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Comparative analysis of epiphytic and endophytic fungal communities in traditional rice seeds

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ABSTRACT

Seed-associated fungal communities play a crucial role in plant health, stress tolerance, and crop productivity, particularly in traditional rice varieties cultivated under low-input systems. This study comparatively analysed the diversity and distribution of epiphytic and endophytic fungi associated with seeds of three traditional rice (Oryza sativa L.) varieties, Mapillai Samba, Karuppu Kavuni, and Karung Kuruvai, using serial dilution and culture-based isolation on Potato Dextrose Agar and Rose Bengal Agar. A total of 101 fungal isolates were recovered, comprising 34 epiphytic and 67 endophytic fungi, indicating a predominance of endophytic associations across all varieties. Karung Kuruvai harboured the highest fungal diversity, followed by Mapillai Samba and Karuppu Kavuni. Morphological and microscopic characterisation revealed diverse fungal genera, including Fusarium, Penicillium, Aspergillus, Alternaria, Trichoderma, Diaporthe, and Bipolaris, with Fusarium being the most dominant. The observed diversity highlights the ecological significance of seed-associated fungi and their potential role in enhancing seed health, stress resilience, and sustainable rice production. However, the study faced challenges such as limitations in culture-dependent methods, which may have underestimated the presence of non-culturable or slow-growing taxa. Additionally, distinguishing morphologically similar species posed difficulties, highlighting the need for molecular tools for more precise identification in future research.

Keywords: *Traditional rice varieties, Seed-associated fungi, Epiphytes, Endophytes, Fungal diversity, Culture-based isolation, Morphological characterization.*

Introduction

Fungi play a complex and indispensable role in plant health, functioning both as mutualistic symbionts and opportunistic or pathogenic agents within agricultural ecosystems. In rice (*Oryza sativa* L.), the complex interactions between fungal communities and plant tissues have profound involvement for seed viability, germination success, seedling vigour, nutrient dynamics, stress tolerance, and overall yield stability. Traditional rice varieties have been cultivated and preserved over generations through farmer-led selection processes. These varieties are not only genetically diverse and highly adaptive to local agroclimatic conditions, but they also exhibit innate resistance to a broad spectrum of biotic and abiotic stresses. Importantly, they serve as ecological reservoirs for a wide range of beneficial microorganisms, including epiphytic and endophytic fungi that inhabit various plant compartments such as seeds, roots, stems, and foliage [7,8]. Among these, seed-associated fungi are of particular importance due to their early and sustained influence on plant development. These fungi are generally classified into two major categories: epiphytes, which colonise the seed surface, and endophytes, which reside within the internal tissues of the seed and other plant parts without immediately triggering disease symptoms [2]. Endophytic fungi, in particular, can exhibit dualistic behaviour, acting as either beneficial symbionts or latent pathogens depending on environmental cues, host health, or interspecies competition. Beneficial endophytes contribute to plant fitness through a range of mechanisms, including enhanced nutrient acquisition (e.g., phosphorus, potassium, zinc solubilization), modulation of plant hormone levels such as auxins and gibberellins, induction of systemic acquired resistance (SAR), and competitive inhibition of phytopathogens through the production of antimicrobial secondary metabolites [6,15].

The ecological and agronomic relevance of these fungal communities extends well beyond individual host plants. They are integral to shaping rhizosphere dynamics, influencing plant-soil feedback mechanisms, and stabilising ecosystem functions under variable environmental conditions. As such, the isolation, taxonomic identification, and functional characterisation of fungal isolates from traditional rice varieties offer valuable insights into microbial biodiversity and ecosystem services. These efforts may also lead to the discovery of novel fungal strains with applications in sustainable agriculture, such as biofertilizers, biocontrol agents, or stress-mitigating inoculants that can be harnessed to reduce reliance on synthetic

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agrochemicals [1, 12].

Hence, exploring the functional diversity of seed-borne fungi holds promise for the development of next-generation crop protection strategies that align with agroecological principles. Incorporating beneficial fungi into integrated pest and disease management frameworks can help reduce the incidence of major rice pathogens, enhance resilience to climate-induced stresses, and improve overall crop productivity in an environmentally sustainable manner. Given the accelerating challenges posed by climate change, soil degradation, and the overuse of chemical inputs, leveraging the microbiome of traditional rice varieties represents a promising pathway toward regenerative and climate-smart agriculture [5, 8]. This study focuses on isolating and identifying fungal communities associated with three traditional rice varieties: Mapillai Samba, Karuppu Kavuni, and Karung Kuruvai. The study aims to examine their distribution, classify them based on morphological characteristics, and contribute to understanding their functional significance in seed health and crop improvement.

