Pak. J. Bot., 58(1) January 2026.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2026-1(8)

COMPARATIVE DIVERSITY ANALYSIS OF MICROMYCETES IN THE RHIZOSPHERIC SOIL OF SELECTED CROPS AND VEGETABLES FROM FAISALABAD AND PATTOKI

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Abstract

This study examines the diversity and frequency of micromycetes in the rhizosphere soils of selected crops (*Zea mays, Saccharum officinarum*) and vegetables (*Raphanus sativus, Daucus carota, Solanum lycopersicum, and Solanum tuberosum*) in Faisalabad and Pattoki, Pakistan. Faisalabad's clayey silt and silty sand soil is ideal for crops, while Pattoki's alluvial soil supports both vegetables and crops effectively. A total of 18 fungal species were identified across 5 orders, 8 families, and eight genera, with Eurotiales as the dominant order and *Penicillium* as the leading genus. Key species such as *Aspergillus niger, A. flavus*, and *Penicillium simplicissimum* were prevalent in both locations, while *Penicillium nalgiovense* and *Rhizoctonia solani* were exclusive to Faisalabad, and *Rhizopus oryzae* and *Talaromyces flavus* were reported from Pattoki only. This comparative analysis is crucial for understanding the influence of soil composition on microbial diversity, shedding light on its impact on soil fertility, plant health, and agricultural sustainability. It provides a basis for efficient soil management, disease prevention, and improved crop productivity, while also contributing to microbial ecology research with applications in biocontrol, organic farming, and environmental conservation.

Key words: Fungal diversity; Soil analysis; Microscopy

Introduction

Pakistan's varied geography, from tropical plains to the cold Himalayas, supports diverse ecosystems, yet its fungal diversity remains largely unexplored (Raza *et al.*, 2022). Soil plays a crucial role in sustaining microorganisms, including fungi that contribute to nutrient cycling and disease resistance (Meena *et al.*, 2023; Hussain *et al.*, 2016; Ambrazaitienė *et al.*, 2013). Cultivation strategies and environmental conditions significantly influence soil microbiota, affecting fungal communities and their interactions with plant roots. Despite the global estimate of 1.5 to 5.1 million fungal species, only about 150,000 have been identified (Gautam *et al.*, 2022), emphasizing the need for further research on their impact on soil fertility and crop health.

Fungi contribute to essential soil processes such as humus formation, organic matter decomposition, and detoxification, supporting soil structure and plant health (Korneikova, 2018). However, soil-borne fungal pathogens pose significant agricultural challenges by reducing crop productivity and quality (Parveen *et al.*, 2021; Manici *et al.*, 2014; Bareja *et al.*, 2013). Opportunistic fungi can also affect humans and animals (Krupodorova *et al.*, 2023), while species like *Aspergillus niger* and *Penicillium polonicum* lead to economic losses in agriculture (Khalil *et al.*, 2019; Gautam *et al.*, 2011). Understanding fungal distribution and diversity in different soils is key to developing disease prevention strategies and improving crop yields.

The rhizosphere, a dynamic zone around plant roots, plays a vital role in nutrient cycling, disease resistance, and plant health (Huang *et al.*, 2014). Micromycetes, which can be beneficial or harmful for plant growth, are particularly important for study (Dighton, 2016). In Pakistan, staple crops like sugarcane (*Saccharum officinarum*) and maize (*Zea mays*), along with vegetables such as carrots (*Daucus carota*), radishes (*Raphanus sativus*), tomatoes (*Solanum lycopersicum*), and potatoes (*Solanum tuberosum*), face threats from soil-borne fungal diseases (Husnain *et al.*, 2020; Rehman *et al.*, 2020; Mahmood *et al.*, 2019, 2017; Ahmad *et al.*, 2012). This study examines fungal diversity in Faisalabad and Pattoki, two agriculturally significant regions with different soil compositions (Ahmad *et al.*, 2020). Identifying dominant fungal species in these areas can help optimize soil management, support sustainable farming practices, and enhance long-term agricultural productivity (Smith & Read, 2008; Van der Heijden *et al.*, 2008). The findings will contribute to enhancing soil fertility, improving crop resilience, and promoting sustainable agriculture (Raza *et al.*, 2022).

