



Molecular Identification of Fungal Pathogens Isolated from Bt. Cotton Grown in Saline Soils of Akola and Buldhana Districts, Maharashtra, India

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ABSTRACT

Cotton (*Gossypium* spp.) is one of the world's most important fiber crops, but its productivity is severely affected by soil salinity and fungal diseases, particularly in the saline-sodic cotton-growing regions of Akola and Buldhana districts in the Vidarbha region of Maharashtra. The present study aims to isolate and identify fungal phytopathogens associated with Bt cotton grown in saline soils. The 32 samples were collected from infected cotton plants from saline-affected fields, and fungal pathogens were isolated on Potato Dextrose Agar (PDA). Three fungal pathogens were identified on the basis of morphological and microscopic examination and molecular characterization. The most frequently recovered species were *Aspergillus niger* (52%) *Fusarium oxysporum* (26%), *Rhizopus arrhizus* (22%).

Keywords: Cotton, Saline Soil, Fungal pathogen, Rhizosphere Sustainable Agriculture

INTRODUCTION

Cotton Production and Economic Importance:

Gossypium spp. of cotton remains the world's most important natural fiber crop, contributing significantly to the global agricultural economies and textile industries. Globally, cotton is cultivated across approximately 33 million hectares, producing around 120 million metric tons of kapas annually, with India, China, and the United States being the leading producers (ICAC 2024). India holds the second-largest position globally in cotton production, cultivating cotton across 12.9 million hectares with an estimated production of 36.4 million bales (252 kg each) during 2024-25 (PIB, 2025).

The Akola and Buldhana districts, located in the Vidarbha region of Maharashtra, are among the major cotton-producing areas of the state, where black cotton (Vertisol) soils provide favorable conditions for the cultivation of cotton. The Purna Valley saline tract, recognized as one of the largest inland saline regions in central India, extends across several villages in the Akola and Buldhana districts (Konde *et al.*, 2024; ICAR-CSSRI, 2024). Despite the economic importance of cotton cultivation in these districts, comprehensive information on fungal pathogen

diversity and microbial ecology in the saline cotton-growing soils of Akola and Buldhana are limited.

Salinity Stress in Cotton Ecosystems: Saline soils are defined as those with an electrical conductivity of the saturation soil extract (EC_e) greater than 4 dS/m at 25°C, whereas sodic soils possess high exchangeable sodium percentages (ESP > 15) along with varying salinity levels (FAO, 2024). Maharashtra, particularly the Vidarbha region, contains significant areas of salt-affected soils that adversely influence agricultural productivity (Sharma & Minhas, 2020). Salinity profoundly influences the biological properties of soil by altering the microbial community composition, reducing the diversity and abundance of beneficial microorganisms, and favoring the proliferation of salt-tolerant microorganisms, including certain phytopathogenic fungi (Egamberdieva et al., 2022).

Fungal Diseases of Cotton: Cotton crops are susceptible to numerous fungal pathogens that cause substantial yield and fiber quality losses under favorable environmental conditions. Among these, Fusarium wilt, caused by *Fusarium oxysporum* and *F. vasinfectum*, is one of the most destructive soil-borne diseases affecting cotton. It is characterized by chlorosis, vascular discoloration, wilting, stunted growth, and plant death. The pathogen produces durable chlamydospores that enable long-term survival in the soil, making disease management particularly difficult (Davis et al., 2006; Kirkpatrick & Rothrock, 2001). Verticillium wilt, caused by *Verticillium dahliae*, is a major vascular disease that affects cotton. The pathogen survives in the soil as melanized microsclerotia and infects plants through root colonization, immune suppression, and toxin production. Despite advances in biological control, effective and consistent biocontrol agents remain limited (Chen et al., 2023; Zhang et al., 2025). Root rot, caused by *Rhizoctonia solani*, is a destructive soil-borne disease that affects cotton seedlings and mature plants. The pathogen survives for long periods in soil and plant debris as sclerotia and mycelia, resulting in persistent disease pressure (Brown & Carling, 2013; El-Shabrawy et al., 2022). Alternaria leaf spot, caused by *Alternaria alternata*, reduces photosynthetic capacity and lint yield by inducing leaf spots, blight, and premature leaf drop (Sharma et al., 2025). *Aspergillus* and *Penicillium* species are commonly associated with cotton rhizosphere soils and may exhibit opportunistic pathogenicity under saline

conditions, where plant defence mechanisms are weakened (Egamberdieva et al., 2022).

