



RESEARCH ARTICLE

Isolation and identification of indigenous entomopathogenic fungi infecting brown plant hopper on rice from Andhra Pradesh, India

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Abstract

The brown planthopper *Nilaparvata lugens* (Stål) is a highly destructive pest of rice. Among the promising eco-friendly alternatives for insect pest management is the use of entomopathogenic fungi. The present study aimed to isolate and identify, both morphologically and molecularly, strains of entomopathogenic fungi infecting brown planthopper populations in rice fields of Nellore district, Andhra Pradesh, India. Two fungal strains were isolated and morphologically characterised: one belonging to *Beauveria bassiana* and the other to *Metarhizium anisopliae*. Molecular identification revealed sequence identity values greater than 99 % with reference strains of *B. bassiana* and *M. anisopliae* in public databases. Phylogenetic analysis further confirmed that the obtained sequences clustered within the respective clades of *B. bassiana* and *M. anisopliae*, validating the morphological and molecular identification.

Keywords: *Beauveria*, brown planthopper, entomopathogenic fungi, *Nilaparvata lugens*, *metarhizium*

Introduction

Rice (*Oryza sativa* 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 % of global rice cultivation and consumption occurs in Asia. India emerged as both the second-largest producer and the largest exporter of rice in the world, cultivating over 47.8 million hectares with an annual production of around 138 MmT. In India, West Bengal is the leading producer of rice with an average annual production of 15.75 MT. Among the Indian states, Andhra Pradesh ranks fifth in rice production with an area of 2.16 M ha and an average production of 7.49 MT during 2023. However, sustaining productivity amidst increasing pest pressure remains a major challenge for farmers and agricultural scientists. The constraints that limit the production of rice include both abiotic and biotic factors. Among the biotic constraints, insect pests viz., brown planthopper, *Nilaparvata lugens* Stal; gall midge, *Orseolia oryzae* (Wood-Mason); stem borer, *Scirpophaga incertulas* (Walker) and leaf folder *Cnaphalocrocis medinalis* Guenee are causing major yield losses. Among the economically significant pests of rice in tropical and temperate regions of Asia, the *N. lugens* (Hemiptera: Delphacidae) is particularly notorious.

N. lugens is a monophagous herbivore recognised as one of the most destructive insect pests of rice capable of causing 20–80 % yield losses in Asia, through both direct and indirect damage (1). Direct feeding by both nymphs and adults involves sucking phloem sap from the rice plant, leading to physiological stress, yellowing and a characteristic symptom known as “hopperburn,” where the affected plants dry and appear scorched (2, 3). Indirectly, BPH also serves as a vector for several viral diseases, including rice grassy stunt virus (RGSV), rice ragged stunt virus (RRSV) and wilted stunt, significantly compounding its impact on crop health and productivity (4). Frequent outbreaks of BPH have been reported across rice-growing regions in Asia, making it a persistent threat to rice production systems. Conventional methods for managing BPH have relied heavily on chemical pesticides. Over time, however, the injudicious and excessive use of these chemicals failed to deliver effective pest control and instead created a range of serious problems, including environmental degradation, pollution, pest resistance, pest resurgence and secondary pest outbreaks. Growing awareness of these issues, especially their environmental impact, prompted the search for alternative pest-control strategies. This led to the development of integrated approaches and Integrated Pest Management (IPM) emerged with a combination of cultural, mechanical, physical and biological tools. Among these, biological control plays a key role by using beneficial microbes to combat pest problems and protect crops.

More than 700 fungal species-predominantly Deuteromycetes and Entomophthorales representing around 90 genera-are known to infect insects. Numerous fungal pathogens have been documented from major rice pests (5). The high-humidity microclimate of rice fields is much more favourable to the development of these pathogens, making them strong candidates for use in microbial pest management. Among them, *Beauveria bassiana* and *Metarhizium anisopliae* are particularly well-known for their efficacy against a wide range of both sucking and chewing insect pests (6). Research indicates a study on the pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* isolates collected from naturally infected brown planthopper (BPH) cadavers in Omon (Vietnam) and various regions in India (7). The isolates were tested under glasshouse conditions against third instar nymphs and adults of BPH. Locally collected fungal strains exhibited greater virulence compared to exotic isolates. The optimal concentration for *B. bassiana* efficacy was 6×10^{12} spores/ha. For effective field-level suppression of *Nilaparvata lugens*, however, successful deployment of entomopathogenic fungi requires the selection of isolates with high virulence, rapid killing ability and strong environmental persistence within agricultural ecosystems.

Indigenous fungal strains tend to perform better under local agro-climatic conditions (8). However, isolating novel strains from the environment is challenging due to ecological disturbances (9). Therefore, isolation from naturally infected insect cadavers is a key method to obtain effective native strains (10). Many strains have been commercially developed and integrated into IPM strategies, showing potential for managing pests like the brown planthopper (11–13). Understanding and utilising native EPF strains helps reveal the regional fungal biodiversity and supports the development of location-specific biocontrol solutions (14). Leveraging indigenous fungal strains may enhance pest suppression under local field conditions. Therefore, focused research is essential to tap into these native fungal resources for pest management.

Materials and Methods

Sampling and isolation of entomopathogenic fungi

Eighteen insect samples showing symptoms of fungal infection, along with adjacent soil samples, were collected from randomly selected rice fields. The insect specimens were placed in paper envelopes, while the soil samples were stored in plastic bags. All samples were properly labelled and kept at room temperature for about 5–7 days until further laboratory analysis. Collections were made from the Nellore and West Godavari districts of Andhra Pradesh, India. (Fig. 1 and Table 1).

