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CHARACTERIZATION OF MYCOFLORA ASSOCIATED WITH THE RHIZOSPHERE OF RICE CROP FROM SELECTED SITES IN DISTRICT GUJRANWALA, PUNJAB, PAKISTAN

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A B S T R A C T

Rice (*Oryza sativa*) belongs to the family Poaceae, and it holds great significance as a staple food crop in Pakistan and worldwide. The impact of mycoflora attacks on the rice crop is particularly critical, as it leads to a reduction in its production within Pakistan. Therefore, it is essential to isolate and identify the mycoflora associated with rice soil in the rich rice-growing regions of Pakistan. To accomplish this, soil samples were collected from two regions: Ghakhar and Rahwali in District Gujranwala, Punjab, Pakistan. The analysis of these soil samples revealed specific characteristics for each region. For Ghakhar, the pH was 8, electrical conductivity (EC) was 8.5 ds m^{-1}, phosphorous content was 45 kg/h, nitrogen content was 65 kg/h, and organic carbon content was 0.6%. Similarly, for Rahwali, the pH was 8.5, EC was 2.5 ds m^{-1}, phosphorous content was 39 kg/h, nitrogen content was 61 kg/h, and organic carbon content was 0.5%. The isolated soil samples were employed to extract mycoflora using the direct plate method. The fungal strains thus obtained were characterized based on their macroscopic and microscopic features. The outcomes revealed the isolation of a total of 11 distinct fungal species from both regions. Among these 11 species, three belonged to the *Aspergillus* genus (*A. niger*, *A. orchraceus*, and *A. flavus*), one was identified as *Alternaria alternata*, another as *Bipolaris sorokiniana*, one as *Aureobasidium pullulans*, one as *Candida albicans*, one as *Fusarium oxysporum*, one as *Penicillium* spp., one as *Rhizopus oryzae*, and the last one as *Trichoderma* spp. This study sheds light on the diversity and prevalence of these 11 fungal species isolated from rice soil. It contributes to our comprehension of the fungal community within the rice cultivation environment. Further detailed studies are required to explore the molecular basis of these findings.

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INTRODUCTION

Fungi, encompassing micromycetes exhibit ubiquity as eukaryotic organisms reliant on organic substrates (Fernández-López et al., 2023). Within numerous ecosystems, they play a pivotal role in preserving functionality by virtue of their presence. Fungi manifest diverse ecological roles, functioning as parasites, saprotrophs, or symbionts in relation to plants and animals (Pergl et al., 2023).

Rice is an important staple food crop globally as well as in Pakistan feeding over half the population and contributing to incomes in Asia and Africa. Approximately 37% of rice yield losses can be attributed to pests and diseases. In more extreme scenarios, the detrimental impact of fungal diseases alone can escalate to yield reductions of up to 80%. (Priyadarshani et al., 2023; Farooq and Fatima, 2022). Punjab, the leading province, accounts for nearly 69% of the total rice production, supporting Pakistan's economy through domestic consumption and export to countries like Saudi Arabia, the UAE, Afghanistan, and Iran (Gul et al., 2022). Sustaining rice production is crucial for ensuring food security and economic stability in Pakistan (Abbas et al., 2022). Rice, being an important food crop is widely affected by different fungal diseases (Habib-ur-Rahman et al., 2022). Diseases such as rice blast, brown spot, bacterial leaf blight, sheath blight, and others can reduce rice yield by 14-18% (Chen et al., 2022). Pathogenic fungi, including Aspergillus and Fusarium species, are associated with the food and food products that can affect both seed viability and food quality (Kortei et al., 2022; Ganesan et al., 2022).

