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Verdant Legacy

Research Article

Isolation of the Culturable Fungi from Rhizospheric Zone of Different Crops from Selected Sites of Lahore and Kasur

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Abstract

During this research work diversity of isolated micromycetes was analyzed from two different crop soil zones (*Sorghum bicolor*, *Saccharum officinarum*, *Zea mays*, and *Oryza sativa*) of Lahore and Kasur. A total of 35 species of these fungi were isolated and identified, belonging to nine orders, eleven families, and thirteen genera. The majority of the isolated fungi belong to the order *Eurotiales*. Phytopathogenic nature of fungi was found dominant as compared to the saprophytic after knowing their status by literature. The highest diversity of isolated fungi was found in the Kasur district, while the lowest diversity was found in Lahore. *Aspergillus carbonarious, Aspergillus ochraceus*, *Rhziocotina solani*, *Candida albicans*, *Penicillium oxalicum are* found in the district of Lahore. In contrast, *Penicillium citrinium*, *Penicillium verruculosum*, *Mucor circinelloides*, *Aspergillus versicolor*, *Botrytis cinerea*, *Aspergillus parasiticus*, *Nigrospora sphaerica*, *Penicillium roqueforti* were found in the district of Kasur; whereas *Aspergillus niger, Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus candidus*, *Aspergillus nidulans*, *Penicillium chrysogenum*, *Rhziocotina* sps, *Rhizopus microsporus*, *Rhizopus stolonifera*, *Rhizopus oryzae*, *Curvularia lunata*, *Mucor hiemalis*, *Rhizopus oligosporus*, *Trichoderma citrinoviride*, *Exserohilum turcicum*, *Fusarium oxysporium*, *Fusarium monoliforme*, *Fusarium graminearum*, *Trichoderma hazrianum*, *Phytopthora cinnamomi*, *Aspergillus awamori* were isolated from both the districts (Lahore and Kasur). The findings of this study have opened the door for more research by utilizing the power of soil microorganisms for increasing agricultural productivity and environmental management. Indigenous data about these fungi is scarce and these findings are a step to fill this gap.

Keywords: Fungal strains, Micromycetes, Phytopathogenic nature, Rhizosperhic Soil, Serial dilution,

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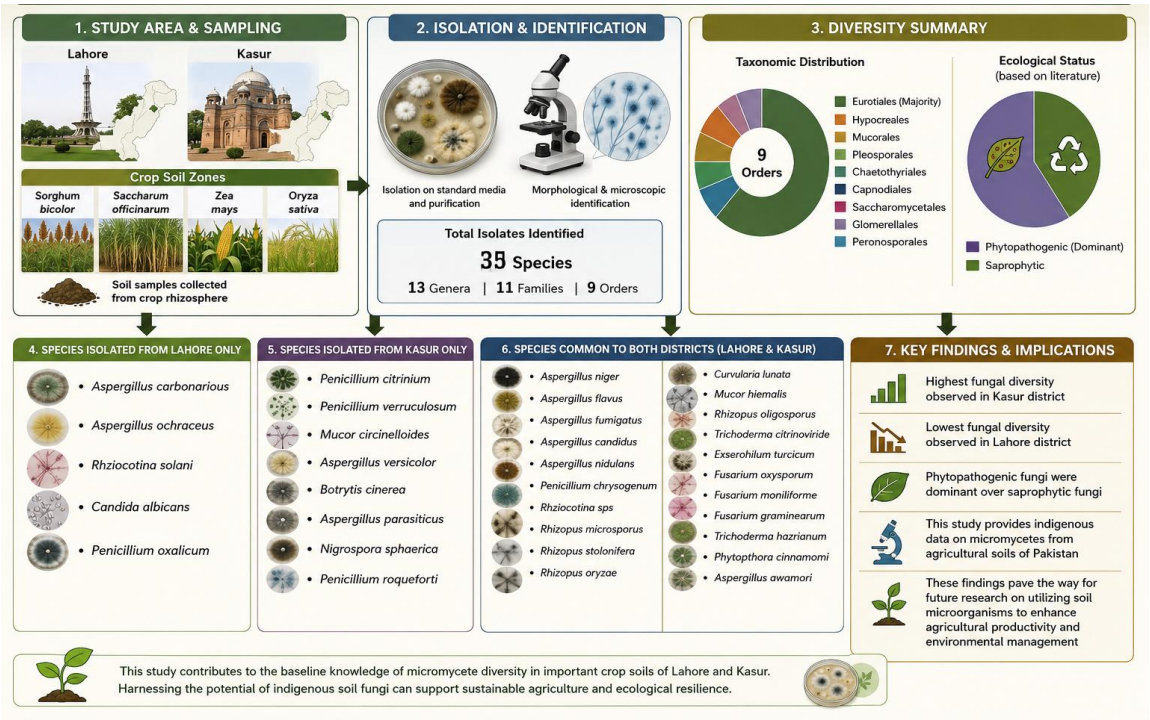
Graphical Abstract:



1: Introduction

Soil is the top layer of the earth a diverse spectrum of bacteria, organic components, gasses, liquids, and minerals, and rocks. The presence of microflora affects the fertility of the soil and its biological activity (Vehra *et al.,* 2015). One gram of soil is containing 105-106 fungus cell. Numerous significant ecological processes, includes nutrient mineralization and carbon sequestration through plant growth are influenced by soil fungi (Landínez *et al.,* 2020). Soil borne pathogens are adapted to survive and grow in bulk soil but rhizosphere is the playground where a fungus establishes parasitic and symbiotic relationship with plants (Raza *et al.,* 2013). A large decline in farming land can occur due to soil-borne pathogenic microflora (Termorshuizen and Jeger, 2008). Micromycetes have their pathogenic effects on plants and their shoots. In plants apical growth occurs due to the activity of micromycetes, they proliferate in the direction of the meristem (Ignatova *et al.,* 2014). It is possible for many of the micromycetes that live in plant roots to benefit their hosts nutritionally (Ioana *et al.,* 2019). Diagnosing plant diseases primarily aims to identify pathogens in a quick, accurate, and trustworthy manner. Early fungal pathogen detection is essential for minimizing or stopping the spread of disease and putting in place efficient management strategies (Dayarathne *et al.,* 2023). The largest biodiversity of the soil micro flora inhabits the globe. The relationship between fungal and plant diversity has been demonstrated by recent research. Therefore, the biodiversity and ecosystem functioning hypothesis suggests that increases in soil fungus community may potentially be advantageous for plant stress tolerance (Bollmann *et al.,* 2022).The biodiversity of soil is an enormous ecosystem includes a diverse array of species including insects, nematodes, fungi, bacteria, and earthworms. The interactions between these creatures impact how the soil ecosystem functions. Because soil biodiversity has important connectionsto many other fields, like agriculture and climate change, it is becoming increasingly important to investigate in the environmental sciences.

Indeed, a variety of ecosystem services providedby soil biodiversity and function have an impact on human well-being, both directly and indirectly (Orgiazzi *et al*., 2015). Though it is evident that very few species have yet been isolated or reported from soil, it is challenging to determine the precise number and range of species present in the soil due to the restricted methods for detecting and isolating fungi from soil. The majority of fungus are quite picky, making it difficult to identify exactly which onesare present in a soil sample. Additionally, only vegetative mycelium is available for in-depth investigation because, while some soil-dwelling fungi grown in culture, it is frequently not yetpossible to germinate resting structures like spores (Bridge *et al*., 2001).

Several biological processes in soils measured in response to plant diversity in certain investigations. The challenges in researching microbial diversity, particularly in soil, are at least partially to blame for the dearth of knowledge in this field (Kowalchuk *et al.,* 2002). Research on fungal biodiversity in agricultural plants' rhizospheres and its interactions with microorganisms is not enough to understand the mechanism of soil microbes.

Zea mays L (Maize) is a vital staple crop grown all over the globe, significant economically for cattle, and plays a critical role in the nourishment of both people and animals (Nafady *et al.,* 2014). Maize is the most popular cereal in Pakistan and is cultivated twice a year (Maqbool *et al.,* 2019). Up to 90 percent of maize seeds in Pakistan found to have fungal infections, with an average of 71.1% from healthy seeds and 83.7% from damaged grains. The fact that fungi produce poisons that pose a risk to human and animal health adds to their significance. Survey of literature shows that several fungi viz., *Alternaria alternata, Aspergillus* spp., *Bipolaris maydis, Fusarium moniliforme, Fusarium* spp., *Cephalosporium* spp., *Helminthosporium* spp.,*Mucor* spp. and *Penicillium* spp., reported from maize seed (Niaz and Dawar, 2009).

Saccharum officinarum (sugarcane) is a key crop, is farmed for ethanol (Romao-Dumaresq *etal.,* 2016). Sugarcane is the second-largest cash crop in Pakistan. It supplies the raw materialsfor the agro-based sector and is Pakistan's first significant source of sugar (Raza *et al.,* 2021).Among the principal viral diseases of sugarcane is mosaic. The virus breaks into sugarcane and then causes systemic illness (Lu *et al.,* 2021). Many diseases that affect sugarcane plants brought on by various bacteria, fungus, viruses, protozoa, or phytoplasma. Among the illnesses that affect sugarcane are grassy shoot, ring spot, mosaic disease, and red rot. *Colletotrichum falcatum* is the source of sugarcane cancer, sometimes referred to as red root. (Went *et al*., 2020).

Oryza sativa (Rice) is a significant food crop as well as a valuable export good. The quality and quantity of the rice crop reduced due to the attacks of various diseases. Rice grain discoloration' is the new yield-reducing threat to rice crops. Due to a decrease in rice output, rice grain discoloration is becoming a major issue in Pakistan and other regions of Asia. However, rice blast has a significant negative impact on global rice yields, with losses ranging from 11.9 to 378.8% in Pakistan and other countries. Surveys of rice fields carried out to evaluate disease prevalence, occurrences, and the gathering of infected samples in order to combat the illness. (Ashfaq *et al.,* 2017).

