

COLLECTION, ISOLATION AND MORPHO-MOLECULAR CHARACTERIZATION OF ENTOMOPATHOGENIC FUNGI, *METARHIZIUM RILEYI* (FARL.) INFECTING FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The present study focuses on collection, isolation and identification of entomopathogenic fungi infecting fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) from various agroclimatic zones of Karnataka. A total of six fungal isolates belonging to the genus *Metarhizium* were collected and isolated from dead fall armyworm larva showing mycosis. Macroscopic features revealed whitish colony, with colony diameter ranging from 2.11 – 5.23 cm, with rough colony texture raised at the centre. Microscopic features revealed ovoid, aseptate, 2 x 3 µm (2–4 x 2–3 µm) conidia arranged in parallel chains and forming dry divergent clusters. Further, molecular confirmation of the fungi using Internal Transcribed Spacer (ITS5 and ITS4) confirmed that they belong to *M. rileyi* species with similarity or identity indexes of 99 %, and which was further confirmed, inferring the phylogenetic tree obtained using MEGA software with bootstraps values up to 97 % to the reference strains from the NCBI. Further, the pairwise distance matrix revealed little or no genetic divergence among the collected isolates. These identified entomopathogenic fungi (EPF) can be further used for eco-friendly management of the invasive pest fall armyworm.

KEYWORDS: Entomopathogenic fungi, *Metarhizium*, Morphology, Molecular, Genetic divergence

INTRODUCTION

Insect pathogenic fungi are remarkably diverse in terms of the number of species and lineages and have a wide host range, and are included in major successful biological pest control programmes (Faria & Wraight, 2007). Fungi cause many diseases in insects and were the first pathogens used for the microbial control of insect pests. One such example is *Metarhizium rileyi* (Farlow) Kepler, SA Rehner and Humber, a cosmopolitan fungal pathogen that infects several species of Lepidoptera, including many important agricultural pests (Vimala *et al.*, 2003). *M. rileyi* was described in 1883 originally as *Botrytis rileyi* (Farlow) and later as *Spicaria rileyi* (Farlow) Charles. Kish *et al.* (1974) redescribed this fungus and transferred it to the genus *Nomuraea* Maublanc. Recently, a new combination was proposed by Kepler *et al.* (2014) moving it to the genus *Metarhizium*.

The parasitic taxa within this order are known to target a wide variety of host organisms, including plants, fungi, nematodes, and insects (Zhang *et al.*, 2018). Furthermore, specific Hypocrealean species demonstrate complex, multi-trophic lifestyles (Hu and Bidochka, 2019). For instance, endophytic insect pathogenic fungi (EIPF) are uniquely adapted to simultaneously colonize host plant tissues as endophytes while acting as lethal pathogens to insects (Branine *et al.*, 2019).

M. rileyi is a dimorphic fungus with yeast-like hyphal bodies and true filamentous growth phase, with a number of isolates isolated in various parts of the world (Humber, 1992). The host range of *M. rileyi* is more restricted than fungi like *B. bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Metchnikoff) Sorokim. *M. rileyi* is mostly limited to lepidoptera larvae, especially in the superfamily Noctuoidea (Suwannakut *et al.*, 2005), although a few beetles, some hemipterans and representatives of a few other arthropod orders are also vulnerable to this fungus (Matter & Sabbour, 2013).

The genetic variability of *M. rileyi* is more strongly related to the host species than to geographic location (Suwannakut *et al.*, 2005). This explains the observation that *M. rileyi* isolates are more virulent against the host species from which they were isolated (Boucias *et al.*, 1982, 2000). Likewise, the period required for conidia germination and growth as well as the completion of the life cycle of the fungus varies according to the strain and host species. Different populations of *M. rileyi* may have great genotypic variability as a function of the recombination in the nuclear genome and in the mitochondria. The expression of this variability may hinder or even impede the monitoring of an isolate after its application in the field, even though the practical results of pest control are achieved.

