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## Isolation and identification of different Rhizospheres fungi of Mansehra region, Pakistan

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#### Abstract

The aim of the current research work was to analyze different fungal species from selected rhizospheres of Mansehra region of Pakistan. The research work was carried out in the Microbiology Laboratory of Hazara University, Mansehra, during April 2016 to August 2016. Soil samples were collected from 6 different locations of Mansehra including Balakoat, Chatar Plan, Dhodial (Hazara University adjacent area), Collage Doraha, Bafa and Oghi. The media used for the isolation and identification included Nutrient agar, Potato Dextrose Agar (PDA) and PDA with peptone. Following inoculation of soil samples, the media was incubated for four days at 25 °C. 10 species of fungal strains were isolated from the soil sample, which included *Cladosporium herbarum*, *Dermatium* spp., *Fusarium oxysporum*, *Mucor hiemalis*, *Alternaria solani*, *Alternaria Alternata*, *Aspergillus flavus* and *Aspergillus niger*. Fungal growth was then purified on nutrient agar medium. The results of this investigation demonstrated that different strains of fungi are present in the soil of Mansehra region.

**Keywords:** Soil borne fungi, rhizospheres, Mansehra region

#### 1. Introduction

The system of the soils is extremely complex, having many constituent playing varied functions mainly due to the activity of soil organisms [1]. Different microorganisms are involved in several biochemical transformation and mineralization activities in soils due to which the soil fertility and plant growth increases. In the activity of soil, micro flora along with the type of cultivation carried out and subsequent crop management can have profound effects [2]. For the soil ecosystem, resident fungi are very vital and play a key role in many essential processes such as elemental release by mineralization and organic matter decomposition [3]. The fungi also constitute major group of organotrophic organisms responsible for the decomposition of organic compounds and their activity contributes in the bio-deterioration and biodegradation of toxic substances in the soil [4]. Fungi are eukaryotic heterotrophic spore bearing organism which have no chlorophyll and they can reproduce asexually and sexually. Most of the fungi producing diseases in human beings and animals directly arise from infected people (anthropophilic organisms), animals (zoophilic organisms), and soil (Geophilic organisms), and indirectly from fomites early in an environment as soil saprophytes [5]. Fungi belonging to the genera *Trichoderma*, *Aspergillus*, *Fusarium*, *Botrytis* and *Rhizopus* were rare or absent from the International Biological Programme (IBP) tundra sites [6]. It was found that microorganisms associated with decay of organic matter in tundra belong to taxonomic groups which are common in other biomes, but the number of taxa and the biomass are usually lower than in other biomes [7].

Antibiotics comprise a chemically various group of small organic molecules of microbial origin that, at low concentrations, are damaging the growth or metabolic activities of other microorganisms [8]. According to Talaro and Talaro [9], Anti-biotics substances produced by natural metabolic processes of some microorganisms can inhibit or destroy other microorganism. Dutta [10] defined antibiotics as the miracle drugs of modern times that act as magic bullets killing down the infective organisms that have attacked the human body and caused infections.

Originally, the term antibiotics rose only to organic compounds, produced by bacteria and which are toxic to other microorganisms [11].