Relationship Between Salinity and Fungal Pathogens: Soil salinity significantly alters rhizosphere microbial communities by reducing the abundance and diversity of beneficial microorganisms while favoring salt-tolerant and stress-adapted fungal taxa. These changes disrupt microbial interactions, weaken the natural biological suppression of pathogens, and increase the risk of soil-borne diseases in cotton plants (Egamberdieva et al., 2022; Liu et al., 2023). The interaction between salinity and fungal pathogens is complex and pathogen dependent. Studies on *Verticillium dahliae* have shown that disease severity is influenced by both soil salinity and the host genetic background. Salt-tolerant cotton cultivars generally maintain greater resistance under saline conditions, whereas susceptible cultivars exhibit increased disease severity as salinity increases (Karademir et al. 2023). These findings suggest that integrating salinity tolerance and disease resistance is essential for sustainable cotton production in salt-affected soils.

Research Gap

Limited Studies on Fungal Pathogen Diversity in Saline Soils of Vidharba: Soil salinity is a major abiotic constraint limiting cotton production worldwide and is becoming increasingly severe in cotton-growing regions of Maharashtra, particularly in the Vidarbha region. Salinity adversely affects plant growth, nutrient uptake, and yield, while altering soil physicochemical properties and rhizosphere microbial communities, including fungal populations associated with plant diseases (Etesami & Jeong 2021). Recent studies have demonstrated that salinity significantly reshapes rhizosphere microbial communities, often reducing the number of beneficial microorganisms and increasing the prevalence of pathogenic fungi (Zhang et al., 2023; Zhang et al., 2022).

However, no comprehensive investigation has systematically characterized the diversity of fungal pathogens in saline cotton soils in Maharashtra using integrated morphological and molecular identification methods. Therefore, the aim of this study is to molecular identification of fungal pathogens isolated from bt. cotton grown in saline soils of Akola and Buldhana districts, Maharashtra

MATERIALS AND METHODS

Study Area and Sample Collection:

The study was conducted in cotton-growing fields in the Akola and Buldhana District of Maharashtra, India. Samples were collected from diseased cotton plants exhibiting wilting, yellowing, leaf drying, and root rot symptoms. Infected plant parts (roots, stems, leaves, flowers, and bolls) were collected in sterile polythene bags and transported to the laboratory for further analyses. A total of 32 samples were collected from 08 sites of Bt. Cotton Grown field. Each sample was carefully separated, labelled, and placed in sterile bags.

Isolation and Identification of Fungal isolates:

Plant tissue segments (0.5–1.0 cm) were surface-sterilized with 70% ethanol (30s) followed by 1% sodium hypochlorite (1 min) and rinsed thrice with sterile distilled water. Sterilized tissues were plated on Potato Dextrose Agar (PDA) and incubated at 25–28°C for 5–7 days. Emerging colonies were sub-cultured to obtain pure cultures of the isolates. Identification was based on colony characteristics and microscopic features using lactophenol–cotton blue stain. The colonies exhibited rapid growth with black conidial heads, globose vesicles, and chains of rough-walled black conidia, which are characteristic of *A. niger* (Pitt and Hocking, 2009), filamentous fungus that forms rapidly growing colonies, initially white to pale pink, which later develop violet to purple pigmentation on potato dextrose agar which are characteristic of *Fusarium* and fast-growing zygomycetous fungus

producing fluffy, cottony colonies that are initially white and later become gray to dark brown or black due to sporangial development which are characteristic of *Rhizopus*.

PCR: Polymerase chain reaction (PCR) was conducted using the oligonucleotide primer pairs ITS5(5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') for *A. Niger*, ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') for *F. oxysporum* and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') for *R. arrhizus*.

Sequencing and analysis:

The PCR amplicons were outsourced for sequencing at Infinite Biotech Institute of Research and Analytics, Sangli. Only closed related fungal strains with the highest sequenced similarity scores were retrieved from GeneBank and considered reliable for species – level identification of fungal pathogens.

RESULTS

In this study, the diversity of fungi associated cotton from saline belt of Akola-Buldhana district was investigated through analysis of the ITS rDNA gene sequences. Thirty-two fungal isolates were successfully recovered from samples collected from saline belt. Three fungal pathogens were identified on the basis of morphological, microscopic examination and molecular characterization.