Sorghum bicolor (Sorghum) is highly prized for its drought resistance and nutritional content,which is comparable to maize, and ranked fifth among the world's top cereal crops. Often referred to as "Jowar," it suffers from harmful diseases such covered smut, head smut, and longsmut, which cause large yield losses in developing nations. Furthermore, mycotoxin production by mold fungus on sorghum seeds can endanger both people and animals (Panchal and Dhal, 2011). The productivity and quality of the sorghum crop decreased by various bacterial, viral, and fungal diseases. Red leaf spot disease in sorghum crops caused by *Colletotrichum graminicola,* which results in significant global losses. The sorghum plant's leaves, stem, and head are all attacked by *C. graminicola.* Red leaf spot is the most harmful disease because it interferes with photosynthetic processes, lowering crop quality (Ali *et al.,* 2021). The goal of the current study is to understand the dynamics of soil mycoflora populationin agricultural fields in districts of Lahore and Kasur

2: Materials and Methods:

2.1: Soil sampling from rhizospheric region of different crops

The soil samples were collected from different areas of Kasur and Lahore, Pakistan, to assess the variety of micromycetes present in the soil of different crops by using random sampling technique (Fig 1). The samples were labeled based on where the place from the collected. Samples were carried to the lab for further examination

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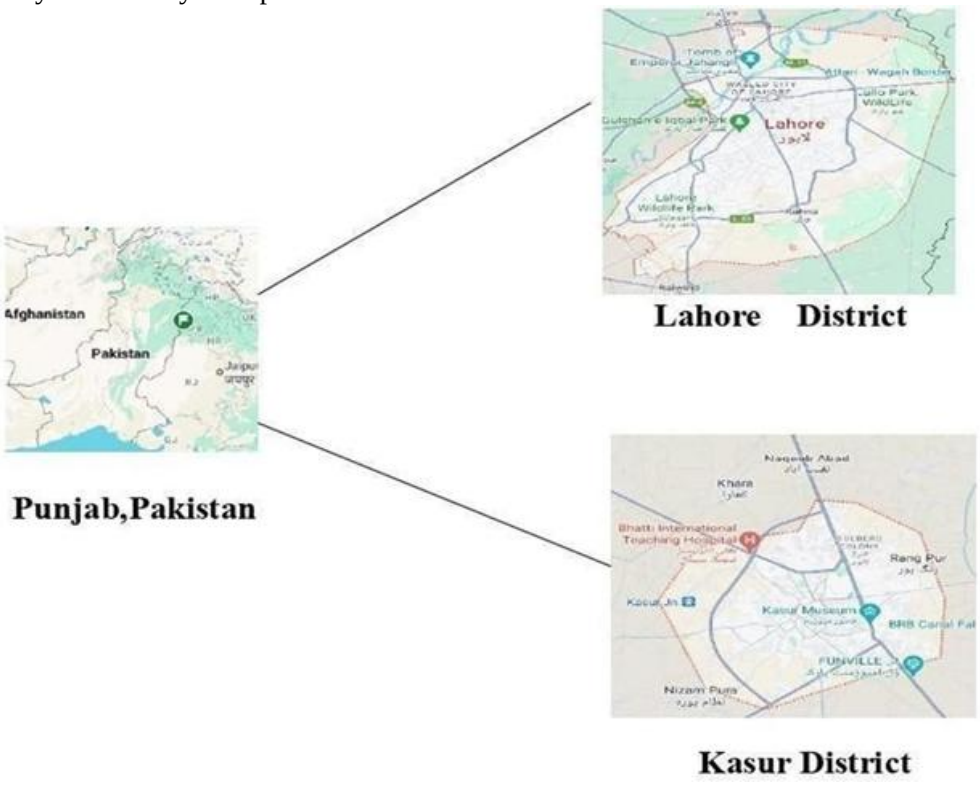

Figure 1: Map showing sampling districts of Lahore and Kasur

2.2: Parameters of Soil Analysis

On soil that had been collected, measurements of chemical parameters such pH, EC, organic matter, phosphorus (P), and potassium (K) were made. To make a solution in a glass container, 5 grams of each soil sample were dissolved in 45ml distilled water. The meter's probe was dipped into the container to measure pH and EC until the reading on the meter stabilized. A 50 mL extractant was used to perform a chemical analysis on a five-gram soil sample. 0.5 M $NaHCO_3$ at pH 8 was used for phosphorus (P), while 1 NH_4OAC at pH 7 was used for potassium (K). Every soil sample from various crops was extracted, and the concentrations of potassium and phosphorus were measured using a spectrophotometer and a flame photometer.

2.3: Media preparation

Potato Dextrose Agar (PDA) medium was used to isolate micromycetes. 20 gram of PDA was added to 500mL distilled water. After that Potato Dextrose Agar (PDA) medium was kept in autoclave for 30 minutes. A small amount of antibacterial capsule was added and left this media for cooling. In Laminar Air Flow transferred this media to the Petri plates.

2.4: Isolation and identification of soil mycoflora serial plate method

Each soil sample (2.0 g) was suspended in 18 ml of sterilized distilled water and mixed thoroughly before being put in serial dilutions of 1.0 ml into sterilized media-filled petri dishes.The dilutions were reproduced three times and incubated at 30 ± 1ºC. After three days of incubation, the total fungal colonies forming unit (CFU)/g soil was determined.

2.5: Identification of isolated micromycetes

For identification of isolated micromycetes macroscopic and microscopic analysis was performed.

2.6: Literature for identification

Growing fungi on plates were be identified by following Watanabe *et al*. (2002) and Pitt *et al*. (2009).

2.7: Statistical Analysis

For diversity analysis of micromycetes, statistical analysis was done on the observed data by using SPSS software.