The advent of synthetic methods has, however, resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganism, or to a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms [12]. It was not until 1940 with the discovery of penicillin, the first, best-known and most widely used antibiotic [13-17]. In 1928 by an English Bacteriologist, late Sir Alexander Fleming that the first clinical trials of penicillin were tried on humans. This antibiotic was obtained from a blue, green mold of the soil called *Penicillium notatum* [18]. Penicillin was discovered accidentally and Alexander Fleming, later on, was able to show its efficacy in laboratory cultures against many diseases producing bacteria. This discovery marked the beginning of the development of antibacterial compounds produced by living organism [19]. Another antibiotic, streptomycin was isolated in 1944 by Waksman, a Microbiologist, from a species of soil bacteria, called *Streptomyces griseus*, particularly *tubercle bacilli*, and has proved to be very valuable against tuberculosis [20]. A vigorous search for more antibiotics was on at this time and in 1947, another antibiotic, Chloromycetin was discovered by Burkholder [21-23]. It was isolated from *Streptomyces venezuelae*. It exhibited powerful action on a wide range of infectious bacteria, both Gram positive and Gram negative [24]. The ability to produce antibiotics has been found mainly in fungi of the group *Aspergillus*, and in a few other bacteria [25]. The *Streptomyces*'s are remarkable for the chemical diversity of antibiotics that they produce [26]. As more antibiotics were discovered, designed and studied, scientists found that they had different properties. Some of these properties include their source, range of activity and their kinds. These were used to classify them [27]. Perhaps one of the few most important discoveries regarding the beneficial use of fungi for humans was the identification in 1928 by Sir Alexander Fleming, that an isolate of *Penicillium notatum* produced a substance capable of killing Gram positive bacteria [28-30]. This compound was subsequently identified as penicillin and was the first member of the  $\beta$ -lactam class of antibiotics to be discovered. These compounds function by inhibiting peptidoglycan synthesis in bacteria and their use has significantly restricted the Gram positive bacteria to cause disease [31-32]. Subsequent to the identification of penicillin production by *Penicillium notatum*, screening experiments revealed that *Penicillium chrysogenum* was the superior producer of penicillin. A typical fermentation yields three types of Penicillin, namely, Penicillin F, Penicillin G and Penicillin V [33-35]. Antibiotics produced by fungi, are widely used in current chemotherapy, especially the penicillin, cephalosporin and fusidic acid, which have antibacterial and antifungal activity [36]. The last three decades are characterized by the novel discoveries of microorganisms capable of producing compounds, as a potential source of new antibiotics [37]. This investigation was aimed at determining the diversity of mesophilic fungi in the soil environment of study area and making an assessment of their antibiotic-producing potentials.

## 2. Materials and methods

This research work was conducted at the Microbiology research laboratory, Department of Microbiology, Hazara University Mansehra, Pakistan

### 2.1 Selection sites

Twenty samples were collected from district Mansehra during

the months of April, May and June 2016 to identify soil fungi present in different areas of district Mansehra including Hazara University area, Baffa, Balakot, Oghi and roadside soil of College Doraha. The samples were collected in sterile polythene bags and were stored at 4 °C for further use.

### 2.2 Collection of soil samples

Samples were collected 10-15 cm after deep pits dug in the area to be sampled. The sample was collected with a surface sterilized towel. Soil was scraped along the wall of pits and collected in sterile polythene bags. Different media used included the Nutrient agar, Potato dextrose agar (PDA) and media, which used PDA, have added ingredients which are essential for the growth of fungi, such as peptone, glucose, calcium carbonate.

### 2.3 Preparation of different media

#### 2.3.1 Potato dextrose agar

The first type of media which was used is Potato dextrose agar (PDA). This was prepared by adding 5 gm calcium carbonate, 5 gm nutrient agar, 5 gm glucose to 1000 ml distilled water, along with 200 gm boiled potatoes. The media was poured into petri plates after adding 10 gm soil to each plate which was then kept at 25 °C in incubator for 4 days.

Potato dextrose agar (PDA) with peptone:

The second media was prepared by adding nutrients like peptone 1 gm, potato 200gm, glucose 5gm, calcium carbonate 5gm, and nutrient agar 5gm.

The Potato dextrose agar (PDA) made after that was poured into plates and soil was also poured into plates. The plates were kept for 4 days with a temperature of 25°C, and growth of fungal species was observed on this media.

#### 2.3.2 Nutrient agar

Nutrient Agar was used as the enrichment medium for the growth of microorganisms.

Medium was prepared by adding 32gms of dehydrated powder in 1000 ml of distilled water. The prepared media was poured into ten petri plates, then 10 gm collected soil was added to each plate. pH was adjusted by the electrical pH meter at 7.4 and was boiled to dissolve completely. The samples were subsequently incubated at 25 °C in microwave oven for four days. After 4 days, the individual plate's growth occurring in the plates was observed.

#### 2.3.3 Media sterilization

All media were sterilized by using an automatic autoclave (SANYO) at 121 °C for 15 minutes.

#### 2.3.4 Media pouring and drying

Media was poured into pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media. The species were identified on the basis of their culture characteristics.

## 4. Results

In present research work, various fungal species were identified from different samples collected from selected spots of district Mansehra.

The results of the Physico-chemical properties of soil samples show that soil environment of Balakot, Bafa, Oghi are sandy, while the soil samples from Chatter plane and Hazara

University area are found mostly clay, and the remaining sampling site i.e. College Doraha are loamy soils. Fungal growth occurred in all medium. In Potato dextrose agar media, four species were mainly isolated. These are *Cladosporium herbarum*, *Fusarium oxysporum* and *Mucor*. In PDA with peptone medium, *Alternaria solani*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger* species growth occurred. All the isolated fungal species were purified on nutrient agar.

