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EXPLORING SOIL-BORNE PATHOGENIC FUNGI AFFECTING POTATO CULTIVATION IN GILGIT-BALTISTAN, PAKISTAN

^aRiaz Ahmad, ^aSaleem Shahzad*, ^bMustansar Mubeen, ^cQaiser Shakeel, ^dAqleem Abbas,
^eMuhammad Rafique, ^fSyed A. Hussain, ^aAbdul Q. Rajput, ^gZakir Hussain

^a Pest & Disease Research Lab, Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Pakistan.

^b Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Pakistan.

^c Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

^d Department of Agriculture and Food technology, Karakorum International University, Gilgit-Baltistan, Pakistan.

^e Agriculture Officer (Plant Protection), Tehsil Sahiwal, Pest warning & Quality Control of pesticides, Sargodha, Pakistan.

^f Department of Animal Sciences, Karakorum International University, Gilgit-Baltistan, Pakistan.

^g Integrated pest and disease management laboratory, Department of Agriculture, Gilgit-Baltistan, Pakistan.

ABSTRACT

Soil-borne plant pathogenic fungi cause substantial yield losses in potatoes worldwide. In Gilgit Baltistan (GB), Pakistan, potatoes are the main cash crop and source of revenue. Late blight, early blight, and black scurf are three examples of soil-born pathogenic fungi shown in only a few studies to be substantial biotic restrictions to potato output in GB. Few details exist on the prevalence and severity of the soil-borne diseases that cause potato diseases. Climate factors such as severe and unseasonal rains as well as warmer winter temperatures have aggravated these diseases in recent years. As a result, the current study was done in three districts of GB: Gilgit, Hunza, and Nagar. Soil samples from potato plants and rhizospheres were obtained, and soil-borne plant pathogenic fungi were isolated using serial dilution, baiting technique, and direct plating procedures. Furthermore, fungal morphology was also studied under microscopes as well as identified using standard keys and monographs. Additionally, the synonymy of the isolated pathogenic fungi was also retrieved. A total of eight soil-borne plant pathogenic fungi were identified, namely, *Alternaria alternata*, *Fusarium incarnatum*, *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium Pythium aphanidermatum*, *Macrophomina phaseolina*, *Fusarium udum*, and *Rhizoctonia solani*. *Alternaria alternata*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, and *R. solani* were found in all three districts. In conclusion, these soil-borne potato pathogenic fungi appeared to be the first records from GB.

Keywords: Isolation, identification, potatoes, soil-borne fungi, climatic era, Gilgit Baltistan.

INTRODUCTION

The potato (*Solanum tuberosum* L.) crops belong to the family of *Solanaceae*. It is a major dicotyledonous tuber crop that has been enriched with starch and is most commonly consumed and produced in the world. (Birch *et al.*, 2012; Anwar *et al.*, 2015). Millions of people worldwide rely on potatoes for food and income. China is

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* Corresponding Author:

Email: sshahzad@uok.edu.pk

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the primary producer, with a crop output of 71 million tonnes, accounting for more than 20% of worldwide production. It is a temperate-climate crop with excellent nutritional content. Its significance depends both on raw and processed forms offered to low-income consumers. Potato consists of 79% water, 17% carbohydrates (88% of which are starch), and 2% protein. In Pakistan, potatoes are grown on 187.2 thousand hectares, yielding 3853.9 thousand tons (Anon., 2019; Fiers *et al.*, 2011; Mu *et al.*, 2017). In Pakistan cultivation of potatoes in three seasons: spring, summer, and autumn. Summer crops are cultivated in the mountains, while spring and autumn crops are grown in the plain areas. Gilgit-Baltistan (GB) is a northern mountainous far-flung region of Pakistan. Potato

growing as a cash crop is one of the most significant sources of revenue in GB. Currently, its cultivation has become more widespread in this region due to the introduction of new potato crop species, having an estimated 1400-1600 kilogram per kanal area yield recorded. However, the introduction of high-yielding cultivars that have displaced the region's indigenous variety has increased the prevalence of potato diseases in GB (Ahmad *et al.*, 1995; Arora *et al.*, 2012; Abbas, 2017). Among, the most common potato diseases, soil-borne fungal pathogens or fungi are economically important and cause significant yield losses. Previously black scurf, late, and early blight diseases have been reported, which cause substantial yield losses to potatoes (Abbas, 2017; Hussain *et al.*, 2017; Agrios, 2005). Many of the existing studies lack systematic approaches are available regarding the severity and incidence of potato diseases based on symptomology. There is also an absence of knowledge regarding the causative pathogens responsible for these diseases. Furthermore, no studies have focused on properly isolating and identifying soil-borne fungi, specifically from potato fields. Moreover, it is predicted that these soil-borne fungi will increase as a result of climate change, primarily due to increased precipitation, including heavy and unseasonal rains, elevated instances of flooding, and warmer winter temperatures. Additionally, there is a consistent rise in the monthly maximum temperature in GB. As a result, it is important to understand the diversity and distribution of soil-borne fungi associated with potatoes in the GB to adopt effective management methods and minimize losses resulting from the degradation of tuber quality due to disease. The objectives of this study aimed to isolate soil-borne pathogenic fungi from the potato rhizosphere soil using serial dilution, baiting technique, and direct plating. To identify soil-borne pathogenic fungi through the use of identification keys and monographs and To gain an understanding of the diversity of soil-borne fungi in Gilgit, Hunza and Nagar districts of Gilgit-Baltistan.

MATERIALS AND METHODS

Study Area: The Gilgit-Baltistan is present in the northern part of Pakistan and the Gilgit, Hunza, and Nagar districts are in the GB region with latitudes

ranging from 36.2764°N to 35.8819°N and longitudes ranging from 74.4643°E to 74.7200°E. These districts are located at elevations of 1500, 2500, and 2688 meters, respectively.

The climate in these areas can be classified as arid and semi-arid. Table 1 demonstrates the variation in average temperatures across these districts, ranging from a minimum average temperature of 9.6°C in the Hunza district to a maximum average temperature of 38.2°C in the Gilgit district. Additionally, the average annual rainfall in Gilgit, Hunza, and Nagar is recorded as 120 mm, 126 mm, and 136 mm, respectively.

Collection of soil samples: During the survey, the soil samples were taken from potato fields of three districts of GB i.e. Gilgit, Hunza and Nagar (Figure 1 and Table 1). Five potato fields per district were chosen at random, and samples were taken from the rhizosphere region around potato plants. Soil samples were taken from a depth of 0-6 cm, and 5 samples were taken (500 grams each) were taken from distinct areas within each field. To create a representative composite sample, the soil were thoroughly mixed. Following that, the samples were properly wrapped in sealed polythene bags, labeled, and delivered to the Pest and Disease Research Laboratory, Department of Agriculture and Agribusiness Management., University of Karachi. The purpose of this transportation was to facilitate the isolation of soil-borne fungi.

Preparation of culture media for growth: The following growth media were prepared: Potato Sucrose Agar (PSA), Water Agar (WA), Corn Meal Agar (CMA), Potato Carrot Agar (PCA), Malt Extract Agar (MEA), and Czapek-Dox Agar (CzA), to evaluate the growth of potato fungal pathogens on various growth media. The composition of the growth media used as per standard in the present study.

Isolation of soil-borne fungi: To isolate the soil-borne fungi from the soil antibiotics penicillin (100.000 U L⁻¹) and streptomycin (0.2 gL⁻¹) were used to suppress bacterial growth.