Material and Methods

Soil sampling: Soil samples were taken from selected sites of Faisalabad and Pattoki from September to December 2023. In each locality approximately 600 grams of soil sample was collected from the surface area to 10-15 cm depth near the rhizosphere region of plants. The collected soil samples were brought to the laboratory in sterile polythene bags and stored at 4ºC until further analysis.

Received: 04-10-2024 | Revised: 23-06-2025 | Accepted: 18-07-2025 | Online: 26 08-2025

Isolation and enumeration of fungi from the soil samples: The isolation and enumeration of soil fungi were conducted using a modified soil dilution plate method to assess fungal diversity and abundance. The Potato Dextrose Agar (PDA) medium was prepared by dissolving 42 g of PDA powder in 1 L of distilled water, followed by boiling and autoclaving at 121°C for 15 minutes to ensure sterility. One percent Streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. To prevent contamination, the sterilized medium was aseptically poured into sterile Petri plates within a laminar flow cabinet. Soil solutions from each sample were prepared by suspending 5 g in 20 mL sterile distilled water to facilitate microbial dispersion. To ensure comprehensive fungal recovery, soil samples were inoculated into the medium using different approaches: dry soil was either sprinkled beneath or on top of the agar, while a semi-liquid soil suspension was dispersed in patches, placing soil slurries beneath, on top, or directly into the medium, ensuring diverse fungal growth conditions. These inoculation methods enhance fungal recovery by simulating natural soil colonization dynamics (Domsch *et al.*, 2007; Bridge & Spooner, 2001). Petri dishes were sealed with paraffin film to prevent external contamination and incubated at ambient room temperature (approximately 25-28°C) for 4-7 days. Daily observations were taken to monitor fungal colony development, morphological characteristics, and species diversity.

Identification of isolated micromycetes: Fungal morphology were studied macroscopically. Following parameters were recorded: features like shape (e.g., circular, filamentous), size, diameter, elevation, margin, surface, texture, opacity, and color (e.g., green, black, yellow). These parameters were checked daily as the colony grew. The observation method followed the procedure outlined by Schöneberg *et al*., (2015). For fungal identification, microscopic features of conidiogenous cells such as the shape, size, and color of conidial heads, conidia, mycelium, and vesicles were examined and compared with known species. On the maturing of the colonies, slides were prepared using KOH solution, Trypan blur or Melzer's reagent. Under compound microscope, the anatomical characteristics were observed at 4X. 10X, 40X and 100X. The fungi were identified with the help of literature (Chamekh *et al.*, 2019; Nagamani *et al.*, 2006; Lugauskas & Krasaukas, 2005; Lugauskas, 2002; Aneja, 2001; Pitt & Hocking, 1997; Rabie *et al.,* 1997).

Physico-chemical soil parameters: The chemical parameters analyzed included pH, Electrical Conductivity (EC), organic matter content, and the concentrations of nitrogen (N), phosphorus (P), and potassium (K). To assess pH and EC, a 5-gram soil sample was suspended in 45 mL of distilled water, thoroughly mixed, and allowed to stabilize before obtaining readings. For nutrient quantification, 5 grams of soil and 50 mL of specific chemical reagents were used: phosphorus was extracted with 0.5 M sodium bicarbonate ($NaHCO_3$) at pH 8, while potassium was extracted with 1 N ammonium acetate (NH_4OAc) at pH 7. The concentrations of phosphorus and potassium were subsequently determined using services of central Lab, Lahore College for Women University, Lahore.