Samples collected from Chattar-plane area revealed *Alternaria* and *Aspergillus* species, while from Balakot, *Cladosporium herbarum* was identified. *Fusarium oxysporum* was found in Hazara University PCB ground. *Mucor* was found in Oghi samples. *Dermatium* was found in Bafa.

Table 1 show the area from where fungal species isolated.

**Table 1:** Area wise distribution of fungal species isolated during current study

Area	Fungal species isolated
Chattar-plane	<i>Alternaria</i> and <i>Aspergillus</i> species
Balakot	<i>Cladosporium herbarum</i>
Hazara university PCB ground	<i>Fusarium oxysporum</i>
Oghi	<i>Mucor</i>
Bafa	<i>Dermatium</i>

Table 2 shows the specific medium which are used for the growth of these different fungal species. The medium and the fungal species which grow on their specific medium are described below.

**Table 2:** Different media which are used for the growth of different fungal species

S. No.	Type of Medium	Type of Fungi growth
01	Nutrient agar	For pure culture
02	PDA	<i>Cladosporium herbarum</i> , <i>dermatium</i> , <i>fusarium oxysporum</i> and <i>Mucor</i> Species.
03	PDA with Peptone	<i>Alternaria solani</i> , <i>Alternaria alternata</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> species growth

The growth of different fungal species which appear on different medium are showing below.

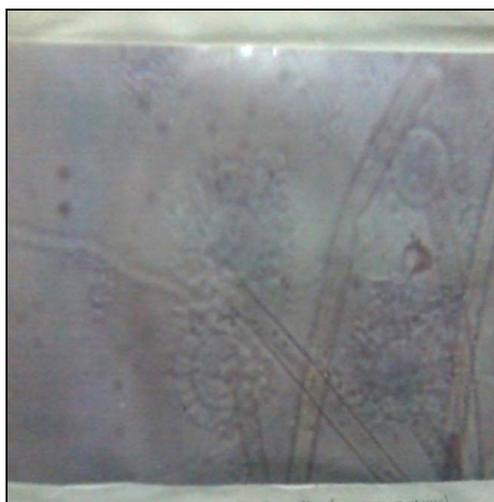
*Mucor fungi* Fig (A), *Aspergillus* (B), *Aspergillus flavus* (C), *Aspergillus niger* (D), *Cladosporium herbarum* (E), *Mucor hiemalis* (F), *Alternaria solani* (G), *Alternaria alternata* (H), *Fusarium oxysporum* (I) and *Dermatium* (J).