The rhizosphere, the area of soil surrounding plant roots or bulk soil due to its unique physical, chemical, and microbial properties, plays a crucial role in influencing plant growth, nutrient uptake, and interactions with soil microorganisms (Xia et al., 2022). Microorganisms in the rhizosphere interact with each other and the environment, forming a specialized microbial ecosystem with specific functions and structures (Mathur and Ulanova, 2022). Soil-borne fungi have both beneficial and detrimental effects on crops, with their composition and abundance impacting crop growth (Dignam et al., 2022). Soil physicochemical characteristics play a role in determining the types of fungal genera present in the soil, with high organic matter content suppressing certain pathogens (Kumar et al., 2022; Richard et al., 2022). Anthropogenic activities such as the use of synthetic fertilizers, pesticides, herbicides, deforestation, and urbanization negatively impact soil microbial diversity and function, affecting soil health and agricultural productivity (Kumare et al., 2022). Azam et al. (2020) isolated *Alternaria* spp., *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp., and *Rhizopus* spp. from the flora of the Pakistan Forest Institute's botanical garden. In addition, they identified four species, namely *Fusarium* spp., *Cladosporium* spp., *Penicillium* spp., and *A. flavus*. Similarly, Abrar et al. (2020) isolated the Aspergillus pakistanicus from soil of Changa Manga forest, Pakistan.

The objective of this study was to comprehensively characterize the micromycetes present in the rhizosphere of rice crops across selected sites in district Gujranwala, Punjab, Pakistan. By focusing on these specific sites, insights were aimed to be gained into the diversity, distribution, and potential roles of these micromycetes in the rice crop ecosystem, contributing to a better understanding of their impact on plant health and soil quality.

MATERIALS AND METHODS

Soil sampling of rice fields

Soil samples were collected from two regions viz. Ghkhar (Latitude: 32.3002 and longitude: 74.138803), Rahwali (Latitude: 32.2479 and Longitude: 74.168035) of Gujranwala during the period of May to October 2022 (Figure 1). To minimize the presence of extraneous microorganisms, the top layer of soil was removed before sampling. A total of 600 g of soil was obtained using a digger and transferred to labeled jars or plastic bags for further analysis.

The samples were properly labeled to indicate the location. Subsequently, the samples were brought to the Mycology Laboratory at Lahore College for Women University (LCWU) for further analysis. To avoid contamination, the culturing of soil samples were subjected for fungal isolation (Ugbede et al., 2021).

Media preparation

For the preparation of Potato Dextrose Agar (PDA) medium 39 g of Potato Dextrose agar was added into 1 L of distilled water and placed in an autoclave for 15 min at 121^{o}C. A small amount of streptomycin was added to the autoclaved media to prevent bacterial contamination. After sterilization, the media was allowed to cool for a period of time. The media was poured gently into the plates near the flame of a spirit lamp in the lamina flow that was kept on during pouring to prevent contamination. The plates were solidified in the laminar air flow cabinet and inverted after solidification (Shahbaz et al., 2023).

Figure 1: Map showing the sampling areas of district Gujranwala.

Isolation and identification of soil mycoflora

Direct plate method

In this study, soil samples (0.01 g) were dispersed in 1 ml of distilled water in a sterilized petri dish. The petri dishes were gently rotated to ensure even distribution of soil particles throughout the medium and allowed to solidify and then incubated at a temperature of 30 ± 1°C for 3-5 days (Bi et al., 2022).

Identification of isolated micromycetes

For the identification of the isolated micromycetes and microscopic analysis, the procedure outlined by Chamekh et al. (2019) was followed.

Physico-chemical soil parameters

The collected soil was subjected for physico-chemical analysis e.g. organic carbon, nitrogen, potassium, electrical conductivity, pH, and salinity from "Soil and Water Testing Laboratory for Research, Bahawalpur" (https://aari.punjab.gov.pk/swtl_bwp) using standard methods (Pan et al., 2013).

Statistical Analysis

For diversity analysis of micromycetes, statistical analysis was done on the observed data by using SPSS software and to illustrate data in tables and graphs, MS Excel was used (Yohannan et al., 2018).