Results

Our study identified 18 micromycete species from agricultural soil samples in Faisalabad and Pattoki, categorizing them into 5 orders, 7 families, and 8 genera (Table 1). The order Eurotiales and family Aspergillaceae were predominant, while others showed lower prevalence. Less-documented families, including Rhizopodaceae, Ceratobasidiaceae, Nectriaceae, Mucoraceae, Saccharomycetaceae, and Talaromycetaceae, were also identified. Among the genera, *Penicillium* was the most abundant, whereas *Rhizopus*, *Aspergillus*, *Rhizoctonia*, *Mucor*, *Talaromyces*, and *Fusarium* were less prevalent (Figs. 1-4). The frequency occurrence analysis showed that *Penicillium pinophilum*, *Aspergillus flavus*, and *A. niger* were the most frequent species at 85%, 80%, and 75% occurrence rates, respectively. In contrast, *Mucor plumbeus* and *Candida albicans* had the lowest occurrence at 30% and 25%. The occurrence time data indicated *Penicillium pinophilum* was the most consistently present species (16 occurrences), followed by *Aspergillus flavus* (15 occurrences), while *Candida albicans* had the least presence (4 occurrences).

Table 1. Distribution of isolated mycoflora.

No.	Fungal species	Region 1	Region 2	Saprophytic or Phytopathogen
1.	*Aspergillus niger*	+	+	Saprophytic
2.	*Aspergillus flavus*	+	+	Phytopathogen
3.	*Rhizopus oryza*	-	+	Saprophytic
4.	*Rhizoctonia solani*	+	-	Phytopathogen
5.	*Candida albicans*	+		Saprophytic
6.	*Penicillium islandicum*	+	+	Saprophytic
7.	*Aspergillus carbonarius*	+	+	Phytopathogen
8.	*Penicillium nalgiovense*	+	-	Saprophytic
9.	*Rhizopus stolonifera*	+	+	Saprophytic
10.	*Mucor hiemalis*	+	-	Saprophytic
11.	*Penicillium funiculosum*	+	+	Saprophytic
12.	*Penicillium pinophillum*	+	+	Saprophytic
13.	*Penicillium simplicissim*	+	+	Saprophytic
14.	*Rhizopus oligosporus*	+	+	Saprophytic
15.	*Fusarium chlamydosporum*	+	-	Phytopathogen
16.	*Talaromyces flavus*	-	+	Saprophytic
17.	*Mucor plumbeus*	-	+	Saprophytic
18.	*Aspergillus candidus*	+	+	Saprophytic