To promote fungal development, the insect samples showing signs of fungal attack were processed using a standardised methodology in which each specimen was placed in a humid chamber and incubated at $28 \pm 1^\circ\text{C}$ (15). The bearing conidia from the insects' body were collected and inoculated in plates with agar culture media, Potato dextrose (PDA-Himedia) and Sabouraud dextrose agar with yeast extract (SDAY) (Himedia) and incubated at $28 \pm 1^\circ\text{C}$ for 5 to 14 days. Soil samples were collected following previously described methodologies (16, 17). From each sample, five serial dilutions (10^{-1} to 10^{-5}) were prepared using sterile distilled water. Aliquots of 0.1 mL from the 10^{-4} and 10^{-5} dilutions were spread onto Petri dishes containing PDA or SDAY using a sterile spatula to ensure even distribution. All plates were incubated at $28 \pm 1^\circ\text{C}$ for 5–14 days.

All procedures involving the direct inoculation of conidia and the dispersion of sterile, distilled water serial dilutions were performed in triplicate. Fungal growth on the culture plates was monitored periodically and microscopic examinations were conducted to identify any colonies of potential interest (18).

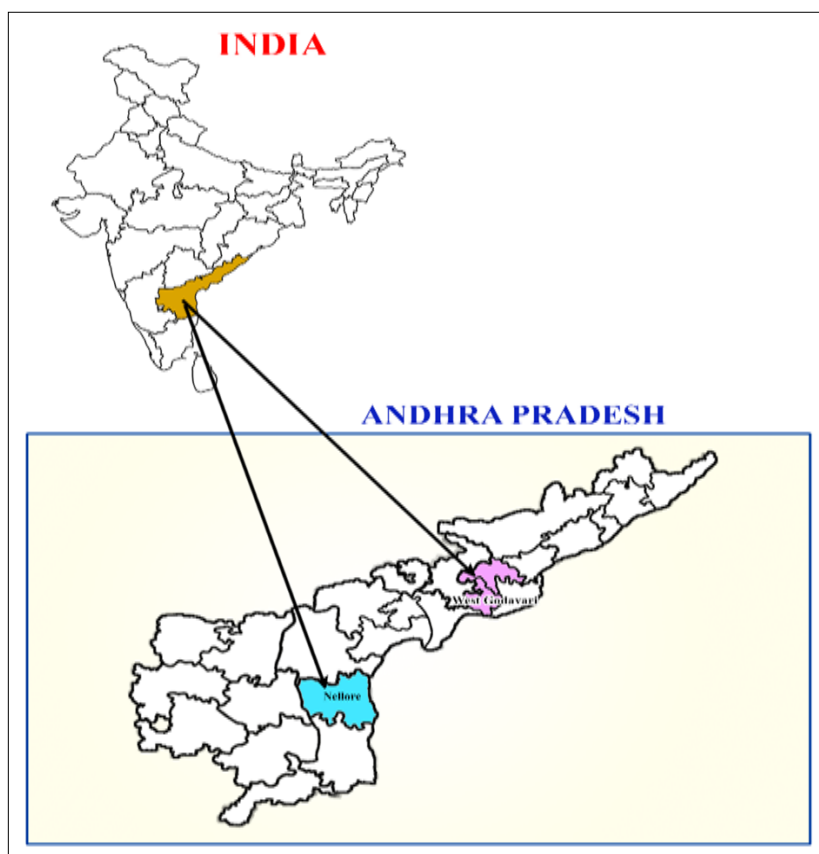


Fig. 1. Survey map showing collection of infected insects of BPH and soils from Nellore and West Godavari districts of Andhra Pradesh, India.

Table 1. Survey locations and details of entomopathogenic fungi-infected insects collected from rice ecosystems of Nellore and West Godavari districts of Andhra Pradesh during *rabi* 2024–25

District	Mandal	Village	Latitude	Longitude	Infected insects collected
Nellore	Nellore rural	Agricultural Research station	14.430195°N	79.99867°E	BPH
		Mullamudi	14.45651°N	79.98019°E	BPH
		Magallapalem	14.44259°N	79.98645°E	--
		Northrajupalem	14.5531°N	79.9887°E	BPH
	Kodavaluru	Ramannapalem	14.54139°N	79.99723°E	--
		Kodavaluru	14.5669°N	80.0034°E	--
		Chinnacherukuru	14.3833°N	80.1000°E	BPH
		Koduru	16.0086°N	81.03441°E	BPH
	T P gudur	Varigonda	16.60999°N	80.72144°E	BPH
		Maruteru	16.631162°N	81.7389°E	BPH
		Neggipudi	16.6366°N	81.7308°E	BPH
		Alamuru	16.7833°N	81.9000°E	BPH
West Godavari	Achant	Koderu	16.60386°N	81.85659°E	---
		Kodamanchili	16.60516°N	81.83343°E	---
		Penumanchili	16.58581°N	81.82376°E	---
		Palakollu rural	16.5333°N	81.7333°E	BPH
	Palakollu	Palamuru	16.5487°N	81.6515°E	---
		Pulapalli	16.5187°N	81.7062°E	---

BPH: brown plant hopper

Morphological identification of entomopathogenic fungi

For the morphological identification of the fungal strains, the culture was grown on SDAY media and macroscopic colony traits were examined first. For each isolate, colony characteristics-including upper and lower surface colouration, shape, texture, margin type, consistency and elevation-were recorded (18–21). To identify the entomopathogenic fungal genera of interest, microscopic features were subsequently assessed, with particular attention to the size and arrangement of hyphae, conidiophores and conidia (18, 20, 21). Microscopic observations and measurements of reproductive structures were conducted at 400 × magnification using a micrometric eyepiece and microphotographs were captured with an OLYMPUS phase-contrast microscope (model BX43, U-TV1X-2). For each structure, 20–30 measurements were taken and the mean and standard deviation values were calculated and reported.

Molecular identification of entomopathogenic fungi

Fungal isolates Bb-NLR and Ma-NLR were molecularly identified based on partial genetic analysis of the internal transcribed spacer (ITS) region of the 18S ribosomal DNA, as it contains highly conserved sequences, allowing universal primers to amplify DNA from almost all fungi. The isolates were first cultured in potato dextrose broth (PDB). After a four-day incubation period, the fungal mycelia were harvested using sterile filter paper and subsequently washed with sterile distilled water to remove residual media before DNA extraction. The washed fungal mats were then gently blot-dried using sterile filter paper. After drying, the mycelial mats were wrapped in sterile aluminium foil and stored at -20 °C until further use for genomic DNA extraction.