RESULTS

The results presented in Figure 2A revealed the frequency occurrence percentage of different isolated mycoflora from two different regions namely Ghakhar and Rahwali of district Gujranwala, Punjab, Pakistan. The highest frequency occurrence % is 90% for *Aspergillus flavus* followed by *Rhizoctonia solani* that had 80%. Similarly, *Cladosporium* and *Alternaria alternata* possessed 70% and 60% respectively. The other mycoflora *A. orchraceus*, *A. niger*, *Penicillium.* spp., *Fusarium oxysporum, F. monoliforme,* and *Mucor* spp*.* exhibited 50%, 40%, 30%, 30%, 20% and 30% respectively. Similarly, *A. flavus* and *Rhizoctonia solani* had occurrence time 9 and 8 respectively. The other fungal species *A. orchraceus*, *A. niger*, *Penicillium.* spp., *F. oxysporum*, *F. monoliforme*, and *Mucor* sp. had the least occurrence time as compared to *A. flavus* and *R. solani* (Figure 2B).

The collected soil was undergone to investigate the physico-chemical features of rice soil. The results revealed that the soil of both regions i.e. Ghakhar and Rahwali showed pH and electrical conductivity 8 and 8.5, 2 ds m^{-1} and 2.5 ds m^{-1} respectively. Similarly, phosphorous was observed 45 kg/h in Ghakhar region while 39 kg/h in Rahwali region. In case of Nitrogen, 65 kg/h was analyzed in Ghakhar and 61 kg/h in Rahwali. The type of soil observed was silt clay loam in both regions. Organic carbon % was observed in medium range 0.6% in Ghakhar and 0.5% in Rahwali (Table 1).

Macroscopic identification of isolated mycoflora

The colony of *A. niger* exhibited a dark-colored appearance, commonly black or dark brown. The growth pattern is usually fast. The texture appeared velvety or fluffy. On the reverse side, colonies remained dark-colored (Figure 3A). For *A. flavus*, colonies were initially white or cream-colored, but they turned yellow, green, or brown as they matured, growth flat, round, radial with concentric zones growing from center to the edges on PDA. Growth rate was slow to moderate. They were velvety or woolly in texture, with a raised center and smooth margins. The reverse side was yellow or brown sometimes with a reddish tint (Figure 3B).

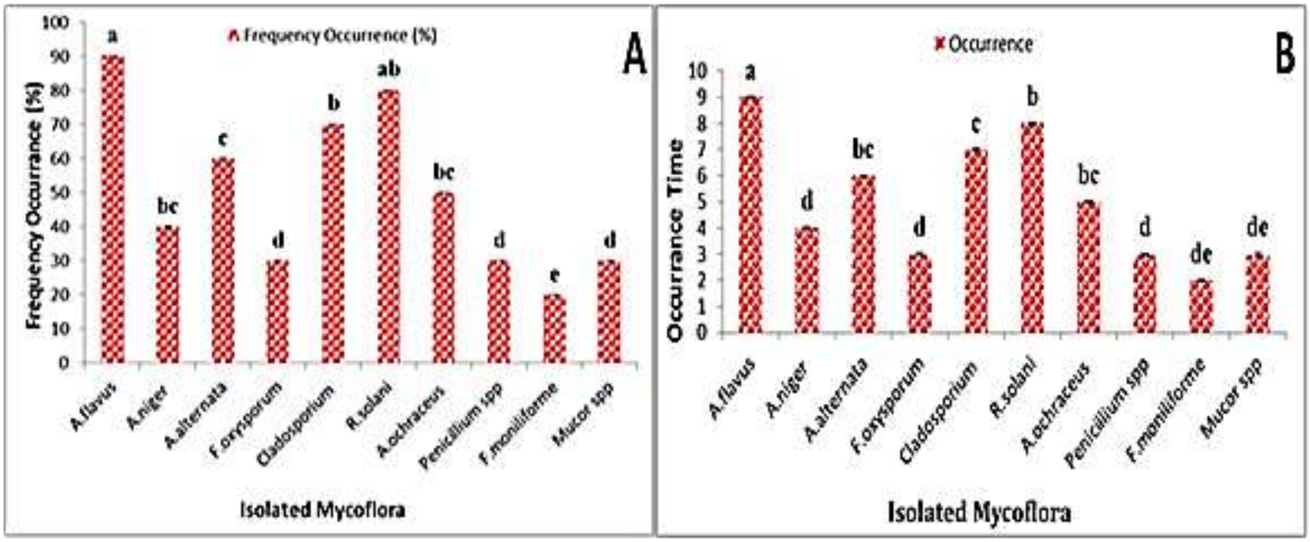

Figure 2: (A) Frequency occurrence (%) and (B) Occurrence time of rice soil isolated mycoflora.

