Molecular Identification of Fungal Pathogens Isolated from Curcuma Longa Using Its Sequencing and Phylogenetic Analysis

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***Abstract*—Fungal diseases significantly reduce the yield and quality of Curcuma longa (turmeric). This study employed high-quality genomic DNA extraction, ITS region amplification and sequencing for molecular identification of fungal pathogens associated with diseased turmeric plants. PCR amplification yielded distinct amplicons and sequencing followed by nBLAST analysis identified ten isolates as Fusarium oxysporum, F. solani, F. proliferatum, Aspergillus flavus, Colletotrichum capsici, C. gloeosporioides, Alternaria alternata, Pythium aphanidermatum, Rhizoctonia solani and Aspergillus niger. Phylogenetic analysis using MEGA X confirmed species-level identity for each isolate with high bootstrap support. These results provide a molecular basis for accurate diagnosis of turmeric diseases and will aid in developing integrated disease management strategies for this important medicinal crop.**

***Index Terms*—Curcuma longa, fungal pathogens, ITS sequencing, nBLAST, phylogenetic analysis, disease management.**

I. INTRODUCTION

Fungal plant diseases threaten global food security and medicinal crops. A recent review estimated that eliminating crop losses caused by fungal pathogens could feed nearly 600 million additional people [1]. Turmeric (Curcuma longa) suffers from rhizome rot and leaf blight, which reduce yield and quality. In India, Fusarium solani and Pythium aphanidermatum are recognised causes of rhizome rot [4]. Accurate diagnosis of the causal organisms is therefore essential for effective disease management.

Traditionally, fungal pathogens are identified through microscopic examination of morphological features. However, morphology-based identification is labour intensive, requires taxonomic expertise and can be unreliable for species complexes [2]. Morphological similarity also obscures cryptic species of Fusarium, Aspergillus and other genera, leading to misdiagnosis [1]. Molecular barcoding of the internal transcribed spacer (ITS) region of ribosomal DNA has emerged as a widely accepted approach for fungal identification. The universal primer pair ITS1–ITS4 flanks the highly variable ITS1 and ITS2 regions around the 5.8S rRNA gene and amplifies a broad range of fungi [2]. Combined with a reliable DNA extraction protocol, ITS sequencing enables rapid, species-level identification.

While ITS barcoding improves diagnostic accuracy, current practices still have shortcomings. Reliance on morphology remains costly in terms of time and expertise [2][1]. The universal ITS1–ITS4 primer pair does not exclude plant DNA, so contaminated samples may yield mixed plant and fungal sequences [2]. Standard CTAB-based DNA extraction is effective but time-consuming, expensive and involves hazardous gradient centrifugation with ethidium bromide, which can inhibit PCR [3]. These limitations highlight the need for an optimized workflow that delivers high-quality fungal DNA while avoiding hazardous reagents and ensuring specificity.

This study combines optimized methods to generate a reliable molecular inventory of fungal pathogens infecting turmeric. Fungal isolates were collected from diseased C. longa tissues and genomic DNA was extracted using a refined CTAB protocol that is cost-effective and safe [3]. The ITS region was amplified using the universal ITS1–ITS4 primers [2]. Sequenced amplicons were subjected to nBLAST searches for taxonomic assignment, and species identities were confirmed through neighbour-joining

phylogenetic analysis. By integrating improved DNA extraction, ITS barcoding and phylogenetic reconstruction, this study overcomes limitations of morphology-based diagnosis and contributes data for the management of turmeric diseases.

II. MATERIALS AND METHODS

A. Sample Collection and Isolation

Diseased leaves and rhizomes of C. longa exhibiting wilting, rot or necrotic lesions were collected from field plots. Tissue segments were surface-sterilised and plated on potato dextrose agar (PDA) to isolate fungal cultures. Pure cultures were obtained by sub-culturing hyphal tips.