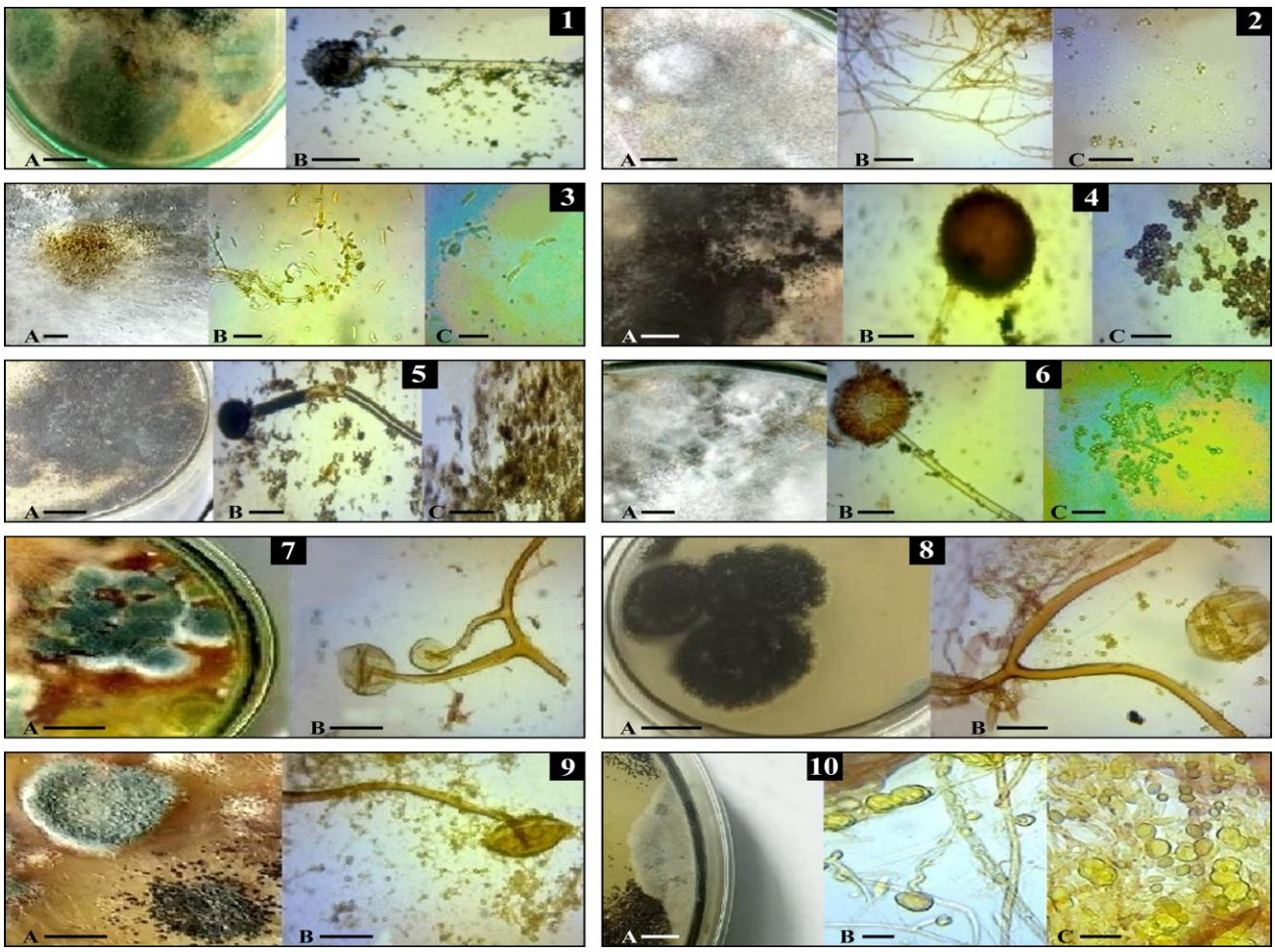

Fig. 1. Diversity of different types of fungi: 1, *Aspergillus flavus*; 2, *Rhizoctania solani*; 3, *Fusarium chlamydosporum*; 4, *Aspergillus carbonarius*; 5, *Aspergillus niger*; 6, *Aspergillus candidus*; 7, *Rhizopus oligosporus*; 8, *Rhizopus stolonifer*; 9, *Rhizopus oryzae*; 10, *Candida albicans*.