3: Results and Discussion

Through combined macroscopic and microscopic characterization (Tables 2 and 3), culturable micromycetes were successfully isolated from rhizospheric soils of rice, sorghum, maize, and sugarcane collected from Lahore and Kasur districts. A total of 35 fungal species were identified (Fig 4 and 5), representing nine orders, eleven families, and thirteen genera. Among the recovered taxa, the genus *Aspergillus* was the most dominant (Fig 3), with *Aspergillus niger* exhibiting the highest abundance. In contrast, species such as *Aspergillus versicolor*, *Botrytis cinerea*, *Mucor circinelloides*, *Penicillium roqueforti*, and *P. verruculosum* were comparatively less frequent (Fig 2).

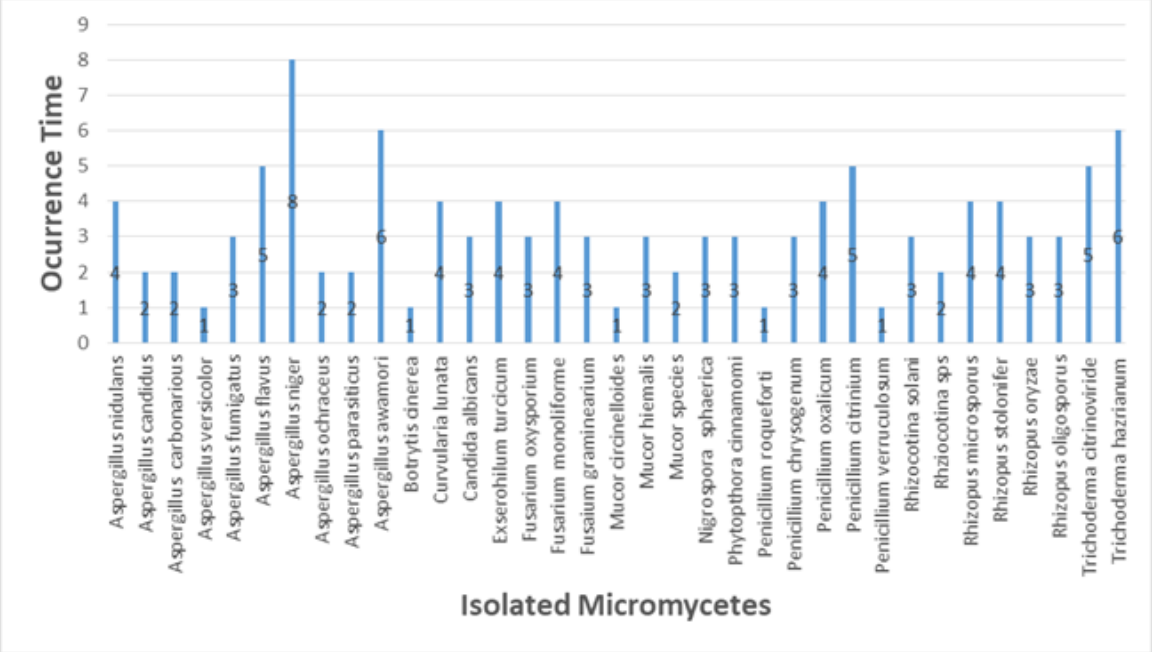

Figure 2. Relative abundance and distribution of isolated micromycete species recovered from agricultural soils of Lahore and Kasur, Pakistan.

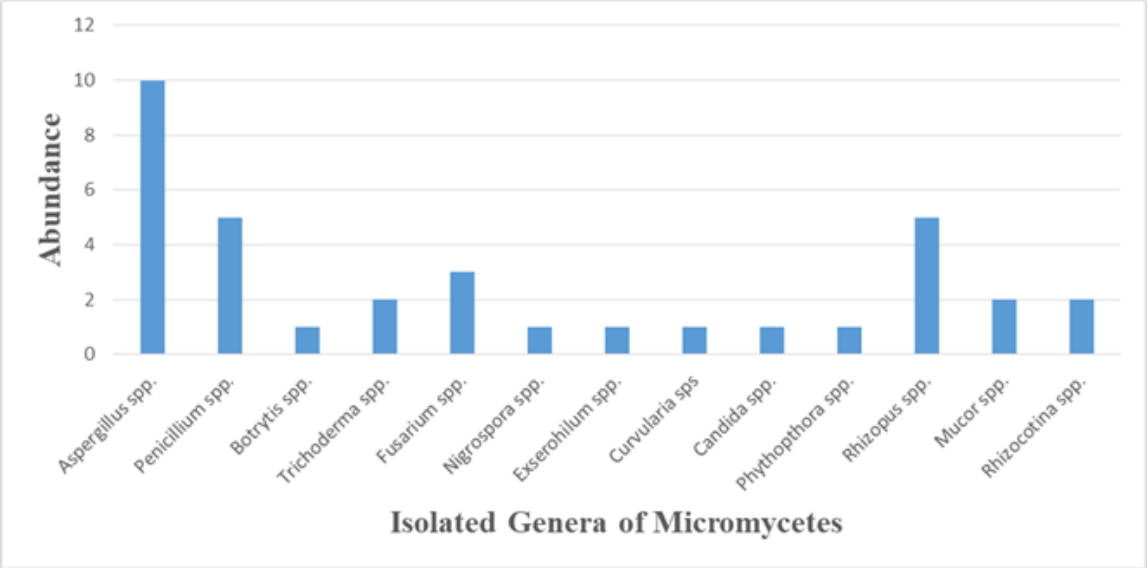

Figure 3. Taxonomic distribution of the isolated micromycete genera

Soils collected from the rhizospheric zones of rice, sorghum, maize, and sugarcane in Lahore and Kasur were analyzed for physicochemical properties (Table 1). The pH of Lahore soils ranged between 7.6–8.8, while Kasur soils exhibited a narrower range of 8.0–8.07. Electrical conductivity (EC) values varied from 632–980 dS m^{-1} in Lahore and 549–640 dS m^{-1} in Kasur. Available phosphorus concentrations were 41–52 kg h^{-1} in Lahore and 41–54 kg h^{-1} in Kasur, whereas potassium levels ranged from 46–64 kg h^{-1} in Lahore and 52–76 kg h^{-1} in Kasur. Both regions were characterized by silt-clay loam soil texture.