Materials and Methods

Freshly harvested seeds from three traditional rice varieties were obtained from farmers' fields located in the Erode district, Tamil Nadu. The experiment was conducted in the Seed Health Laboratory at the Department of Seed Science and Technology at Tamil Nadu Agricultural University, Coimbatore. Upon collection, the seed samples were thoroughly washed, sun-dried, and stored under cold conditions with optimal moisture levels for future research. Before use in experiments, all buffers, distilled water, cleaned glass Petri plates (wiped with ethanol), and media were sterilised by autoclaving at 121°C and 15 psi pressure for 20 minutes, after which they were transferred into the laminar flow chamber for experimental procedures [10].

Sterilization of epiphytes: Seeds weighing 1g were transferred to a laminar flow chamber for further processing. The seeds were rinsed twice with distilled water and further washed with phosphate-buffered saline solution, with slight modifications to the standard protocol given by [9]. From the resulting aliquot, 1 ml was taken and added to 9 ml of distilled water to prepare the stock solution. Serial dilutions were performed up to a 10^{-10} dilution, and fungal isolation was performed using the plate method.

Sterilization of endophytes: Seeds weighing 1g were taken into the laminar flow hood for processing. The seeds were first washed with distilled water, followed by surface sterilisation with 2% sodium hypochlorite for 1 minute. They were rinsed twice with distilled water and subsequently washed with 70% ethanol for 30 seconds. Finally, the seeds were washed thrice with distilled water, crushed, and the resulting aliquot was subjected to serial dilution up to a 10-fold dilution series, and the pour-plate method was then used for fungal isolation [4].

For fungal isolation, two types of media were used: Potato Dextrose Agar (PDA) and Rose Bengal Agar. From the stock solution prepared, 1ml of an aliquot was transferred to two separate petri plates, and the prepared media was poured on each petri plate. The experiment was replicated three times for each medium. The plates were gently swirled to ensure uniform mixing before being covered and sealed with a thin plastic film. The plated samples were then incubated at a temperature of 30°C for seven days (Figure 1). After the incubation period was over, morphologically distinct fungal colonies were selected for subculturing, and identification was performed using microscopic analysis.

Results

Following serial dilution, a substantial number of fungal colonies were observed on the culture plates (Figure 2). Each distinct colony was individually subcultured to obtain and maintain pure isolates. A total of 101 fungal isolates were recovered from the three traditional rice varieties under investigation (Table 1). Of these, 34 isolates were identified as epiphytic fungi, comprising 12 from Mapillai Samba, 10 from Karuppu Kavuni, and 12 from Karung Kuruvai. The remaining 67 isolates were classified as endophytic fungi, with 22 obtained from Mapillai Samba, 21 from Karuppu Kavuni, and 24 from Karung Kuruvai. This distribution highlights a higher prevalence of endophytic fungi across all rice varieties examined, suggesting a significant role for endophytic associations in the microbial ecology of traditional rice cultivars.

Each morphologically distinct fungal colony obtained from the culture plates was subcultured to obtain and maintain pure isolates (Table 2). These purified fungal isolates were subsequently subjected to microscopic examination for preliminary taxonomic identification, based on spore morphology and reproductive structures (Figure 3). The microscopic analysis revealed the presence of diverse fungal genera, including *Fusarium* (36 isolates), *Penicillium* (21 isolates), *Aspergillus* (20 isolates), *Alternaria* (11 isolates), *Trichoderma* (3 isolates), *Diaporthe* (3 isolates), and *Bipolaris* (2 isolates). In addition, five isolates could not be definitively identified based on morphological characteristics alone and were thus classified as unidentified fungi pending further molecular analysis.

Among the identified genera, *Fusarium* was the most dominant, comprising the largest proportion of isolates across all three rice varieties. This predominance suggests that *Fusarium* species are well-adapted to the rice-associated microenvironment and may play significant ecological roles as endophytes, epiphytes, or potential pathogens. The diversity observed highlights the rich fungal community associated with traditional rice cultivars and underscores the importance of further characterisation to elucidate their functional roles and potential applications.

