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**ORIGINAL ARTICLE**

# Isolation and Identification of Soil Mycoflora from Different Populated Area of Bareilly City

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**ABSTRACT**
*A total of seventeen species belonging to thirteen genera of fungi were isolated from soil samples collected from diverse part of city Bareilly during November 2012 to March 2013 at three intervals. The mycoflora were isolated on Yeast powder soluble starch (YpSs) agar added with antibacterial substances streptomycin. Identification and characterization of the mycoflora were made with the help of available literature & photomicrograph. The most common fungal forms were Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Trichoderma viride, Rhizopus oryzae and Botrytis cineria while Curvularia clavata, Fusarium oxysporum, Fusarium solani, Curvularia lunata were unusual frequency of occurrence of the isolates was also evaluated.*
***Key Words:*** *Bareilly, Agricultural Fields, Mycoflora diversity.*

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## INTRODUCTION

Soils are very composite systems, with many components playing diverse functions mainly due to the activity of soil organisms [3]. Soil mycoflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth [14] by biochemical transformation and mineralization activities in soils. Type of cultivation and crop management practices found to have greater influence on the activity of soil mycoflora [20]. Continuous use of chemical fertilizers over a long period may cause imbalance in soil mycoflora and thereby indirectly affect biological properties of soil leading to soil degradation [19]. Fungi are an important component of the soil micro biota [5]. Micro fungi play a focal role in nutrient cycling by regulating soil biological activity [12]. Indirect accumulation in higher trophic level organisms, such as mammals, may cause health problems over time because of the increasing levels of toxic compounds within the body. There are two main reasons that these compounds persist in nature. First, the conditions necessary for their biodegradation are not present. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site. If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavorable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation [8]. The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. In addition to chemical fertilizers and wide range of pesticides shows adverse effect on mycoflora the members and kinds of micro organisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration, pH and temperature etc. The aim of the present investigation is to isolate mycoflora from different fields, and to observe the percentage contribution of different fungi by soil dilution method and soil plate method.

## MATERIALS AND METHODS

### Study site and location:

Bareilly is a district in the northern Indian state of Uttar Pradesh & situated at the bank of river Ramganga. It is located 252 kilometers (157 mi) north of the state capital, Lucknow and 250 kilometers (155 mi) east of the national capital new Delhi. Bareilly is located at 28°10′N 78°23′E. Bareilly

is known to have moderate climate. The low-lying Ganges plains provide fertile alluvial soil suitable for agriculture. Nature of the soil is generally sandy loam, loam, clay loam, silt loam.

**Collection of soil samples:**

The soil samples were collected from six different fields in various locations of Bareilly city. Soil samples of six fields at Bareilly city were collected during Nov. 2012 to July 2013 in three intervals .The soil samples were collected from different fields (up to 15cm depth ) into a small sterilized polythene bags and brought to laboratory for further studies (Table: 1).

**Table -1: collection sites of study**

| Sample No | Place |
|---|---|
| **1.** | Karamchari nagar |
| **2.** | Shastringar |
| **3.** | Sanjaynagar |
| **4.** | University campus |
| **5** | Chaupula |
| **6.** | Rithora |

**PHYSIOCHEMICAL CHARACTERIZATION OF SOIL SAMPLES:**

Physicochemical parameters include pH, water content and temperature etc. microbial population density generally decreases with depth as a function of the availability of organic carbon and molecular oxygen, parameters which typically decrease with depth. Temperature and color of the soil samples was recorded on the spot. pH was measured according to standard procedure as given below (Table 2).

**pH of soil sample**: Soil sample were dried at $60^0$C for 72 h, powdered in pestle and mortar and filtered through 2 mm sieve and the sieved soil were dissolved in distilled water (2.5w/v) and vortexing for 5 minutes at 120 rpm then pH was measured by digital pH meter (Table 2).