Table 1: Physico-chemical properties of rice soil.

S. No.	Region	pH	EC (ds m^{-1})	Type of Soil	% Organic C	P (kg/h)	N (kg/h)
1	Ghakhar	8	2	Silt clay Loam	0.6	45	65
2	Rahwali	8.5	2.5	Silt clay Loam	0.5	39	61

Similarly, the colony color of *A. alternata* showed colonies with a dark-colored appearance, commonly black or brown. The growth pattern was radiating from the center with concentric zones. The growth rate was moderate, and the texture was usually velvety or woolly. On the reverse side, colonies exhibited a dark coloration (Figure 3C).

The colony of *Bipolaris sarokiniana* was dark-colored often ranging from brown to black. The growth pattern showed radiating while the growth rate observed was moderate, and the texture and the reverse side appeared velvety or woolly and dark coloration respectively (Figure 3D). *Candida albicans* colonies appeared creamy-white or pale in color. The growth pattern observed was smooth and creamy and texture was found glistening or moist. On the reverse side, colonies maintained a similar color to the front (Figure 3E). *Fusarium oxysporum* colonies were observed whitish in color with moderate growth rate producing velvety texture and pink/reddish colony on reverse side (Figure 3F).

In case of *Trichoderma* sp., color of colony was seen white or light-colored with fast and vigorously growth pattern producing woolly or velvety texture. On the reverse side colonies were seen white or light colored (Figure 3G). *Aureobasidium pullulans* produced dark-colored colony ranging from dark brown to black with slow growth and velvety or granular texture. On the reverse side, colony was dark coloration (Figure 3H). *A. orcheous* exhibited with a whitish or cream-colored colony with fast and spreading growth pattern. The texture was seen velvety or fluffy, the reverse side, colonies appeared light in color (Figure 3I).

Microscopic Identification

The results regarding microscopic identification of the isolated rice rhizospheric were observed on the basis of hyphae, conidial heads, color, length, vesicle shape, as well as conidia size. The microscopic features of *A. niger* revealed that the hyphae were septate with small, round and abundant conidia while the conidiophores were smooth, elongated showing the conidial heads formed dense clusters of small, round spores (Figure 4A).