The scarcity of field studies, with detailed information about the behaviour of different strains of *M. rileyi* in a variety of environmental conditions after application, is a gap that must be filled. Conducting field evaluations and monitoring natural epizootics can help increase the range of species that can be controlled by of *M. rileyi* and provide new bio-ecological data that will enable more efficient use of this organism.

Despite significant advances in understanding the diverse ecological roles of Hypocrealean fungi, the complex, multifunctional behaviors of the genus *Metarhizium* require further exploration. Accordingly, the present study focuses on the collection of naturally infected insect cadavers from agro-ecosystems, followed by isolation of the associated fungi on appropriate culture media. The recovered isolates were subsequently subjected to detailed morphological and molecular characterization to enable confirmation at the species level which can be further used to evaluate its efficacy against various pest of agriculture importance.

MATERIALS AND METHODS

Samples collection and isolation

Field visits were made around several districts of Karnataka for the collection infected insect cadavers (Figure 1). A total of 21 infected insect samples were collected from all the sites. The collected samples were brought to the insect pathogen laboratory in a sealed vials to avoid any contaminations. Fungus isolation was made following the technique outlined by Senthil *et al.* (2015), with slight modifications. The dead individuals of each species were superficially disinfected using 10 per cent sodium hypochlorite for 3 seconds and subsequently rinsed twice with distilled water. The specimens were then air-dried and placed in Petri dishes lined with wet Whatman paper (No. 1), incubating them at 25°C for 48 hours in the absence of light (Fig 2.). Cadavers that showed fungal growth were selected and transferred to Sabouraud Dextrose Agar (SDA) medium, where they were incubated again at 25°C for 14 days. The single spore method was utilized to achieve purified cultures of each fungal isolate (Figure 2).

Morphological identification

To study the colony characters of the fungus, 6 mm disc of the fungus (grown on PDA) was inoculated on Sabouraud dextrose agar with yeast extract (SDAY) and incubated at 25 ± 2 °C for 7 days. Macroscopic features such as growth and appearance of the colony, pigmentation on reverse of the media were recorded. Micro-morphological features of conidiophores, conidiogenous cells, and conidia of the fungus grown on culture media, and from field collected infected insects were recorded using Leica microscope (Leica DM750) attached with camera (Leica MC170 HD). Conidia and conidiophores were mounted in lactic acid cotton blue and measured using the Leica Application Suite, version 4.12.0 with 10 measurements per structure.

Molecular identification and phylogenetic analysis

One-week old fungus grown on SDAY at 25 ± 1 °C was used to extract total genomic DNA. Fungal mycelia was crushed with sodium lauryl sulphate buffer and kept for incubation at 60°C for 20 min. Further DNA extraction was performed by using Cetyltrimethylammonium bromide (CTAB) with few modifications. The newly formed mycelia was scraped using a sterile slide onto pestle and mortar. Later required quantity of the prepared Sodium Dodecyl Sulfate buffer (0.5M EDTA + 1M Tris HCL + 5M NaCl + SDS) was added to crush the mycelia. Crushed mycelia was transferred to 2 mL eppendorf tubes and incubated at 60 °C for 10 minutes. Next, 280 µL of 5M NaCl and 140 µL of 10% CTAB were introduced, followed by another 10-minute incubation at 60 °C. Afterward, 500 µL of a 24:1 chloroform-isoamyl alcohol mixture was added, and the samples were centrifuged at 10,000 RPM for 12 minutes. The resulting supernatant was carefully transferred to new 1.5 mL Eppendorf tubes, and this extraction process was repeated. Then into the 1.5 mL Eppendorf tube three by fourth isopropanol and one fourth 3M sodium acetate was added and stored in -20. °C overnight. The next day the samples were centrifuged at 10,000 RPM for 12 minutes and the aqueous phase discarded. Further, 1 mL of 70 per cent ethanol was added and centrifuged at 8,000 RPM for 2 minutes, repeating the step twice. The obtained pellets were air dried until the ethanol evaporated and diluted to required concentration using nuclease free water. Once the DNA was extracted, the presence was confirmed by performing genomic DNA gel run with bromophenol blue. For amplification of the DNA Internal Transcribed Spacer (ITS) primer pair ITS5/ITS4 were used (Kumar *et al.*, 2016). Amplification reactions were made in volumes of 25 µl with 10 ng of template DNA. PCR conditions were as followed, one cycle at 94 °C for 5 min was followed by 35 cycles at 94 °C for 40 s, 49 °C for 40 s, 72 °C for 60 s, and then 1 cycle at 72 °C for 10 min. The PCR products were given for sequencing at Medauxin and the obtained sequences were manually trimmed using Bio-Edit software and subjected to BLAST (Basic Local Alignment Search Tool) to identify the significant homology from Gene bank, then the sequences were deposited to gain accession number. Reference ITS sequences of taxonomically recognized species of EPF used in earlier studies were retrieved from the GenBank and were initially aligned using ClustalW multiple alignment tool in BioEdit software. Further MEGA version 11.0 was used to compare the sequences and to construct phylogenetic tree.