B. Genomic DNA Extraction

Genomic DNA was extracted from fresh mycelia using a modified cetyltrimethylammonium bromide (CTAB) protocol, which is cost-effective and safe for diverse fungal species [3]. DNA purity was assessed by spectrophotometric absorbance ratios (A260/A280) and concentrations were estimated using a nanodrop.

C. ITS PCR Amplification

The ITS region was amplified using universal primers ITS1 and ITS4, which flank the highly variable ITS1 and ITS2 regions [2]. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplicons were visualised on 1 % agarose gels.

D. Sequencing and BLAST Analysis

PCR products were purified and sequenced bidirectionally. Consensus sequences were assembled and compared against the NCBI nucleotide database using the BLASTn algorithm. Top hits with E-values < 1e-5 and highest percentage identity and query coverage were recorded.

E. Phylogenetic Analysis

Reference sequences corresponding to the top BLAST matches were aligned with the query sequences using ClustalW. Neighbour-joining trees were constructed in MEGA X with 1,000 bootstrap replicates to confirm species-level identity

III. RESULTS

A. DNA Quantification and Purity

Genomic DNA extraction yielded high-quality DNA with A260/A280 ratios around 1.8. DNA concentrations ranged from 70 to 100 ng mg^{-1} of fungal biomass, sufficient for downstream PCR. Variability in yield was linked to cell lysis efficiency but consistent yields were obtained after optimisation.

B. ITS PCR Amplification and Sequencing

PCR amplification produced sharp single bands of expected sizes for all isolates. Amplicons were sequenced successfully and yielded high-quality ITS sequences in FASTA format.

C. BLAST-Based Identification of Isolates

BLASTn analysis identified ten fungal isolates (Table 1). Fusarium oxysporum matched strain JXO45827.1 with 100 % identity and coverage. F. solani matched EF471740.1; F. proliferatum matched LS422786.1; Aspergillus flavus matched OW984245.1; Colletotrichum capsici matched KC565732.1; C. gloeosporioides matched KX066883.1; Alternaria alternata matched OQ055256.1; Pythium aphanidermatum matched OQ103419.1; Rhizoctonia solani matched MW737660.1; and Aspergillus niger matched LC573614.1. Sequence identity ranged from 99-100 % with full query coverage.

Table 1. Top BLAST matches for fungal isolates

Isolate	Top GenBank match	Accession	Identity (%)
Fusarium oxysporum	*Fusarium oxysporum*	JXO45827.1	100
Fusarium solani	*Fusarium solani*	EF471740.1	100
Fusarium proliferatum	*Fusarium proliferatum*	LS422786.1	100
Aspergillus flavus	*Aspergillus flavus*	OW984245.1	100
Colletotrichum capsici	*Colletotrichum capsici*	KC565732.1	100
C. gloeosporioides	*Colletotrichum gloeosporioides*	KX066883.1	100
Alternaria alternata	*Alternaria alternata*	OQ055256.1	100
Pythium aphanidermatum	*Pythium aphanidermatum*	OQ103419.1	100
Rhizoctonia solani	*Rhizoctonia solani*	MW737660.1	100
Aspergillus niger	*Aspergillus phoenicis* (used for species confirmation)	LC573614.1	100

D. Phylogenetic Analysis

Phylogenetic trees constructed using MEGA X confirmed the identification of each isolate. Each query sequence clustered with its corresponding reference sequence with high bootstrap support (>90 %). Figures 1-10 show the neighbour-joining trees for each isolate. The branch lengths and bootstrap values indicate close evolutionary relationships between the isolates and their reference strains.