Table 1. Districts were sampled during the present study in Gilgit-Baltistan

Districts	Latitude	Longitude	Elevation	Annual precipitation	Highest average temperature	Lowest average temperature	Average Humidity
Gilgit	35.8819° N	74.4643° E	1,500 m	120 mm	38.2°C	17°C	29%
Hunza	36.3167° N	74.6500° E	2,500 m	126 mm	36°C	9.6°C	27%
Nagar	36.2764° N	74.7200° E	2,688 m	136 mm	36.2°C	11°C	38%

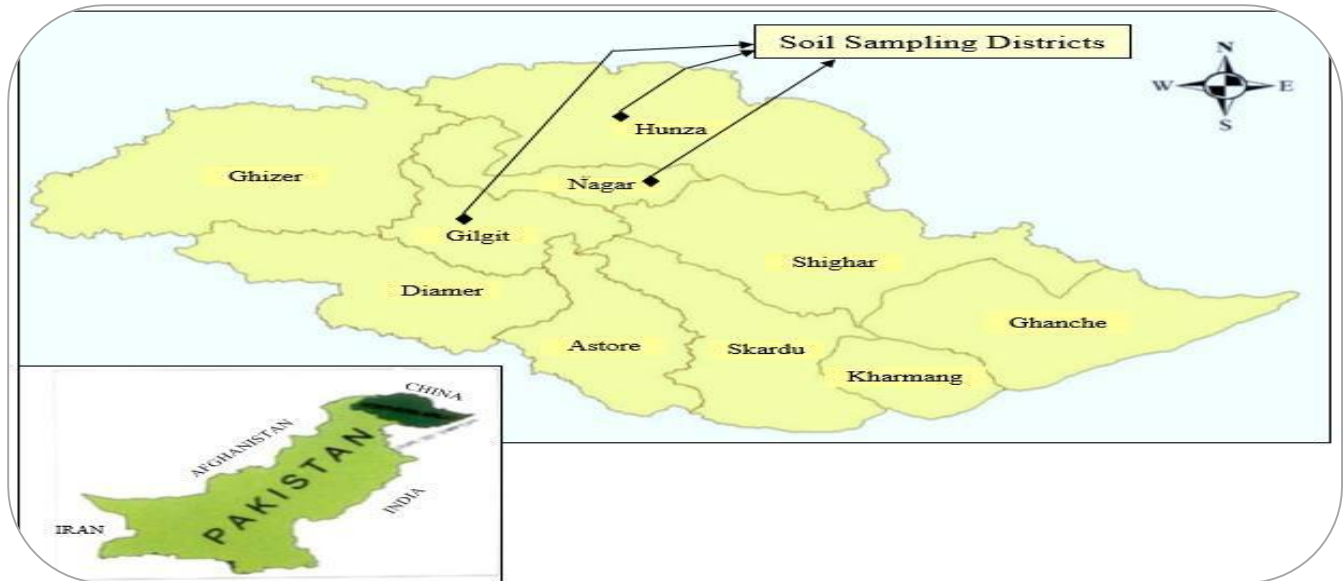


Figure 1. The soil sampling geographical location of the three districts of Gilgit-Baltistan

Serial dilution method: Waksman and Fred (1922) described the method of serial dilution, in which to obtain a 1/10 dilution, one gram of soil samples was mixed with 9 ml of sterile water. A series of (1/100, 1/1000, and 1/10,000) dilutions have been obtained by diluting 1 ml in 9 ml of autoclave water with a sterile pipette. On PSA-containing medium plates, one ml of aliquot sample from each dilution was added and dispersed with a sterile bent glass rod on three replicate plates. The colonies were transferred to Petri plates containing PSA for purification after incubating the plates at room temperature (20-25°C) for 3-5 days.

Direct plating method: This method was used to isolate fungi from soil and roots. On one side of a petri-plate containing agar medium, a small amount of air-dried soil was added. One milliliter of autoclave water was added to the soil, which was then distributed on a medium with the help of a bent sterilized glass and incubated at room temperature for 1-2 days. For purification, the fungi growing on the agar surface were transferred to other petri plates using PSA or cornmeal agar (CMA).

Baiting method: Petri dish method: In a polythene bag, a soil sample was submerged with autoclaved water, and mixed thoroughly, and allowed for 10-20 minutes to achieve a paste-like consistency (Harvey, 1925). A sterile teaspoon was used to insert a soil sample on one side of the sterilized Petri dish, and 10- 15 ml of autoclaved water was poured into the Petri dishes. Three grass blades (3 cm) were placed on each Petri-plates, one near the soil and the other two away from the soil. The plates

were incubated at room temperature (25-30°C) for 6-8 days. The mycelial growth on baits was rinsed with sterilized autoclave water and transferred to a fresh, two new baits were added, and daily monitoring was performed in a sterilized Petri dish half-filled with sterile autoclaved water. After 2-3 days, the baits were moved to antibiotic-amended PSA and CMA media plates, and CMA media was used for purification.

Bating with potato slices: A soil sample of 30-50 g in a plastic bag was sufficiently wet but not so wet that it puddled. Three to four mm thick slices of disinfected potato-tuber were placed on top of the soil in each plastic bag for 24 hours at room temperature (25-30°C). The potato slices are placed on water agar after being rinsed with tap water to remove soil. After 24 hours, the plates were inspected under a compound microscope for the presence of coenocytic hyphae, which were then moved to new agar plates for purification.

Morphological studies of fungi: Morphological fungal examinations were performed on a pure fungus culture that was 5-7 days old. On a microscopic slide, a little piece of growth from a colony has been placed in a drop of lactophenol and covered with a cover slip. In the situation of hyaline fungus, lactophenol and cotton blue were utilized. Fungi characteristics such as mycelial septation, branching pattern, colony form, and color were observed. After calibrating the compound microscope with an ocular and a stage micrometer, mycelial width, and size of the spores was measured. The diameter of fungal mycelium on Petri plates on

different solid media was measured by drawing two lines at right angles to each other and passing through the middle of the culture plates. The developing colony's diameter was calculated as the average of the two diameters of fungal development.

Preservation of Cultures: The stock cultures were cultivated on potato dextrose agar (PDA) slants and stored in the refrigerator at 4-5°C. The fungi were subcultured at regular intervals to keep them alive.

Identification of fungi: Fungal structures (such as hyphae branching, color, septate, nonseptate, conidiophores, conidia, sporangia, and sporangiophores, among others) have been studied using an Olympus BX51 compound microscope by using taxonomic keys for each taxon. The

keys of Domsch *et al.*, (1980), Barnett and Hunter (1972), Dick (1990), Ellis (1971-1976), and Raper and Fennell (1965) were used for identification of fungi up to species level. The website <http://www.speciesfungorum.org> was followed for synonym.

RESULTS

Isolation of soil-borne pathogenic fungi strains and genera: 67 soil-born pathogenic fungal strains belonging to five genera and eight species were isolated from five potato fields in three districts of the GB (Figure 2). Approximately 35% of the total isolated strains belong to the prominent fungal genera such as *Alternaria*, followed by *Fusarium* (30%), *Rhizoctonia* and *Pythium* (10.05%), and *Macrophomina* (3.35%).

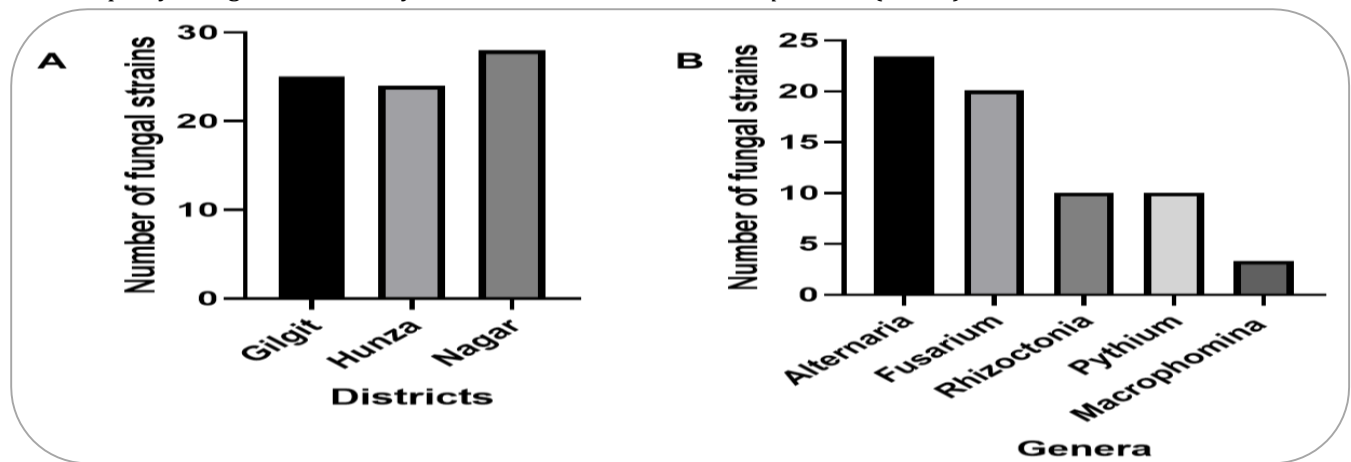


Figure 2. Isolated soil born pathogenic fungal strains and genera in the three districts

Identification of soil-born fungal pathogens: The eight-potato soil-born pathogenic fungal species were *Alternaria alternata*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Fusarium culmorum*, *Fusarium udum*, *Macrophomina phaseolina*, *Rhizoctonia solani*. Among these *Alternaria solani*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, and *R. solani* were found in all three districts as shown in Table 2. Among these fungal

pathogens, seven fungal pathogens such as *Alternaria alternata*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Fusarium culmorum* *Alternaria alternata*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Rhizoctonia solani* were found common in all the three districts as shown in Table 2. *Fusarium culmorum*, was only isolated and identified from district Gilgit as shown in Table 2.