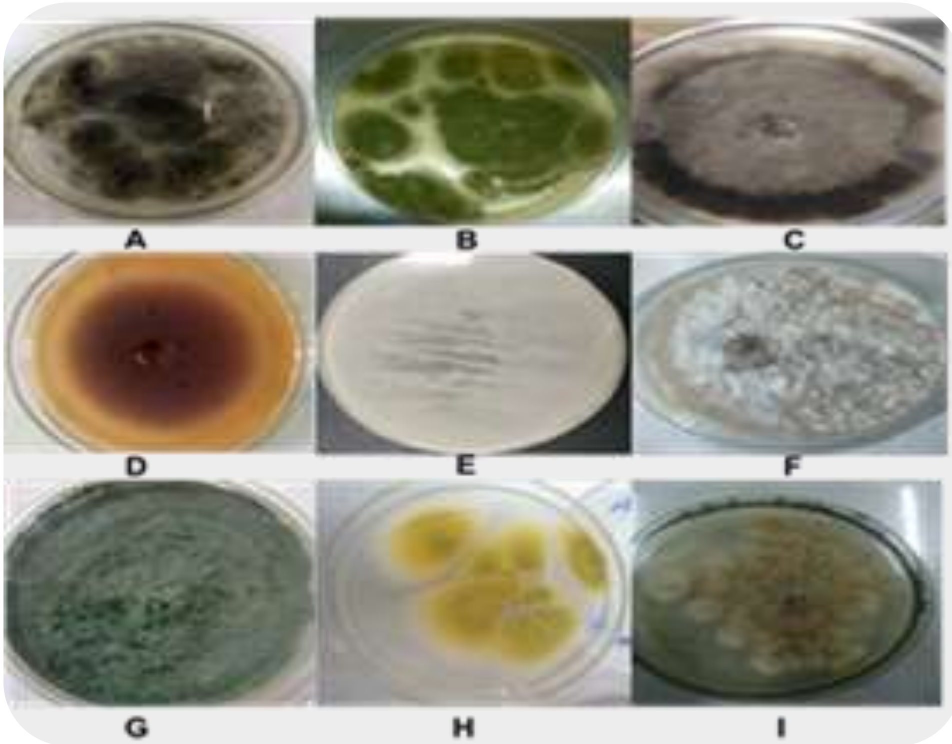

Figure 3: Macroscopic identification: (A) *Aspergillus niger*, (B) *Aspergillus flavus*, (C) *Alternaria alternata*, (D) *Bipolaris sarokiniana*, (E) *Candida albicans*, (F) *Fusarium oxysporum*, (G) *Trichoderma sp*., (H) *Aureobasidium pullulans*, and (I) *Aspergillus orcheous*.

A. flavus showed hyphae with typically septate spores along with smooth, double walled cylindrical, unbranched conidiophore. Conidial head dense cluster of small, round spores conidia small and round, pale green, or smooth forming long chains (Figure 4B). *A. alternata* exhibited septate hyphae producing small oval shaped conidia along smooth conidiophores. The conidial heads were seen as dense clusters of small, round spores (Figure 4C). Similarly, the hyphae of *B. sarokiniana* were septate producing conidia as small, elongated, and abundant in the colonies while conidiophores appeared smooth with the conidial heads that appeared dense clusters of small, elongated spores (Figure 4D).

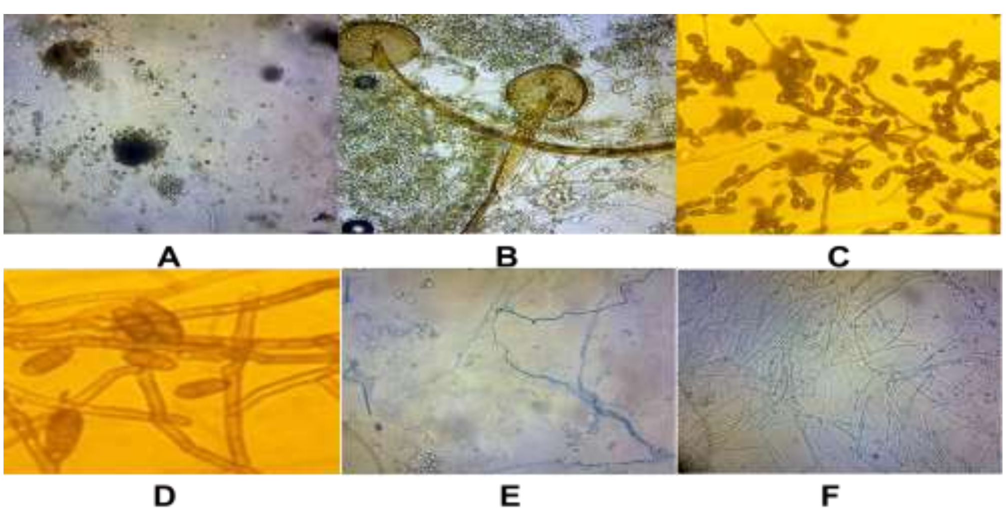

Figure 4: Description of the microscopic features of mycoflora isolated from rice soil.

C. albicans exhibited the hyphae that seen as pseudohyphae with small conidia along with elongated conidiophore. Conidial head appeared at the tip of the conidiophore as dense clusters (Figure 4E). In case of *F. oxysporum*, the hyphae were observed septate with small conidia as its main type of spore along with smooth conidiophores and dense conidial head (Figure 4F).