**Table -2: Physiochemical parameter of soil samples**

| Sample | color | temperature | pH |
|---|---|---|---|
| **K1** | black | $26.1^0$C | 8.61 |
| **K2** | gray | $27.5^0$C | 8.55 |
| **K3** | black | $28.1^0$C | 8.62 |
| **SH1** | brown | $26.2^0$C | 8.51 |
| **SH2** | brown | $26.8^0$C | 8.53 |
| **SH3** | black | $28.3^0$C | 8.52 |
| **SA1** | black | $26.4^0$C | 8.44 |
| **SA2** | black | $26.8^0$C | 8.43 |
| **SA3** | black | $27.8^0$C | 8.43 |
| **C1** | brown | $26.4^0$C | 8.55 |
| **C2** | black | $26.2^0$C | 8.54 |
| **C3** | black | $28.8^0$C | 8.53 |
| **R1** | black | $25.3^0$C | 8.71 |
| **R2** | black | $25.7^0$C | 8.73 |
| **R3** | black | $27.2^0$C | 8.74 |
| **U1** | brown | $26.2^0$C | 8.52 |
| **U2** | brown | $26.6^0$C | 8.54 |
| **U3** | brown | $28.7^0$C | 8.53 |

**K-** Karamchari Nagar, **SH-** Shastri Nagar, **SA-** Sanjay Nagar, **CH-** Chaupula, **R-** Rithora, **U-** University Campus.

**ISOLATION OF FUNGI FROM THE SOIL SAMPLES**:

The soil micro fungi were enumerated by two methods, namely Soil Dilution [4] and soil plate method [1] on a growth media such as yeast powder starch soluble Agar (Ypss).

**Soil dilution plate method** (Waksman, 1922): 1gram of soil sample was suspended in 10ml of double distilled water to make microbial suspensions ($10^{-1}$ to $10^{-5}$). Dilution of $10^{-3}$, $10^{-4}$ and $10^{-5}$ were used to isolate fungi. 1 ml of microbial suspension of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile yeast powder starch soluble Agar (YpSs). One percent streptomycin solution was added to the medium before pouring into petriplates for preventing

bacterial growth. The Petri dishes were then incubated at 28±20$^0$C in dark. The plates were observed everyday up to three or four days.

**Soil plate method** (Warcup, 1950): About 0.005g of soil was scattered on the bottom of a sterile petridish and molten cooled (40-45°C) agar medium (YpSs) was added, which was then rotated gently to disperse the soil particles in the medium. The Petri dishes were then incubated at 28 ± 20$^0$C in dark for three days.

**IDENTIFICATION OF THE SOIL FUNGI:**

Fungal morphology were studied macroscopically by observing colony features (Color and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, Conidiophores and arrangement of spores [13].The fungi were identified with the help of literature [6,1] and photomicrograph.

**STATISTICAL ANALYSIS:**

The number of colonies per plate in 1g of soil was calculated. The percent contribution of each isolate was calculated by using the following formula:

$$\% \text{ contribution} = \frac{\text{Total no. of CFU of an individual species} \times 100}{\text{Total no. of CFU of all species}}$$

** CFU-Colony Forming Unit*

**RESULTS & DISCUSSION**

The Soil pH, organic content and water are the main factors affecting the fungal population and diversity [2]. The organic carbon, nitrogen, phosphorus, potassium are important for fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot [18]. It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high [6, 7] has reported that environmental factors such as pH, moisture, temperature, organic carbon, organic, nitrogen play an important role in the distribution of mycoflora. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture [15, 4].During the investigation period 161 fungal colonies of 8 fungal species were observed (Table 3). The maximum fungal species belongs to ascomycotina and Zygomycotina were observed. In our findings, *Aspergillus niger* (13.04%), *Aspergillus flavus* (13.04%), *Aspergillus fumigates* (13.6%), *Aspergillus terrus* (10.5%), *Aspergillus nidulance (9.3%), Botrytis cineria (5.59%), Rhizopus oryzae (8.07%), and Trichoderma viride* (8.07%) were found. *Aspergillus fumigates* (13.6%), *Aspergillus niger* (13.04%), *Aspergillus flavus* (13.04%), *Aspergillus terrus* (10.5%) were the dominant species, they show vigorous growth and were found in large numbers. The frequency of mycoflora in different fields were found to be regulated by many factors like temperature, humidity, vegetation, organic and inorganic materials, soil type and texture. The fungi were mostly observed in month of June to September due to suitable temperature and humidity.