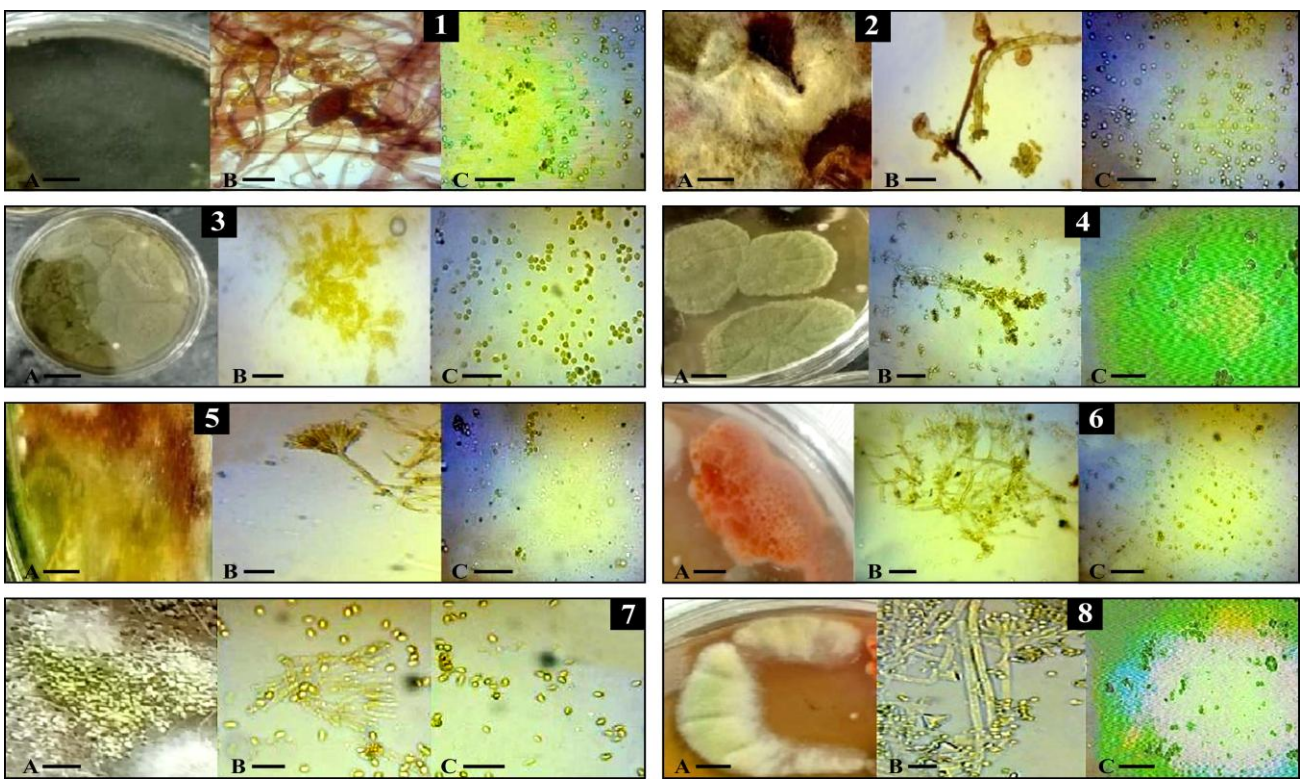

Fig. 2. Diversity of different types of fungi: 1, *Mucor plumbeus*; 2, *Mucor hiemalis*; 3, *Penicillium simplicissimum*; 4, *Penicillium funiculosum*; 5, *Penicillium pinophilum*; 6, *Penicillium islandicum*; 7, *Talaromyces flavus*; 8, *Penicillium nalgiovense.*