Table 2. Identified soil-borne pathogenic fungi from three Districts: Gilgit, Hunza, and Nagar.

S. No	Fungi Name	Gilgit	Hunza	Nagar
1	<i>Alternaria solani</i>	+	+	+
2	<i>Fusarium culmorum</i>	+	-	-
3	<i>Fusarium incarnatum</i>	+	+	+
4	<i>Fusarium oxysporum</i>	+	+	+
5	<i>Fusarium udum</i>	-	+	+
6	<i>Macrophomina phaseolina</i>	+	-	+
7	<i>Pythium aphanidermatum</i>	+	+	+
8	<i>Rhizoctonia solani</i>	+	+	+

+ = Present, - = Absent

Morphological study of identified fungi: *Alternaria alternata*

***alternata*:** The colony exhibits a brown to black color, occasionally appearing grey, with a diameter of approximately 5.1 cm after 7 days of growth. Conidiophores are pale brown, smooth, and exhibit a straight or flexuous morphology. Reached lengths of up to 67 μm and measure 2-5 μm in thickness. Conidia are formed at the conidiophore apical fertile area, forming long

or branched chains. They are oval, ellipsoidal, or obclavate in shape, with a short cylindrical beak. The conidia display a pale to golden brown coloration, with up to 1-3 or multiple oblique and longitudinal septa and having up to 7 transverse septa. The general length of the conidia ranges from 18-40 μm , while the broadest part measures 7-15 μm in thickness. The beak of the conidia is approximately 2-4 μm thick (see Figure 3 A-D).

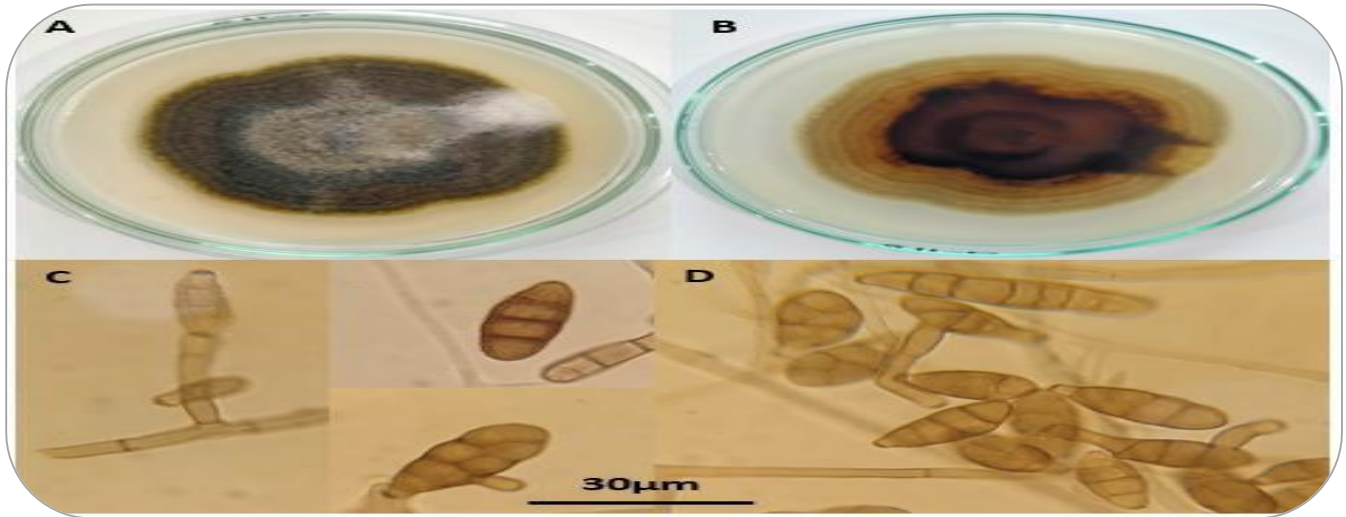


Figure 3. *Alternaria alternata*: (A-B) Culture plate, front and back, (C) Conidiophore containing conidia, (D) Conidia.

***Fusarium culmorum*:** The culture of *Fusarium culmorum* exhibits rapid proliferation, resulting in the formation of abundant mycelium. Initially, the mycelium appears pale white but eventually develops red pigmentation within the agar. Macroconidia are produced from monophialides and possess relatively thick walls, with the broadest point observed at the midpoint. The ventral side of the macroconidia is nearly straight, while the dorsal side

exhibits slight curvature. The apical cell of the macroconidia is rounded and blunt, while the basal cell is notched and typically contains three septa. However, some conidia may have up to four septa. The macroconidia can reach lengths of up to 36 μm and measure 3-4 μm in width at the midpoint. Microconidia are absent in this species. Chlamydospores, on the other hand, are abundant and form relatively quickly (see Figure 4).

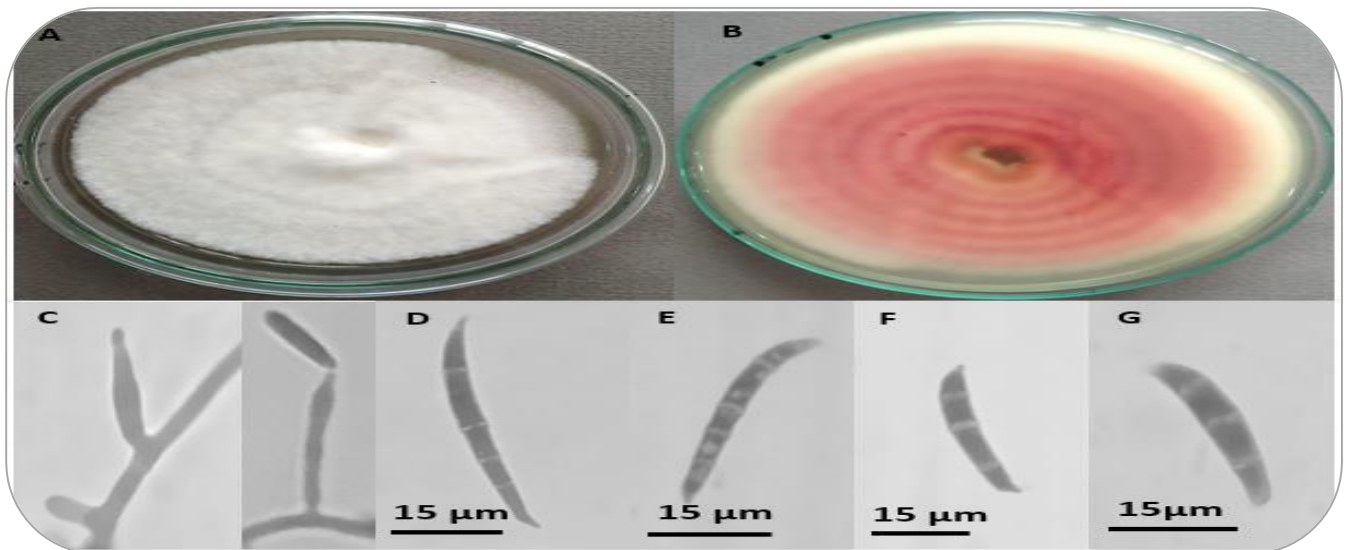


Figure 4. *Fusarium culmorum*: (A-B) Culture plate, front and back (C) Monophialides (D-E) Conidia.

***Fusarium incarnatum*:** The culture of the organism grows rapidly, displaying abundant aerial mycelium. Initially, the mycelium appears white but gradually changes to orange or brown with age. After 4 days of growth, the colony reaches a diameter of 8.4 cm. Conidiogenous cells are observed to be both mono- and polyphialidic. Within the aerial mycelium, mesoconidia are abundant. They typically

exhibit a straight ventral surface and a curved dorsal surface. The apical cells of the mesoconidia are curved to point, while the basal surface takes on a foot-shaped appearance. Mesoconidia can reach up to 32 µm lengths and contain 3-4 septa. Microconidia, on the other hand, are scarce and obovate to pyriform in shape, usually possessing one septum (Figure 5).