**Table-3: Frequency of mycoflora in different places at Bareilly city**

| S. NO. | Place | Average No. of total colonies | Average no. of individual colonies | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Aspergillus* | | | | | *Botrytis cineria* | *Trichoderma viride* | *Rhizopus Oryzae* |
| | | | An | Afl | Afu | Ani | At | Bc | Tv | Ro |
| **1** | Karamchari Nagar | 30 | 2 | 4 | 5 | - | 3 | 2 | 2 | 1 |
| **2** | Shastringar | 28 | 3 | 2 | 4 | 3 | 4 | 3 | 3 | 2 |
| **3** | Sanjaynagar | 25 | 4 | 3 | 3 | 2 | - | - | 1 | 2 |
| **4** | University campus | 29 | 3 | 3 | 4 | 3 | 2 | 2 | 3 | 3 |
| **5** | Chaupula | 26 | 4 | 4 | 3 | 4 | 3 | 1 | 1 | 2 |
| **6** | Rithora | 23 | 5 | 5 | 3 | 3 | 5 | 1 | 3 | 3 |
| total | | 161 | 21 | 21 | 22 | 15 | 17 | 9 | 13 | 13 |
| % Contribution | | | 13.04 | 13.04 | 13.6 | 9.3 | 10.5 | 5.59 | 8.07 | 8.07 |

An - *Aspergillus niger* Ro- *Rhizopus oryzae* Afu- *Aspergillus fumigatus* Tv- *Trichoderma viride*
Afl- *Aspergillus flavus* Bo- *Botrytis cineria* Ani- *Aspergillus nidulans* At- *Aspergillus terrus*