RESULTS AND DISCUSSION

Collection and isolation

In the present study a total of six isolates belonging to genera *Metarhizium* were collected and isolated from fall armyworm, *Spodoptera frugiperda* across geographical locations of Karnataka (Table 1). Among which two isolates were collected from Chitradurga (UASB-SF1 and UASB-SF3) districts and one isolate each from Bangalore (*M. rileyi* UASB SF2), Mandya (*M. rileyi* UASB SF5), Hassan (*M. rileyi* UASB SF8) and Chikballapur

(UASB SF9). Similar studies conducted by Ranadev *et al.* (2023) in eastern dry zone and southern dry zone of Karnataka, resulted in isolation of 81 isolates of entomopathogenic fungi from soil and infected cadavers, belonging to various genera and identified two species *Isaria fumosorosea* and *Hirsutella thompsonii* as effective entomopathogenic fungi against mites.

Morphological identification

From the samples collected across various districts of Karnataka, all fungal strains showing typical characteristics of entomopathogenic fungi were isolated. Of these fungi, all the six isolates resembled to *M. rileyi* species.

The isolates presented white colonies that after three weeks of incubation developed conidiophores in compact green clusters, typical macroscopic characteristics of *Metarhizium* (Figure 3). In the microscopic observations, the strains presented reproductive structures and conidia with morphology, size, and color typical of *M. rileyi*. In the microscopic observations, all the isolates presented reproductive structures and conidia with morphology, size, and color typical of *Metarhizium*. The isolates exhibited a slower growth with white colony and elongated conidia (Table 1). Based on all the above-mentioned morphological characters, the fungi were identified with the help of the keys given by Humber (2012). Mongkolsamrit *et al.* (2020) also described *Metarhizium* as the genus which involves beautiful branching pattern of the conidiophores, which are densely inter-wound, rounded to conical apices alignment of the conidiogenesis cells which are arranged in a dense hymenium. The conidia are without cross walls, therefore, termed as aseptate, and are of pale or bright green or yellow-green colour (Leadmon *et al.*, 2020). They are ovoid or cylindrical in the form of chains which are usually aggregated into prismatic or cylindrical columns or even a solid mass of parallel chains (Bridge *et al.*, 1993). The morphological identification (employing macroscopic and microscopic information) allowed the identification of the isolates to the genera and species levels. All the fungal strains obtained corresponded to microorganisms of the Phylum *Ascomycota*, class *Sordariomycetes*, and family *Clavicipitaceae* confirming them as isolates belonging to genus *Metarhizium* (Humber, 2005, 2012).

Molecular identification of entomopathogenic fungi

The PCR products of the ITS regions of the rDNA of the isolated entomopathogenic fungi generated fragments of approximately 600 bp (Table 2).