DNA extraction was done as per the standard method with a few modifications (22). The fungal mats were homogenised in a sample extraction buffer composed of 0.15 M NaCl, 50 mM Tris-HCl, 50 mM EDTA, three per cent SDS and one per cent β-mercaptoethanol and CTAB with a pre-sterilised pestle and mortar and transferred to sterile Eppendorf tubes. After vortexing for a few minutes, the mixture was incubated for 1 hr at 65 °C in a water bath. For about 10 min at 10000 rpm, the samples were centrifuged. The supernatant obtained from this was taken in a separate tube and equal volumes of phenol: chloroform (1:1) were added. After adding, again centrifugation for 10 min at 10000 rpm was done. Supernatant was collected and to this chloroform: isoamyl alcohol (24:1) was added in equal volume and centrifuged for 10 min at 10000 rpm. To

get the DNA precipitate, the supernatant obtained from the above centrifuged product was taken and to this 0.6 volume (e.g. 300 µL then 0.6 300 =180 µL) of ice-cold isopropanol and 0.1 volume of Sodium acetate (3M) (pH 5.2) were added and incubated at -20 °C for 30 min. Then the centrifuge tubes with DNA precipitate were spun at 13000 rpm for 20 min at 4 °C and from this DNA pellet was collected and the supernatant was discarded. The obtained DNA pellet was dried in an airtight chamber and dissolved in 100 µL sterile distilled water and to this 1 µL RNase (10mg/mL) was added. Then the Eppendorf tubes were stored at -20 °C for further use.

The concentration and quality of DNA were estimated using a Nanodrop Spectrophotometer at 260 nm (ND-2000C Spectrophotometer-Thermo scientific). The presence of DNA was verified by running a DNA sample (2 µL) from each isolate mixed with 2 mL of 1 % loading dye on 1 % agarose gel along with a 50 bp ladder (TrackIt™ 50 bp Thermo Fisher Scientific).

PCR amplification was at ITS region of 18S rDNA, using ITS-1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'- TCCTCCGCTTATTGATATGC -3') primers (23). The PCR mixture consisted of molecular-grade water, a commercial 2× PCR mastermix, small measured amounts of the ITS1 and ITS4 primers and genomic DNA at an approximate concentration of 250 ng/µL. Amplification was performed using a thermal cycling program that began with an extended high-temperature activation step, followed by repeated cycles of denaturation, primer annealing and DNA extension. After the final elongation phase, the reaction was held at a moderate temperature before concluding.

The resulting PCR amplicons were then purified using a Polyethylene Glycol-based precipitation approach (24), after which they were prepared for downstream sequencing.

PCR amplified DNA was evaluated by 1 % agarose gel (W/V) electrophoresis. The purified PCR product was eluted and sequenced using an automated DNA sequencing platform. Sequence assembly, nucleotide alignment and percent identity matrix analyses were carried out using BioEdit version 7.0 software (25). The nucleotide sequences of *fungal isolates* obtained were compared with reference sequences retrieved from the NCBI GenBank database. A phylogenetic tree (phylogram) was constructed using the neighbour-joining method with 1000 bootstrap replications in MEGA 11.0 software to assess the evolutionary relationships of the isolates with related strains (26).

Results

Morphological identification of entomopathogenic fungi

Two fungal strains were successfully isolated from the infected cadavers of BPH. After culture on SDAY plates at $25 \pm 1^\circ\text{C}$ for 10 days, one fungal strain formed white powdery colonies with a cottony to velvety texture and dense aerial mycelial growth. The colonies were uniformly round with a flat elevation, indicating even radial expansion. The colony reached a diameter of approximately 6.5 cm, displaying a consistent circular growth pattern. The microscopic observations exhibited hyaline, smooth-walled conidia that were globose to sub-globose in shape and borne singly on zig-zag-shaped rachis of conidiogenous cells. The conidia were smooth-walled, hyaline and measured $2.3\text{--}3.0\text{ }\mu\text{m}$ (SD 0.1 to 0.4) in length and $2.0\text{--}2.5\text{ }\mu\text{m}$ (SD 0.2 to 0.6) in width (Fig. 2a–2b).

The other fungal strain displayed colonies ranging from light green to dark green, with a compact, granular surface texture. The colonies were round with flat elevation, expanding evenly across the SDAY plate surface. After incubation for 10–12 days at $25 \pm 1^\circ\text{C}$, the colony reached a diameter of approximately 5.8 cm, showing moderate radial growth. Microscopic examination revealed conidia that were cylindrical to ellipsoidal in shape, arranged in chains on verticillate conidiophores and showing bright green pigmentation. The conidia measured between $6.4\text{--}7.2\text{ }\mu\text{m}$ (SD 0.6 to 0.9) in length and $3.4\text{--}3.6\text{ }\mu\text{m}$ (SD 0.5 to 0.9) in width (Fig. 2c–2d).

Molecular identification of entomopathogenic fungi

For molecular identification, the partial ITS sequence of the fungal isolates was amplified and sequenced. The obtained sequences were 553 bp (gene bank accession number: PV 803533) and 539 bp (gene bank accession number: PV 803534) and shared 99.65 and 100 % similarity with *B. bassiana* and *M. anisopliae* strains, respectively. Phylogenetic analysis also showed that the tested fungal strains clustered with the strains of *B. bassiana* and *M. anisopliae* with a bootstrap support of 99 % inferred by the Neighbour-Joining (NJ) method (Fig. 3). Combining the results of morphological and molecular characterisation, the isolated fungal strains were identified as *B. bassiana* and *M. anisopliae*, named as Bb-NLR and Ma-NLR, respectively.

