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Isolation and Characterisation of Dominant Fungal Species from Bathinda, Punjab, India

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ABSTRACT

Background: Fungi play crucial roles in ecosystem functioning and have significant environmental and biotechnological applications. This study aimed to assess fungal diversity and identify predominant species from environmental samples collected in Bathinda, Punjab, India.

Methods: Environmental samples were collected from various locations, including rhizospheric soils and infected plant tissues. Fungal species were isolated using standard culturing techniques and identified based on colony morphology and microscopic characteristics. Representative isolates were selected for molecular identification through 18S rRNA gene sequencing, and sequences were submitted to GenBank for confirmation and phylogenetic analysis.

Results: A total of 25 fungal isolates were recovered and classified into multiple genera, including *Aspergillus*, *Penicillium*, *Curvularia*, *Fusarium*, *Alternaria*, *Cladosporium*, *Rhizopus*, *Talaromyces*, *Botrytis*, *Purpureocillium*, *Acremonium*, and *Trichoderma*. Frequently isolated species included *Aspergillus niger*, *A. nidulans*, *A. fumigatus*, *Penicillium citrinum*, *P. oxalicum*, *P. bilaiae*, *Purpureocillium sodanum*, and *Aspergillus ochraceopetaliformis*. Rhizospheric soil samples yielded isolates such as *Purpureocillium sodanum*, *Trichoderma* spp., *Acremonium implicatum*, *Talaromyces pinophilus*, and *A. ochraceopetaliformis*, while infected plant tissues contained less frequently isolated genera such as *Botrytis*, *Alternaria*, *Fusarium*, *Bipolaris*, and *Curvularia* spp.

Conclusion: Fungal diversity in the agro-climatic region of Bathinda, Punjab, remains poorly documented. Notably, *Purpureocillium sodanum*, and *Aspergillus ochraceopetaliformis* were identified for the first time in Punjab, India. Their isolation from the rhizosphere of healthy plants highlights their potential ecological and biotechnological significance. The findings provide baseline data for future studies on fungal biodiversity, plant–microbe interactions, and sustainable agricultural applications in this underexplored region.

Keywords: *Fungal diversity, Rhizospheric fungi, Pathogenic fungi, Purpureocillium sodanum*

1. INTRODUCTION

Fungi represent one of the most diverse and ecologically significant groups of organisms in the environment, with roles ranging from nutrient cycling to plant pathogenesis and symbiosis [1]. In agricultural systems, fungal species can act both as destructive pathogens and beneficial agents, promoting plant health [2]. The identification and monitoring of fungal populations in different agro-ecological zones are therefore essential for understanding their ecological roles, mitigating crop losses, and exploring their potential applications in biocontrol.

Bathinda, located in the semi-arid region of Punjab, India, is an important agricultural area characterised by diverse cropping systems [3]. However, the fungal diversity of this region, particularly the composition of culturable fungi from both healthy rhizospheres and infected plant tissues, remains largely undocumented. The isolation and identification of dominant fungal species in such regions are critical for managing plant health and identifying potential fungal bioresources.

Traditional methods such as colony morphology and microscopic examination provide a preliminary basis for fungal identification, while molecular tools, particularly DNA sequencing of conserved regions such as the 18S rRNA gene, offer

precise taxonomic resolution and insight into phylogenetic relationships. The integration of both morphological and molecular approaches facilitates accurate species identification and enhances our understanding of fungal ecology.

The present study was undertaken to isolate and identify dominant fungal species from environmental samples collected across various locations in Bathinda, Punjab, including riversides, roadsides, and agricultural fields. The investigation aimed to assess fungal diversity using morphological characteristics and to validate the identity of representative isolates through molecular techniques, thereby contributing to the foundational knowledge of fungal biodiversity in this region.

2. MATERIALS AND METHODS

Sample collection

Fungal samples were collected from various environmental locations across Bathinda, Punjab, including roadsides, riversides, and agricultural fields. Diseased plant parts—such as leaves, bark, and fruits—were sampled from visibly infected trees. In addition, rhizospheric soil samples were collected from the root zones of healthy trees and crop plants at multiple locations to capture the diversity of beneficial and naturally occurring fungi. All samples were placed in sterile ziplock bags, properly labelled, and transported to the Akal University, Botany laboratory under cool conditions for further analysis.

Isolation and Identification of Fungal Strains

Fungal isolation from plant and soil samples was carried out using standard mycological techniques. Small portions of diseased plant tissues—including leaves, bark, and fruits—were surface sterilized using 1% sodium hypochlorite, rinsed in sterile distilled water, and aseptically placed onto pre-poured Potato Dextrose Agar (PDA) plates [4]. Similarly, 1 g of rhizospheric soil from healthy trees and crop plants was suspended in 5 ml of sterile distilled water, serially diluted, and plated on PDA using the spread plate method. All plates were incubated at 25 ± 1°C for 5–7 days [5].