The results of the identity analysis of the sequences obtained for six isolates morphologically identified as *Metathizium* allowed confirming that they belonged to *M. rileyi* species with similarity or identity indexes of 99%. Therefore, it was possible to verify the similarity at molecular level for all the six isolated strains of entomopathogenic fungi. All the fungal isolates made a cluster with *M. rileyi* sequences with Bootstrap values of 97% (Figure 4). The phylogenetic tree included six UASB isolates, namely UASB-SF9 (PV248708.1), UASB-SF8 (PV770124.1), UASB-SF5 (PV770219.1), UASB-SF1 (PV248922.1), UASB-SF3 (PV767507.1), and UASB-SF2 (PZ437994.1), together with reference sequences of *Metarhizium rileyi* retrieved from GenBank including FAW-L2 (OR826620.1), CBS 807.71 (MH860365.1), KNU-Gunwi 2B (LC591851.1), KNU-Ye (ON114181.1), 936 (LR792766.1), 1972 (LR792765.1), DT2011N7 (KX641194.1), and *M. rileyi* (KY436756.1). Among the UASB isolates, UASB-SF9, UASB-SF8 and UASB-SF5 occurred in the upper portion of the *M. rileyi* assemblage and grouped with the isolate FAW-L2, whereas UASB-SF1, UASB-SF3 and UASB-SF2 occurred in the lower portion of the same lineage and grouped with CBS 807.71, KNU-Gunwi 2B. Thus, although the six isolates were distributed at different positions within the *M. rileyi* lineage, they remained associated with the same species group, indicating intraspecific sequence variation among the UASB isolates (Table 3 and 4). Whereas, all the *Metarhizium rileyi* isolates, had a clear grouping when compared to the other species of the *Metarhizium*.

Intraspecific genetic divergence analysis revealed low sequence variation among the *Metarhizium rileyi* UASB isolates and GenBank references (Table 3), with maximum-likelihood distances ranging from 0.0000 to 0.0203 substitutions per site. UASB-SF1, SF2, SF3, SF5 and SF8 showed complete sequence identity, while SF9 differed by only 0.0017 substitutions per site from SF1–SF3. Several reference sequences, including 1972 (LR792765.1), 936 (LR792766.1) and FAW-L2 (OR826620.1), also showed zero divergence from most UASB isolates. The highest distances were observed between CBS 807.71 (MH860365.1) and selected reference sequences, reaching 0.0196 substitutions per site. Overall, the low evolutionary distances indicate limited sequence variation and support the close genetic affiliation of the UASB isolates with *M. rileyi*.

Whereas, interspecific genetic divergence analysis based on ITS sequences revealed substantially greater genetic distances among *Metarhizium* species than within *M. rileyi* (Table 4). The distance between *M. rileyi* UASB-SF1 and *M. rileyi* CBS 807.71 was only 0.0017 substitutions per site, whereas distances between *M. rileyi* and other *Metarhizium* species ranged from 0.1378 to 0.2591 substitutions per site. The lowest interspecific divergence was observed with *M. flavoviride* (0.1378), while the highest was with *M. acridum* (0.2591). *M. cylindrosporium* showed a distance of 0.1802, whereas *M. brunneum* and *M. anisopliae* showed higher distances of 0.2473 and 0.2586, respectively. *Cordyceps javanica* exhibited the greatest divergence from *M. rileyi* (0.2967 substitutions per site). Overall, the markedly higher interspecific distances compared with the intraspecific variation support the distinct genetic identity of the UASB *M. rileyi* isolates.

The classical taxonomy employs diverse morphological criteria for the determination of the different fungal genera. Currently, molecular biology techniques are used to identify and characterize the genetic variability of entomopathogenic fungi. The most precise way to determine the levels of diversity and intraspecific genetic structure is through the use of molecular markers (Becerra *et al.*, 2007). Molecular tools are being widely used for the recognition of fungal species, the ITS region of the rDNA is considered the official DNA barcode for fungi since it has hypervariable sequences that allow recognition both at the species level and at lower levels (Schoch

et al., 2012). In the present work, the sequencing of the ITS regions of the rDNA allowed the corroboration of the molecular taxonomic identity of the five fungal strains; and the alignment and phylogenetic analysis confirmed the taxonomic identity of the strains analyzed.