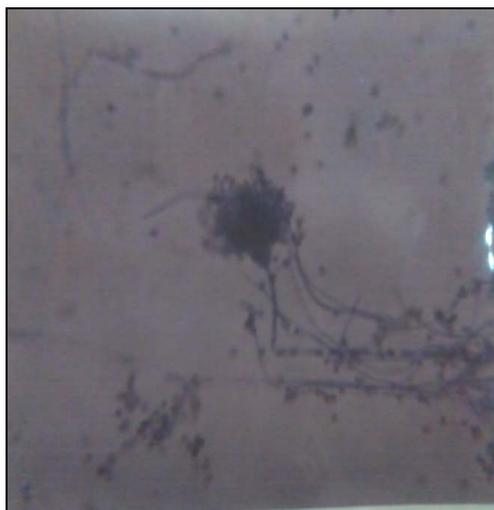
**Fig (A):** *Mucor fungi*



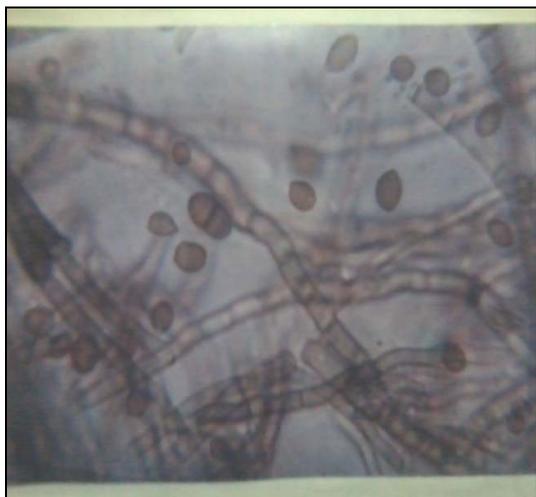
**Fig (B):** *Aspergillus*



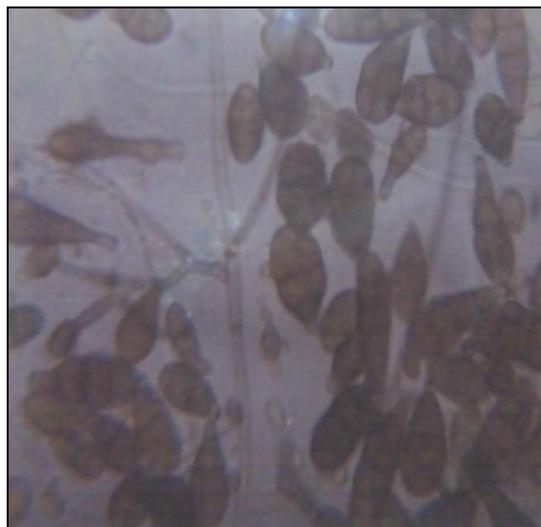
**Fig (C):** *Aspergillus flavus*



**Fig (D):** *Aspergillus niger*



**Fig E:** *Cladosporium herbarum*



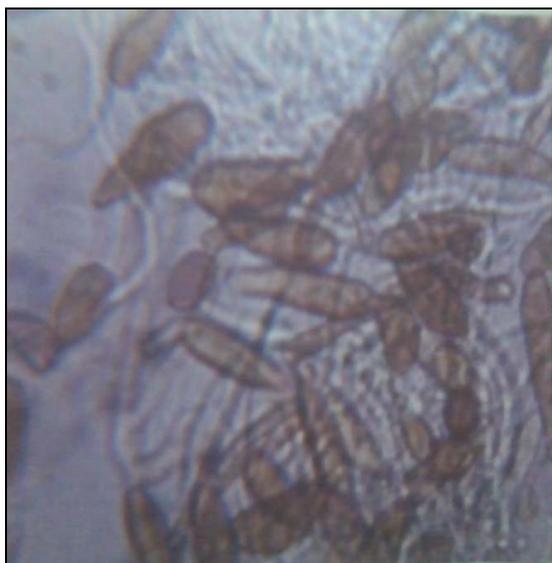
**Fig (H):** *Alternaria alternate*



**Fig (F):** *Mucor hiemalis*



**Fig (I):** *Fusarium oxysporum*



**Fig (G):** *Alternaria solani*



**Fig (J):** *Dermatium*

## 5. Discussion

In this study, survey and screening of the soil fungi were carried out at Hazara University from soil samples collected from different adjacent areas. The study aim was to collect mycoflora from different area and to identify them by application of different techniques and to find out the prevalence of the mycoflora in different habitats. During current study, samples collected from Chattar-plane area revealed *Alternaria* and *Aspergillus species*, while from Balakot, *Cladosporium herbarum* was identified. *Fusarium oxysporum* were found in Hazara University PCB ground. *Mucor* was found in Oghi samples. *Dermatium* was found in Bafa. Our study correlates with the previous study [38,39]. They explained that different environmental factors and more nutrition elements increase the fungal growth frequency in the soil samples. Our study correlate with the study occurrence of *Aspergillus* and *Fusarium* at the sites of Wazirabad and Mandi Bahudin regions was high on both soil and root samples followed by *Penicillium*, *Mucor*, *Alternaria*, *Trichoderma*, *Rhizoctonia*, *Chaetomium*, *Geotrichum* and *Phytophthora*. *Aspergillus* and *Fusarium* shows from root and soil samples of Wazirabad and Mandi Bahudin regions [40]. These are comparable with another relevant research work on the soil mycoflora in different crop fields the most common among them viz; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus nidulans* were isolated and characterized [41].

The results indicate that these great differences occurred due to the geographical location, fungal growth media as well as due to applying different sampling methods. [42-43] also investigated the variations among different fungal species. They also revealed that the variation in results occurred due to variable growth media, different locality and due to the implementation of different methods. From the recent studies conducted at the beginning of the 21<sup>st</sup> century indicate that during the winter season the microbial community of Arctic soil is dominated by saprotrophic fungi [44] but fungal activity throughout the growing season cannot be neglected because of its important role in supplying Arctic plants with mineral nutrients [45].

## 6. Conclusion

The present research work shows that different biologically important fungi are present in the collected soil samples. Almost all the collected samples showed the presence of various species. From this work it is concluded that soil is a good niche for fungus. We can easily isolate various medicinal and industrial important fungus species from the soil.

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