Emerging fungal colonies were subcultured to obtain pure cultures, which were then characterised based on colony morphology and microscopic features, following established taxonomic keys and literature [6-10].

The percentage frequency of each fungal species was calculated using the given formula [11]

$$\text{Frequency (\%)} = \frac{\text{No.of observations in which spp.appeared}}{\text{Total no.of observations}} \times 100$$

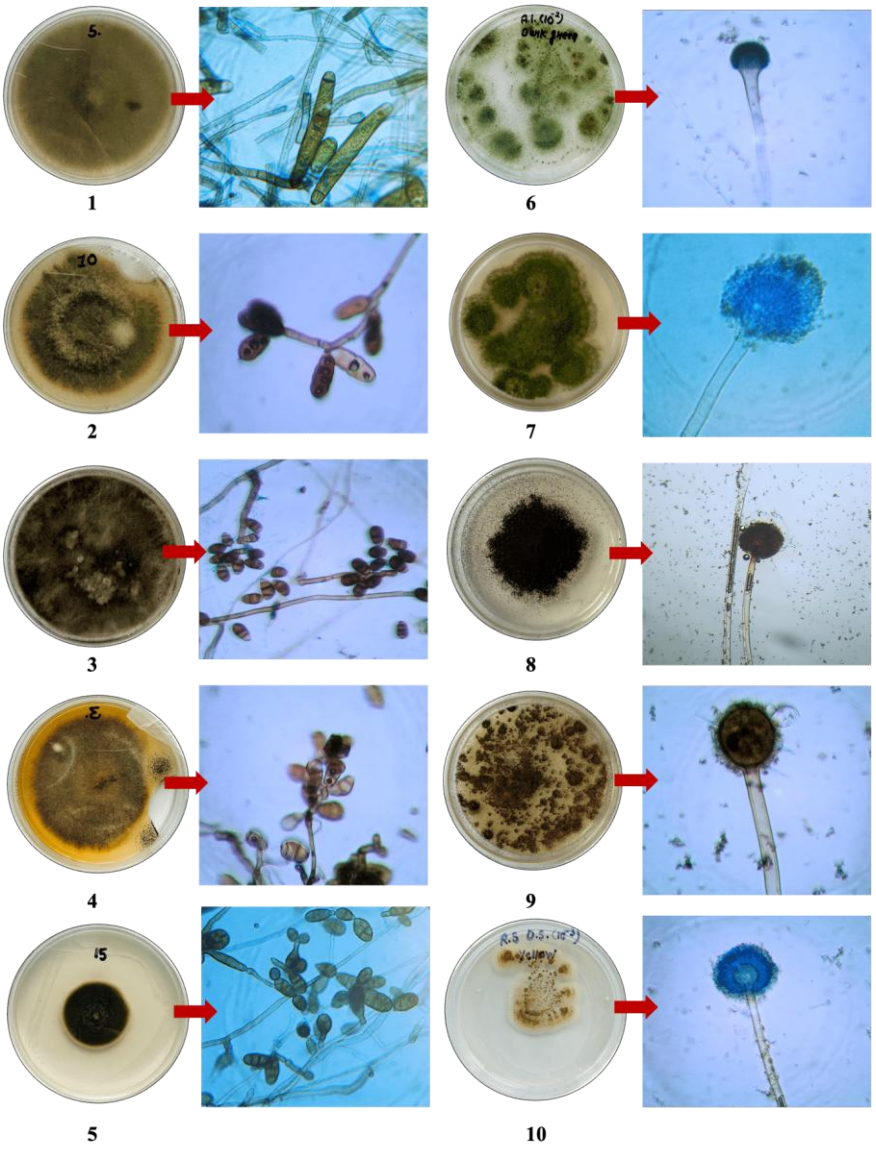
Molecular Identification of fungal isolates

Representative and frequently occurring fungal isolates were selected for molecular identification to confirm morphological characterizations. These isolates were outsourced to Barcode Biosciences Pvt. Ltd., Bangalore, India, for the Internal Transcribed Spacer (ITS) region of the 18s rRNA. The confirmed sequences were submitted to GenBank, and accession numbers were obtained for future reference.

3. RESULTS

A total of 24 different fungal species were isolated from fruit and vegetable samples collected from Bathinda, Punjab, India, and were identified based on their cultural and morphological characteristics. These included features such as colony diameter, texture, pigmentation (both obverse and reverse), hyphal structure, type and branching of conidiophores, and morphology of conidia (Figure 1).