Schoch *et al.* (2012) established the nuclear ribosomal ITS region as the universal DNA barcode for fungi, while previous studies on *Metarhizium* demonstrated that ITS sequences provide useful resolution for distinguishing species and major lineages. In particular, Driver *et al.* (2000) and Pantou *et al.* (2003) amplifying the ITS1-5,8S-ITS2 region of *Metarhizium* strains found sufficient genetic variability in these markers to differentiate clades of different species within the genus *Metarhizium*.

The grouping of the UASB isolates with *M. rileyi* sequences from geographically diverse origins is also consistent with previous molecular studies of this species. For example, studies of *M. rileyi* associated with *Spodoptera frugiperda* have used ITS-based phylogenetic analysis to confirm species identity and demonstrate genetic differentiation among isolates (Peng *et al.*, 2024). In one such study, isolates from different geographical regions formed separate subgroups within the broader *M. rileyi* lineage, suggesting considerable genetic variation within the species. Similarly, recent analyses of *M. rileyi* isolates have demonstrated that isolates belonging to the same species can exhibit measurable genetic differentiation and form distinct phylogenetic clusters (Ramos *et al.*, 2024). It is important to note that molecular techniques do not replace or eliminate the need of use classical morphological taxonomy tools, but instead, they should be used to complement the studies to achieve a complete, accurate and sufficiently descriptive identification (Hyde *et al.*, 2010). Consequently, a procedure of morphological and molecular identification for biocontrol agents is ideal because when applying a biological control system, it is essential a good identification procedure of the fungal isolates that are being used as a way to measure the efficiency of the system (Coates *et al.*, 2002). The strains of entomopathogenic fungi obtained in the present study will be used later by researchers working in biocontrol to evaluate the entomopathogenic efficiency against different plague insects of agricultural and forestry crops. The possible application of entomopathogenic fungi in agricultural and forestry crops of Misiones could generate advantages in the health of the crops in a medium and long term, as well as add value to the crops by including techniques of biological management of pests in a sustainable manner.

CONCLUSIONS

A total of six fungal isolates belonging to the genus *Metarhizium* were isolated from various locations across Karnataka. The macro and microscopic characters depicted by these isolates confirmed upto the genus *Metarhizium* and further molecular characterization delimited them to the species *Metarhizium rileyi*. The entomopathogenic fungal strains obtained in this study will later be utilized further to test their entomopathogenic potential against various pest insects affecting agricultural and forestry crops and for development of a suitable product that can be used for Integrated Pest Management.

ACKNOWLEDGEMENT

I sincerely thank the Anusandhan National Research Foundation (ANRF) and the Confederation of Indian Industries (CII), Government of India, under the Prime Minister's Fellowship for Doctoral Research. I am also deeply grateful to the Department of Agricultural Entomology at the University of Agricultural Sciences, College of Agriculture, Bangalore, for providing the essential facilities to carry out this study.

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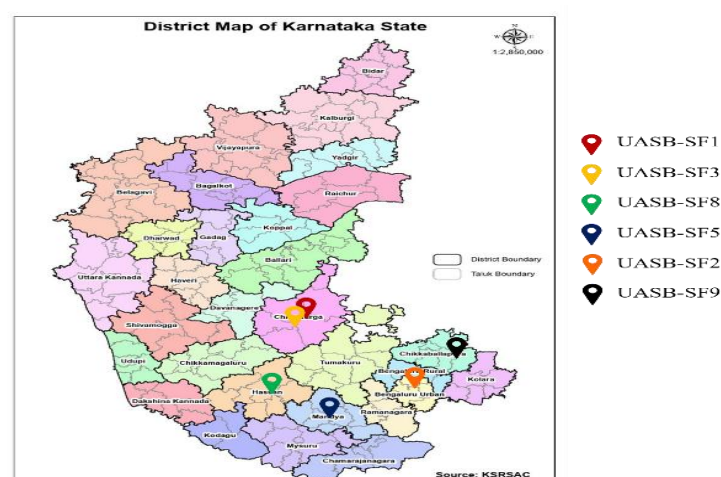


