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Isolation and Identification of soil born fungi from agricultural fields of Dehradun, India

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Abstract
Most of the soil born fungi are pathogens with the number of more than 8,000 species which cause diseases to agricultural crops. The agricultural plants are susceptible to these soil borne diseases that is the most concerning part. These fungi present a common threat to both agricultural production and the health of healthy and immuno-compromised individuals. Taken together, these relative fungi can cause huge economic losses to agriculture, loss of food for consumption, and serious, often fatal diseases in humans and animals. In the present study the soil samples were collected from different agricultural fields of Dehradun (Uttarakhand), India. The isolation of fungal isolates was done on SDA media by following serial dilution and spread plate technique at temperature and incubation period of 30 ^{0}C and 24 hours respectively. Based on the morphological and gram staining results a total of 9 different species of soil borne fungi were isolated, among which *Rhizopus* and *Mucor* were dominant. Further it was found that 3 different species of *Aspergills* viz. *A. versicolor*, *A. glaucus* and *A. flavus* were also identified. All three species of *Aspergillus* are considered as pathogens that damage the agronomically important crops and *A. flavus* is able to cause diseases in economically important crops, such as maize and peanuts, and to produce potent mycotoxins. It was concluded that the soils of the agricultural fields have a good diversity of fungal species and some of the isolates were pathogenic as per the available scientific literature. Hence the research provides the early alarm for the presence of pathogenic fungi that can be detrimental to economically important crops of the regions and thus the loss can be prevented by taking early preventive measures.

Keywords: soil born fungi, agricultural fields, isolation, serial dilution, *Aspergillus*, crops

Introduction
Soil provides home for the growth of all living organisms I,e plants, animals and microorganisms. Soil has moisture holding capacity and it maintains adequate aerations. Among all microorganisms grown in soil fungi grows dominantly (Ali-Shtayeh and Jamous, 2000; Rane and Gandhe, 2006) [17]. Soil harbors fungi and other macro and microorganisms viz bacteria, worms, beetles and plants etc. Soil, the mantle of weathered rock contains nutrients and minerals in addition to organic matter (Sylvia *et al*., 2005) [19]. Soil habitats are full of life and the soils which are rich in keratinous material are the most conductive for the occurrence and growth of keratinophilic fungi (Ingham, 2007) [12]. Soil habitats can be different and the characteristics of soil affect what lives in it.
Fungi belong to kingdom Myceteae and are cosmopolite in distribution. They can grow in any habitat where life is possible. The microscopic cells of fungi grow usually as long strands or threads called hyphae. The hyphae push their way between soil particles, roots and rocks. The significant characteristics of fungi are that, they are eukaryotic, heterotropic or non photosynthetic, lack tissue differentiation and their cell wall is composed of chitin or other polysaccharides and they propagate by spores sexually and/or asexually (Benson, 2002) [4]. Fungi play important services in the fields of water dynamics, nutrient cycling, and disease suppression and they also play important role as decomposers along with bacteria in the soil food web. Decomposers, mutualists and pathogens are the three general categories of soil fungi created on the bases of how they get energy. Decomposers help in breaking down of dead organic matter, mutualistic fungi colonize the plant roots and provide nutrients to plant in exchange for shelter and carbon (an energy source). Pathogens are responsible for diseases or death of living organism on which they colonize and feed (Tugel *et al*., 2000; Brady and Weil, 2002) [20].

Material and Methods
Study area
Dehradun, the Capital state of Uttarakhand (77°34` to 81°2`E longitude and 28°53`N to 31°27`N latitude) is located in the foothills of Himalaya. The climate of Dehradun is moderate and same as that of other North Indian cities that is cool winters, warm summers, rainy monsoons and a balmy spring. The climate of Dehradun also

depends upon the altitude, the higher we go, the colder we will feel. During the summers, the temperature ranges between 36°C and 16.7°C. In winters, the temperature lies in between 23.4°C and 5.2°C. Dehradun receives the rainfall of 2073.3mm annually.

Though the study was conducted in District Dehradun (77°45` to 78°15`E and 30°00`N to 30°35`N). The investigation covers 4 different sites of Dehradun shown in fig (1). The soil samples were collected from different agricultural fields of these sites. Fields like rice, wheat, sugarcane and mustard were selected for research purpose.

Methodology

The agricultural fields of Shakti Vihar, Chandarbani, Harbajwala and ISBT of Dehradun were randomly selected for study course. Soil samples were collected from different agricultural fields of these sites. Possible information regarding the moisture, colour and fertility of soil were obtained from local population and by observation.

The soils of rice and wheat fields were black in colour, light moistured and easy to dug but sugarcane fields contain brown and totally dry soils. The soils of mustard fields were black, wet or loamy type and crumbly. Further details are given below in table (Table 1).