Table 1. Physicochemical characteristics of agricultural soil samples collected from different crop rhizospheres.

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Chemical Parameter	**CROP SOIL SAMPLES**							
	KASUR				**LAHORE**			
	Rice	**Corn**	**Sugarcane**	**Sorghum**	**Rice**	**Corn**	**Sugarcane**	**Sorghum**
pH	8.07	8.6	8.5	8	8.7	8.8	7.6	8.5
(EC) (µS/ cm)	640	635	590	549	628	980	798	632
(P) (mg/kg)	54	43	41	44	52	44	42	41
(K) (mg/kg)	71	52	53	76	64	61	46	63

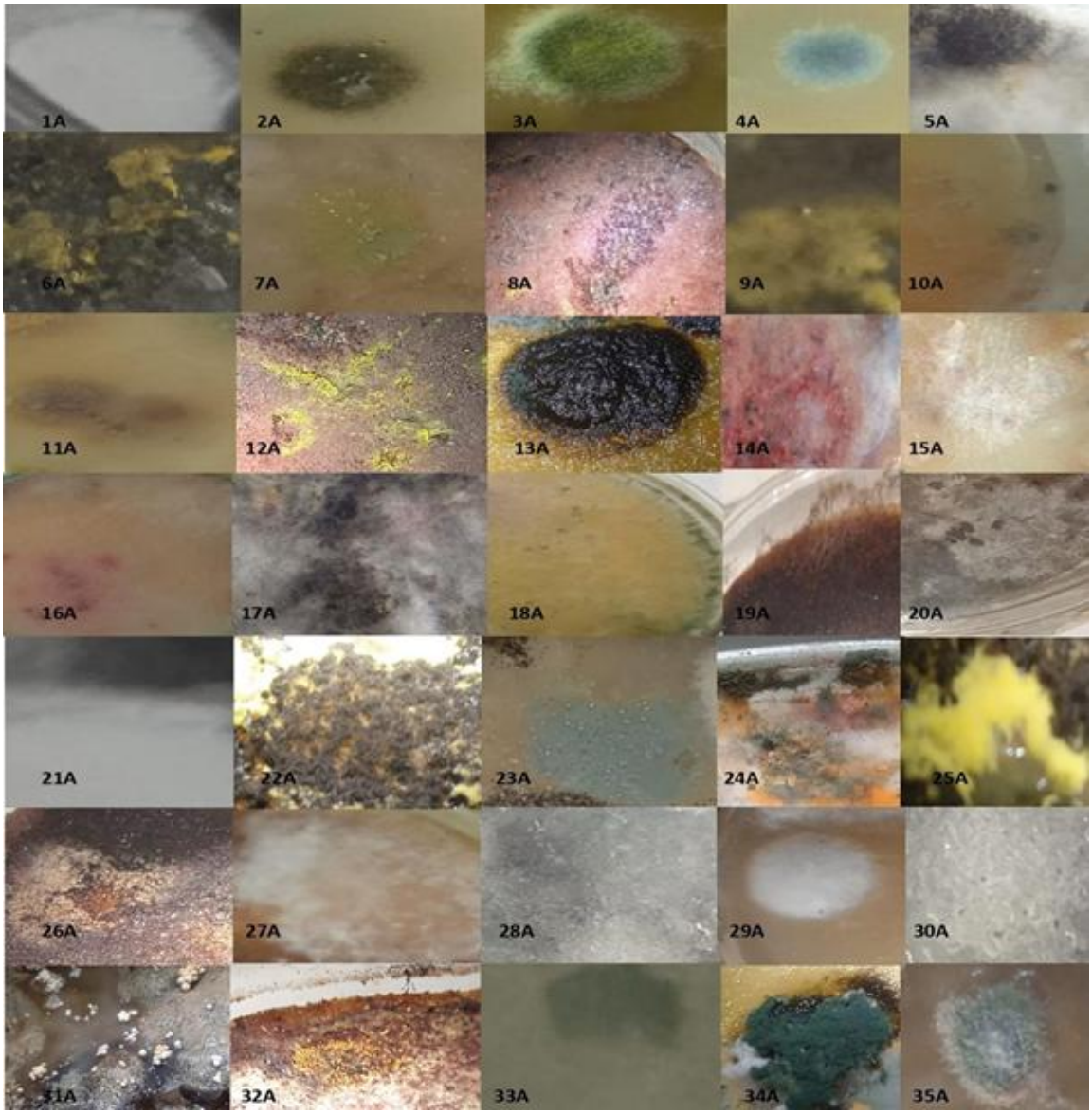

Figure 4. Colonies of isolated fungi: 1A (*Aspergillus candidus*), 2A (*Aspergillus carbonarious), 3A (Aspergillus versicolor*), 4A (*Aspergillus fumigatus*), 5A (*Aspergillus flavus*), 6A (*Aspergillus niger*), 7A (*Aspergillus ochraceus*), 8A (*Aspergillus parasiticus*), 9A (*Aspergillus awamori*), 10A (*Botrytis cinerea*), 11A *(Curvularia lunata*), 12A (*Candida albicans*), 13A (*Exserohilum turcicum*), 14A (*Fusarium Oxysporium*), 15A (*Fusarium monoliforme*), 16A (*Fusarium graminearium*), 17A (*Mucor circinelloides*), 18A *(Mucorhiemalis*), 19A (*Mucorspecies*), 20A (*Nigrospora sphaerica*), 21A (*Phytopthora cinnamomi*), 22A (*Penicillium roqueforti*), 23A (*Penicillium chrysogenum*), 24A (*Penicillium oxalicum*), 25A (*Penicillium citrinium*), 26A (*Penicillium verruculosum*), 27A (*Rhizopus microspores*), 28A (*Rhiz,opus stolonifer*), 29A (*Rhizopus oryzae*), 30A (*Rhizopus oligosporus*), 31A (*Rhizocotina solani*), 32A (*Rhizocotina* sp.), 33A (*Trichoderma citrinoverdie),* 34A (*Trichoderma hazrianum*), 35A (*Aspergillus nidulans*).

Table 2. Macroscopic characteristics of isolated micromycetes.

isolated Micromycetes	Colony Color	Reverse Colony	Growth	Texture
Aspergillus candidus	White	Pale yellow	moderate	Cottony
Aspergillus carbonarious	dark brown	pale yellow	uniform	cottony
Aspergillus versicolor	blue green	yellow to brown	medium	velvety