Figure 1. Geographical location of the *Metarhizium rileyi* isolates collected during the study



Figure 2. Collection and isolation of *Metarhizium rileyi* isolates a,b,c- Infected fall armyworm cadavers, d,e- cadavers kept for sporulation, f,g- Isolation of the fungi on PDA

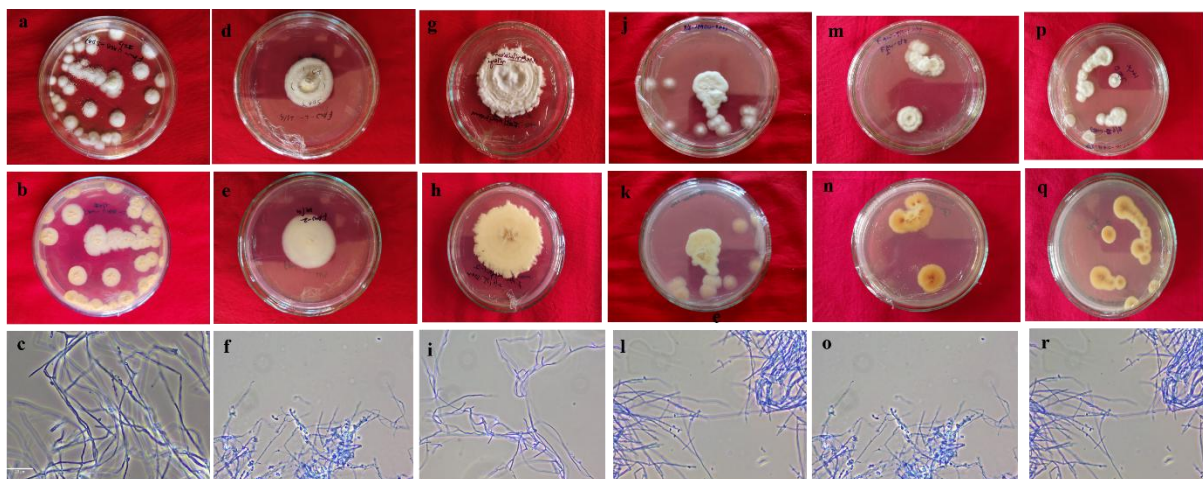


Figure 3. Macroscopic and microscopic characteristics of *Metarhizium rileyi* isolates on PDA. a, b & c – UASB SF1; d, e, f- UASB SF2; g, h, i,-UASB SF3; j, k, l-UASB SF5; m, n, o-UASB SF8; p, q, r-UASBS SF9