A. orcheous exhibited the septate hyphae with small oval shaped conidia along with smooth, elongated conidiophores producing the conidial heads appeared as oval-shaped spores (Figure 5A). For *Trichoderma* spp., the hyphae were seen septate with small oval shaped conidia. Conidiophor**es** appeared elongated with conidia at their tips. Conidial head was observed as dense clusters of conidia that were seen small, asexual spores (Figure 5B). *A. pullulans* revealed septate hyphae with abundant spores producing elongated conidiophore. Conidial heads were seen as dense clusters of conidia with small, oval-shaped conidia (Figure 5C). *R. solani* produced microscopic features as septate hyphae without conidiophore along with small, pale, green pink conidia (Figure 5D). *R. oryzae* exhibited septate hyphae and spores were produced within specialized structures called sporangia. The sporangia were seen round or oval-shaped (Figure 5E).

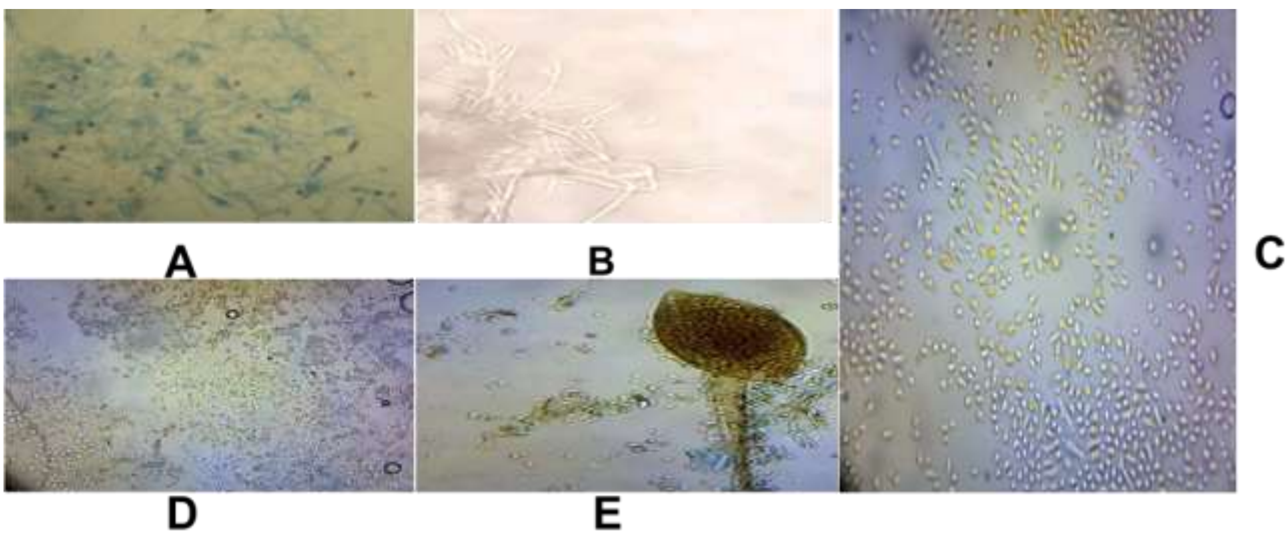

Figure 5: Description of the microscopic characteristics of fungal flora isolated from rice soil.

DISCUSSION

In this study, we conducted a survey and screening of soil micromycetes from two different regions i.e. Ghakhar and Rahwali of district Gujranwala. The primary objective was to gather mycoflora from above mentioned two regions and identify them using macroscopic and microscopic features. Our findings revealed the presence of different fungal species in specific areas. In the Ghakhar region, we identified *Alternaria solani*, *Candida albicans*, *Fusarium solani*, *Penicillium sp*, *Rhizoctonia solani*, *Rhizopus oryzae*, whereas *Aureobasidium pullulans*, *Fusarium graminearum*, *Trichoderma* sp., *Fusarium monoliforme*, *Bipolaris sarokiniana* were found in Rahwali. Our study aligns with previous research conducted by Basilico et al. (2007) in which they observed that environmental factors and nutrient availability influenced fungal growth frequencies in soil samples. In line with the work of Raza et al. (2013), we also isolated and characterized common fungal species in different crop fields, such as *A. flavus*, *A. niger* and *A. oryzae*. The variations observed in our results can be attributed to geographical location, fungal growth media, and differences in sampling methods, as demonstrated by Liu et al. (2020), who investigated variations among different fungal species. Recent studies at the beginning of the 21st century have shown that during the winter season, saprotrophic fungi dominate the microbial community in Arctic soil, as observed by Dobashi et al. (1988). These findings emphasize the importance of considering various factors and methodologies when studying soil mycoflora to obtain comprehensive and accurate results. *A. niger, A.*