Figure 1: The phylogenetic tree demonstrates that the analyzed fungal isolate belongs to *Rhizopus arrhizus* and shares a very close evolutionary relationship with previously reported strains. The very small genetic distances and tight clustering confirm species-level identification using ITS sequence analysis. Therefore, the molecular data strongly support the classification of the isolate as *Rhizopus arrhizus*.

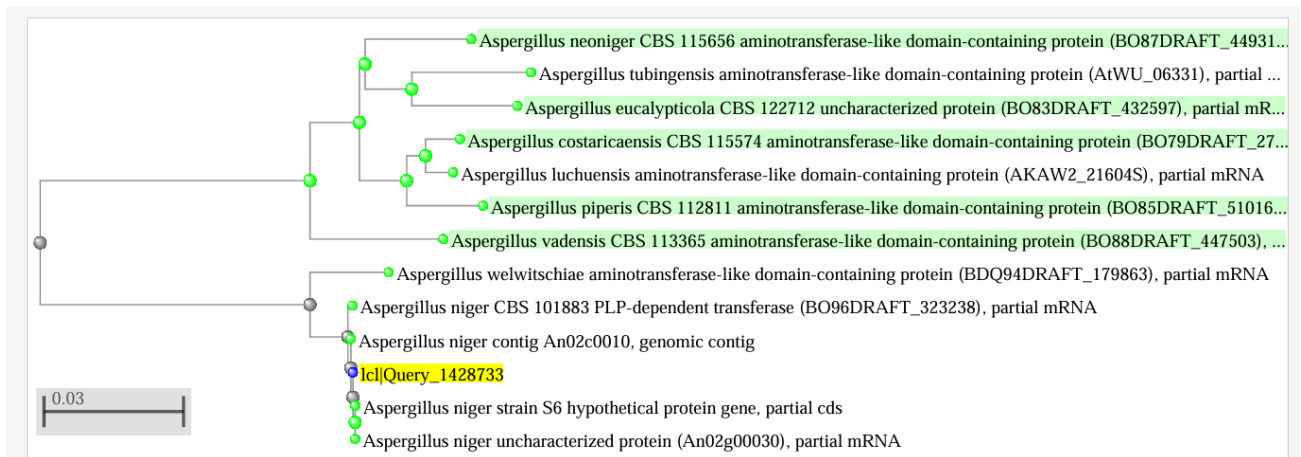


Figure 2: Phylogenetic tree of the aminotransferase-like protein sequence obtained from the query sequence (Icl|Query_1428733) and homologous sequences from different *Aspergillus* species. The tree shows that the query sequence clusters closely with *Aspergillus niger* sequences, indicating a close evolutionary relationship and suggesting that the protein belongs to the conserved aminotransferase family within the *Aspergillus* section *Nigri*. The scale bar (0.03) represents the number of amino acid substitutions per site.