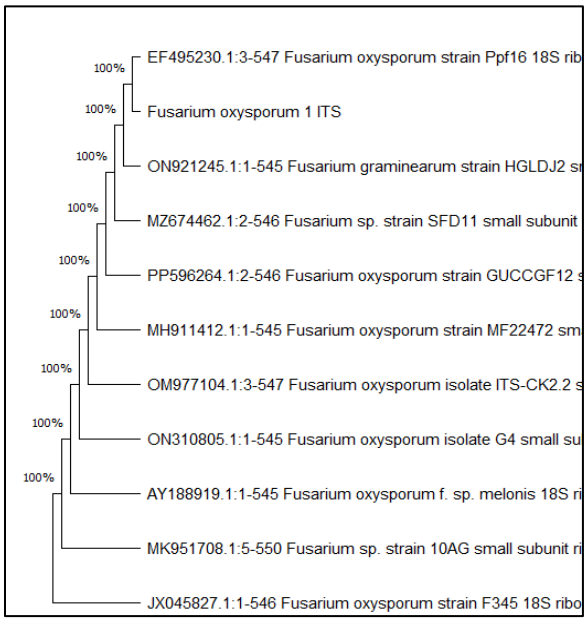

Figure 1 Confirmation of identified strain using phylogenetic tree of *Fusarium oxysporum* as *Fusarium oxysporum* 1 ITS

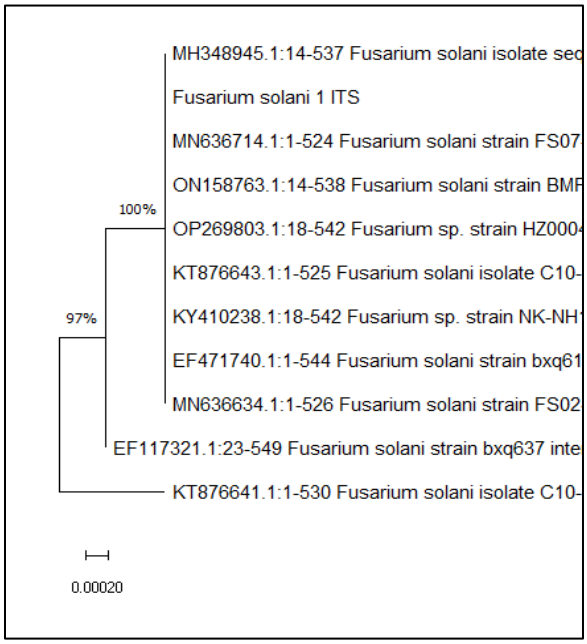

Figure 2 Confirmation of identified strain using phylogenetic tree of *Fusarium solani* as *Fusarium solani* 1 ITS

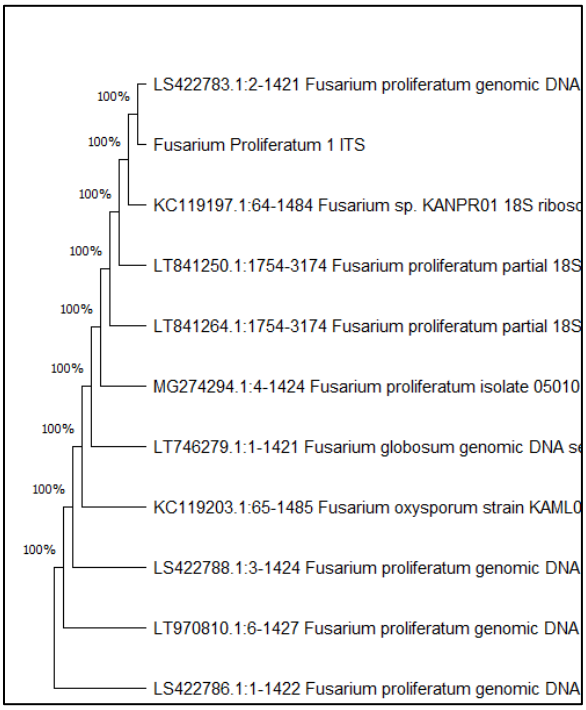

Figure 3 Confirmation of identified strain using phylogenetic tree of *Fusarium proliferatum* as *Fusarium proliferatum* 1 ITS