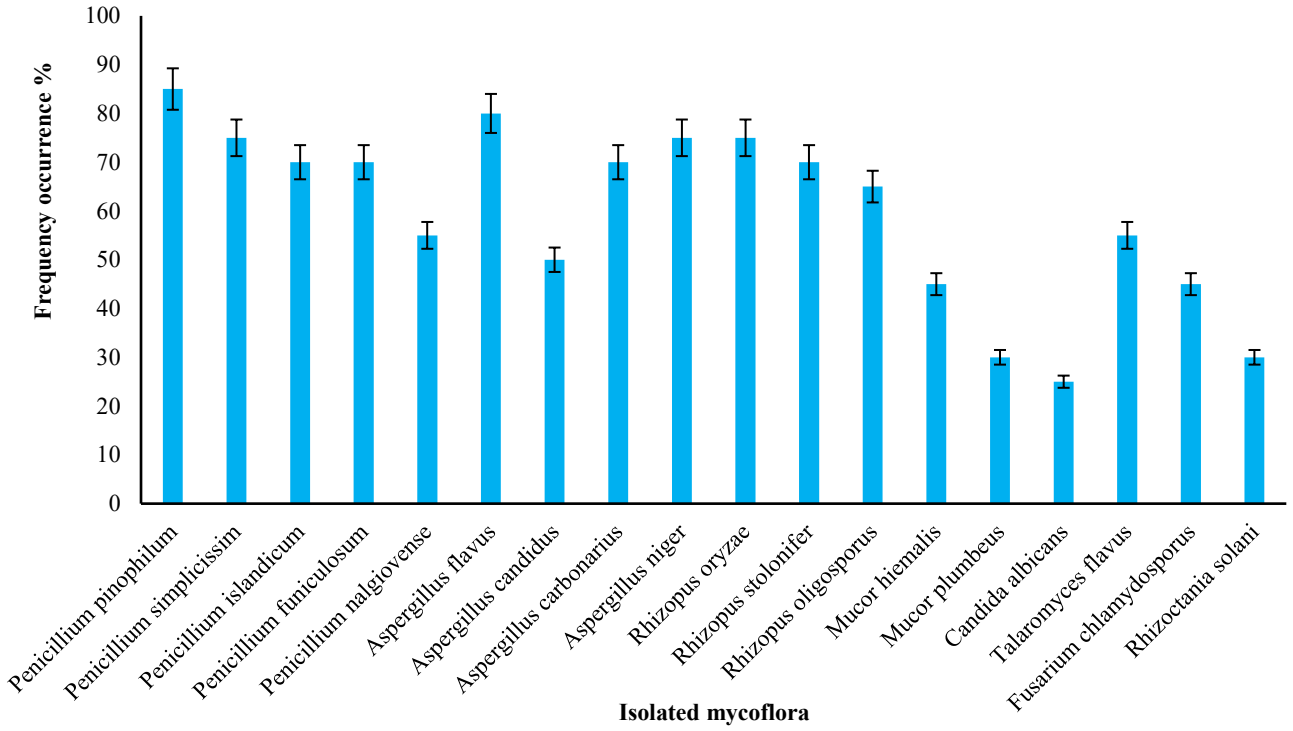

Fig. 3. Frequency occurrence percentage of isolated mycoflora.

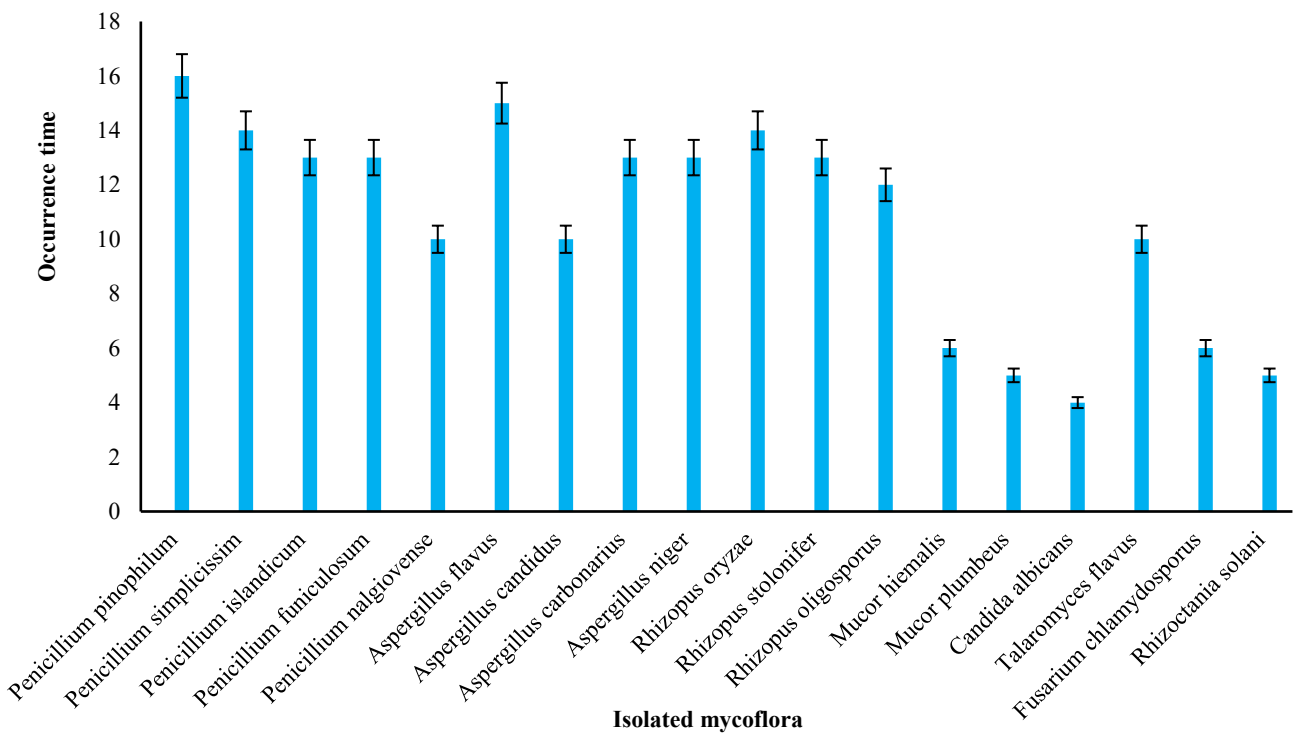

Fig. 4. Occurrence time of isolated mycoflora.