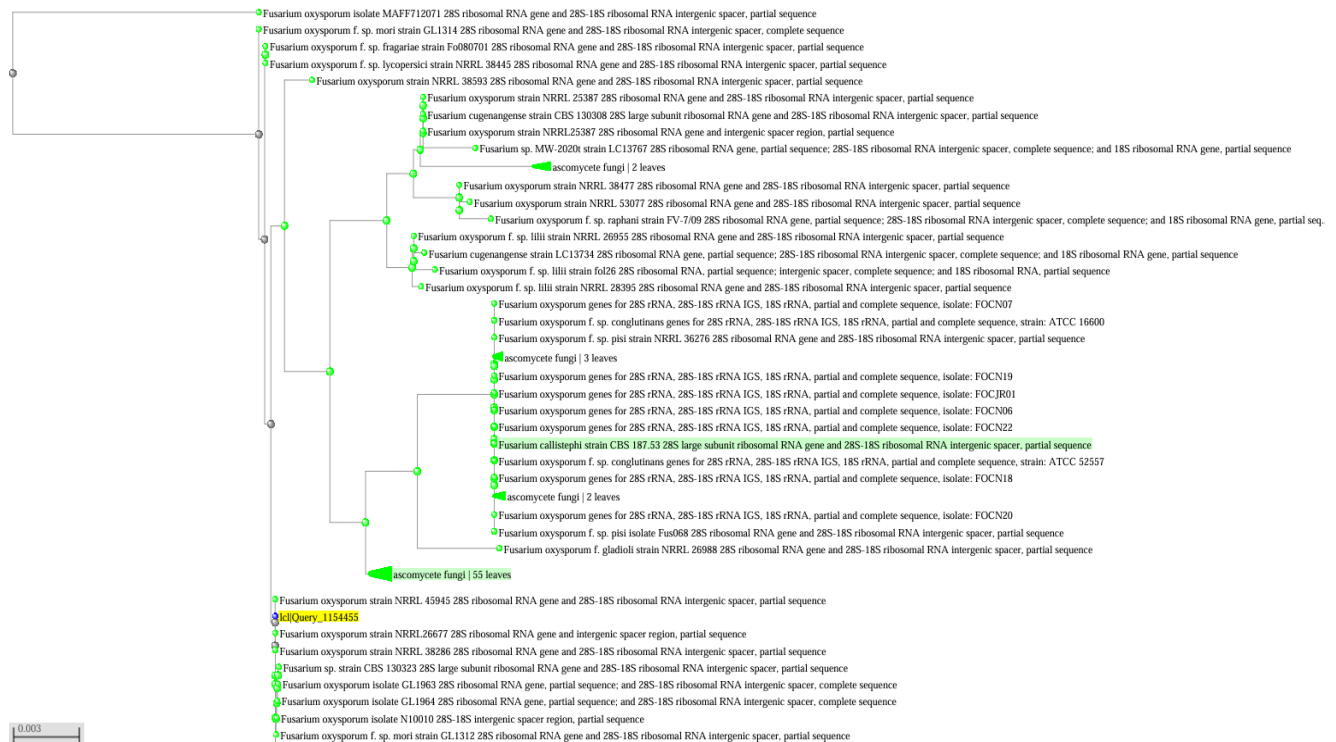


Figure:3 The phylogenetic analysis confirms that the unknown isolate (IQuery_115455) belongs to the *Fusarium oxysporum* species complex. Its close association with authenticated *F. oxysporum* strains indicates a high degree of sequence similarity and supports its taxonomic identification. However, because the ITS region has limited discriminatory power within the *F. oxysporum* species complex, the phylogeny does not permit reliable identification of the isolate to the forma specialis or pathogenic race level. Therefore, additional molecular markers (such as *TEF1-α*, *RPB2*, or β -tubulin genes) and pathogenicity tests are recommended for precise classification.

Table: 1 The fungal isolate **A1 (Seq89_A1_NC160534)** was identified by sequencing approximately **2000 bp of the 18S rRNA gene**. BLAST analysis against the NCBI database showed the highest similarity with *Aspergillus niger*.

Sr. No.	Strain	Aim	Primer	NCBI BLAST (Type Strain)	Remarks
1	Seq89_A1_NC160534 A1	Identification of 18srRNA gene ++ Strain received From Rupali Deshmukh	907R (2000bp)	SequenceID: NR_134694 Taxon ID: <i>Aspergillus niger</i> Identities: 1952/2000(99.99%) Ref: Int.J.Syst.Evol. Microbiol.65(PT3), 857-863(2015) SequenceID: NR_134694 Taxon ID: <i>Aspergillus niger</i> Identities: 1952/2000(99.99%) Ref: strain WB1	Strain showed closest Homology with <i>Aspergillus niger</i> (Closer to Multiple species) ++ Genus/genera is/are Comprised of large Number of species, hence 18srRNA may not resolve species identity. ++ Biochemical/phenotypic tests /whole genome sequencing analysis might resolve species level identity. Kindly refer latest literature related to this genus taxonomy at https://lpsn.dsmz.de/Li St of Prokaryotic Names with Standing In Nomenclature (LPSN), which provides comprehensive information on The nomenclature of prokaryotes.

Table 2. The fungal isolate **A1 (Seq88_A1_NC160533)** was identified by sequencing approximately **2000 bp of the 18S rRNA gene**. NCBI BLAST analysis showed the highest sequence similarity (**99.99%**) with *Fusarium oxysporum*.

Sr. No.	Strain	Aim	Primer	NCBI BLAST (Type Strain)	Remarks
1	Seq88_A1_NC160533 A1	Identification of 18srRNA gene ++ Strain received From Rupali Deshmukh	907R (2000bp)	Sequence ID: NR_134693 Taxon ID: <i>Fusarium oxysporum</i> Identities: 1936/2000(99.99%) Ref: Int.J.Syst.Evol. Microbiol.65(PT3), 857-863(2015) ++	Strain showed closest Homology with <i>Fusarium oxysporum</i> (Closer to Multiple species) ++ Genus/genera is/are Comprised of large Number of species, hence 18srRNA may not resolve species identity.