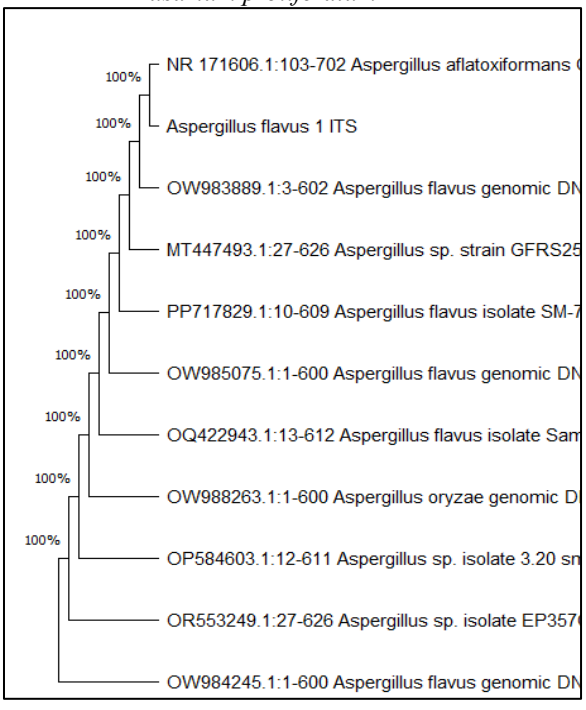

Figure 4 Confirmation of identified strain using phylogenetic tree of *Aspergillus flavus* as *Aspergillus flavus* 1 ITS

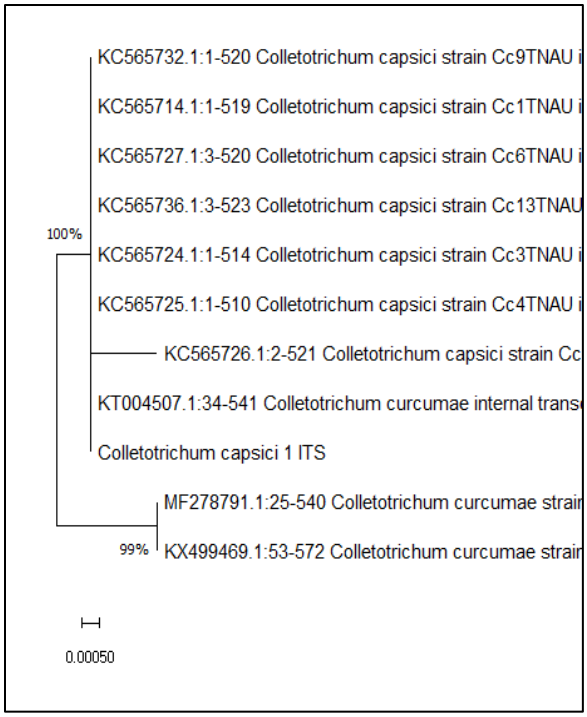

Figure 5 Confirmation of identified strain using phylogenetic tree of *Colletotrichum capsici* as *Colletotrichum capsici* 1 ITS