Discussion

With the rapid development of insecticide resistance, biocontrol agents as an alternative to chemical pesticides against BPH are urgently needed. In the present study, we isolated and identified two native fungal isolates from infected BPH cadavers from the rice ecosystem. Based on the morphological and molecular characterisation, the isolated fungal strains were identified as strains of *B. bassiana* and *M. anisopliae*.

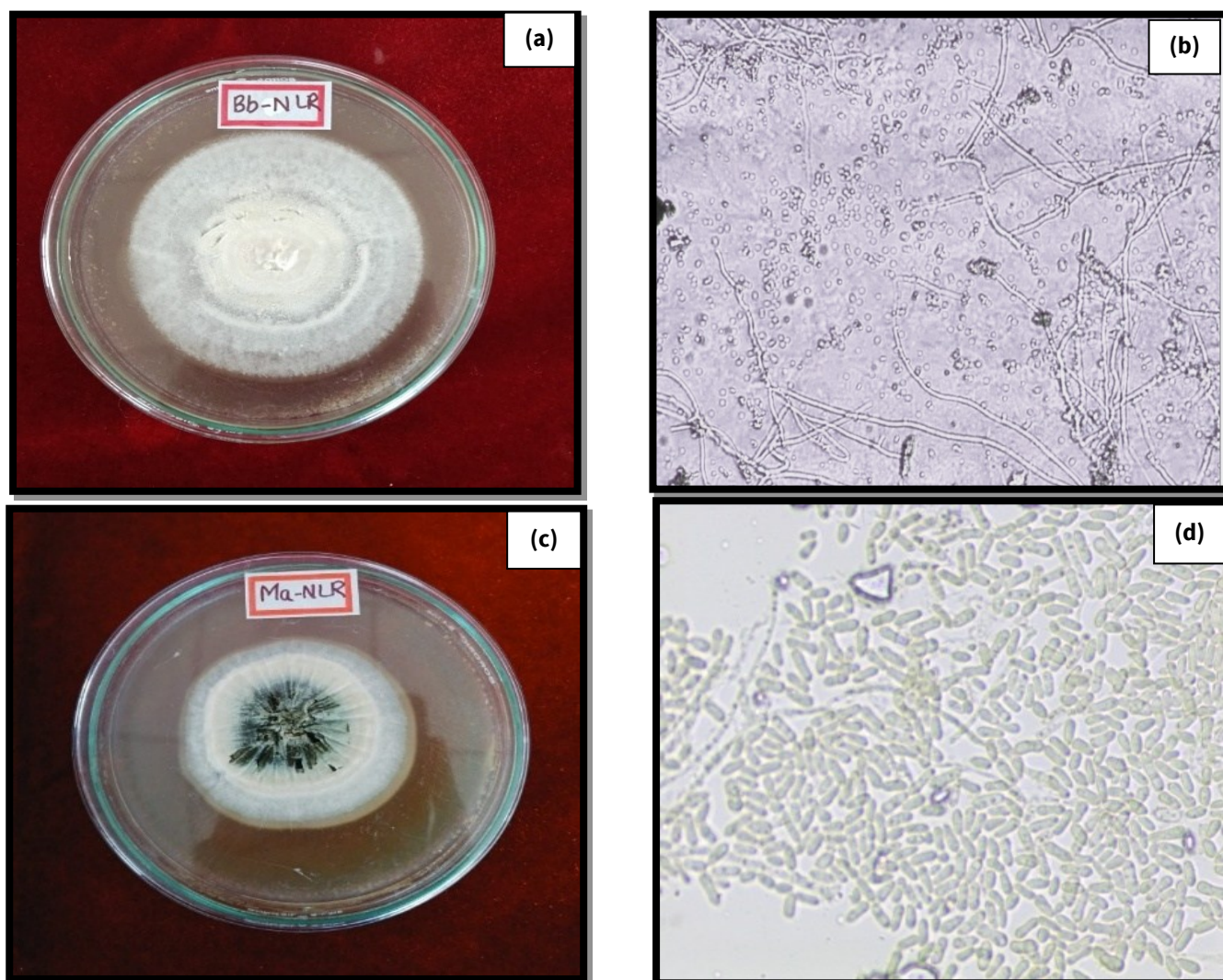


Fig. 2. Macroscopic and microscopic photographs of fungal isolates: (a) Morphology of *B. bassiana* colony on PDA medium, (b) *B. bassiana* conidia at 40X, (c) Morphology of *M. anisopliae* colony on PDA medium, (d) *M. anisopliae* conidia at 40X.