				SequenceID: NR_134693 Taxon ID: <i>Fusarium oxysporum</i> Identities: 1936/2000(99.99%) Ref: strain WB1	++ Biochemical/phenotypic tests/whole genome sequencing analysis might resolve species level identity. Kindly refer latest literature related to this genus taxonomy at https://lpsn.dsmz.de/Li of Prokaryotic names with Standing in Nomenclature (LPSN), which provides comprehensive information on the nomenclature of prokaryotes.
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Table 3. The fungal isolate **A1 (Seq91_A1_NC160536)** was identified by sequencing approximately **2000 bp of the 18S rRNA gene**. NCBI BLAST analysis showed the highest sequence similarity (**99.99%**) with *Rhizopus arrhizus*.

Sr. No.	Strain	Aim	Primer	NCBI BLAST (Type Strain)	Remarks
1	Seq91_A1_NC160536 A1	Identification of 18srRNA gene ++ Strain received From Rupali Deshmukh	907R (2000bp)	SequenceID: NR_134696 Taxon ID: <i>Rhizopus arrhizus</i> Identities: 1937/2000(99.99%) Ref: Int.J.Syst.Evol. Microbiol.65(PT3), 857-863(2015) ++ SequenceID: NR_134696 Taxon ID: <i>Rhizopus arrhizus</i> Identities: 1937/2000(99.99%) Ref: strain WB1	Strain showed closest Homology with <i>Rhizopus arrhizus</i> (Close to Multiple species) ++ Genus/genera is/are Comprised of large Number of species, hence 18srRNA may Not resolve species identity. ++ Biochemical/phenotypic tests/whole genome Sequencing analysis Might resolve species Level identity. Kindly refer latest Literature related to This genus taxonomy at https://lpsn.dsmz.de/Li of Prokaryotic Names with Standing In Nomenclature

					(LPSN), which provides comprehensive Information on The nomenclature of prokaryotes.
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The most frequently recovered species were *Aspergillus niger* (52%) *Fusarium oxysporum* (26%), *Rhizopus arrhizus* (22%). Phylogenetic relationships among the fungal isolates and their closest reference sequences were shown in figure 1,2 and 3 (Table 1, 2 and 3).

Discussion

Cotton (*Gossypium* spp.) is one of the world's most economically important fiber crops; however, its productivity is significantly constrained by soil salinity and fungal pathogens, particularly in saline agroecosystems. The present study investigated the fungal diversity associated with Bt. cotton cultivated in the saline belts of Akola and Buldhana districts of Maharashtra using molecular identification based on 18S rRNA/ITS sequence analysis and phylogenetic characterization. A total of 32 fungal isolates belonging to three genera were recovered from the surveyed fields. Among these, *Aspergillus niger* was the most predominant species, accounting for 52% of the isolates, followed by *Fusarium oxysporum* (26%) and *Rhizopus arrhizus* (22%). The predominance of *A. niger* suggests that saline soil conditions may favor its establishment and survival. Previous studies have reported that species of *Aspergillus* possess considerable tolerance to osmotic and salt stress, enabling them to colonize saline environments effectively. The occurrence of *F. oxysporum* and *R. arrhizus* further indicates a diverse fungal community capable of surviving under adverse soil conditions, with potential implications for cotton health and productivity.

Phylogenetic analyses confirmed the taxonomic identity of all isolates. The *Aspergillus* isolates clustered closely with authenticated *A. niger* reference strains, while *Rhizopus* isolates formed a highly conserved clade with *R. arrhizus*, indicating low intraspecific variation. The *Fusarium* isolates grouped within the *Fusarium oxysporum* species complex

(FOSC), although ITS sequences were insufficient to distinguish different *formae speciales*, highlighting the need for additional markers such as **TEF1- α** and **RPB2** for higher phylogenetic resolution.

Overall, the study demonstrates that *A. niger*, *F. oxysporum*, and *R. arrhizus* are the dominant fungi associated with Bt. cotton grown in saline soils of Akola and Buldhana. Molecular and phylogenetic characterization provides accurate species identification and establishes a foundation for future research on fungal pathogenicity, salinity adaptation, and the development of effective disease management strategies for sustainable cotton production in salt-affected regions.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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