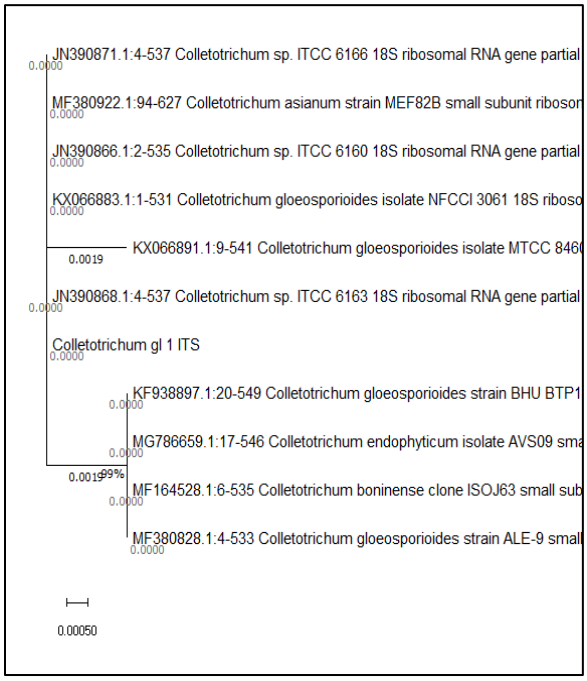

Figure 6 Confirmation of identified strain using phylogenetic tree of *Colletotrichum gloeosporioides* as *Colletotrichum gloeosporioides* 1 ITS

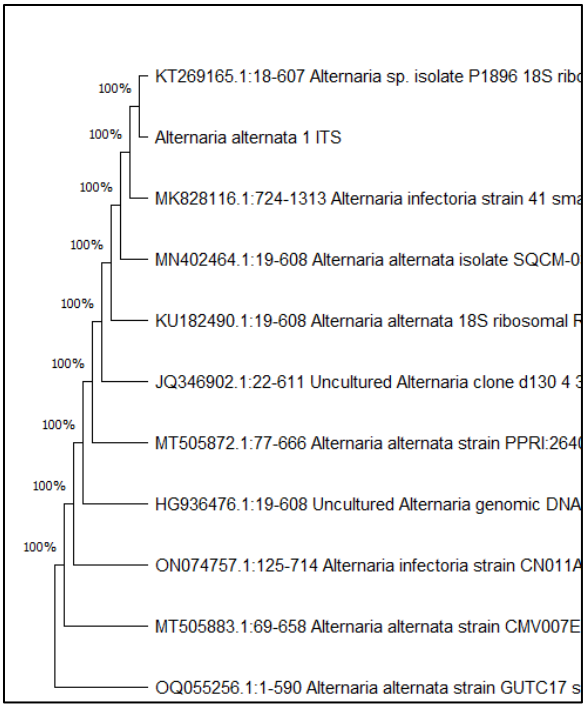

Figure 7 Confirmation of identified strain using phylogenetic tree of *Alternaria alternata* as *Alternaria alternata* 1 ITS

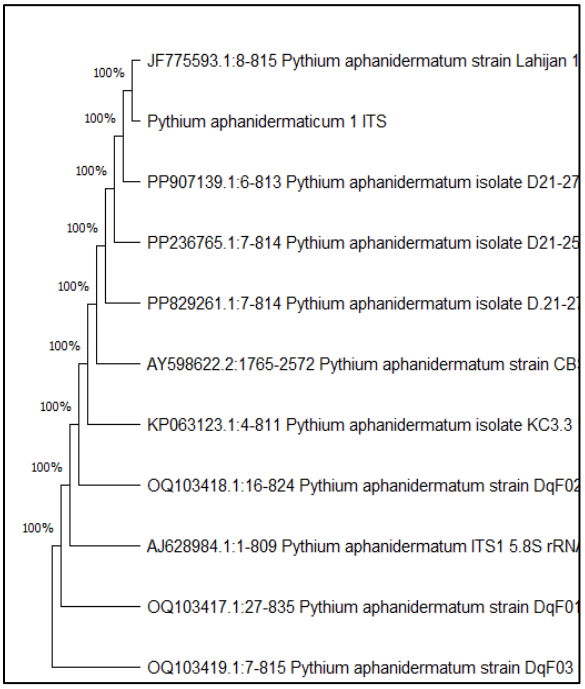

Figure 8 Confirmation of identified strain using phylogenetic tree *Pythium aphanidermatum* as *Pythium aphanidermaticum* 1 ITS