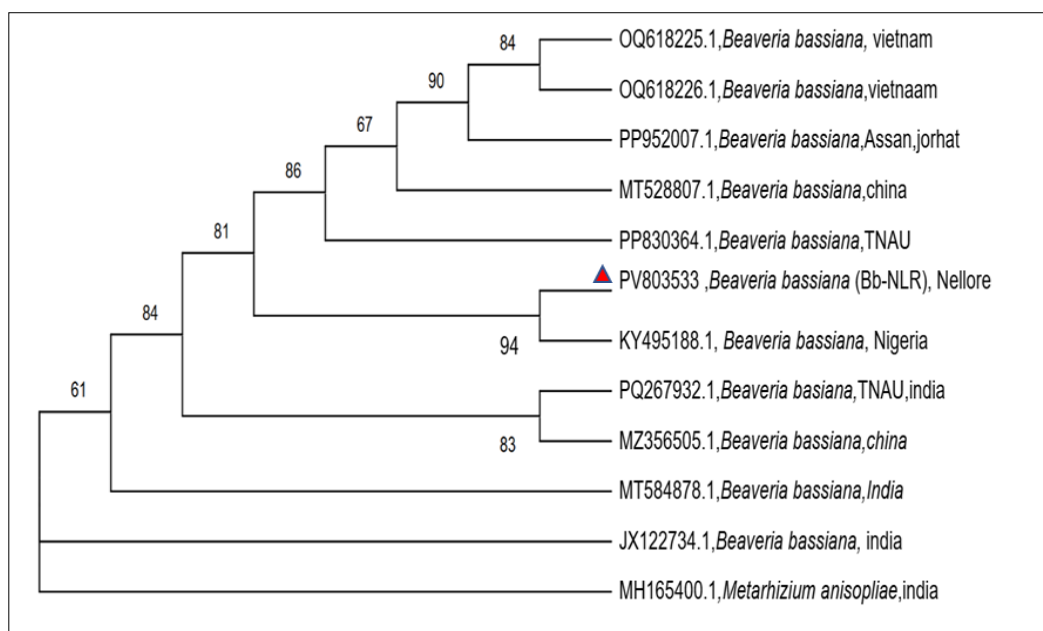


Fig. 3. Phylogenetic tree showing the evolutionary relationship of *Beauveria bassiana* isolate Bb-NLR based on ITS region (18S rDNA) sequences constructed using the Neighbor-Joining method ▲ Isolated EPF in current investigation

The morphological studies of the present investigation were consistent with the observations made regarding the macroscopic characters of *Beauveria* isolates, which exhibited a white colony colour, powdery to cottony texture and a round shape (27). Microscopic examination of *Beauveria* isolates revealed globose to sub-globose conidia with hyaline hyphae. (28) studied the characters of *B. bassiana*, which showed dispersed growth patterns, white to yellowish colony colour, smooth powdery to cotton texture, raised to flat elevation and round shape and globose to subglobose conidia with hyaline hyphae. Research identified *B. bassiana* based on the morphological characteristics as follows: colonies appear white, with cylindrical, hyaline, septate hyphae approximately 3.5 μm wide (29). The conidiophores are either single or branched, arising abundantly from vegetative cells and bearing globose to flask-shaped conidiogenous cells (3–5 $\times$ 3–7 μm). These cells possess a well-developed rachis up to 20 μm long and 1–1.5 μm wide. Conidia are produced at the thread-like apex of the phialides on zigzag-shaped branchlets resembling a cyme and are globose (1–4 μm) to oval (1.5–5 $\times$ 1.0–3.0 μm), smooth and hyaline. *B. bassiana* isolate exhibited typical septate hyphae and conidiogenous cells (4.7–7.3 μm (SD 0.5 to 0.8) $\times$ 2.0–2.4 μm (SD 0.2 to 0.4) with broad bases and tapered tips. These features align with classical descriptions by (17, 18). (30) studied the morphological characters of *B. bassiana* and reported that the average size of *B. bassiana* spores (length $\times$ width) was 1.87 $\times$ 1.60 μm .

The other strain of the *Metarhizium* genus displayed colonies ranging from light green to dark green, with a compact, granular surface texture. The colonies were round with flat elevation, expanding evenly across the SDAY plate surface. Research indicates that Potato Dextrose Agar, the colony of *M. anisopliae* was whitish yellow and turned to dark green when matured and it was slow growing with floccose mycelium (31). Research observed that colony borders of all the isolates of *M. anisopliae* were regular (29). 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 categorised as oval, round, or elongated based on their length and width.

Molecular identification of entomopathogenic fungi

Accurate identification is crucial when using entomopathogenic fungi (EPF) for insect pest control. Morphological characteristics alone are insufficient for distinguishing fungi at the species level. Research indicates that genera such as *Lecanicillium*, *Simplicillium*, *Beauveria* and *Isaria* share similar morphological traits, making species-level identification unreliable (33). Because these genera possess only a limited number of distinguishable morphological features, reliance solely on morphology leads to imprecise classification. Therefore, molecular sequence data are essential for accurate species identification (34). The present study aimed to precisely identify the two EPF isolates previously shown to infect the brown planthopper.

Based on the results of the identity analysis of the sequences obtained for the fungal isolate, Bb-NLR allowed confirming that they belonged to *B. bassiana* species with an identity index of 99.65 % sequence identity with *B. bassiana* strain CGAIPFBS-012 (KY495188.1), along with strong matches to other *B. bassiana* strains, including MZ356505.1, PP952007.1, OQ618225.1 and OK036438.1. On the other hand, with respect to the fungal isolate, Ma-NLR identity indexes of 100 % to *M. anisopliae* isolate TMBMAVTL (MT229077.1) and shared complete alignment with other *M. anisopliae* sequences such as MZ482015.1, MZ479677.1, GQ88737.1 and MN727141.1. (Table 2). To enhance the molecular identification of these isolated strains, the phylogenetic relationships of *Beauveria* and *Metarhizium* were assessed through independent phylogenetic reconstructions. The alignment covered 571 positions and a phylogenetic tree was constructed using the Neighbour-Joining (NJ) method under the Maximum Composite Likelihood model in MEGA11. The NJ tree, rooted with *M. anisopliae* (MH165400.1) as the out group, as given in the image, placed Bb-NLR firmly within the *B. bassiana* clade with robust bootstrap support, confirming its species identity. The strong bootstrap support and high sequence similarity (≥ 99 %) indicate that Bb-NLR matches the genomic characteristics and evolutionary lineage of *B. bassiana*, as defined by the phylogenetic species concept (Fig. 3). This species-level confirmation is consistent with ITS1-5.8S-ITS2 comparisons reliably supporting *Beauveria* species identification (35).

