

## Isolation and identification of fungi from soil sample of different localities of agricultural land in Dehradun



### Biotechnology

**KEYWORDS :** mycoflora , Aspergillus, fusarium , Rhizopus, lacto phenol

**Neha Saini**

Department of Botany, Uttaranchal college of biomedical sciences & Hospital.

**Sandeep Dhyani**

Department of Botany, Uttaranchal college of biomedical sciences & Hospital.

**Divya Dimri**

Department of Biotechnology, Uttaranchal college of biomedical sciences & Hospital.

### ABSTRACT

The mycoflora were isolated by using soil dilution technique and soil plate technique on Potato Dextrose Agar medium supplemented by suitable antibiotics i.e streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. *Aspergillus* was dominant in soil sample followed by *fusarium* and *Rhizopus*. The most common specie which are successfully identified among them was *Aspergillus niger*. *Aspergillus niger* was identified, based on their morphological and biochemical characteristics such as conformation by lacto phenol cotton blue staining and Microscope .

### INTRODUCTION

The English word fungus is directly adopted from the Latin word fungus (mushroom), used in the writings of *Horace* and *Pliny*. This in turn is derived from the Greek word sphongos , which refers to the macroscopic structures and morphology of mushroom and molds. Soil is a very species rich habitat containing all major groups of microorganism like bacteria, algae, protest, and fungi. Fungi constitute a group of microorganisms that are widely distributed in environment especially in soil (Boer *et al.*, 2005). The great majority of fungal species have at least one part of their lifecycle in soil (Bridge and Spooner, 2001). The occurrence of fungi varies with depth of soil (Baruah, 1982). *Aspergillus* and *Rhizopus*, are generally found on the superficial layer of soil whereas *Penicillium* and *Alternaria* found in deep soil.

Since the 1940s, fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases and insect pests. Many species produce bioactive compounds called mycotoxins, such as alkaloids and polyketides, that are toxic to animals including humans. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases (e.g., rice blast disease) or food spoilage can have a large impact on human food supplies and local economies. However, little is known about the true biodiversity of Kingdom Fungi but about 5% of these having been formally classified phylogenetically.

### Material and Methods

#### Site study

Dehradun, is the district capital of Uttarakhand. Doon Valley, part of district Dehradun (latitude – 29°58' and 30°32' N and longitude – 77°35' and 78°20'E) comprises of 2 main river basins, namely, the Ganga river basin and the Yamuna river basin.

#### Method of collection of soil sample:

The soil samples from three different agricultural lands in three different localities of Dehradun were collected (Table: 1). The soil sample was collected using augers by digging up to 12cm deep and was immediately scooped into sterile polythene bags using the hand trowel. The samples were collected from three spots in each site and were brought to lab for further study. Then the soil sample from three localities were mixed together in order to obtain a representative sample.

**Table 1: Agricultural soil samples collected from different places in Doon Valley**

Sample No.	Agricultural field	Locality
1	Wheat	Sewla khurd
2	Paddy	Sewla kala
3	Wheat	Subhashnagar

#### Physico-chemical analysis of soil:

The collected soil was characterized for its physico-chemical properties (Table: 2). The physico-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH and moisture content were analyzed. The soil texture was determined by wet sieving technique (Barbour *et al.*, 1980). Soil pH was determined using pH meter with a glass electrode in mixed soil water 1:2 ratio and by electrometric method, following Brady (1990). Moisture content of soil sample was calculated by oven drying the soil and determining the weight loss (Garrett, 1963)

**Table-2: Physico-chemical properties of soil samples collected from different agricultural fields of Doon valley**

S.No.	Crop field	Place	Soil colour	pH	Moisture content
1	Wheat	Sewla Khurd	Yellowish brown	6.0	50%
2	Paddy	Sewla Kala	Yellowish brown	5.5	80%
3	Wheat	Subhashnagar	Yellowish brown	5.1	55%

#### ISOLATION OF FUNGI FROM THE SOIL:

The soil microorganisms were isolated using soil dilution method on media such as Potato dextrose agar.

#### Soil Dilution plate agar:

One gram of soil was added into the tube containing 9mL of sterile distilled water to obtain 1/10 (stock solution) and a series of 1/100, 1/1000, 1/10,000, and 1/100,000 dilutions was prepared by adding 1mL of solution to 9 ml of sterile distilled water respectively (Waksman & Fred., 1922). One mL suspension from each dilution was transferred onto Potato Dextrose Agar (PDA) (Johnston & Booth, 1983) media.