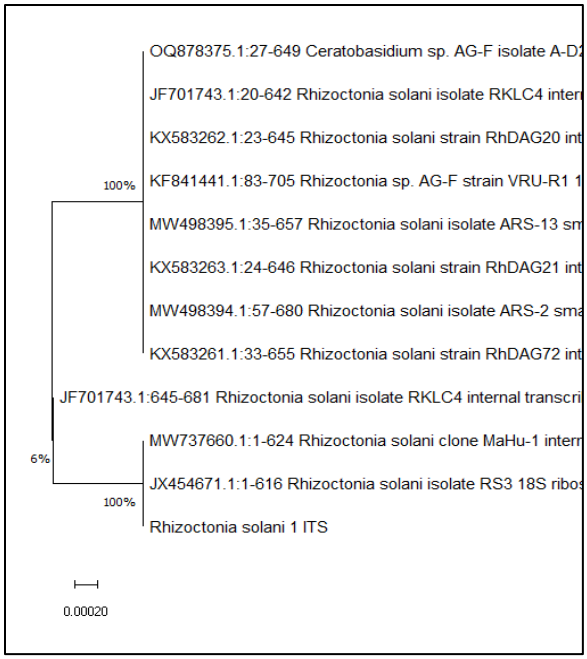

Figure 9 Confirmation of identified strain using phylogenetic tree of *Rhizoctonia solani* as *Rhizoctonia solani* 1 ITS

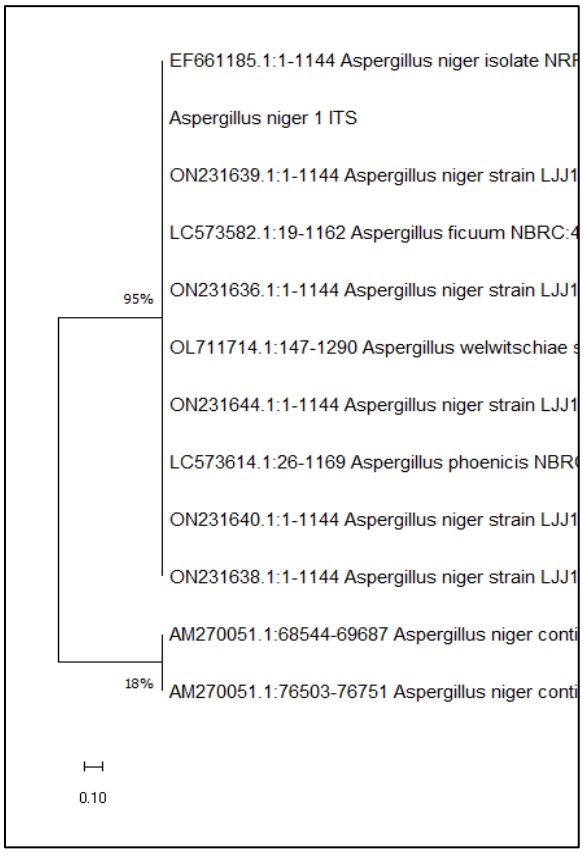

Figure 10 Confirmation of identified strain using phylogenetic tree of *Aspergillus niger* as *Aspergillus niger* 1 ITS

IV. DISCUSSION

Accurate identification of fungal pathogens is essential for understanding disease epidemiology and developing effective control strategies. In this study we identified ten fungal species associated with diseased turmeric plants using ITS sequencing and phylogenetic analysis. The dominance of Fusarium species corroborates earlier reports of Fusarium causing wilt and rot diseases on a wide range of host plants [5]. F. solani and Pythium aphanidermatum are known pathogens of turmeric rhizome rot in India [4], and their presence in this study underscores the importance of managing soil-borne inoculum.

Colletotrichum gloeosporioides and C. capsici cause anthracnose and leaf spot diseases on many crops and are widely disseminated pathogens [6][7]. The detection of Alternaria alternata, a ubiquitous saprophytic and plant-pathogenic fungus [9], suggests that opportunistic foliar pathogens also colonise turmeric. Aspergillus flavus and A. niger are common environmental fungi capable of producing mycotoxins, posing postharvest contamination risks [8]. Rhizoctonia solani, a soil-borne fungus with a broad host range [11], was also isolated, indicating potential seedling and stem rot issues in turmeric cultivation.