Table 01: Soil PH, colour and texture of different crop fields of study sites

Sample Sites	Crop field	Soil PH	Soil Colour	Soil Texture
1- Chandrabani	Rice	7.67	Light grey	Clay
	Sugarcane	7.59	Dark grey	Sandy loam
	Mustard	7.02	Dark brown	Sandy Clay
2- Harbajwala	Rice	7.84	Black	Wet Clay
	Sugarcane	6.88	Brown	Sandy loam
3- ISBT	Mustered	7.62	Black	Sandy clay
4- Shaktivihar	Rice	5.43	Light black	Clay
	Mustered	5.43	Dark grey	Wet clay
	Wheat	6.61	Light black	Sandy loam

During study course the 4 different sites (fig 1) were chose and these sites include 9 different agricultural fields. The agricultural fields, rice, mustard, wheat and sugarcane were selected for the purpose of sampling. Three samples were collected vertically from each field, from the surface of soil and at various depths viz, 0-5cm, 05-10cm and 10-15cm. These samples were mixed to make a composite sample. This composite sample was then used for the isolation of fungi. Soil samples were collected by using the method of Brown (1958) [6].

In the laboratory the samples were kept in oven for 24 hours to get dry samples. Then the dried soil was grinded and sieved to remove large debris and stones to obtain soil samples with small particles. The soil was then processed in an isolated process of fungi. First serial dilution was done by adding one gram of each soil sample to 9ml of sterile distilled water in an autoclaved test tube. The test tube was shaken well and a serial dilution up to (10^{-3}) were made by following the same method. One ml of (10^{-3}) dilution was poured in each petri dish containing prepared Sabouraud Dextrose Agar media (SDA media). Pouring was done with the help of micropipette and each sample made by three replication plates and then incubated at 32^0C for 15 days.

After long wait of 15 days good colonies of fungi were grown in petri plates. These fungi were then mounted on a clean glass slide, stained with methylene-cotton blue to observe and detect fungal structures (Basu,1980), before observing under microscope the slide was covered with a cover slip and then examined under microscope and identified on the basis of their colony morphology and spore characteristics. (Ronhede *et al.,* 2005 and Rajankar *et al.,* 2007) [18, 16].

Results and discussion

The above areas (fig.1) of District Dehradun represents a rich diversity of fungal flora. The present study was an attempt to know about diversity of mycoflora found in the said sites. For this reason these areas were surveyed to collect soil samples.

During the course of study 09 different species of fungi belonging to 04 different families were documented which are pathogenic in nature and cause diseases to agricultural crops (Table 2). The families which were isolated are *Nectriaceae, Saccharomycetaceae, Mucoraceae and Trichocomaceae* among these *Mucoraceae* was dominant and the species belonging to these families *are Fusarium oxysporum, Candida albicans, Rhizopus sp, Mucor sp., Aspergillus flavus, Aspergillus glaucus, Aspergillus versicolor* and *Aspergillus niger* respectively

The diversity of fungi in any soil depends on various factors such as pH, organic content, moisture and texture of soil (Neha *et al*, 2016) [14] The Physicochemical analysis of soil showed that pH of soil of different site ranging from 5.4 to 7.8 and soil textures determined the fungal population and their diversity in agricultural fields of Dehradun.

Table 2: Fungal species isolated from the soil collected from different parts of Dehradun

Name of the fungus	Family	Colour of colony	Number of colonies
Rhizopus sp.	*Mucoraceae*	White	19
Mucor sp.	*Mucoraceae*	Black	17
Penicillium	*Trichocomaceae*	Blue	1
Candida albicans	*Saccharomycetaceae*	White	11
Fusarium oxysporum	*Nectriaceae*	White	4
Aspergillus flavus	*Trichocomaceae*	Green	3
A. glaucus	*Trichocomaceae*	Green	5
A. versicolor	*Trichocomaceae*	White yellow	7
A. niger	*Trichocomaceae*	Brown	2

The main aim of current research was to isolate and identify the soil born fungi from different agricultural fields of Dehradun (fig 01). In this research the soil samples from different areas were collected and followed by serial dilution. Finally, pour on the SDA media. Then after 15 days the isolated colonies of fungus were identified.

Here the interesting and rare case we found was many incubated petri plates were filled with different types of fungi in each single petri plate (fig 05). These different fungi differ in morphological view and also each fungal colony had different colour which is seen in very rare cases. Species like *Aspergillus*, *penicillium*, *Mucor* etc were grown in common petri plate which is clearly seen in figure 05.

The soil of different area varies accordingly in colour, texture, drainage, moisture content, pH, organic matter and micro or macro nutrients, these all parameter of soil affecting the diversity of mycoflora (Guleri *et al*, 2012) [11]. The nutrients of the soil are affected by soil pH due to reactions with soil particles and other nutrients (Wright *et al*, 2009*)*. Generally the number of species of fungi decrease with the increase of depth of soil as the necessory organic matter present on the surface of soil required for fungal growth (Guleri *et al*, 2016).