Table 2. Results of the nearest fungi taxon BLAST homology ITS1, 5.8S and ITS4 of rDNA in NCBI (<https://www.ncbi.nlm.nih.gov/>)

Isolate	Type	Genebank accession number	Location of Isolate	% similarity
<i>Bb-NLR</i> (ARS, Nellore rural, A. P. India) No. accession/host/ size PV803533 / <i>Nilaparvata lugens</i> Stal./ 553 bp	<i>Beauveria bassiana</i> strain CGAIPFBS-012	KY495188.1	Nigeria	99.65
	<i>Beauveria brongniartii</i> strain TF6	JX122734.1	India	99.65
	<i>Beauveria bassiana</i> strain n67	MZ356505.1	China	99.65
	<i>Beauveria bassiana</i> isolate PDCPGS-Bb	PP952007.1	Jorhat, Assam	99.65
	<i>Beauveria bassiana</i> isolate BB1	OQ618225.1	Vietnam	99.65
	<i>Beauveria bassiana</i> isolate BC9	OQ618226.1	Vietnam: Central Highlands	99.65
	<i>Beauveria bassiana</i> isolate SBI-Bb01	MT584878.1	India	99.65
	<i>Beauveria bassiana</i> clone EF158	MT528807.1	China	99.65
	<i>Beauveria bassiana</i> isolate TNAU	PP830364.1	Tamil Nadu, India (TNAU)	99.64
	<i>Beauveria bassiana</i> isolate TNAU ENT BB DP3	PQ267932.1	Dharmapuri, Tamil Nadu, India	99.47
<i>Ma-NLR</i> (Chinnacherukuru, Thotapalli Gudur, A. P., India) No. accession/host/ size PV803534 / <i>Nilaparvata lugens</i> Stal./ 539 bp	<i>Metarhizium anisopliae</i> isolate TMBMAVTL	MT229077.1	Karnataka, India	100.00
	<i>Metarhizium sp.</i> isolate UGJKCS10	PQ788221.1	USA: Minnesota	100.00
	<i>Metarhizium anisopliae</i> isolate FM-03	MG825184.1	China	100.00
	<i>Metarhizium anisopliae</i> isolate ERUMA003	OQ186753.1	India	100.00
	<i>Metarhizium anisopliae</i> isolate MA-02	OR731303.1	Gujarat, India	99.82
	<i>Metarhizium anisopliae</i> isolate TNAU	PP776585.1	Tamil Nadu, India (TNAU)	99.82
	<i>Metarhizium sp.</i> isolate TCD 008	KX451122.1	Brazil	99.82
	<i>Metarhizium anisopliae</i> strain VKMA007	MH165400.1	India	99.82
	<i>Metarhizium anisopliae</i> isolate TNAU	PP768165.1	Tamil Nadu, India (TNAU)	99.82
	<i>Metarhizium anisopliae</i> isolate 5B	KX255642.1	Coimbatore	99.65

TNAU: Tamil Nadu Agricultural University, AP: Andhra Pradesh

Thus, Bb-NLR is conclusively a *B. bassiana* isolate and the NJ phylogenetic topology agrees with previously reported *Beauveria* clade structures. Bootstrap analyses supported the tight clustering of Ma-NLR with the *M. anisopliae* group. The evolutionary distance between Ma-NLR and its nearest reference strain was effectively zero (0.000 substitutions/site), indicating minimal genetic divergence. Given that $\geq 99\%$ ITS sequence identity is a standard species threshold, Ma-NLR is unequivocally assigned to *M. anisopliae* (Fig. 4). The resulting NJ topology, supported by strong bootstrap values, mirrors the known species boundaries established by prior studies (36, 37). Hence, the phylogenetic analysis, using appropriate *Beauveria* spp. as the designated outgroups, confirms Ma-NLRs' placement within *M. anisopliae*.

Fungal isolates of the present study exhibiting high phylogenetic relatedness and $>99\%$ sequence similarity in the 18S rDNA region to their respective reference strains can be classified as the same species. Ribosomal DNA markers, particularly 18S rDNA, are widely used for species identification and for elucidating phylogenetic relationships among organisms (38). Research reported about the molecular phylogenetic tree using the ITS-rDNA sequences of isolates employing the neighbour-joining method with MEGA-X-10.1.8 software and performing 1000 bootstrap replications (39). The phylogenetic tree provided strong support for a clade of *B. bassiana* isolates with a bootstrap value of 90%.

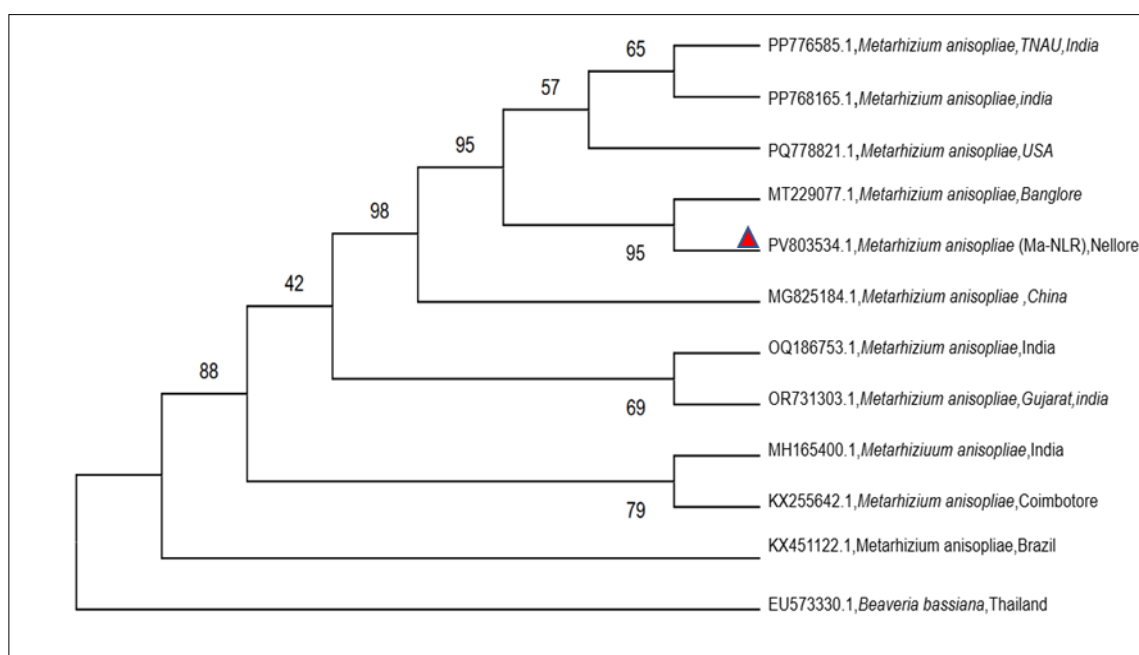


Fig. 4. Phylogenetic tree showing the evolutionary relationship of *Metarhizium anisopliae* isolate Ma-NLR based on ITS region (18S rDNA) sequences, constructed using the Neighbor-Joining method ▲ Isolated EPF in current investigation.