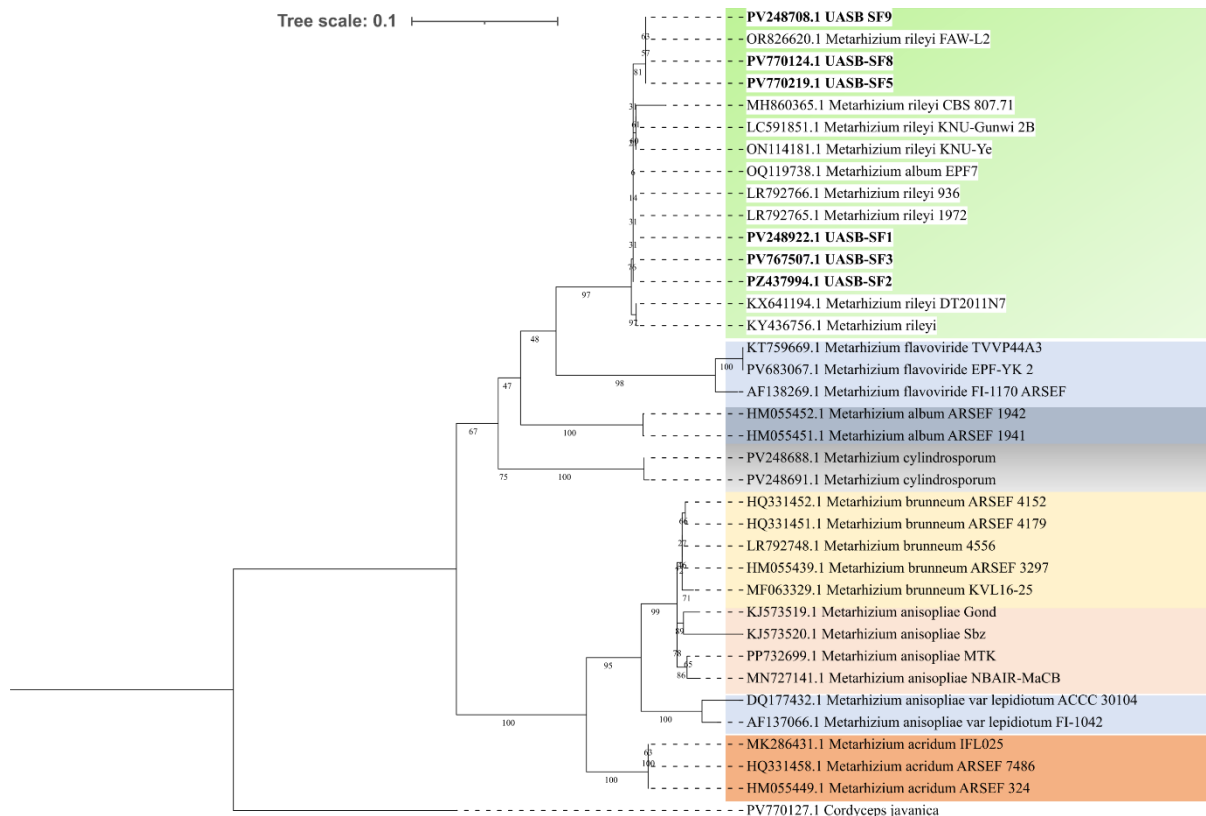


Figure 4. *Metarhizium* consensus tree of phylogenetic relationships. Tree based on the alignment of the ITS1-5.8S-ITS2 region of the rDNA of fungal strains together with sequences obtained from GenBank (NCBI). Bootstrap test with 1000 replications and the Tamura-Nei model for the calculation of genetic distance. The red circle indicates the Bootstrap value obtained for the group of interest. In bold are the entomopathogenic fungi isolated and identified in this work. The boxes with different colors show the varieties in the *Metarhizium* species.

Table 1. Genus level identification of entomopathogenic fungal isolates based on macroscopic and microscopic characteristics

Fungal isolate	Morphological characters							
	Mycelial growth (SDA) (cm)	Colony colour	Colony texture	Surface and topography	Margin	Pigmentation	Conidia length (L*B) µm	Other important characters
<i>Metarhizium rileyi</i> UASB-F1	3.31	Whitish	Rough	Raised center at	Irregular	pale yellow to orange-brown	3.2–4.1x2–3	Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-F2								Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-F3	4.21	Whitish	Rough	Raised center at	Irregular	pale yellow to orange-brown	3.5–4.5X2–3.1	Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-F5	3.38	Whitish	Rough	Raised center at	Irregular	pale yellow to orange-brown	3.3–4x1.9–2.8	Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-F8	3.76	Whitish	Rough	Raised center at	Irregular	pale yellow to orange-brown	3.3–4.x2–3	Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-CHNT1	2.71	Whitish to yellow	Rough	Concentric circles	Irregular	pale yellow to orange-brown	3.2–4.2x2.1–3.3	Conidia arranged in parallel chains
<i>Metarhizium rileyi</i> UASB-MLW1	5.23	Whitish	Smooth	Flat	regular	pale yellow to orange-brown	3.5–4.5X3.1–3.5	Conidia arranged in parallel chains