Figure 1. Microscopic morphology of 25 fungal isolates observed under 40× magnification. The images are numbered 1 to 25 (right side), corresponding to the fungal species listed in Table 1.


1
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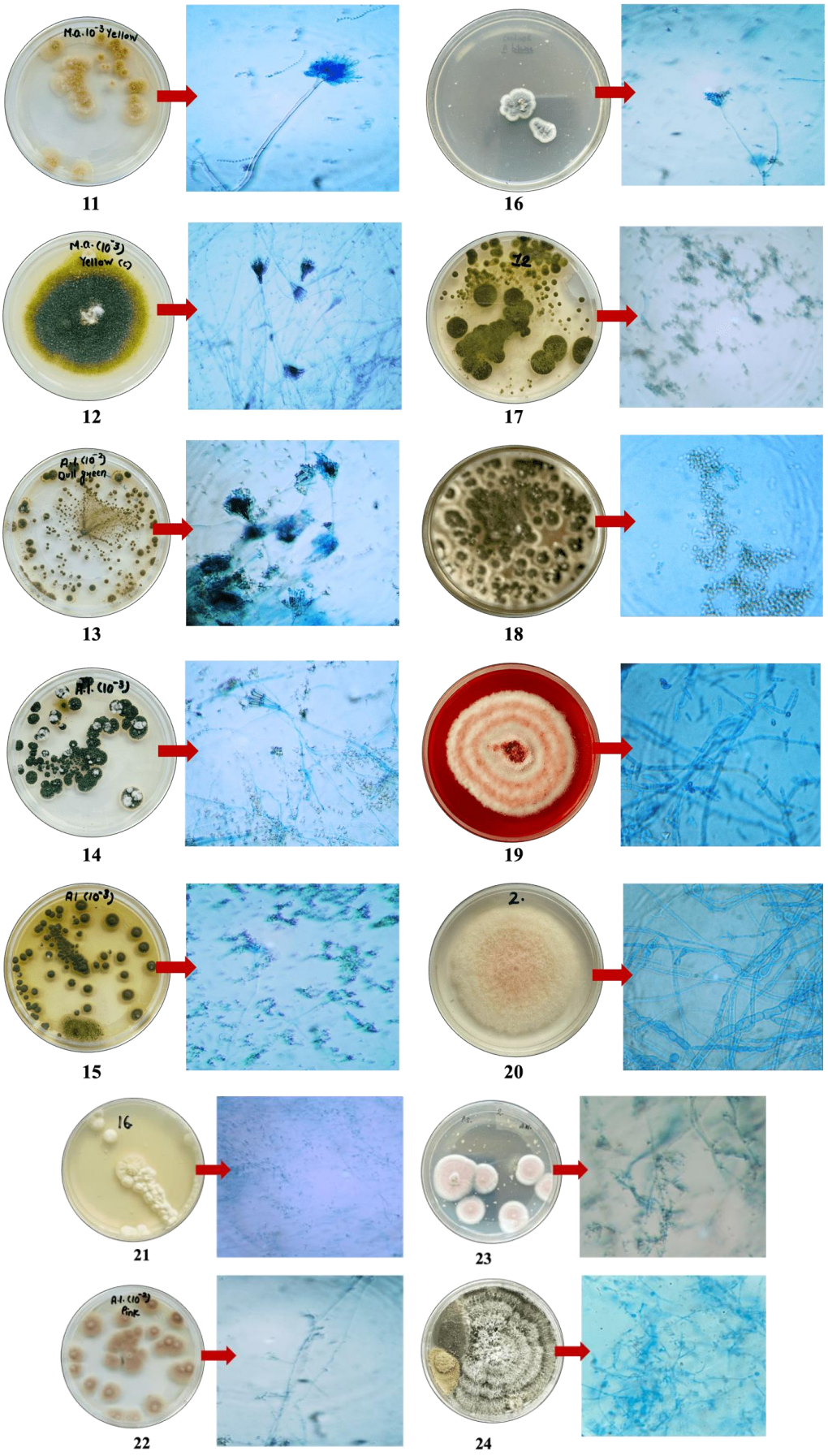
M.a.10-3 Yellow
11
16
M.a.(10-3) Yellow (c)
12
17
A.f.(10-3) Dull green
13
18
A.f.(10-3)
14
19
A.f.(10-3)
15
20
16
21
23
A.f.(10-3) Pink
22
24