Aspergillus fumigatus	yellow green	cream color	moderate	velvety
Aspergillus flavus	yellow green	cream color	slow	creamy
Aspergillus niger	black	cream color	rapid	silky
Aspergillus ochraceus	yellow green	cream color	slow	creamy
Aspergillus parasiticus	yellow green	cream color	rapid	velvety
Aspergillus awamori	brown	cream color	medium	powdery
Botrytis cinerea	white to dark gray	white	moderate	wooly
Curvularia lunata	brown to black	dark brown	fast	threadlike
Candida albicans	creamy to yellow	cream color	Rapid	creamy
Exserohilum turcicum	olive brown	pale yellow	moderate	silky
Fusarium Oxysporium	whitish/pale color	pinkish to red	moderate	wooly
Fusarium monoliforme	white	pink or reddish	medium,	cottony
Fusarium graminearium	white	reddish.	fast	wooly
Mucor circinelloides	white	pale brown	fast	granular
Mucor hiemalis	black	grey	fast	wooly
Mucor species	black	cream colored	vigorous	hairy
Nigrospora sphaerica	White to dark brown	cream colored	fast	creamy
Phytopthora cinnamomi	white	cream colored	fast	woolly
Penicillium roqueforti	olive brown to black	cream-colored	rapid	velvety
Penicillium chrysogenum	yellowish green	light yellow.	rapid	cottony
Penicillium oxalicum	grayish green	lighter than top	rapid	granular
Penicillium citrinium	green to orange	yellow or tan	moderate	powdery
Penicillium verruculosum	black	lighter than top	moderate	granular
Rhizopus microsporus	white	brownish	rapid	velvety
Rhizopus stolonifer	grayish brown	pale to black	rapid	wooly
Rhizopus oryzae	cream color	grey	vigorous,	wooly
Rhizopus oligosporus	grey	pale yellow	fast	cottony
Rhizocotina solani	White to brownish	cream colored	rapid	powdery
Rhizocotina sps	White to brownish white	cream colored.	vigorous	granular
Trichoderma citrinoverdie	blue to zinc color	light colored	fast	woolly
Trichoderma hazrianum	yellowish green color	cream colored.	rapid	fluffy
Aspergillus nidulans	greenish blue	yellow colored	fast	velvety