Table 2. Isolates and their NCBI accession number

Sl. No	Isolate	NCBI Accession number
1.	<i>Metarhizium rileyi</i> UASB-SF1	PV248922
2.	<i>Metarhizium rileyi</i> UASB-SF2	PZ437994
3.	<i>Metarhizium rileyi</i> UASB-SF3	PV767507
4.	<i>Metarhizium rileyi</i> UASB-SF5	PV770219
5.	<i>Metarhizium rileyi</i> UASB-SF8	PV770124
6.	<i>Metarhizium rileyi</i> UASB-CHNT1/SF9	PV248708

Table 3. Intraspecific pairwise genetic divergence matrix for UASB isolates and genbank *M. rileyi* references

Isolates / Taxa	1	2	3	4	5	6	7	8	9	10
UASB-SF1	—									
UASB-SF2	0.0000	—								
UASB-SF3	0.0000	0.0000	—							
UASB-SF5	0.0000	0.0000	0.0000	—						
UASB-SF8	0.0000	0.0000	0.0000	0.0000	—					
UASB-SF9	0.0017	0.0017	0.0017	0.0000	0.0000	—				
1972 (LR792765.1)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	—			
KNU-Ye (ON114181.1)	0.0017	0.0017	0.0017	0.0022	0.0018	0.0034	0.0016	—		
FAW-L2 (OR826620.1)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0069	0.0052	—	
CBS 807.71 (MH860365.1)	0.0017	0.0017	0.0017	0.0022	0.0018	0.0034	0.0196	0.0164	0.0193	—
936 (LR792766.1)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000	0.0016	0.0069	0.0196
DT2011N7 (KX641194.1)	0.0034	0.0034	0.0034	0.0000	0.0000	0.0034	0.0033	0.0050	0.0086	0.0186
KY436756.1	0.0051	0.0051	0.0051	0.0000	0.0000	0.0051	0.0049	0.0066	0.0086	0.0203
KNU-Gunwi_2B (LC591851.1)	0.0017	0.0017	0.0017	0.0022	0.0018	0.0034	0.0016	0.0000	0.0017	0.0098

(Note: Values represent Maximum Likelihood pairwise evolutionary distances measured in substitutions per site. GenBank accession numbers are provided in parentheses)

Table 4. Matrix of Interspecific pairwise genetic distances among *Metarhizium rileyi* taxa and 6 related species based on internal transcribed spacer (ITS) gene sequences, in addition to the out group

Isolates / Taxa	1	2	3	4	5	6	7	8	9
<i>M. rileyi</i> UASB-SF1	—								
<i>M. rileyi</i> CBS 807.71	0.0017	—							
<i>M. flavoviride</i> TVVP44A3	0.1378	0.1322	—						
<i>M. album</i> ARSEF 1942	0.1755	0.2007	0.2173	—					
<i>M. cylindrosporum</i> UASB-M1	0.1802	0.1845	0.2047	0.1799	—				
<i>M. brunneum</i> ARSEF 4152	0.2473	0.2496	0.2691	0.2486	0.2786	—			
<i>M. anisopliae</i> Gond	0.2586	0.2564	0.2777	0.2735	0.2912	0.0156	—		
<i>M. acridum</i> ARSEF 324	0.2591	0.2908	0.2693	0.2182	0.2646	0.1187	0.1237	—	
<i>C. javanica</i> UASB-IJ2	0.2967	0.2939	0.2658	0.3637	0.3381	0.3041	0.3228	0.3197	—

Note: Values represent Maximum Likelihood pairwise evolutionary distances measured in substitutions per site. The native isolate UASB-SF1 was utilized as the representative taxon for the collected *M. rileyi* strains due to the absence of intraspecific genetic divergence ($d = 0.0000$) among the UASB-SF isolates. Strain UASB-IJ2 (*Cordyceps javanica*) was included as an outgroup to root the analysis.