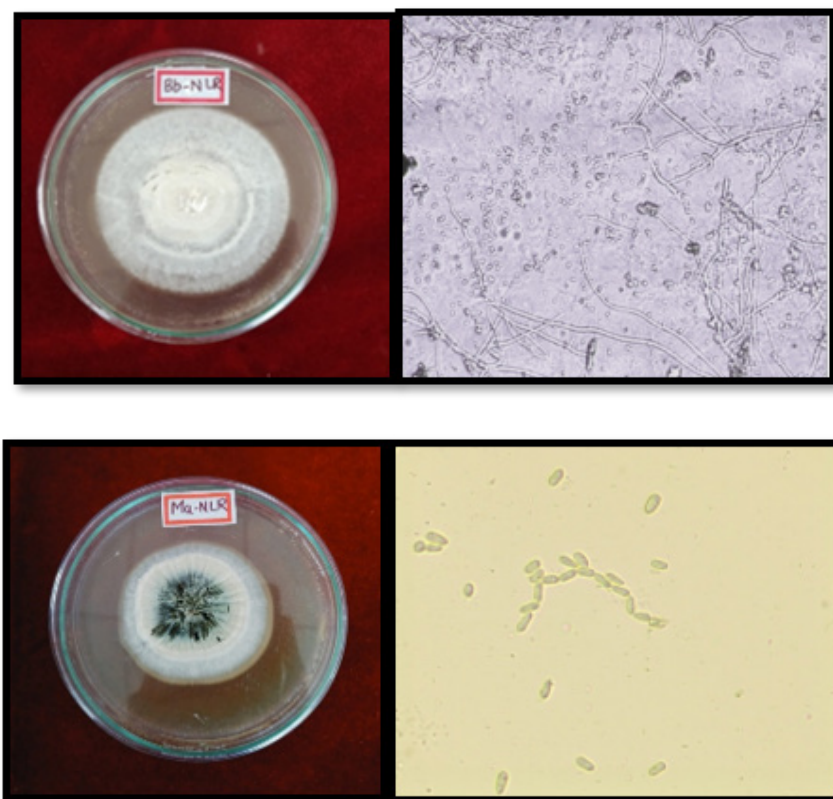


Fig. 3. Macroscopic and microscopic identification of fungal isolates (a) Morphology of *Beauveria bassiana* colony and (b) *B. bassiana* conidia (Bb-NLR) at 40X (c) Morphology of *Metarhizium anisopliae* colony (d) *M. anisopliae* conidia (Ma-NLR) at 40X

The Ma-NLR isolate displayed colonies with light to olive-green pigmentation, compact and granular in surface texture, and reached a diameter of approximately 5.8 cm after 10–12 days of incubation. Microscopic observation revealed cylindrical conidia arranged in chains on verticillate conidiophores. The conidia were smooth-walled, green and measured 6.4–7.2 μm in length and 3.4–3.6 μm in width. These morphological characteristics are consistent with previous reports for *Metarhizium anisopliae*, including the observations made by Elham *et al.* (2018), who reported similar traits on Potato Dextrose Agar. Bai *et al.* (2015) observed that colony borders of all the isolates of *M. anisopliae* were regular. During sporulation, the colour of colonies became yellow green or olivaceous green to dark green. The average spore width was 2.10– 4.10 μm , while the average spore length was 3.20– 7.69 μm . The spores were categorized as oval, round, or elongated based on their length and width.

The present work presents information of indigenous entomopathogenic fungi associated with brown plant hopper, *N. lugens* in rice ecosystem of Nellore district, Andhra Pradesh. Two native strains of entomopathogenic fungi were isolated from the infected BPH cadavers. According to their macroscopic and microscopic traits, one isolate corresponded to *B. bassiana* species and one isolate corresponded to *M. anisopliae* species. No entomopathogenic fungi were recovered from soil samples despite multiple isolations. The native isolates have potential for use in eco-friendly rice pest management strategies.

Table 2. Macroscopic characteristics of isolated entomopathogenic fungal isolates

Isolate code	Colony colour	Colony shape	Elevation	Surface texture	Colony diameter (cm)	Growth pattern
Bb- NLR	White, powdery	Round	raised	Cottony to velvety	6.5	Circular
Ma-NLR	Light to olive green	Round	Flat	Granular, compact	5.8	Circular

Table 3. Microscopic characteristics of isolated entomopathogenic fungal isolates

Isolate code	Conidial shape and colour	Conidial arrangement	Spore length (µm) (40X)	Spore width (µm)(40X)	Spore wall	Conidiogenous structure
Bb NLR	Globose, hyaline	Singly borne	2.3–3.0	2.0–2.5	Smooth, hyaline	Zig-zag rachis, denticulate
Ma-NLR	Cylindrical	Chains	6.4–7.2	3.4–3.6	Smooth, green	Verticillate conidiophores

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