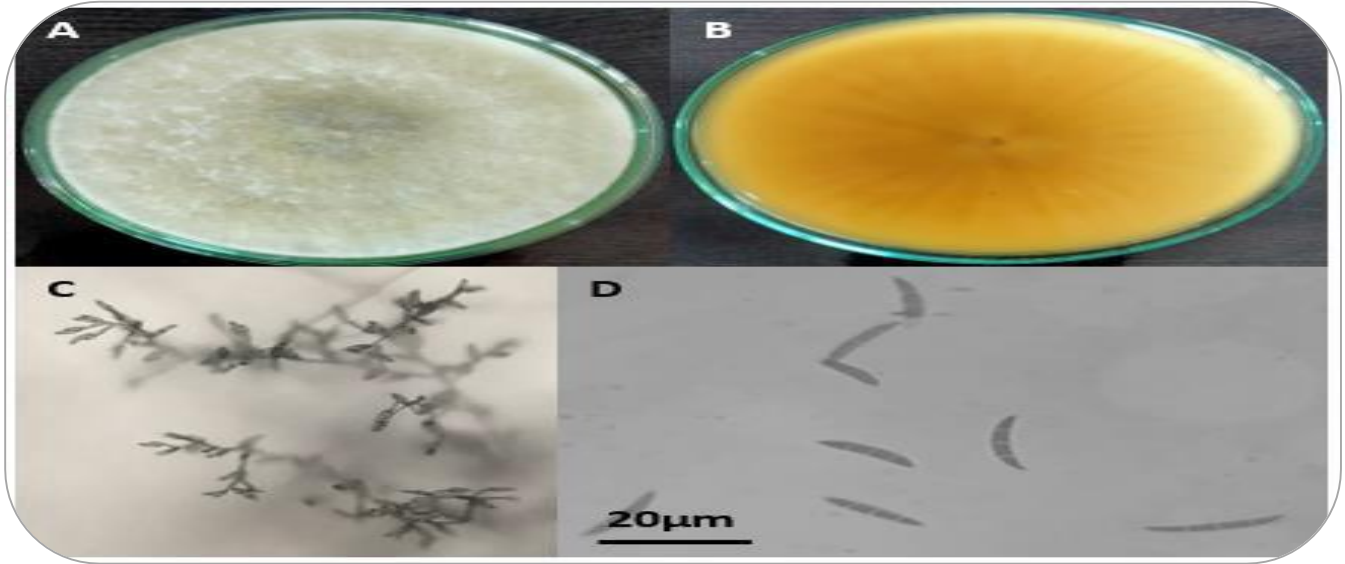


Figure 5. *Fusarium incarnatum*: (A-B) Culture plate front and back (C) Conidiogenous cells, (D) Conidia.

***Fusarium oxysporum*:** Cottony white aerial mycelium abundant, violet, magenta, or dark violet color produced in agar. The colony diameter is 5.3 cm in 7 days. Macroconidia short to medium length, thin-walled, straight or slightly curved, slender up to 37 × 2 µm long. The apical cell is curved or tapered, hooked slightly, while

the basal cell foot form has 3 septate. Microconidia up to 10 × 2 µm, oval and elliptical, usually non-septate, seen on false heads. Short conidiogenous cells that are prominent in the aerial mycelium, monophialidic 5-13 × 2 µm. Chlamydospores are rough or smooth, formed individually or in pairs (Figure 6).

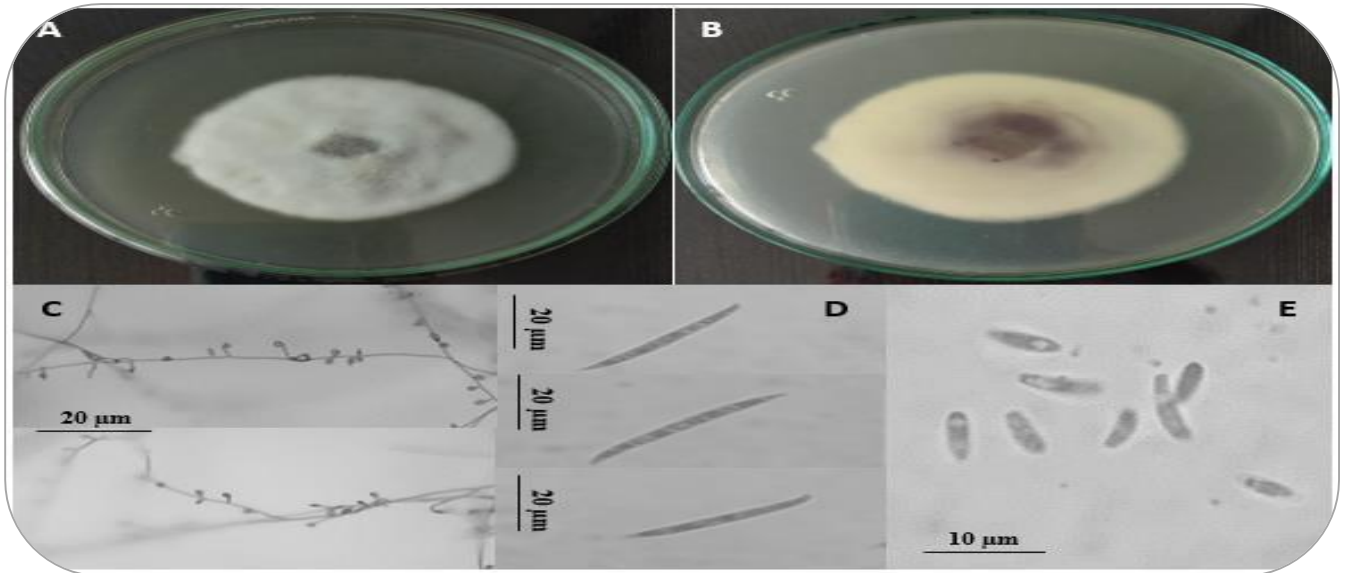


Figure 6. *Fusarium oxysporum*: (A-B) Culture plate, front and back, (C) Monophialides, (D) Macroconidia, (E) Microconidia.

Fusarium udum: The colony color was white initially, then became purple to pink due to the pigments production in the agar medium. The colony diameter was about 7.3 cm in eight days. Up to 5-10 μm long monophialidic conidiogenous cell. Conidia grow on fake heads. Macroconidia is 25-35 μm long and 3 μm wide,

with a thin-walled, curved to hooked apical cell and a foot-shaped, three-septate basal cell. Up to 12 μm lengths fusiform, oval to reniform, with 0-1 septate macroconidia. Chlamydospores are present, smooth-walled, and usually intercalary in the hyphae, occurring in chains or pairs (Figure 7).

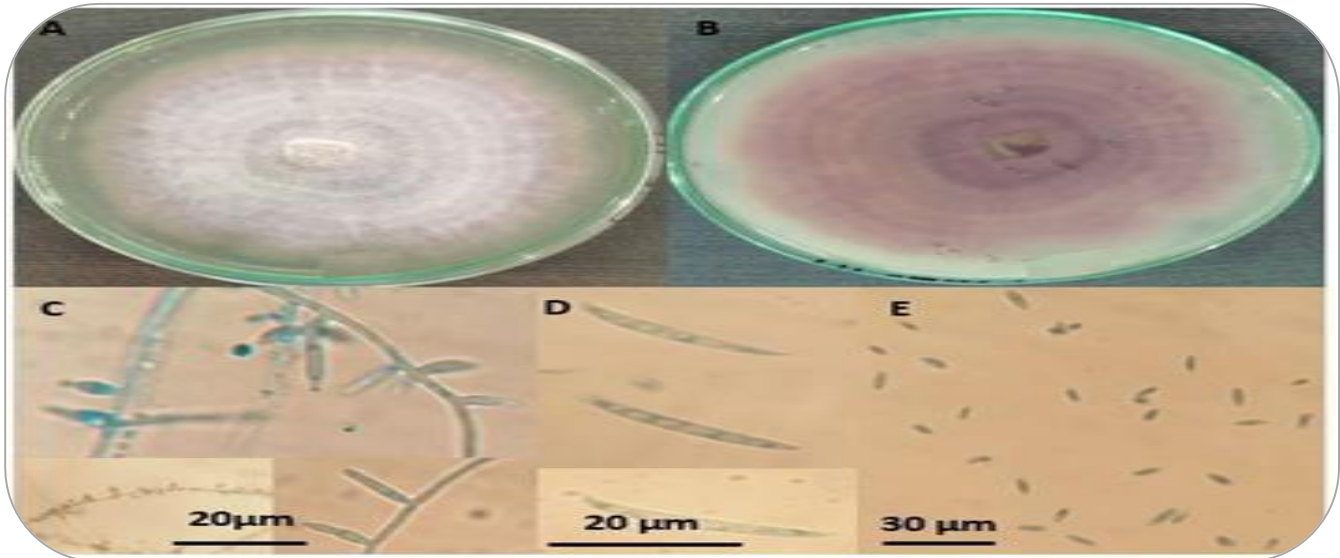


Figure 7. *Fusarium udum*: (A-B) Culture plate, front and back, (C) Monophialides, (D) Macroconidia, (E) Microconidia. ***Macrophomina phaseolina***: The colony was white at first, then greyish, then black as it matured; mycelium was initially light brown and septate, white fluffy, but this altered as it matured. The diameter of the hyphae ranged from 2-6.5 μm . Dark black sclerotia with various sizes up to 55-110 μm (Figure 8).