flavus, and *A. oryza*e are the most frequently occurring fungi in our study with 9, 6, and 5 occurrences respectively. *R. oryzae*, *Penicillium* sp., *R. solani*, *F. oxysporum*, *F. monoliforme*, *F. solani*, *F. graminearum*, *A. solani*, *B. sarokiniana*, *Trichoderma* sp., *A. pullulans*, and *A. alternata* are also present. In our current study, we identified *A. flavus* from fungal cultures isolated from maize and soil samples collected in several districts in Kenya, including Makueni, Nyeri, Bungoma, and Uasin Gishu. Among all, the isolated fungal species, *A. flavus* showed the highest incidence, accounting for 90% of the isolates. The variation in *A. flavus* occurrence among different species could be attributed to the diverse environmental conditions prevailing in those districts. According to the findings of Massomo, (2020), crops grown in warm climates have a higher risk of being infected by aflatoxin-producing fungi, particularly during periods of drought and elevated temperatures. In the case of Makueni, the region experiences temperatures ranging from 25 to 35°C and drier conditions, which create favorable conditions for *A. flavus* infection in crops. These findings suggest that the occurrence of these fungi in the samples is not unusual. *B. sorokiniana* is a fungal pathogen that can infect a wide range of crops, including rice. Previous research has shown that *B. sorokiniana* can cause significant yield losses in rice if not managed properly (Singh et al., 2022). Maryani et al. (2019) isolated the *Fusarium* species that supports our study. Similarly, the isolation of fungal flora from soil by Kadaifciler (2017) is quite similar to our study. Sachdev et al. (2023) isolated *R. solani*, *Fusarium* sp. and *A.niger.* The research conducted by Dolatabad et al. (2017*)*, *R. solani* and *Fusarium* spp. and *A. niger* were isolated that is similar to our isolated mycoflora.

CONCLUSION

The present study described a survey of two regions i.e. Ghakhar and Rahwali of Gujranwala to isolate the fungal diversity from rice soil. The study's findings revealed a total of 11 distinct fungal species that were isolated from both the regions of Ghakhar and Rahwali. Within this group, three species of *Aspergillus* (*A. niger*, *A. orchraceus*, and *A. flavus*), one species of *Alternaria* (*Alternaria alternata*), one species of *Bipolaris* (*B. sorokiniana*), one species of *Aureobasidium* (*A. pullulans*), one species of *Candida* (*C. albicans*), one species of *Fusarium* (*F. oxysporum*), one species of *Penicillium* (*P.* sp), one species of *Rhizopus* (*R. oryzae*), and one species of *Trichoderma* (*Trichoderma* sp.) were successfully identified. In summary, this research shed significant light on the diverse range of fungal species present in the soil samples collected from the rice-abundant regions of Ghakhar and Rahwali, situated in the Gujranwala district of Punjab, Pakistan. This comprehensive exploration holds noteworthy implications for enhancing our comprehension of mycoflora diversity within these specific regions. Moreover, it contributes to the broader understanding of the various factors that impact the health and productivity of rice crops.

AUTHORS' CONTRIBUTIONS

NF conceived the study, collected soil samples and performed the experiment, wrote the original manuscript, and prepared the draft; SS critically reviewed the data, acted as a supervisor and provided lab facilities; MS reviewed the manuscript and prepared the final draft.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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