Classical fungal taxonomy relies on a variety of morphological criteria to differentiate genera. Today, however, molecular biology techniques are extensively applied to identify and characterise the genetic variability of entomopathogenic fungi. Among these approaches, molecular markers provide the most accurate means of assessing diversity and intraspecific genetic structure (40). Molecular tools have become fundamental for species recognition and the ITS region of rDNA is considered the official DNA barcode for fungi due to its hypervariable sequences, which enable identification at both the species and subspecies levels (41, 42). In the present study, sequencing of the ITS regions confirmed the molecular taxonomic identity of the two fungal isolates and subsequent alignment and phylogenetic analysis further validated the classification of the strains examined.

Conclusion

This study reports the entomopathogenic fungi associated with brown planthopper (BPH) infestations in rice fields of the Nellore district, Andhra Pradesh, India. Two native fungal strains were isolated from infected BPH specimens. Based on macroscopic and microscopic characteristics, two isolates were identified as *Beauveria bassiana* and one as *Metarhizium anisopliae*. Amplification and sequencing of the ITS1–5.8S–ITS4 region, followed by comparison with molecular databases and phylogenetic analysis, confirmed the taxonomic identity of these strains. Further, conduct of field trials and development of shelf-stable formulations of *B. bassiana* and *M. anisopliae* is needed for practical field-level application.

Authors' contributions

TSR executed the experiment and collected the samples. IP conceptualised and supervised the experiment, analysed the data and prepared the manuscript. All authors read and approved the final manuscript. Acharya N G Ranga Agricultural University, Lam, Guntur, Andhra Pradesh, India has provided funding for the entire study. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

References

- Balachiranjeevi CH, Prahalada GD, Mahender A, Jamaluddin M, Marfori-Nazarea CM, Vinarão R, et al. Identification of a novel locus, BPH38(t), conferring resistance to brown planthopper (*Nilaparvata lugens* Stål) using early backcross population in rice (*Oryza sativa* L.). *Euphytica*. 2019;215:185. <https://doi.org/10.1007/s10681-019-2506-2>
- Rao YK, Bentur JS, Pasalu IC. Rice brown planthopper: Ecology, field problems and management. *Indian J Plant Prot*. 2003;31(1):1–9.
- Backus EA, Serrano MS, Ranger CM. Mechanisms of hopperburn: An overview of insect taxonomy, behavior and physiology. *Annu Rev Entomol*. 2005;50:125–51. <https://doi.org/10.1146/annurev.ento.49.061802.123310>
- Helina S, Sulandari S, Hartono S, Trisyono YA. Detection and analysis of protein profile on rice infected by stunt virus with different severity on Ciherang and Situ Bagendit varieties. *Indones J Plant Prot*. 2019;23(1):116–24. <https://doi.org/10.22146/jpti.36549>
- Rombach MC, Humber RA, Roberts DW. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of *Nilaparvata lugens* and *Nephotettix* spp. (Homoptera: Delphacidae and Cicadellidae). *Mycotaxon*. 1986;26:87–92. <https://doi.org/10.5962/p.418873>
- Malekan N, Hatami B, Ebadi R, Akhavan A, Radjabi R. Evaluation of entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* on different nymphal stages of greenhouse whitefly *Trialeurodes vaporariorum* under greenhouse conditions. *Biharean Biol*. 2015;9(2):108–112.
- Huynh VN, Nhan NT, Hung PQ, Khang VT, Loc NT. Studies on some entomogenous fungi to control brown planthopper in rice. *Omonrice*. 1999;7:149–57.
- Jackson MA, Jaronski ST, Dunlap CA. Ecological factors in the inundative use of fungal entomopathogens. *Biocontrol*. 2000;45:159–85. https://doi.org/10.1007/978-90-481-3966-8_12
- Dong T, Zhang B, Jiang Y, Hu Q. Isolation and classification of fungal whitefly entomopathogens from soils of the Qinghai-Tibet Plateau and Gansu Corridor in China. *PLoS One*. 2016;11(5):e0156187. <https://doi.org/10.1371/journal.pone.0156087>
- Dayanti AK, Sholahuddin, Yunus A, Subositi D. First record of entomopathogenic fungi on autumn leaf caterpillar (*Doleschallia bisaltide*). *IOP Conf Ser Earth Environ Sci*. 2018;142(1):012034. <https://doi.org/10.1088/1755-1315/142/1/012040>
- Atta S, Rizwan M, Ullah MI, Khan AR. Pathogenicity of entomopathogenic fungi against brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). *Egypt J Biol Pest Control*. 2020;30(1):1–7.
- Reddy AV, Devi RS, Dhurua S, Reddy DVV. Study on the efficacy of some entomogenous fungi against brown planthopper, *Nilaparvata lugens* Stål in irrigated rice. *J Biopest*. 2013;6(2):139–43. <https://doi.org/10.57182/jbiopestic.6.2.139-143>
- Trizelia T, Rahma H. Virulence of five isolates of the entomopathogenic fungus *Metarhizium anisopliae* against brown planthopper (*Nilaparvata lugens*). *J Proteksi Tanaman*. 2023;7(2):127–33. <https://doi.org/10.25077/jpt.7.2.127-133.2023>
- Quesada-Moraga E, Navas-Cortés JA, Maranhao EAA, Ortiz-Urquiza A, Santiago-Álvarez C. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycol Res*. 2007;111(8):947–66. <https://doi.org/10.1016/j.mycres.2007.06.006>
- Teixeira AC, Faria MR. Protocols for isolation and characterization of entomopathogenic fungi. *Biol Control*. 2010;53(2):195–200.
- Lacey LA, Solter LF. Initial handling and diagnosis of diseased invertebrates. In: Lacey LA, editor. *Manual of techniques in invertebrate pathology*. 2nd ed. Academic Press; 2012. p. 1–13. <https://doi.org/10.1016/B978-0-12-386899-2.00001-4>
- Schapovaloff ME, Alves SB, Lopez Lastra CC. Occurrence of entomopathogenic fungi in soils of agricultural crops in Argentina. *Rev Soc Entomol Argent*. 2015;74(3–4):119–26.
- Humber RA. Entomopathogenic fungal identification. Ithaca: USDA-ARS Plant Protection Research Unit; 2005 [cited 2026 Mar 15]. Available from: <https://www.ars.usda.gov/arsuserfiles/80620520/apswkshoprev.pdf>
- Samson RA, Evans HC, Latgé JP. Atlas of entomopathogenic fungi. New York: Springer-Verlag; 1988. p.1–187. https://doi.org/10.1007/978-3-662-05890-9_1
- Kirk PM, Cannon PF, Minter DW, Stalpers JA. *Ainsworth & Bisby's dictionary of the fungi*. 10th ed. Wallingford (UK): CAB International; 2008. <https://doi.org/10.1079/9780851998268.0000>