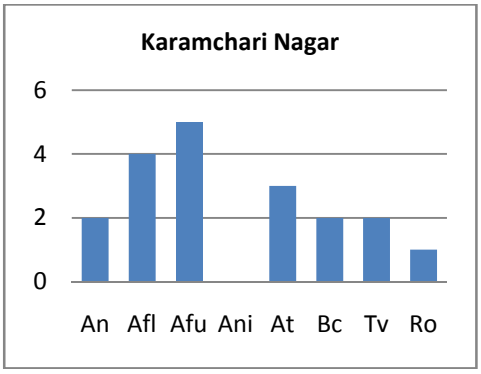


**Chart-1:** % contribution of mycoflora At karamcharinagar

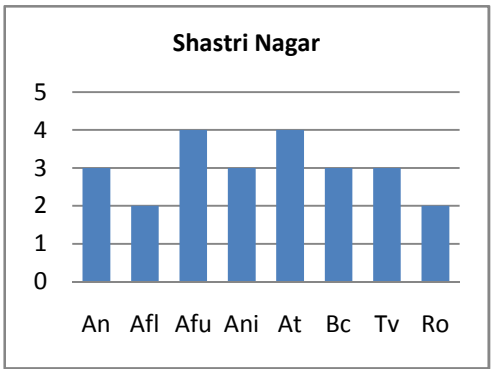


**Chart-2:** % contribution of mycoflora at Shastri nagar

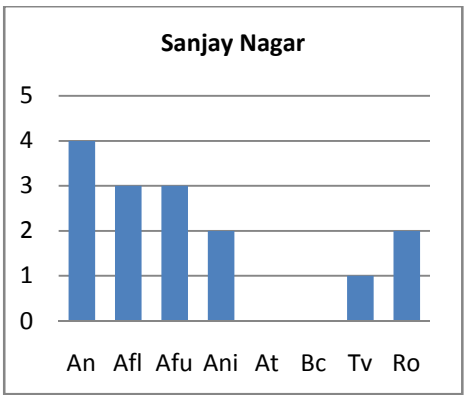


**Chart-3:** % contribution of mycoflora At sanjay nagar

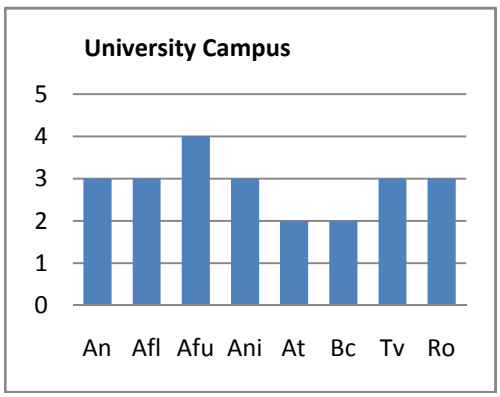


**Chart-4:** % contribution of mycoflora at university campus

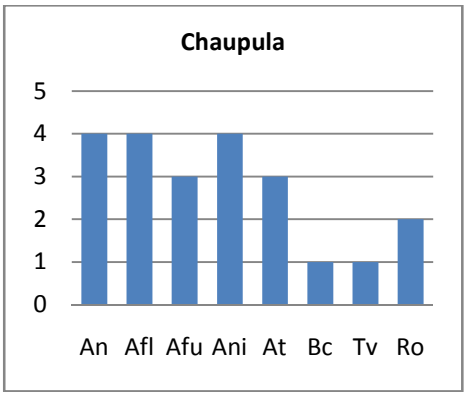


**Chart-5:** % contribution of mycoflora At chaupula

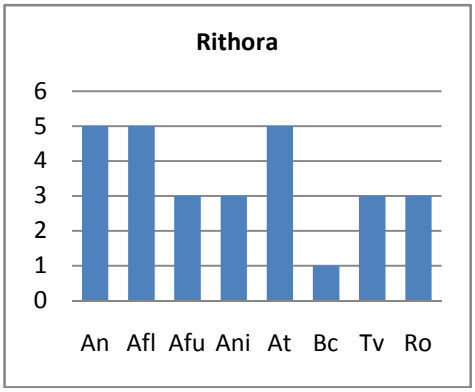


**Chart-6:** % contribution of mycoflora at rithor

**Percent contribution of mycoflora in six different fields at Bareilly- An** *-Aspergillus niger,* ***Afl****-Aspergillus flavus* , **Afu**- *Aspergillus fumigatus,* **Ani**- *Aspergillus nidulans,* **At**- *Aspergillus terreus,* ***Tv****-Trichoderma viride,* **Bc-** *Botrytis cineria*

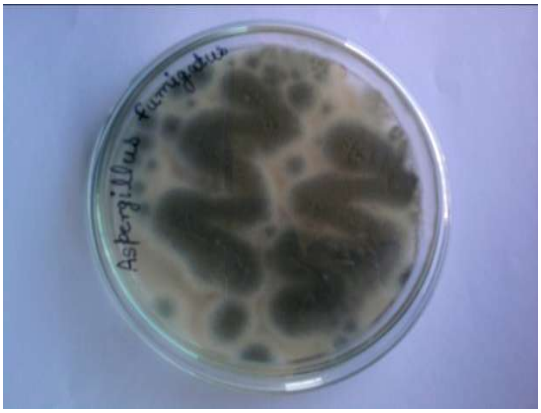


**Fig-1:** *Aspergillus fumigatus*

**Fig-2:** *Aspergillus nidulans*

**Fig-3:** *Aspergillus terreus*

**Fig-4**: *Botrytis cineria*

**Fig-5:** *Rhizopus oryzae*

**Fig-6:** *Trichoderma viride*

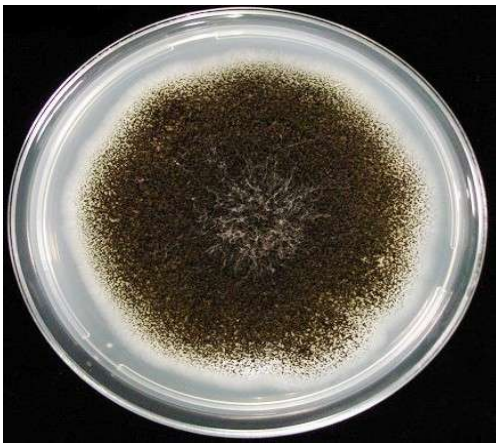

**Fig-7:** *Aspergillus niger*

**Fig-8**: *Aspergillus flavus*

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