The fungal culture was raised by using spread plate technique, 0.1ml of diluted sample was plated in a sterile petri plates, containing selective media i.e. PDA. 1% streptomycin solution was added to the medium before pouring into petri plates for preventing bacterial growth. The plates were incubated at 37°C for 48 hrs. After incubation colonies were examined under the microscope for ascertaining the identity of fungi with the help of lactophenol staining.

#### Purification of isolated fungi :-

The morphologically different colonies from the medium were picked up and purified. For purification, streak plate technique

was followed. Repeated streaking was done to obtain morphologically homogenous colonies.

The isolated fungi were purified by point inoculating them on plates containing PDA (Potato dextrose agar) medium. The fungi were purified by repeated point inoculation. The purity of the isolated fungus was confirmed by microscopic examination of the culture at

400X magnification using light microscope. After ensuring purity, the cultures were sub cultured on PDA slants and allowed to grow for a period of 5-7 days and subsequently stored at 4°C as stock cultures.

#### Identification of fungi:-

Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue which was observed under compound microscope for the conidia, conidiophores and arrangement of spores .

On the basis of their colony and morphological characteristics, the fungi were identified. Lacto phenol cotton blue stain was used as mounting fluid. The slides were observed under microscope and fungi were identified. The morphological characteristics evaluated included colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc. The characteristics were compared with the standard description of, 'A manual of Soil Fungi', by Gilman, (1957), 'Industrial Mycology' by Onions et al. (1981) and 'Compendium of Soil Fungi' by Domsch and Gams (1980).

#### Result and discussion:

Various factors are responsible for the fungal diversity. The common fungi found *Aspergillus*, *Rhizopus* and *Fusarium*. A fungal species of belonging to the phyla ascomycota, and genera *Aspergillus* (Eurotium) were successfully identified after staining with lactophenol cotton blue based on their morphological characters and microscopic analysis. Rests of the strains were not identified owing to the lack of sporulating structures under present incubation conditions. On PDA *Aspergillus* colonies attained 5.5cm in diameter in seven days, with white to pale yellow margin and black conidial heads. Conidiophores attained a length upto 3.0 cm and width of 15.0 cm and were observed with white to pale walled with hyaline at the base which became brownish at the apex.

Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture. Physico-chemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures determined the fungal population and their diversity in agricultural fields of Dehradun. During the investigation the maximum fungal species belongs to Ascomycotina (Figure: 1) and the genera *Aspergillus* (*sp Aniger*)

In the present study species of *Aspergillus* are not only dominant but also common in soil under study. PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. *Aspergillus* was the only genus that was distributed in all the types, indicating that it adapts easily to different environments well; followed by *Fusarium* (Figure: 2) and *Rhizopus*. The later genera have rare distribution with special requirement.

The Properties like organic matter, pH and moisture content etc., affects the density and diversity of microbes in the soil. From the present study it is clear that pH of soil samples generally alkaline. The soil pH of different type of soil samples in this study was nearly natural, favouring microbial growth. Common

species found in upper were rarely found in deeper soils. The majority of the taxa showed changes in quantity with soil depth. Rama Rao (1970), Domch and Gams (1972) suggested that species of *Aspergillus* are more common in tropical soils. In the present study the species of *Aspergillus* are not only dominant but common to the soil under study.

The moisture content in soil acts as solvent and is essential for microbial functioning. A certain minimum level of organic matter and moisture content is essential to ensure the presence of an active microbial population in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms.

Total 3 fungal genera were isolated from the soil sample, but the most dominant is *Aspergillus* (Figure: 3). The genus *Aspergillus* includes about 160 species and out of these 44 species were reported in India.



Figure: 1

Figure: 2



Figure: 3

#### Conclusion:

The present study undertaken to comprehend the hyphomycetous fungal diversity. Diversity was found to be higher in the agricultural fields and garden soils as compared to the unattended barren land. This indicates utilization of superior quality of soils for plantations. The studies also suggest that agricultural soil samples and especially the garden soil need amendment. Among the various genera of Hyphomycetes as *Aspergillus* was the only genus that was dominant followed by *Rhizopus sp* and *Fusarium sp*. The reason behind genera with rare distribution of *Fusarium* and *Rhizopus* is lack of nutrition which means less organic matter in the soil

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