Among the identified species, *Aspergillus niger* was found to be the most dominant with a frequency of 36.7%, followed by *Aspergillus fumigatus* (30%) and *Aspergillus nidulans* (26.7%), *Aspergillus flavus*, *Trichoderma viride*, and *Penicillium citrinum* (20.0% each). These dominant isolates showed distinct macroscopic and microscopic features. *A. niger* produced black powdery colonies with off-white reverse, septate brownish hyphae, and radiate thick-walled conidiophores bearing roughened globose conidia. *A. nidulans* formed olive floccose colonies with yellowish reverse, biseriate vesicles, and echinulate subglobose conidia. *A. flavus* exhibited yellow-green powdery colonies with pale brown reverse and radiate conidiophores with finely roughened globose to subglobose conidia.

Trichoderma viride developed woolly green colonies with cream reverse, and produced short, branched phialides with clustered globose to oval conidia. *Penicillium citrinum* had characteristic blue-green velvety colonies with bright yellow reverse, and branched metulae and phialides bearing smooth, globose to subglobose conidia. Other commonly encountered species included *Penicillium bilaiae* (19.3%), *Fusarium oxysporum* (16.0%), *Alternaria alternata* (15.3%), *Penicillium expansum* (14.7%), *Penicillium oxalicum* and *Fusarium solani* (14.0% each), and *Bipolaris spicifera* (14.0%). Less frequently isolated species were *Curvularia spp.* (0.7%), *Botrytis cinerea* (8.7%) and *Acremonium implicatum* (8.0%) (Table 1).

Table 1. Morpho-cultural characteristics and occurrence frequency (%) of 25 fungal species isolated from infected plant tissues and rhizospheric soils. Observations include colony diameter, texture, colour (surface and reverse), hyphal structure, conidiophore and conidia morphology.

Name of the fungus	Colony diameter	Colony Texture	Colony colour	Colony Reverse	Characters of hyphae	Conidiophore	Conidia	Occurance frequency %
Bipolaris spicifera	2-3 cm	Velvety to floccose	Dark olive brown	Dark brown to blakish	Septate, brown	Straight, branched	Ellipsoidal, septate	14
Bipolaris sorokiniana	2-3 cm	Woolly to cottony	Dark grey to brownish	Dark brown to blakish	Septate, brown	Simple, brown	Fusiform, multicelled	9.3
Curvularia lunata	3-4 cm	Woolly	Dark brown to black	Black to olive brown	Septate, brown	Branched, geniculate	Crescent shaped, multicelled	10.7
Curvularia spp.	3-4 cm	Floccose	Brown to olive black	Dark olive to black	Septate, brown	Simple to geniculate	Fusiform, septate	0.7
Curvularia geniculata	3-4 cm	Woolly to cottony	Dark grey	Dark brown	Septate, brown	Geniculate	Curved, multicellular	8.0
Alternaria alternata	2-3 cm	Floccose	Dark Brown to Blackish	Dark brown to black	Septate, branched	Simple, brown	Club-shaped, muriform	15.3
Aspergillus fumigatus	5-6 cm	Powdery to granular	Smoky grey to olive green	White to light brown	Septate, hyaline	Uniseriate, thick-walled vesicles	Globose to subglobose, smooth	30
Aspergillus nidulans	5-6 cm	Velvety to powdery	Yellowish to parrot green	Pale yellow to brownish	Septate, brownish	Radiate, thick-walled	Globose, roughened	26.7

Aspergillus niger	6-7 cm	Granular to floccose	Black with white margin	Pale yellow to cream	Septate, hyaline	Biserriate vesicle	Subglobose, echinulate	36.7
Aspergillus flavus	5-6 cm	Granular to woolly	Yellow to green	Yellow to golden brown	Septate, hyaline	Radiate, rough walls	Globose to subglobose, finely roughened	18.7
Aspergillus ochraceopetaliformis	4-5 cm	Velvety	Pale yellow to ochre	Light ochre to yellowish brown	Septate, hyaline	Uniseriate vesicle	Roughened, echinulate	8
Aspergillus terreus	4-5 cm	Powdery	Cinnamon brown	Light brown	Septate, hyaline	Columnar heads with hemispherical vesicle	Smooth, globose to ellipsoidal	10
Talaromyces pinophilus	3-4 cm	Floccose	Yellow to greenish with white margin	Reddish to brownish	Septate, hyaline	Branched with metulae and phialides	Globose to subglobose	8
Penicillium expansum	4-5 cm	Velvety to granular	Bluish green	Plae yellow to greenish	Septate, hyaline	Brush-like branched structure	Subglobose, smooth	14.7
Penicillium oxalicum	4-5 cm	Velvety	Bluish green with white edge	Pale yellow to dull green	Septate, hyaline	Branched, penicilli with phialides	Ellipsoidal, smooth	14
Penicillium citrinum	3-4 cm	Velvety	Dull to bluish green	Pale cream to yellowish	Septate, hyaline	Branched, metulae and phialides	Globose to subglobose, smooth	20
Penicillium bilaiae	4-5 cm	Cottony to powdery	Pale green	Pale yellowish to colourless	Septate, hyaline	Branched with short phialides	Subglobose to cylindrical	19.3
Cladosporium	2-3 cm	Velvety to suede like	Olive black	Dark olive to black	Septate, dark brown	Branched, tree-like	Ovoid to lemon-shaped	9.3
Botrytis cineria	2-3 cm	Fluffy cottony	Olive green with margin	Yellowish brown	Septate hyaline	Branched tree like	Oval to ellipsoid	8.7
Fusarium solani	2-3 cm	Cottony	White with pink to orange	Pinkish to orange	Septate, curved	Simple or branched	Curved, blunt apical cells	14