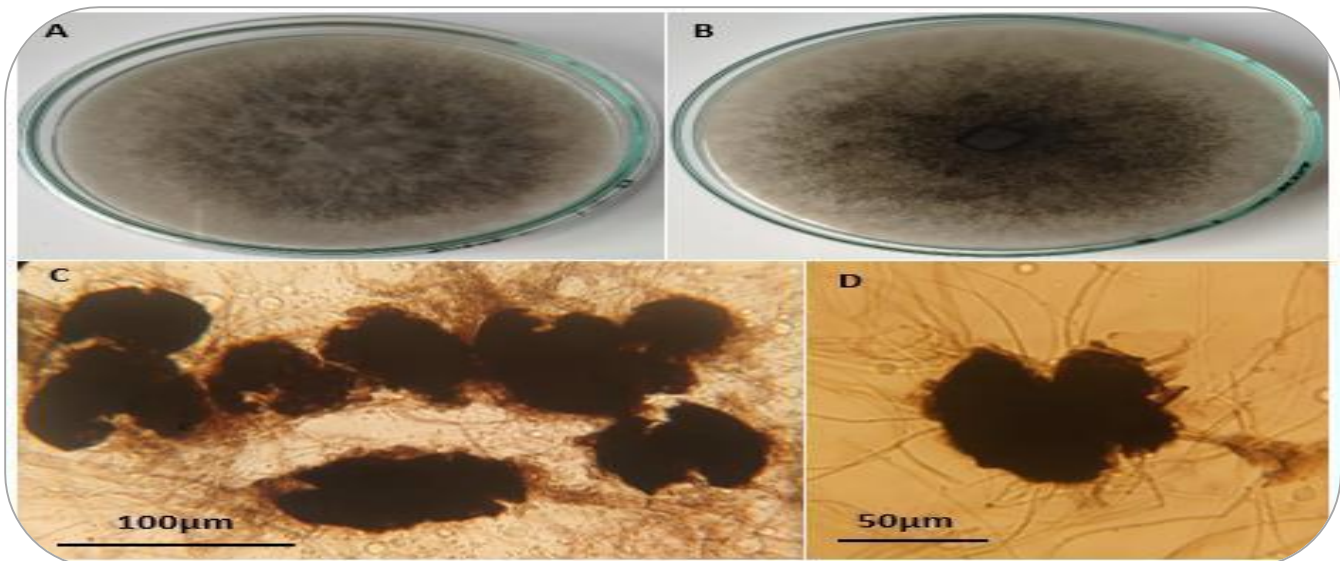


Figure 8. *Macrophomina phaseolina*: (A-B) Culture plate, front and back, (C-D) Sclerotia and Hyphae on agar culture. ***Pythium aphanidermatum***: The mycelium was hyaline branched and septate, up to 7-8 μm wide. The colony was cottony white; aerial mycelium with no noticeable pattern. Sporangia were filamentous, toroid with different widths and lengths, formed abundantly in water culture. Globose oogonia, terminal and smooth, 18-23 (av.19) μm , oospore 15.5-18 (av. 16.7), ooplast 8.5-11 (av.9.75) μm in diameter. Antheridia are diclinous, hardly monoclinal, primarily intercalary, and make wide apical contact with the oogonium and 1-2 per oogonium. Oospores were aplerotic (Figure 9).

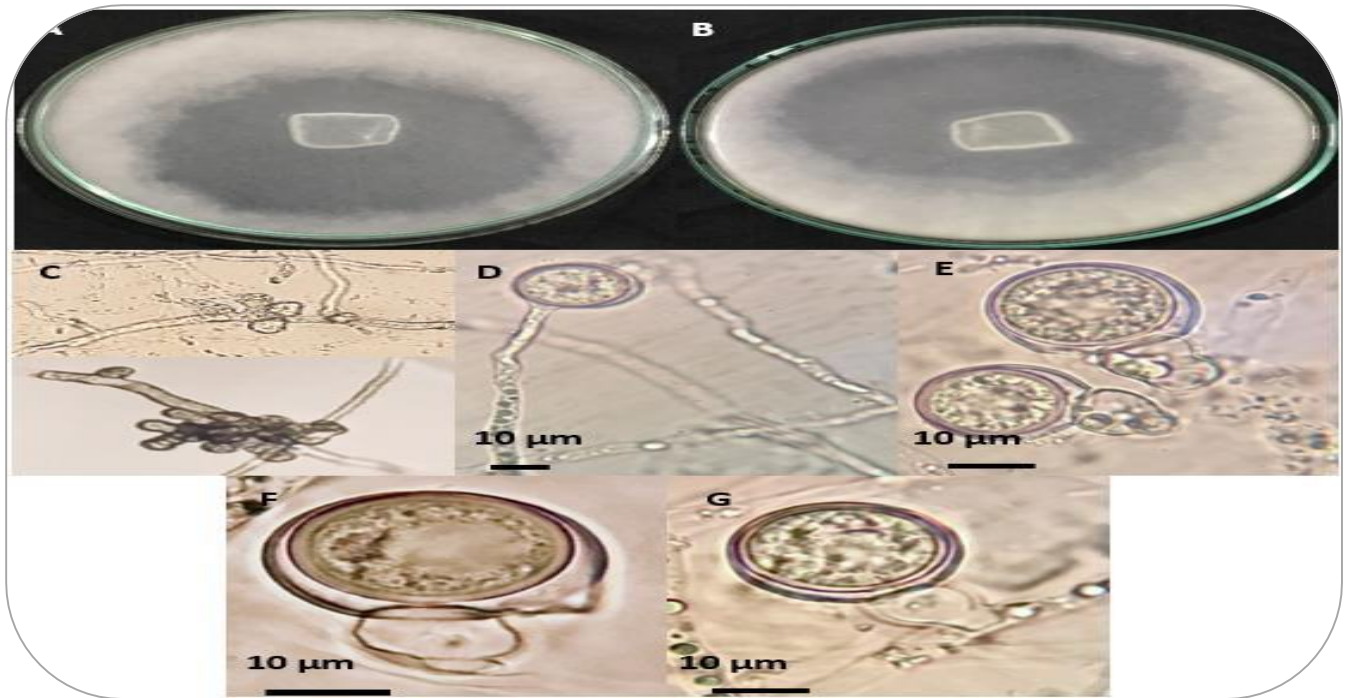


Figure 9. *Pythium aphanidermatum*: (A) Culture plate, front and back, (C) sporangia toruloid, (D-G) Antheridia, oogonia, oospores.

***Rhizoctonia solani*:** The colony was initially light grey, then became golden brown or dark brown with plentiful mycelial growth. In three days, the fungus had grown to a diameter of 7.8 cm. Mycelium had right-angle branching with no septum at the origin, but a septum was generated in the branch some distance from the origin. At the base

of each branch, there was a tiny constriction. There were no conidia or conidiophores found. The hyphal length ranged from 52.5 to 160 μm. with the majority of the hyphal length being between 125 and 137.5 μm. The hyphal diameter ranged from 5-6.5 μm. Sclerotia were formed after 7- 9 days of incubation (Figure 10).