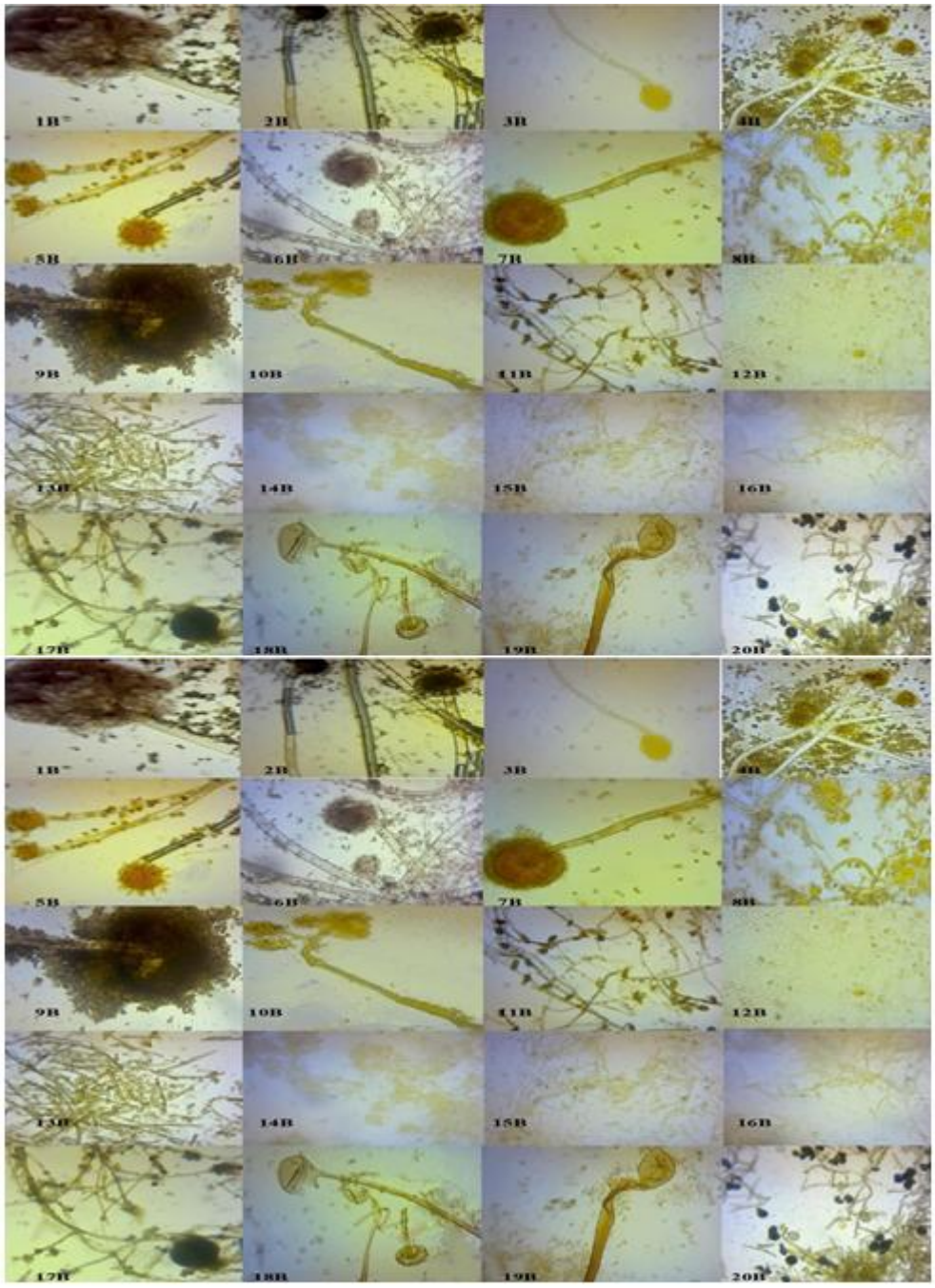

Figure 5. Microscopic images of isolated fungi: 1B (*Aspergillus candidus*), 2B (*Aspergillus carbonarious), 3B (Aspergillus versicolor*), 4B (*Aspergillus fumigatus*), 5B (*Aspergillus flavus*), 6B (*Aspergillus niger*), 7B (*Aspergillus ochraceus*), 8B (*Aspergillus parasiticus*), 9B (*Aspergillus awamori*), 10B (*Botrytis cinerea*), 11B *(Curvularia lunata*), 12B (*Candida albicans*), 13B (*Exserohilum turcicum*), 14B (*Fusarium Oxysporium*), 15B (*Fusarium monoliforme*), 16B (*Fusarium graminearium*), 17B (*Mucor circinelloides*), 18B *(Mucorhiemalis*), 19B (*Mucorspecies*), 20B (*Nigrospora sphaerica*), 21B (*Phytopthora cinnamomi*), 22B (*Penicillium roqueforti*), 23B (*Penicillium chrysogenum*), 24B (*Penicillium oxalicum*), 25B (*Penicillium citrinium*), 26B (*Penicillium verruculosum*), 27B (*Rhizopus microspores*), 28B (*Rhiz,opus stolonifer*), 29B (*Rhizopus oryzae*), 30B (*Rhizopus oligosporus*), 31B (*Rhizocotina solani*), 32B (*Rhizocotina sps*), 33B (*Trichoderma citrinoverdie),* 34B (*Trichoderma hazrianum*), 35B (*Aspergillus nidulans*).