Table 2. Different parameters of soil samples.

Name of plants	pH	EC	Phosphorus	Potassium
Raphanus sativus	8.7-8.9	517 to 693 µS/cm	53-57 mg/kg	96-98 mg/kg
Saccharum officinarum	8.5-8.7	636 to 760 µS/cm	7-36 mg/kg	50-45 mg/kg
Daucus carota	8.6-8.75	567 to 604 µS/cm	42-44 mg/kg	52-54 mg/kg
Zea mays	7.6-8.7	518 to 785 µS/cm	45-50 mg/kg	75-76 mg/kg
Solanum lycopersicum	8.5-8.7	639 to 981 µS/cm	44-46 mg/kg	60-62 mg/kg
Solanum tuberosum	8.5-8.7	787 to 801 µS/cm	41-43 mg/kg	43-47 mg/kg

Phytopathogenic mycoflora were isolated from the rhizosphere of *Saccharum officinarum*, *Zea mays*, *Daucus carota*, *Raphanus sativus*, *Solanum tuberosum*, and *Solanum lycopersicum*. These included *Aspergillus carbonarius*, *A. flavus*, *Rhizoctonia solani*, and *Fusarium chlamydosporum*. The macroscopic and microscopic characteristics of *Aspergillus flavus* revealed rapid growth, yellow-green colonies, and septate hyphae with spherical conidia. *Aspergillus carbonarius* formed black colonies showing moderate growth, and rough-walled jet black conidia. *Rhizoctonia solani* was characterized by irregular, cottony growth with non-traditional conidiophores, while *Fusarium chlamydosporum* displayed yellow-brown colonies with distinctive septate macroconidia and abundant chlamydoconidia.

Additionally, saprophytic mycoflora were isolated, including *Penicillium* spp., *Aspergillus niger*, *A. candidus*, *Rhizopus oligosporus*, *Candida albicans*, *Talaromyces flavus*, and *Mucor* spp. *Aspergillus niger* formed dark colonies with dense conidial heads, while *Aspergillus candidus* exhibited white, granular colonies with spherical conidia. *Rhizopus oligosporus* showed cobweb-like growth with spherical sporangiospores. These findings provide valuable insight into fungal diversity in agricultural soils and its implications for plant health and soil management. *Rhizopus stolonifer* displayed rapid fibrous growth, producing dark brownish-black colonies, with unbranched sporangiophores and striate-walled sporangiospores. *Rhizopus oryzae* was characterized by its fast-growing cottony colonies, developing spherical sporangia with brown, angular sporangiospores. *Candida albicans* formed smooth, creamy colonies with pseudohyphae and small conidia.

The study also identified *Mucor plumbeus* with rapidly spreading pale grey colonies, possessing spiny-walled dark sporangia and spheroidal sporangiospores. *Mucor hiemalis* developed dense yellowish colonies with spherical sporangia and kidney-shaped hyaline spores. Additionally, *Penicillium simplicissimum* formed velutinous green colonies, characterized by rough-walled conidiophores and ellipsoidal conidia. *Penicillium funiculosum* exhibited rapid growth with velvety dull green colonies and closely appressed phialides producing smooth-walled conidia.

Lastly, *Penicillium pinophilum* developed floccose dark green colonies with vesiculate conidiophores bearing subspheroidal conidia. Several species, including *Mucor plumbeus, Penicillium simplicissimum, P. funiculosum,* and *P. pinophilum*, are the new records for fungal diversity in Pakistan. These findings shall contribute to the broader understanding of fungal ecology, plant-pathogen interactions, and soil microbial diversity in agricultural regions.

Soil parameters analysis: The chemical analysis of soil samples collected from Faisalabad and Pattoki were assessed for the following parameters: pH, Electrical Conductivity (EC), phosphorus (P), and potassium (K), influencing soil quality and crop productivity (Table 2). pH values, crucial for nutrient availability, ranged from 7.6 to 8.9, the highest pH was recorded in *Raphanus sativus* (radish) fields and the lowest in *Zea mays* (Maize) fields. Most samples were within FAO's recommended range (6.5-8.5), though some exceeded, indicating alkaline characteristics. EC, a measure of soil salinity and ion concentration, varied widely, with *Solanum lycopersicum* (tomatoes) fields showing the highest EC (981 µS/cm), surpassing the FAO's acceptable limit of 250 µS/cm. Elevated EC levels suggested accumulation of salts such as sodium, chloride, and nitrate, which could affect plant health.