Present study also showed that different physico-chemical properties of soil of any crop affect the growth of mycoflora. So it is necessary to study the physico-chemical properties of soil and relation of mycoflora.

The dominancy as per colony count and species count are shown below (fig 02).

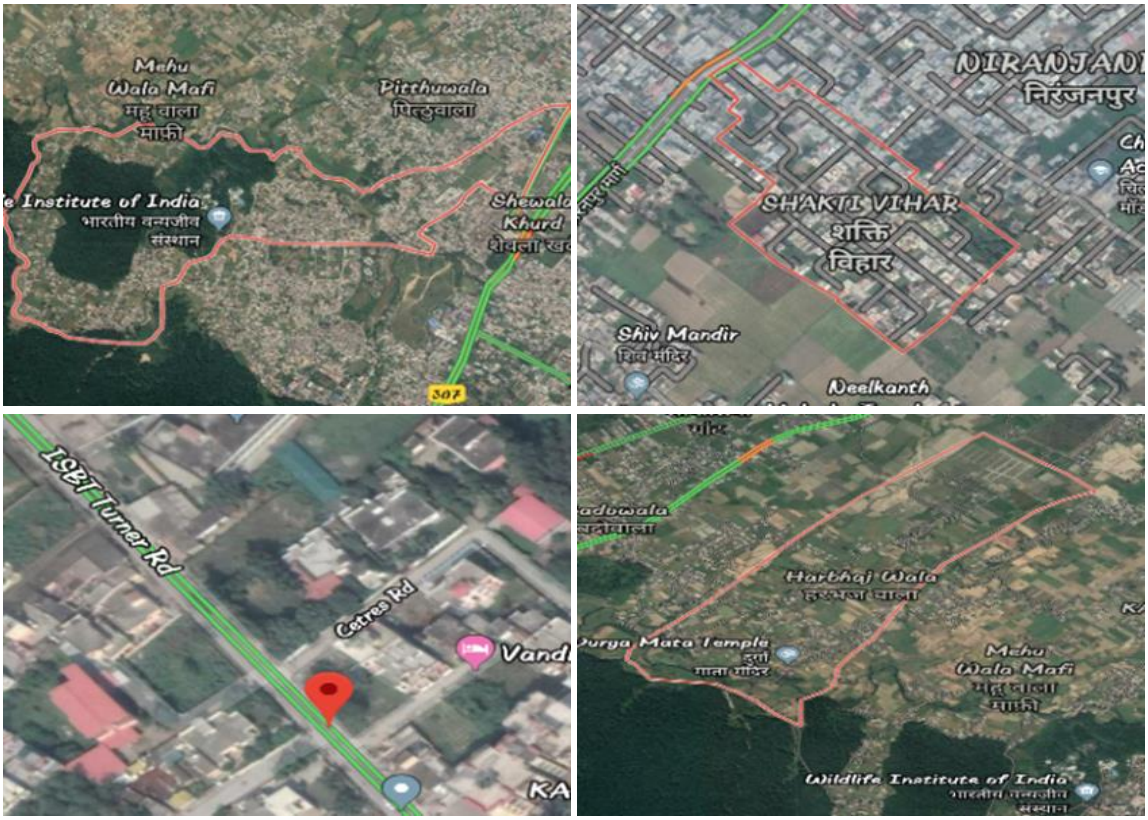

Fig 1: Map of Study Sites

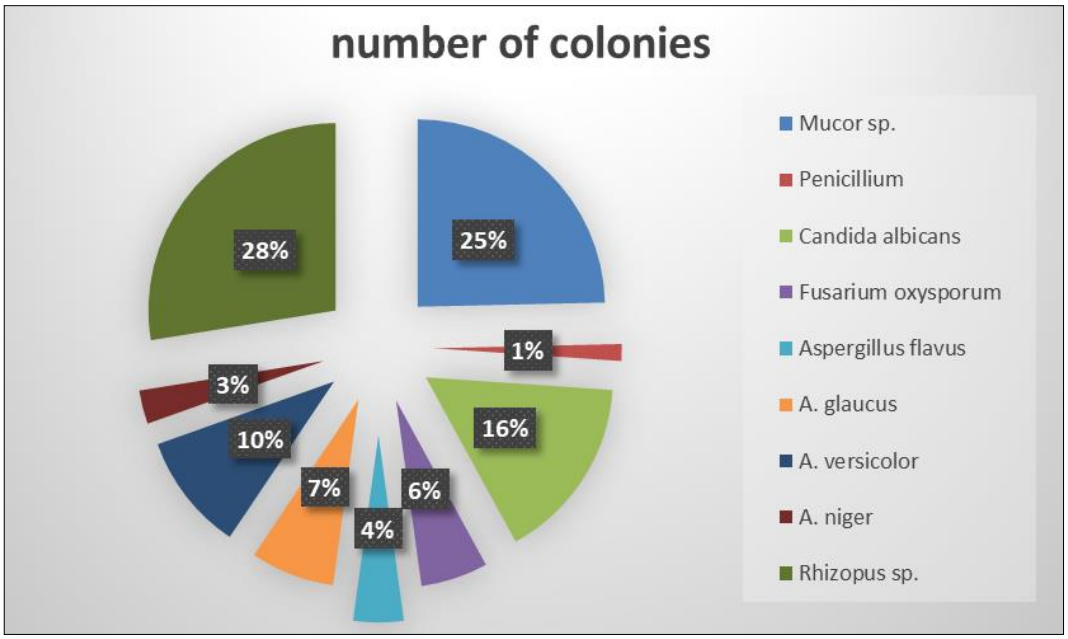

Fig 2: Graph showing dominant species as per colony count

Conclusion

The agricultural sector is of vital importance for the region. In earlier studies from Uttarakhand the agricultural soils were found to have rich diversity of fungal species that was found to have positive relation with the depth and organics of the soil samples (Guleri *et al.*,2016). During our study course we isolated 09 different species of fungi belonging to 04 different families. The dominating family of the area was *Trichocomaceae* but as per colony count *Mucor sp.