Discussion

The current study aimed to explore the diversity and distribution of fungal communities associated with three traditional rice varieties, Mapillai Samba, Karuppu Kavuni, and Karung Kuruvai, through serial dilution and culture-based isolation techniques. The distribution of isolates revealed a notable predominance of endophytic fungi compared to epiphytic fungi. This trend suggests a strong endophytic colonisation in traditional rice varieties, which may be indicative of mutualistic relationships that have evolved. Endophytes are known to confer various benefits to their host plants, including enhanced stress tolerance, growth promotion, and pathogen resistance. The higher number of endophytes across all three rice varieties highlights their potential significance in plant health and resilience, especially under traditional and possibly low-input farming systems, where such symbiotic interactions may play a critical role conferred by [8, 11].

Among the three rice varieties, Karung Kuruvai exhibited the highest total number of fungal isolates, followed by Mapillai Samba and Karuppu Kavuni. This distribution may reflect varietal differences in morphology, physiology, or microenvironmental conditions that influence fungal colonisation. Notably, each rice variety harboured both epiphytic and endophytic fungi, underscoring the microbial complexity associated with different plant compartments. The relatively even distribution of epiphytes and endophytes across varieties suggests that traditional rice cultivars maintain a balanced fungal community structure, which could be leveraged for sustainable crop improvement strategies suggested by [16].

The higher diversity of endophytes, particularly in Karung Kuruvai and Mapillai Samba, may also imply an adaptive advantage in hosting beneficial microbes capable of enhancing nutrient uptake, growth, and disease resistance. These findings are consistent with previous studies that highlight the ecological importance of fungal endophytes in crop health and productivity. The isolation and preservation of these fungal communities provide a valuable resource for future bioprospecting, including the identification of novel bioactive compounds or biocontrol agents [12].

Microscopic examination of fungal isolates revealed a diverse community encompassing several morphologically distinct genera, underscoring the complex fungal ecosystem associated with traditional rice varieties. Based on spore morphology and colony characteristics, *Fusarium* emerged as the most dominant genus, comprising over one-third of all isolates. This prevalence is consistent with earlier reports highlighting *Fusarium* spp. as common endophytes and opportunistic pathogens in cereals. While some *Fusarium* species are known for their pathogenicity and mycotoxin production, others function as endophytes that may enhance plant fitness under stress conditions.

The dominance of *Fusarium* across all three rice varieties suggests its ecological versatility and potential dual role as both a plant associate and a latent pathogen, meriting further molecular and pathogenicity characterisation. A similar result led by [8, 15].

Penicillium and *Aspergillus* were also well represented, with 21 and 20 isolates, respectively. Both genera are ubiquitous in soil and plant-associated environments and are known for their ability to produce a wide range of secondary metabolites, including antimicrobial compounds. Their frequent isolation in this study points to their potential role in natural plant defence mechanisms or nutrient cycling within the plant microenvironment [4].

Other genera, such as Alternaria, Trichoderma, *Diaporthe*, and *Bipolaris*, were less prevalent but are notable due to their diverse ecological functions. Alternaria and *Bipolaris* are often associated with foliar diseases, although some species can exist as non-pathogenic endophytes. Trichoderma spp., although represented by only three isolates, are of particular interest due to their well-documented antagonistic properties against plant pathogens and potential application in biocontrol. The presence of *Diaporthe*, a genus known for its dual lifestyle as an endophyte and a pathogen, also adds complexity to the fungal community structure observed [3, 13].

Table 1. Distribution of epiphytes and endophytes in various traditional rice varieties

S.No.	Rice variety	Epiphytic Isolates	Endophytic Isolates
1.	Mapillai Samba	12	22
2.	Karuppu Kavuni	10	21
3.	Karung Kuruvai	12	24
	Total	34	67
	Grand Total	101	

Table 2. Distribution of fungal species in various healthy traditional rice varieties

S. No.	Fungus	Mapillai Samba	Karuppu Kavuni	Karung Kuruvai	Total No. of isolates
1.	*Aspergillus sp.*	8	5	7	20
2.	*Fusarium sp.*	13	12	11	36
3.	*Penicillium sp.*	4	9	8	21
4.	*Bipolaris spp*	-	-	2	2
5.	*Alternaria sp.*	5	2	4	11
6.	*Diaporthe sp.*	1	1	1	3
7.	*Trichoderma sp.*	-	1	1	3
8.	Other unidentified	2	2	2	6