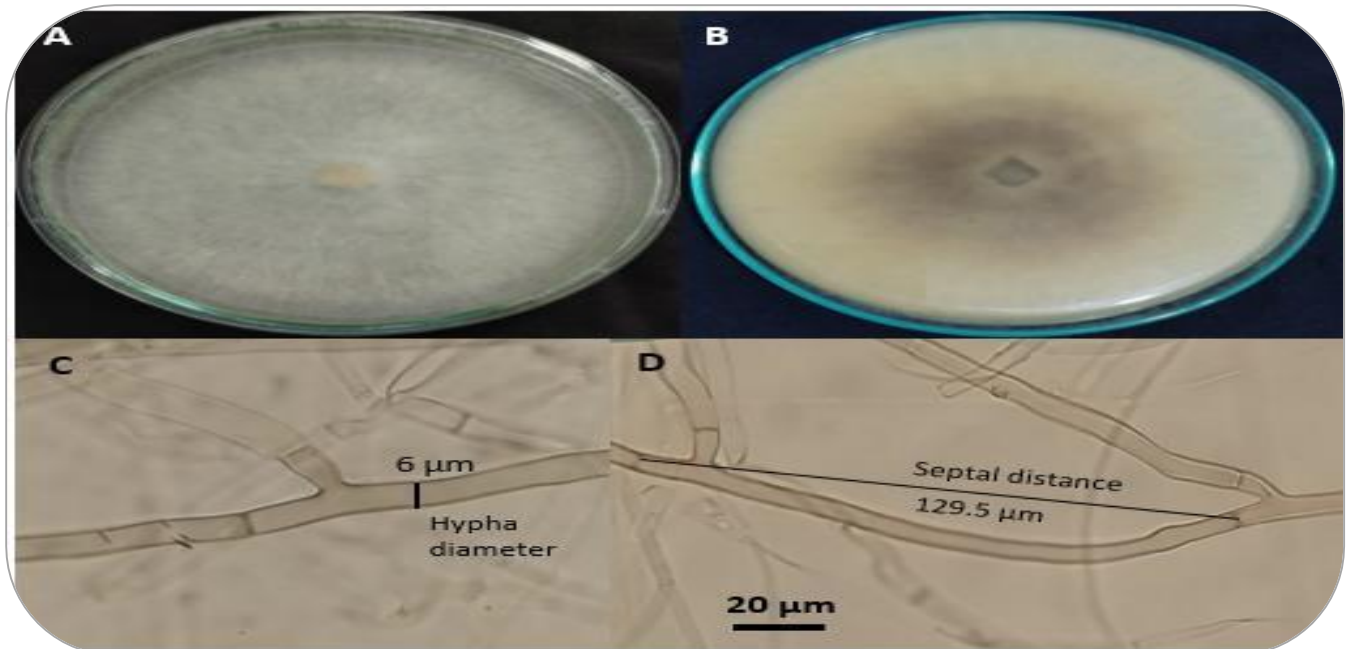


Figure 10. *Rhizoctonia solani*: (A-B) Culture plate, front and back, (C) Hyphal diameter and right angle branching, (D) Inter-septal distance.

DISCUSSION

Plant diseases have been an ongoing challenge since the dawn of agriculture. These diseases have a significant impact on the quality of human life and history is undeniable. The devastating consequences of the late blight of potatoes caused by *Phytophthora infestans* created severe famine, starving and killing millions of people in Ireland. In recent years, the effects of climate change triggered by global warming have exacerbated the rapid spread of plant diseases and severity. These pathogens have emerged as serious concerns to global food security. Detecting and monitoring plant diseases has become critical for ensuring agricultural sustainability (Abbas *et al.*, 2018). In this study, conducted the isolation of diverse soil-borne pathogenic fungi from three districts in GB, Pakistan. The most prevalent genera identified were *Alternaria*, *Fusarium*, *Rhizoctonia*, *Macrophomina*, and *Pythium*. Consistently found these genera in all potato-cultivated areas across the three districts of GB and *Rhizoctonia solani* was isolated which causes black scurf disease in potatoes Hussain *et al.* (2017) researched various valleys in Central Karakorum National Park (CKNP) and discovered that *Rhizoctonia solani* was the most problematic disease. According to the survey, the condition was most prevalent in Haramosh (45.40 14.62) and least prevalent in Bagrote (63.85 9.06). *R. solani* is a common fungal pathogen in Pakistan's potato-producing agro-ecological zones. It widely distributed pathogen affecting potato production, tuber quality, and other economically important crops. The suffering potato tubers exhibit deformation, cracking, and pitting, resulting in poor product quality (Bhutta *et al.*, 2004; Khan *et al.*, 1995; Rauf *et al.*, 2007). *Macrophomina phaseolina*, a root pathogenic fungus isolated during the current research, causes root rot, stem rot, charcoal rot, and damping-off in over 600 plant species, with 67 hosts noticed from Pakistan alone (Shahzad and Ghaffar, 1986; Shahzad *et al.*, 1988). *Fusarium avenaceum*, *Fusarium oxysporum*, and *Fusarium solani* induce Fusarium wilt (Peters *et al.*, 2008). The presence of *Fusarium* spp. in potato fields in the GB indicates a potential threat to potato production in the region. *Pythium* is one of the largest oomycetes genera, with around 160 species (Chen *et al.*, 2017). The species of this genus are widespread all over the world, have extensive host ranges, and

cause economically significant diseases in crop plants (Martin and Loper, 1999). Potato infections are caused by *Pythium* spp. degrading seeds and tubers in the field, following harvest, and during storage (Salas and Secor 2001). During the current investigation, *Pythium aphanidermatum* was isolated from among the *Pythium* spp. *P. aphanidermatum* produces leak (watery wound rot) on potato tubers, resulting in severe tuber loss, whereas *P. spinosum* frequently infects plant seeds and young roots, producing wilting, root rot and damping-off diseases. (Daami-Remadi *et al.*, 2012; Hendricks and Roberts, 2015; Plaats-Niterink, 1981). *Alternaria* spp. are widely distributed in soil and organic waste that is decomposing. These microbes are saprophytic, endophytic, and pathogenic. *Alternaria solani* and *Alternaria alternata* significantly reduce potato production and cause massive financial losses to the global farming community (Aslam *et al.*, 2019; Abbas, 2018). In the present study, *Alternaria alternata* was isolated from the three districts of GB. However, we could not isolate and identify *A. solani* using standard keys and monograph.

CONCLUSION

In conclusion, we have isolated eight soil-born fungal pathogens of potatoes in this study. Among these eight soil-born fungal pathogens, five such as *Alternaria solani*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Pythium aphanidermatum*, and *R. solani* were found in all three districts. These five seem to be potential pathogens of potato crops in GB. This information will help to take effective measures against the diseases caused by these pathogens. The present study also has drawbacks because we have relied on morphology to identify soil-born pathogenic fungi. Morphological approaches to fungal systematics may not always work well for species-level classification. Morphological traits can frequently be deceiving due to hybridization, cryptic speciation, and convergence of evolution. Moreover, endosymbiotic fungal strains do not always sporulate in culture, leaving no phenotypic attributes that can be used to identify them based on morphology. Future research directions and potential control measures that could help mitigate the impact of these fungi on potato production in GB are as follows:

Molecular Identification: To overcome the limitations of relying solely on morphological

characteristics for fungal identification, future studies should incorporate molecular techniques, such as DNA sequencing, for accurate and reliable identification of soil-borne pathogenic fungi. This approach would enable species-level classification and help differentiate closely related or cryptic species. Pathogenicity Studies: It is important to do pathogenicity investigation on the found fungi, specifically *A. solani*, *F. incarnatum*, *F. oxysporum*, *P. aphanidermatum*, and *R. solani*. Such studies would determine the specific virulence factors and mechanisms these pathogens employ to infect potato plants. This information is crucial for developing targeted control strategies.

Disease Management Strategies: Develop integrated disease management strategies to mitigate the impact of soil-borne fungal pathogens on potato crops. This could include a combination of cultural practices, such as crop rotation, planting resistant cultivars, and implementing proper sanitation measures and chemical and biological control methods. Integrated pest management approaches should be tailored to the specific pathogens prevalent in different regions of GB.

Surveillance and Monitoring: Establish a systematic surveillance and monitoring program to continuously assess the distribution, incidence, and severity of soil-borne pathogenic fungi across different regions of GB. Regular monitoring would enable the timely detection of emerging pathogens and the evaluation of disease dynamics, providing valuable data for decision-making and intervention strategies.

Soil Health Management: Emphasize soil health management practices to improve the suppressive capacity of soils against soil-borne pathogens. Implementing organic amendments, cover cropping, and biological soil amendments could enhance the natural antagonistic microflora and improve overall soil health, reducing the prevalence and impact of soil-borne diseases.

Capacity Building and Awareness: Foster collaboration and knowledge sharing among researchers, extension services, and farmers to build capacity in the identification, management, and prevention of soil-borne fungal pathogens. Conduct workshops, training programs, and awareness campaigns to disseminate information and promote best practices for disease management.

Regional Exploration: Expand the scope of research to explore different regions of GB for soil-borne pathogenic fungi isolation and identification. This comprehensive approach would provide a more accurate understanding of these pathogens' distribution patterns and diversity, enabling targeted interventions in specific areas. By focusing on these future research directions and implementing effective control measures, the agricultural community in GB can enhance potato crop resilience, minimize yield losses, and sustainably manage soil-borne pathogenic fungi, contributing to the overall improvement of potato production in the region.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. All authors declared no conflict of interest.