* were dominant. The study was concluded that the soils of agricultural fields have a good diversity of fungal species and some of the species are pathogenic as per the available scientific literature. The research provides an early alarm for the presence of pathogenic fungi that can be determental to economically important crops of the region. Thus, the loss can be prevented by taking early preventive measures.

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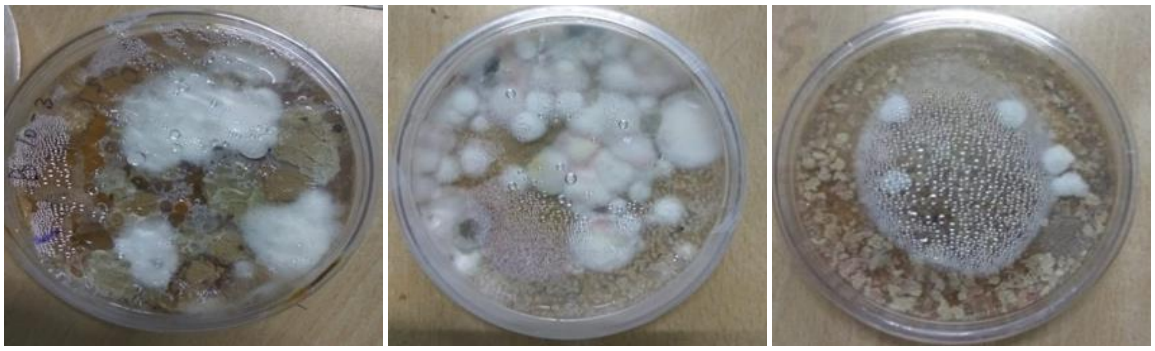
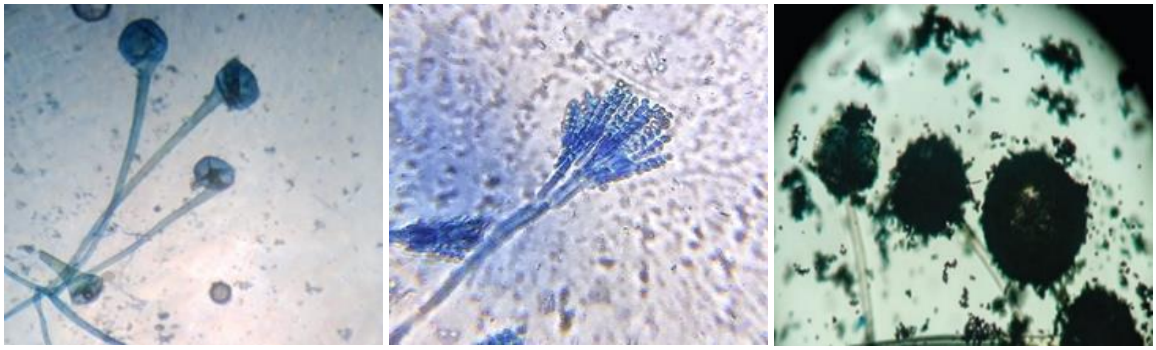


Fig 3: The morphological view of incubated petri plates showing fungal growth

Fig 4: Many petri plates were filled with mixed fungi which is found in rare cases

Fig 5: Streaking plates of isolated fungal colonies



Mucor spp. *Penicillium spp.* *Aspergillus flavus*

Fig 6: Microscopic picture

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