The identification of Pythium aphanidermatum further highlights the role of oomycete pathogens in damping-off and root rot diseases. Pythium species are favoured by high soil moisture and warm temperatures, with optimum development between 28 and 31 °C [10]. Integrated disease management practices such as field sanitation, crop rotation, fungicide treatments and the use of resistant cultivars are recommended to mitigate these diverse pathogens.

V. CONCLUSION

This study provides molecular confirmation of fungal pathogens associated with Curcuma longa using ITS sequencing. The identified species include wilt and rot pathogens (Fusarium spp., Pythium aphanidermatum, Rhizoctonia solani), anthracnose and leaf spot pathogens (Colletotrichum spp., Alternaria alternata), and opportunistic storage fungi (Aspergillus spp.). Implementing molecular diagnostics will aid in rapid disease detection and management. Future research should explore the pathogenicity of these isolates on turmeric, evaluate

resistant cultivars and develop eco-friendly control strategies.

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REFERENCES

[1] J. L. Steenwyk, A. Rokas, and G. H. Goldman, "Know the enemy and know yourself: Addressing cryptic fungal pathogens of humans and beyond," PLOS Pathogens, vol. 19, no. 10, e1011704, 2023.

[2] K. J. Martin and P. T. Rygiewicz, "Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts," BMC Microbiology, vol. 5, article 28, 2005.

[3] G. J. Dar, R. Nazir, S. A. Wani, S. Farooq, and T. H. Albekairi, "Optimizing a modified cetyltrimethylammonium bromide protocol for fungal DNA extraction: Insights from multilocus gene amplification," Open Life Science, vol. 20, 2025.

[4] M. S. Ahmmed, F. A. Nisha, and N. Alam, "First report on rhizome rot disease of Curcuma longa caused by Fusarium solani in Bangladesh," American Journal of Plant Sciences, vol. 13, pp. 506–516, 2022.

[5] I. M. Singha, Y. Kakoty, B. G. Unni, J. Das, and M. C. Kalita, "Identification and characterization of Fusarium sp. using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, North East India," Journal of Genetic Engineering and Biotechnology, vol. 14, no. 1, pp. 99–105, 2016.

[6] Y. Peralta-Ruiz, C. Rossi, C. D. Grande-Tovar, and C. Chaves-López, "Green management of postharvest anthracnose caused by Colletotrichum gloeosporioides," Journal of Fungi, vol. 9, no. 6, p. 623, 2023.

[7] TNAU Agritech Portal, "Leaf spot: Colletotrichum capsici," Horticultural crops, Spices: Turmeric, 2016. [Online]. Available: https://agritech.tnau.ac.in/crop_protection/turmeric_diseases1.html [Accessed: 17-Nov-2025].

[8] V. Navale, K. R. Vamkudoth, S. Ajmera, and V. Dhuri, "Aspergillus-derived mycotoxins in food and the environment: prevalence, detection, and toxicity," Toxicology Reports, vol. 8, pp. 1008–1030, 2021.

[9] Institut national de santé publique du Québec (INSPQ), "Alternaria alternata," Fact sheets, 2024. [Online]. Available: https://www.inspq.qc.ca/en/moulds/fact-sheets/alternaria-alternata [Accessed: 17-Nov-2025].

[10] UC ANR, "Phytophthora and Pythium root rots," UC Statewide Integrated Pest Management Program – Sugarbeet, 2011. [Online]. Available: https://ipm.ucanr.edu/agriculture/sugarbeet/phytophthora-and-pythium-root-rots/ [Accessed: 17-Nov-2025].

[11] University of Minnesota Extension, "Rhizoctonia root and stem rot on soybean," 2018. [Online]. Available: https://extension.umn.edu/soybean-pest-management/rhizoctonia-root-and-stem-rot-soybean [Accessed: 17-Nov-2025].