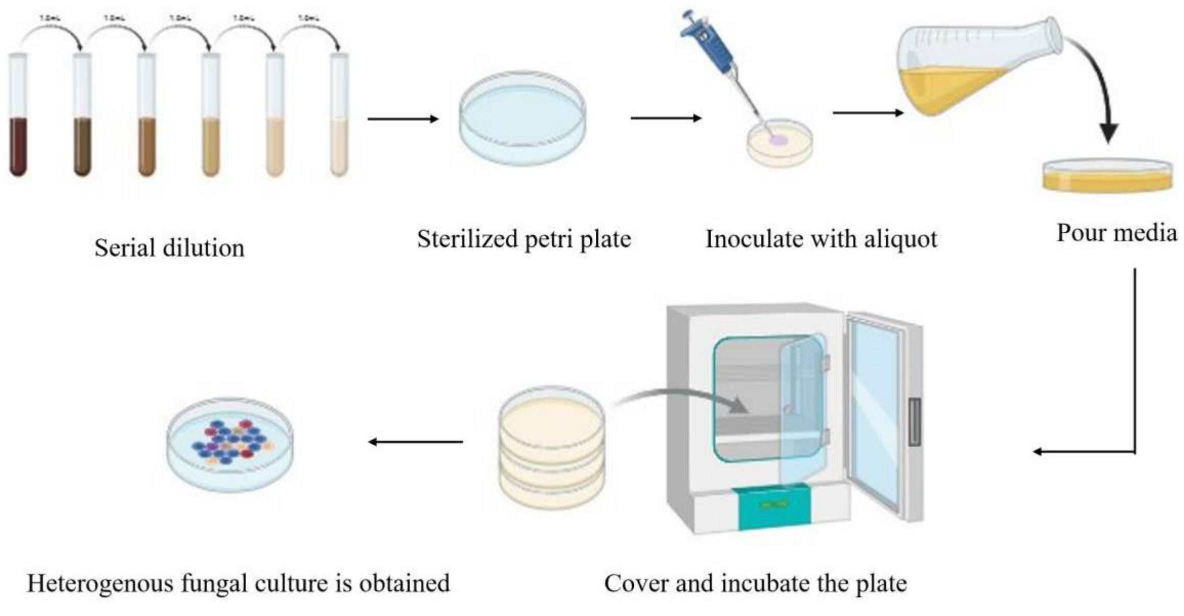
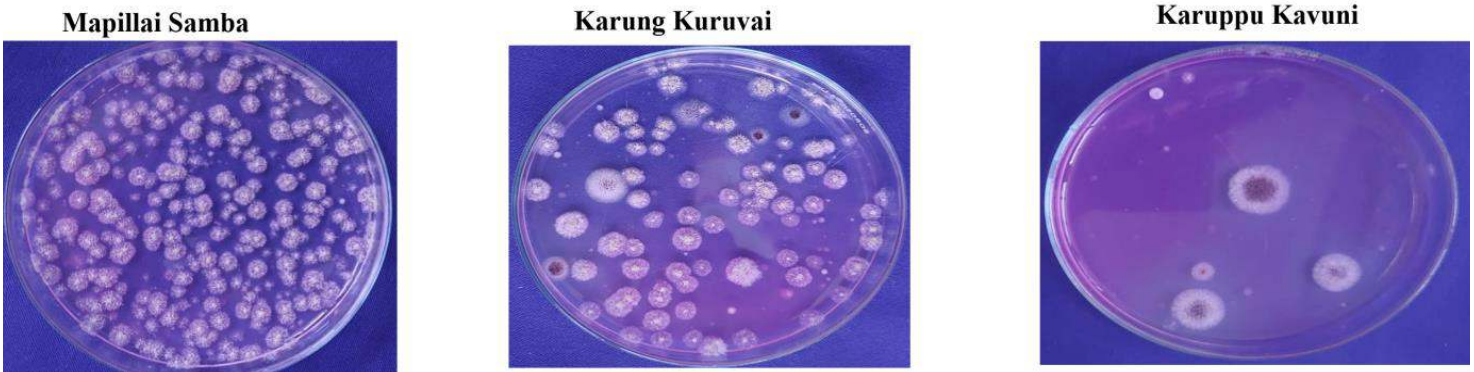