Table 3. Microscopic characteristics of isolated Micromycetes

Isolated Micromycetes	Hyphae	Conidiophores	Conidia
Aspergillus candidus	septate	colorless flat walls,	brown to black circular
Aspergillus carbonarious	septate	smooth walls	black globose
Aspergillus versicolor	septate	hyaline, erect, slender	round, plane double walled
Aspergillus fumigatus	septate	hyaline, foot at base	round, smooth wall
Aspergillus flavus	septate	erect, slender, plane,	yellowish brown, round
Aspergillus niger	septate	Slender, transparent stipe.	ellipsoidal, dark brown
Aspergillus ochraceus	septate	unbranched, slender,	small spherical, uneven
Aspergillus parasiticus	septate.	Long, slender, unbranched	yellowish brown color
Aspergillus awamori	septate	plane, columnar	cylindrical, even surface
Botrytis cinerea	septate	septate, pale brown in color	ovoid, unicellular, hyaline
Curvularia lunata	septate	bent from apex, septate	round light to pale brown
Candida albicans	absent	not present	Not present
Exserohilum turcicum	septate	Long, slender, unbranched	dark brown, sigmoid
Fusarium oxysporium	septate	smooth, hyaline	fusiform, circular
Fusarium monoliforme	septate	smooth, hyaline	bean-shaped, fusiform
Fusarium graminearium	septate	even, hyaline	size short, long curved,
Mucor circinelloides	septate	elongated, branched	hyaline, ellipsoidal,
Mucor hiemalis	aseptate	transparent, branched	yellow to dark brown
Mucor sp.	aseptate	unbranched, bear sporangia	circular form
Nigrospora sphaerica	septate	smooth, hyaline	smooth, clusters, black
Phytopthora cinnamomi	septate	ellipsoid or ovoid	small, oval bodies
Penicillium roqueforti	septate	hyaline, bi-verticillate,	ellipsoidal
Penicillium chrysogenum	septate	slender, long, bi-verticillate	produce on phialide
Penicillium oxalicum	septate	upright structure	green to brown color
Penicillium citrinium	septate	birverticillate, solitary	globose, produce in chains
Penicillium verruculosum	septate	erect, slender terverticillate	smooth or subglobose
Rhizopus microsporus	aseptate	columnar	globose brown flat wall
Rhizopus stolonifer	aseptate	unbranched, hyaline	oval, brown, flat walled
Rhizopus oryzae	aseptate	Long, slender, unbranched	spherical, smooth-walled
Rhizopus oligosporus	aseptate	erect, selender, subglobose	uneven, subglobose spore
Rhizocotina solani	septate	not present	thick-walled
Rhizocotina sps	septate	not present	formed in chains,
Trichoderma citrinoverdie	septate	elongated, cylindrical	oval-shaped, asexual spores
Trichoderma hazrianum	septate	elongated	ovoid, yellowish green
Aspergillus nidulans	septate	short un-even walled	spherical, yellowish brown

The present study provides a comparative account of culturable fungal diversity in the rhizospheric soils of rice, sorghum, maize, and sugarcane from selected sites in Lahore and Kasur, Punjab. A total of 35 species representing 13 genera were isolated and identified through morphological and microscopic analyses. Distinct differences in fungal composition were observed between the two districts. Species such as *Aspergillus carbonarius*, *A. ochraceus*, *Candida albicans*, *Penicillium oxalicum*, and *Rhizoctonia solani* were restricted to Lahore soils, whereas *Aspergillus parasiticus*, *A. versicolor*, *Botrytis cinerea*, *Mucor circinelloides*, *Nigrospora sphaerica*, *Penicillium citrinum*, *P. roqueforti*, and *P. verruculosum* were confined to Kasur. Several taxa, including *Aspergillus awamori*, *A. candidus*, *A.*

flavus, *A. fumigatus*, *A. nidulans*, *A. niger*, *Curvularia lunata*, *Exserohilum turcicum*, *Fusarium* spp., *Mucor hiemalis*, *Penicillium chrysogenum*, *Phytophthora cinnamomi*, *Rhizoctonia* spp., *Rhizopus* spp., and *Trichoderma* spp. were recovered from both districts, indicating their widespread distribution in agroecosystems of Punjab.

Among the isolated taxa, *Aspergillus* species were predominant, with ten representatives recorded. District-specific occurrence of *A. carbonarius* and *A. ochraceus* in Lahore, and *A. parasiticus* and *A. versicolor* in Kasur, highlights ecological differentiation across sites. The isolation of *Botrytis cinerea* from maize rhizosphere in Kasur corroborates earlier reports of its phytopathogenic potential on diverse hosts, including *Punica granatum* in Mexico (Bardas et al., 2009). Similarly, *Nigrospora sphaerica*, detected once in Kasur soils, has been associated with leaf spot of date palm and seed infections in *Lens esculenta* in Pakistan (Alam et al., 2020). The recovery of *Phytophthora cinnamomi* from rice and sugarcane soils in both districts is noteworthy, given its role in shisham decline in Pakistan (Gill et al., 2002). *Exserohilum turcicum*, isolated from rice and sorghum soils, is consistent with its documented pathogenicity in maize, causing northern leaf blight in Azad Kashmir (Razzaq et al., 2019). The occurrence of opportunistic taxa such as *Candida albicans*, isolated from rice and maize soils, reflects their adaptability to diverse ecological niches. Its repeated isolation underscores its ecological significance, complementing previous reports from wheat crops in Pakistan (Begum et al., 2018). Collectively, these findings highlight the dual roles of rhizospheric fungi as both saprophytes and phytopathogens, influencing crop health and productivity. The district-specific distribution patterns suggest that edaphic factors, crop type, and microclimatic conditions may shape fungal community structure. This baseline documentation contributes to understanding the ecological diversity of soil mycoflora in Punjab and underscores the need for further molecular characterization to resolve cryptic diversity and assess functional roles in agroecosystem sustainability

Author contribution: Conceptualization SS, Methodology **JZ**, Softwar **HA,** Formal analysis **JZ** and , Investigation **JZ**, Data curation **SH,** Writing original draft **JZ and SS** writing review and editing **SS, HA, SH and FN**

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Declaration on the Use of Artificial Intelligence (AI):

The authors declare that any artificial intelligence (AI)-assisted technologies used during the preparation of this manuscript were employed solely to improve language, grammar, readability, or formatting. All scientific content, including the study design, data collection, analysis, interpretation of results, and conclusions, was conceived, verified, and approved by the authors. The authors accept full responsibility and accountability for the accuracy, originality, and integrity of the manuscript.

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