21. Humber RA. Identification of entomopathogenic fungi. In: Lacey LA, editor. Manual of techniques in invertebrate pathology. 2nd ed. Academic Press; 2012. <https://doi.org/10.1016/B978-0-12-386899-2.00006-3>
22. Lee SB, Taylor JW. Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: A guide to methods and applications. San Diego: Academic Press; 1990. p.282–87. <https://doi.org/10.1016/B978-0-12-372180-8.50038-X>
23. White TJ, Bruns T, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: A guide to methods and applications. San Diego: Academic Press; 1990. p.315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
24. Hiraishi A, Shin YK, Ueda Y, Sugiyama J, Nishiyama K, Komagata K. Phylogenetic relationships among species of the genus *Acinetobacter* based on the 16S rRNA gene sequences. Int J Syst Bacteriol. 1995;45(4):623–9.
25. Hall T. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.
26. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–4. <https://doi.org/10.1093/molbev/msw054>
27. Kirubhadharsini BL, Nakkeeran S, Kennedy JS, Manoharan T. Morphological and molecular characterization of entomopathogenic fungi isolated from different *Beauveria bassiana* isolates from insects in India. Green Farming. 2017;4:940–44.
28. Gebremariam A, Chekol Y, Assefa F. Phenotypic, molecular and virulence characterization of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from soil samples of Ethiopia for development of mycoinsecticide. Heliyon. 2021;7(5):e07091. <https://doi.org/10.1016/j.heliyon.2021.e07091>
29. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. St Paul (MN): American Phytopathological Society; 1999.
30. Slavimira A, Draganova D, Takov I. Naturally occurring entomopathogenic fungi on three bark beetle species (Coleoptera: Curculionidae) in Bulgaria. Pestic Phytomed. 2010;25(1):59–63. <https://doi.org/10.2298/PIF1001059D>
31. Elham MSH, Pong KK, Lee GEL, Azmi WA. Occurrence of entomopathogenic fungus *Metarhizium anisopliae* isolated from island, BRIS and coastal soils of Terengganu, Malaysia. J Sustain Sci Manag. 2018;13(5):179–89.
32. Bai NS, Sasidharan TO, Remadevi OK, Dharmarajan P, Pandian SK, Balaji K. Morphology and RAPD analysis of certain potentially entomopathogenic isolates of *Metarhizium anisopliae*. J Microbiol Biotechnol Res. 2015;5(1):34–40.
33. Lim SY, Lee S, Kong HG, Lee J. Entomopathogenicity of *Simplicillium lanosoniveum* isolated in Korea. Mycobiology. 2014;42(4):317–21. <https://doi.org/10.5941/MYCO.2014.42.4.317>
34. Imoulan A, Ibnsouda SK, El Meziane A. Molecular characterization and effectiveness of native entomopathogenic *Beauveria bassiana* strains against adults of Mediterranean fruit fly (*Ceratitidis capitata*). J Biosci Biotechnol Discov. 2016;1(1):6–16. <https://doi.org/10.31248/JBBD2016.008>
35. Rehner SA, Buckley E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia. 2005;97(1):84–98. <https://doi.org/10.3852/mycologia.97.1.84>
36. Henry T, Iwen PC, Hinrichs SH. Identification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. J Clin Microbiol. 2000;38(4):1510–15. <https://doi.org/10.1128/JCM.38.4.1510-1515.2000>
37. Shenoy BD, Jeewon R, Hyde KD. Impact of DNA sequence data on the taxonomy of anamorphic fungi. Fungal Divers. 2007;26:1–54.
38. Bich GA, Castrillo ML, Kramer FL, Villalba LL, Zapata PD. Morphological and molecular identification of entomopathogenic fungi from agricultural and forestry crops. Floresta Ambiente. 2021;28(2):e20180180. <https://doi.org/10.1590/2179-8087-floram-2018-0086>
39. Sayed SM, Ali EF, Arnaouty SAE, Mahmoud SF, Amer SA. Isolation, identification and molecular diversity of indigenous isolates of *Beauveria bassiana* from Taif region, Saudi Arabia. Egypt J Biol Pest Control. 2018;28:47. <https://doi.org/10.1186/s41938-018-0054-z>
40. Becerra RA, Rangel RE, Rodriguez AA. Genetic variability and population structure using molecular markers. J Genet Mol Biol. 2007;18(2):123–34.
41. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci USA. 2012;109(16):6241–6. <https://doi.org/10.1073/pnas.1117018109>
42. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One. 2012;7(7):e40863. <https://doi.org/10.1371/journal.pone.0040863>

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