Figure 1. Fungal isolation using the pour plate method



Mapillai Samba

Karung Kuruvai

Karuppu Kavuni

Epiphytic fungi from Mapillai Samba

Epiphytic fungi from Karung Kuruvai

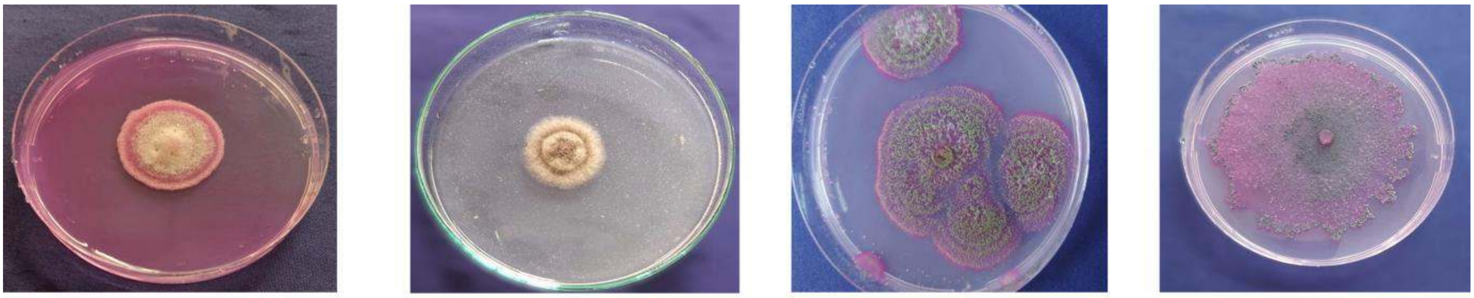
Epiphytic fungi from Karuppu Kavuni

Endophytic fungi from Mapillai Samba

Endophytic fungi from Karung Kuruvai

Endophytic fungi from Karuppu Kavuni

Figure 2. Fungal isolates obtained from different traditional rice varieties following serial dilution



Fusarium colonies

Alternaria colonies

Aspergillus colonies

Trichoderma colonies

Fusarium colonies under microscope

Alternaria colonies under microscope

Aspergillus colonies under microscope

Trichoderma colonies under microscope

Figure 3. Fungal isolates obtained from different traditional rice varieties following serial dilution

Conclusion

This study demonstrates that traditional rice varieties harbour a diverse range of seed-associated fungal communities, with a clear predominance of endophytic fungi over epiphytic forms. Among the three varieties assessed, Karung Kuruvai exhibited the highest fungal diversity, followed by Mapillai Samba and Karuppu Kavuni, indicating varietal differences in microbiome composition. The isolation and identification of genera such as *Fusarium, Penicillium, Aspergillus, Alternaria, Trichoderma, Diaporthe,* and *Bipolaris* highlight both pathogenic and potentially beneficial fungal groups coexisting within the seed environment, with *Fusarium* emerging as the dominant genus. These findings underline the ecological importance of seed-associated fungi in influencing seed health, germination potential, and adaptive responses under low-input cultivation systems.

Although the study encountered challenges associated with culture-dependent isolation,particularly the potential underestimation of non-culturable or slow-growing fungal taxa, it nevertheless contributes valuable baseline information on seed-associated mycobiota in traditional rice varieties. By documenting culturable epiphytic and endophytic fungi and identifying dominant genera through morphological and microscopic assessments, the research provides an essential reference point for future comparative studies. Additionally, the difficulty in distinguishing morphologically similar species emphasizes the importance of integrating molecular tools such as DNA barcoding or ITS sequencing. This recognition itself is a significant contribution, as it outlines a clear methodological pathway for advancing fungal identification accuracy and improving taxonomic resolution in subsequent research.

Future scope of study

The findings of this study provide a foundation for advancing research on seed-associated fungal communities in traditional rice varieties. Future work should integrate molecular identification techniques, such as ITS sequencing and metagenomic approaches, to overcome the limitations of culture-dependent methods and capture a more comprehensive profile of both culturable and non-culturable taxa. Exploring the functional roles of dominant and beneficial endophytic fungi, particularly those with potential biocontrol or plant growth–promoting traits, may offer valuable applications for developing bioinoculants or seed treatments tailored for low-input and organic farming systems. Additionally, controlled greenhouse and field studies are required to evaluate the influence of specific endophytes on seed germination, disease suppression, and stress tolerance under biotic and abiotic challenges. As traditional rice varieties continue to gain importance in climate-resilient agriculture, harnessing their native seed microbiome may contribute to sustainable crop improvement strategies, reduced chemical input dependency, and the development of microbial solutions that support future food and ecological security.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' Contribution

Rashmi Jha- writing of the manuscript, V. Manonmani- reviewing, editing and proofreading, K. Sundaralingam- reviewing and editing, S. Vanitha- reviewing and editing, M. Gnanachitra- proofreading, reviewing and editing and T. Kalaiselvi- proofreading, reviewing and editing,

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