REFERENCES

- Abbas, A. 2017. First report of *Alternaria* blight of potatoes in Nomal valley, Gilgit- Baltistan Pakistan. *Applied Microbiology Open Access*, 3: 137.
- Abbas, A., S.A. Syed and A. Moosa. 2018. Emerging plant diseases of Gilgit- Baltistan (GB) Pakistan: A review. *Agricultural Research and Technology*, 18: 1-6.
- Abdollahzadeh, J., A. Javadi, E.M. Goltapeh, R. Zare and A.J.L. Phillips. 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 25: 1-10.
- Abdul Haq, M. 1996. *Oomycetes fungi from Bajaur Agency, FATA, Pakistan*. M.Sc. Thesis. Department of Botany, University of Karachi.
- Abdul Haq, M. 2014. *Studies on biodiversity of Oomycetes of Bajaur Agency, Fata*, Ph.D. Thesis. Department of Botany, University of Karachi, Karachi.
- Abo-El-Dahab, M.K., S.A. El-Kazazz, A.A. Shoeib and M.A. El-Sheikh. 1992. Biochemical changes in citrus fruits infected with *Botryodiplodia theobromae*. *Journal of Agricultural Sciences, Mansoura University. (Egypt)*, 17: 3524- 3532.
- Agrawal, T. and A.S. Kotasthane. 2012. Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. *Springer Plus*, 1:

- 73-83.
- Agrios, G.N. 2005. Plant pathology, Academic Press, San Diego.
- Ahmad, I. 1998. Emergence of diseases and their impact on seed potato production. Research paper presented at workshop on disease free seed potato in Northern Areas. Agriculture Department, Northern Areas, Gilgit.
- Ahmad, I., M.H. Soomro, S. Iftikhar and M. Aslam. 1991. *Investigation on powdery scab of potato in Pakistan*. CDRI-PSPDP, PARC, Islamabad, Pakistan, 1-2.
- Ahmad, I., S. Iftikhar, M.H. Soomro, S. Khalid and A. Munir. 1995. *Diseases of potato in Northern Areas during 1992*. CDRI-PSPDP, PARC, Islamabad, Pakistan, 38.
- Ahmed, S., S.H. Iqbal and A.N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmad Mycological Society of Pakistan, Department of Botany, University of Punjab, Lahore 54590, Pakistan. 248.
- Allain-Boulé, N., C.A. Lévesque, C. Martinez, R.R. Bélanger and R.J. Tweddell. 2004. Identification of *Pythium* species associated with cavity-spot lesions on carrots in eastern Quebec. *Canadian Journal of Plant Pathology*, 26: 365-370.
- Anonymous. 2009. *Survey Report 2008-2009*. Department of Agriculture, Gilgit- Baltistan.
- Anonymous. 2019. Agricultural statistics of Pakistan. Ministry of National food security and research, Islamabad, Pakistan.
- Anwar, D., D. Shabbir, M.H. Shahid and W. Samreen. 2015. Determinants of potato prices and its forecasting: A case study of Punjab, Pakistan. University library of Munich, Germany.
- Anwar, S., F.A. Khan and A. Rahman. 2019. Impact of Karakorum highway on land use and agricultural development of Gilgit-Baltistan, Pakistan. *Sarhad Journal of Agriculture*, 35: 417-431.
- Arora, J., S. Goyal and K.G. Ramawat. 2012. Co-evolution of pathogens, mechanism involved in pathogenesis and biocontrol of plant diseases: an overview. *Plant Defense: Biological Control*. Springer Dordrecht, pp. 3-22.
- Aslam, S., H. M. U. Aslam, A. Abbas, M. A. Ali, M. W. Alam, L. Amrao and M. L. Gleason. 2019. First report of leaf spot of spinach caused by *Alternaria alternata* in Pakistan. *Plant Disease*. 103: 1430-1430.
- Azhar, H., M.S. Awan, F. Morari, S.M. Iqbal and N.U.H. Syed. 2014. Spatial analysis of potato black scurf disease distribution using GIS and variability of *Rhizoctonia solani* isolates in Central Karakoram National Park Gilgit-Baltistan, Pakistan. *International Journal of Biosciences*, 4: 17-27.
- Barnett, H.L. and B.B.G. Hunter. 1972. *Illustrated genera of imperfect Fungi*. Burgess Public Corporation. Minneapolis. 241.
- Bhutta, A.R., M.Q. Muhammad and J.I. Hussain. 2004. Pathological survey of potato crop in Northern areas, 2003. *FSC and RD/AKRSP, DOA, Islamabad*, 109.
- Birch, P.R., G. Bryan, B. Fenton, E.M. Gilroy, I. Hein, J.T. Jones and I.K. Toth. 2012. Crops that feed the world 8: Potato: are the trends of increased sustainable production. *Food Security*, 4: 477-508.
- Chakraborty, R. 2016. Resistant starch a new weapon against disease fighting of potato eater: A focused review. *Asian Journal of Pharmaceutics*, 10: 192-195.
- Chen, J.J., L.I. Lü, W.W. Ye, Y.C. Wang and X.B. Zheng. 2017. *Pythium cedri* sp. nov. (*Pythiaceae*, *Pythiales*) from southern China based on morphological and molecular characters. *Phytotaxa*, 309: 135-142.
- Chen, J.L., S.Z. Sun, C.P. Miao, K. Wu, Y.W. Chen, L.H. Xu and L.X. Zhao. 2016. Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*. *Journal of Ginseng Research*, 40: 315-324.
- Da Silva, J.A.T., E.V. de Medeiros, J.M. da Silva, D.D.A. Tenório, K.A. Moreira, T.C.E.D.S. Nascimento and C. Souza-Motta. 2016. *Trichoderma aureoviride* URM 5158 and *Trichoderma hamatum* URM 6656 are biocontrol agents that act against cassava root rot through different mechanisms. *Journal of Phytopathology*, 164: 1003-1011.
- Daami-Remadi, M., I. Dkhili, H. Jabnoun-Khiareddine and M. El Mahjoub. 2012. Biological control of potato leak with antagonistic fungi isolated from compost teas and solarised and non-solarised soils. *Pest Technology*, 6: 32-40.
- Desjardins, A.E. 2006. Fusarium mycotoxins:

- chemistry, genetics, and biology. American Phytopathological Society, USA.
- Dewan, M.M. and K. Sivasithamparam. 1988. Occurrence of species of *Aspergillus* and *Penicillium* in roots of wheat and ryegrass and their effects on root rot caused by *Gaeumannomyces grami* var. *tritici*. Australian journal of botany, 36: 701- 710.
- Diab, M.M., I. Khalil, N.A. Dawood and E.M. El-Assiuty. 1984. Ear and grain rot of maize caused by *Botryodiplodia theobromae* pathogens in Egypt. Minufiya. Journal of Agricultural Research, 9: 129-138.
- Dick, M.W. 1990. Keys to Pythium. University of Reading, Reading, Berks., UK.
- Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil fungi. Volume* Academic Press Limited, London.
- Ellis, M.B. 1971. *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute. U.K.
- Ellis, M.B. 1976. *More dematiaceous hyphomycetes*. Commonwealth Mycological Institute. Kew. England.
- Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora diseases worldwide*. American Phytopathological Society, USA.
- FAO. 2016. Food and Agriculture Organization. United Nations, New York, United States.
- Fiers, M., V. Edel-Hermann, C. Héraud, N. Gautheron, C. Chatot, Y. Le Hingrat and A.P.S.C. Steinbe. 2011. Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France. Mycologia, 103: 1230-1244.
- Gilman, J.C. 2001. A Manual of Soil fungi, 2nd Indian edition. John Wiley and Sons Inc, 17: 336-341.
- Haggag, W.M., M.S.M. Abou Rayya and N.E. Kasim. 2007. First report of a canker disease of walnut caused by *Botryodiplodia theobromae* in Egypt. Plant disease, 91: 226-226.
- Harvey, J.V. 1925. A study of the water molds and *Pythium* occurring in the soils of chapel hill. Journal of the Elisha Mitchell Scientific Society, 41: 151-164.
- Hendricks, K.E. and P.D. Roberts. 2015. First Report of *Pythium spinosum* as a pathogen of watermelon and in association with a dieback of watermelon in Southwest Florida. Plant Health Progress, 16: 77-79.
- Hooker, W.J. 1981. *Compendium of potato diseases*. International Potato Center.
- Hussain, A., S.W. Khan, M.S. Awan, S. Ali, Q. Abbas, Z. Ali and S. Ali. 2017. Potato black scurf, production practices and fungi toxic efficacy of *Rhizoctonia solani* isolates in hilly areas of Gilgit-Baltistan, Pakistan. Pakistan Journal of Botany, 49: 1553-1560.
- Jacobs, R. 2002. *Characterisation of Botryosphaeria species from mango in South Africa*. Master's thesis. Pretoria, South Africa: University of Pretoria.
- Kamal, P. and M.J. Nasir. 1998. The Impact of the Karakorum Highway on the land use of the Northern Areas. Karakoram-Hindu Kush-Himalaya: Dynamics of Change (Part-I), Culture Area Karakoram Scientific Studies, 4: 303-318.
- Khalid, S., S. Iftikhar, A. Munir and I. Ahmad. 2000. Potato diseases in Pakistan. *PARC. Islamabad*, 165.
- Khan, R.A., S. Iftikhar, A. Rafi, S. Riaz and I. Ahmad. 1995. Distribution and incidence of tuber diseases of potato in Swat valley. In: *Research and development of potato production in Pakistan. Proceedings of the National Seminar held at NARC, Islamabad, Pakistan 23-25 April, 1995*. 169-172. Pak- Swiss Potato Development Project, Pakistan Agricultural Research Council.
- Khanzada, M.A., A.M. Lodhi and S. Shahzad. 2004. Mango dieback and gummosis in Sindh, Pakistan caused by *Lasiodiplodia theobromae*. Plant Health Progress, 5: 13.
- Kokab, A.A. and A.E. Smith. 1989. *Marketing potatoes in Pakistan, Pakistan-Swiss Potato Development Project*. Pakistan Agriculture Research Council, Islamabad, Pakistan.
- Kozakiewicz Z. 1989. *Aspergillus* species on stored products. *Mycological Papers*. 161: 1-188.
- Lodhi, A.M. 2007. *Taxonomic studies on oomycetous fungi from Sindh*. Ph.D. Thesis Department of Agriculture, University of Karachi, Karachi.
- Louis, B., P. Roy, D.W. Sayanika and N.C. Talukdar. 2013. *Aspergillus terreus* Thom a new pathogen that causes foliar blight of potato. *Plant Pathology and Quarantine*, 3: 29-33.
- Majeed, A. and Z. Muhammad. 2018. Potato production in Pakistan: challenges and prospective management strategies-a review. Pakistan

- Journal of Botany, 50: 2077-2084.
- Martin F.N. and J.E. Loper. 1999. Soil-borne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. *Critical Review Plant Science.*, 18:111-181.
- Matarese, F., S. Sarrocco, S. Gruber, V. Seidl-Seiboth and G. Vannacci. 2012. Biocontrol of *Fusarium* head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*. *Microbiology*, 158: 98-106.
- Melo, I.S., J.L. Faull and R.S. Nascimento. 2006. Antagonism of *Aspergillus terreus* to *Sclerotinia sclerotiorum*. *Brazilian Journal of microbiology*, 37: 417-419.
- Mirza, J.H. and M.S.A. Qureshi. 1978. *Fungi of Pakistan*, 311. University of Agriculture, Faisalabad, Pakistan.
- Mu, T. and H. Sun. 2017. Progress in research and development of potato staple food processing technology. *Journal of Applied Glycoscience*, 64: 51-64.
- Mundkur, B.B. 1938. *Fungi of India*. Supplement I. *Council of Agricultural Research*, 54.
- Newsham, K.K., A.H. Fitter and A.R. Watkinson. 1995. Arbuscular mycorrhizae protect an annual grass from root pathogenic fungi in the field. *Journal of ecology*, 83: 991-1000.
- Oerke, E.C., H.W. Dehne, F. Schonbeck and A. Weber. 1994. *Crop Production and Crop Protection Estimated Losses in Major Food and Cash Crops*. Elsevier Science BV.
- Pereira, A.B. and C.C. Shock. 2007. A review of agrometeorology and potato production. *Guide to Agricultural Meteorology (GAMP)*. World Meteorological Organization, Geneva, Switzerland.
- Peters, R.D., C. MacLeod, K.A. Seifert, R.A. Martin, L.R. Hale, C.R. Grau and S. Mac Innis. 2008. Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato, cereal and forage crops. *American Journal of Potato Research*, 85: 367.
- Plaats-Neterink, A.J.V. 1981. Monograph to the genus *Pythium*. *Studies in mycology No. 21*. Central-bureau voor Schimmelcultuur, Baarn. 242.
- Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae*. In: *Bibliotheca Mycologica*. J. Cramer, Berlin.
- Rahman, A., N.A. Khan, S. Khan and S. Qasim. 2013. Impacts of CRBC on agricultural production of district D.I Khan: An ex-post evaluation. *Journal of Science and Technology*, 37: 37-51.
- Raper, K.B. and D.I. Fennell. 1965. *The genus Aspergillus*. Williams and W. Wilkins Co., Baltimore, USA.
- Rauf, C.A., M. Ashraf and I. Ahmad. 2007. Occurrence and distribution of black scurf of potato in Pakistan. *Pakistan Journal of Botany*, 39: 1341.
- Rodríguez-Gálvez, E., E. Maldonado and A. Alves. 2015. Identification and pathogenicity of *Lasiodiplodia theobromae* causing dieback of table grapes in Peru. *European journal of plant pathology*, 141: 477-489.
- Salas, B. and G.A. Secor. 2001. Leak. In: *Compendium of potato diseases*, 2nd ed. W.R. Stevenson, R. Loria, G.D. Franc, and D.P. Weingartner (Eds.) 30-31. St. Paul, MN: APS.
- Shahzad, S. and A. Ghaffar. 1986. *Macrophomina phaseolina* on some new hosts. *FAO Plant Protection Bulletin*, 34: 163.
- Shahzad, S., A. Sattar and A. Ghaffar. 1988. Additions to the hosts of *Macrophomina phaseolina*. *Pakistan Journal of Botany*, 20: 151-152.
- Sheikh, A.H. and A. Ghaffar. 1992. Effect of fungicide on the survival of sclerotia of *Macrophomina phaseolina* at different soil manure levels and its infection on guar (*Cyamopsis tetragonoloba*). *Proceeding of National Symposium on Status of Plant Pathology in Pakistan*, Department of Botany, University of Karachi, Karachi, Pakistan, p. 267-271
- Siddaiah, C.N., N.R. Satyanarayana, V. Mudili, V.K. Gupta, S. Gurunathan, S. Rangappa and R.K. Srivastava. 2017. Elicitation of resistance and associated defense responses in *Trichoderma hamatum* induced protection against pearl millet downy mildew pathogen. *Scientific reports*, 7: 1-18.
- Singh, J. and L. Kaur. 2016. *Advances in potato chemistry and technology*. Academic press.
- Spooner, D.M., K. McLean, G. Ramsay, R. Waugh and G.J. Bryan. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the national academy of sciences*, 102: 14694-14699.

Vasudeva, R.S. 1960. The fungi of India. ICAR, New Delhi. P. 552.

Waksman, S.A. and E.B. Fred. 1922. A tentative outline of the plate method for determining the number of micro-organisms in the soil. *Soil Science*, 14: 27- 28.

Weightman, B. A. 2011. *Dragons and tigers: a geography of South, East, and Southeast Asia*. John Wiley and Sons.

White, J.G., M.E. Stanghellini and L.M. Ayoubi. 1988. Variation in the sensitivity to metalaxyl of *Pythium* spp. isolated from carrot and other sources. *Annals of applied biology*, 113: 269-277.

Younis, M., M.A. Khan, S.T. Sahi and R. Ahmad. 2009. Genotypic variations among different potato lines/varieties for tolerance against late blight disease. *Pakistan Journal of Phytopathology*, 21: 13-17.

Zaheer, K. and M.H. Akhtar. 2016. Potato production, usage, and nutrition-a review. *Critical reviews in food science and nutrition*, 56: 711-721.

Contribution of Authors:

Riaz Ahmad	:	Investigation, methodology and data collection.
Saleem Shahzad	:	Supervision and resources.
Mustansar Mubeen	:	Original draft writing.
Qaiser Shakeel	:	Software and validation.
Aqleem Abbas	:	Conceptualization, methodology, GIS mapping and formal analysis.
Muhammad Rafique	:	Editing and revision.
Syed A. Hussain	:	Data collection and methodology.
Abdul Q. Rajput	:	Investigation, methodology and data collection.
Zakir Hussain	:	Technical assistance.