Phosphorus and potassium availability were also examined, highlighting variations across samples. Phosphorus levels ranged from 7-57 mg/kg, with *Raphanus sativus* fields containing the highest concentration. Potassium, essential for plant metabolism, varied between 43-98 mg/kg, with *Raphanus sativus* showing the highest levels and *Solanum tuberosum* the lowest. The findings emphasize the influence of soil chemistry on plant growth and reinforce the necessity for soil management strategies to maintain optimal nutrient levels for sustainable agriculture.

Discussion

Our study provides a comparative diversity analysis of micromycetes in the rhizospheric soil of selected crops and vegetables from Faisalabad and Pattoki, revealing a rich fungal community with variations in species prevalence. We identified total 18 different micromycetes. *Penicillium* emerged as the dominant genus, represented by five species, with *Penicillium pinophilum* having the highest occurrence at 85%. Other *Penicillium* species, including *P. simplicissimum*, *P. islandicum*, *P. funiculosum*, and *P. nalgiovense*, exhibited moderate prevalence across the study regions. Notably, some species, such as *P. pinophilum* and *P. simplicissimum*, had not been previously reported in the rhizosphere of any crop in Pakistan, suggesting novel fungal diversity in agricultural soils. These findings aligned with studies by Smith & Read (2008) and Van der Heijden *et al*., (2008), which emphasized the critical role of rhizospheric fungi in nutrient cycling and plant health.

Our analysis also observed significant occurrences of *Aspergillus* species, with *Aspergillus flavus* being the most dominant phytopathogen, accounting for 80% frequency, followed by *A. carbonarius*, *A. niger*, and *A. candidus*. *A. niger*, exhibiting both saprophytic and phytopathogenic traits, had a high occurrence rate, matches with previous research by Saleemi *et al*., (2010) on fungal presence in *Oryza sativa* rhizosphere. Similarly, *Rhizopus* and *Mucor* species showed moderate prevalence, with *Rhizopus stolonifer* (70%) and *Rhizopus oligosporus* (65%) frequently appearing across soil samples. Other saprophytic fungi, including *Candida albicans* and *Talaromyces flavus*, exhibited lower occurrence rates but contributed to microbial diversity in rhizospheric soils.

The study further compared soil physicochemical parameters with fungal diversity, revealing that pH and electrical conductivity (EC) had an influence on microbial composition. Soil pH in the study regions ranged from 7.6 to 8.9, remaining largely within FAO-recommended limits (6.5-8.5), with *Raphanus sativus* fields exhibiting the highest alkalinity. Elevated EC levels, particularly in *Solanum lycopersicum* fields (639-981 µS/cm), exceeded FAO standards indicating potential soil contamination. These findings parallel reports by Qudsia *et al*., (2017), which highlight the impact of excessive salts on microbial diversity and plant health. Additionally, phosphorus and potassium concentrations remained within acceptable limits, ensuring adequate soil fertility for sustaining crop production. This study identified 18 micromycete species in the rhizosphere soils of Faisalabad and Pattoki, with *Penicillium* as the dominant genus and notable differences in fungal diversity between the two regions. Soil

composition and physicochemical parameters significantly influenced microbial diversity.

Our findings reinforces the significance of soil microbial communities in agricultural ecosystems, contributing to ongoing research by documenting dominant and rare fungal species, essential for developing sustainable soil management strategies. New species records from Pakistan underscores the need for further studies to explore fungal biodiversity, soil fertility, and ecological balance in agricultural regions. This comparative analysis enhances knowledge of soil microbiology and supports effective agricultural practices in Faisalabad and Pattoki. Concludingly, this study highlights the influence of soil composition on micromycete diversity in the rhizosphere of selected crops and vegetables in Faisalabad and Pattoki, Pakistan. Our findings provide a basis for improved soil management and ecological research.

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