			center					
Fusarium oxysporum	2-3 cm	Fluffy to cottony	White to pinkish	Pinkish white	Septate, fusiform	Branched	Curved, pointed both ends	16
Acremonium implicatum	1-2 cm	Cotton y smooth	Pale white	Pale to colorless	Septate, hyaline	Unbranched slender phialides	One-celled, slimy clusters	8
Purpureocillium sodanum	5-6 cm	Cotton y to powder y	White to lilac pink	Pale lilac to pink	Septate, hyaline	Verticillate conidiophor es	Cylindrical to ellipsoidal	12
Purpureocillium lilacinum	4-5 cm	Cotton y to powder y	lilac	Lilac to pink	Septate, hyaline	Verticillate conidiophor es	Elliptical, smooth	12.7
Trichoderma viride	6-7 cm	Floccos e to compac t	Dense green with white margin	Pale yellow to colourle ss	Septate, hyaline	Short branched phialides	Globose to oval, clustered	20

Nine unidentified isolates of these 24 species were subjected to molecular identification using 18S rRNA gene sequencing. The DNA sequences obtained were compared with those available in the NCBI database to confirm species identity. The sequence data of these nine fungal isolates were successfully submitted to the GenBank database, along with their corresponding accession numbers (Table 2).

Table 2. List of Fungal Isolates with Their Corresponding Accession Numbers

Fungus	**Accession number**
Purpureocillium sodanum	PV269825
Talaromyces pinophilus	PV269826
Aspergillus ochraceopetaliformis	PV269827
Alternaria alternata	PV269828
Penicillium oxalicum	PV269829
Penicillium bilaiae	PV269830
Penicillium expansum	PV269831
Penicillium citrinum	PV269832
Acremonium implicatum	PV269833

4. DISCUSSION

This study provides a comprehensive overview of the culturable fungal diversity present in the agro-climatic region of Bathinda, Punjab, a region previously underexplored in this context. A total of 25 fungal isolates were obtained from both infected plant tissues and rhizospheric soils of healthy plants, representing a broad taxonomic range across several ecologically and industrially important genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and others.

The high frequency of *Aspergillus* and *Penicillium* species aligns with prior studies emphasizing their ubiquitous presence in soil and plant-associated environments (Cuadros-Orellana et al., 2013). Notably, *Trichoderma* spp., *Purpureocillium* spp.

and some of the *Aspergillus* spp. are known for their roles in biodegradation and biocontrol, respectively, supporting their ecological and potential agricultural importance [12-13].

The recovery of fungal isolates from both healthy and diseased plant environments underscores the dual ecological roles fungi can play—as pathogens, symbionts, or saprophytes depending on environmental context and host interactions. These findings lay the groundwork for future investigations into the functional roles of these fungi in soil health, plant disease, and sustainable agriculture.

5. CONCLUSION

This study presents the first systematic documentation of culturable fungal diversity in the Bathinda region of Punjab. The identification of 25 fungal isolates, including novel regional records, underscores the richness of fungal biodiversity in both rhizospheric and diseased plant environments. The identification of *Purpureocillium sodanum* and *Aspergillus ochraceopetaliformis* for the first time in Punjab, India, marks a significant contribution to regional biodiversity records. Their presence in rhizospheric soils of healthy plants suggests potential beneficial roles, possibly in plant growth promotion or soil nutrient cycling, although further functional studies are required.

The findings offer a valuable baseline for further ecological and functional studies, particularly concerning plant–microbe interactions and the potential use of beneficial fungi in sustainable agriculture.

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