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Isolation and Identification of Soil Fungi of St. Pious X Degree and PG College, Hyderabad

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Abstract
Fungi are one of the most important groups of organisms. Fungi play an important role in many aspects of human life, including medicine, food and farming. 10-fold serial dilution was performed and 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions were taken into consideration for isolation. The media used for the isolation of fungi was PDA (Potato Dextrose Agar) medium. After the inoculation, the plates were incubated at $28^0C \pm 2$. The colonies were observed after 3-4 days of incubation. The criteria for identification was colony morphology followed by staining. The fungal organisms were identified using lactophenol blue staining. The different types of micro-organisms observed were *Aspergillus sps, Fusarium, Trichoderma, Cladosporium, Alternaria and Mucor.* In the present study an attempt was made to isolate the native fungal micro-organisms of our campus as the microflora around the rhizosphere and varies from genus to genus and it is specific to the plant environment and the results interpreted some significant observations which focus on the applied aspects of micro-organisms which hold immense importance in the allied fields.

Keywords
Fungi, Serial dilution, PDA media, LPB staining and biogas effluent will be further used for vermicomposting as well as generation of electricity.

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1. INTRODUCTION
Soil is an important component of the earth's ecosystem and acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients in the soils. Soil has tremendous range of available range of niches, habitats. The soil acts as an excellent source of culture media for the wide growth of bacteria, fungi, protozoans and algae etc. Soil can effectively remove impurities, kill disease agents and degrade contaminants, the latter property is called as natural attenuation. Soils often plant physical strength, air, water, temperature moderation, nutrients and protection from toxins. Soils help in making the nutrients available to the plant by the degradation of organic matter into simpler forms and aids in growth of various types of microbial flora. Plants, animals, fungi and bacteria affect the soil formation. Microorganisms including fungi and bacteria, effect the chemical exchanges between roots and soil and act as a reserve for nutrients in the soil in the soil biological hotspot called "rhizosphere".
In addition to other microbes, Fungi are metabolically complex and play a pivotal role in the breakdown of the complex polymers associated with the litter and other soil-borne organic debris (R. Michael Miller and Michael s. Fitzsimons, 2011). Fungi are fundamental for the functioning of soil ecosystem (Warcup JH 1951). Especially in forest and agricultural soils, they

play a major role in many essential processes such as organic matter decomposition, elemental release by the mineralization. Micro fungi play a focal role in the nutrient cycling by regulating soil biological activity (Arunachala, *et, al 1997)*. Fungi grow on diverse habitats in the nature and are cosmopolitan in the distribution and it requires several specific elements for the growth and reproduction.

The most important component of the soil fungi is their biomass, with biomass being directly proportional to the hyphal length. The amount of fungal biomass in the soil represents a significant pool of available nutrients, with its turnover having important consequences for carbon and nutrient cycling(Kjoller and Struwe,1982).Fungi responds dynamically to the environmental conditions and gazing pressures.

The chemical changes occur as a result of humification of organic matter. The resultant mineralization of various organic compounds (phosphorus, sulphur and nitrogen) provides the plants with forms of nutrition that are readily available for the uptake. The turnover of the microbial populations also results in the release of nutrients (Jeyanthi and Rebecca. L *et.al* 2012). The traditional assessment of the soil contamination is based on the regular routine of comparison of allowable threshold values with the results of monitoring. This approach is even a required action in environmental agencies, agricultural administration and managing organization. Very often, solving a a particular problem concerning the soil contamination or respective decision making is based solely on single results and not on a more generalized model about the state of the soil contamination in a certain region. This environmental strategy makes it possible to detect relationships between the chemical pollutants and specific soil parameters, between sampling sites and therefore, to achieve a stratification of the pollution.(Md.Mehedi Hasan Magnet 2013).The present investigation was carried out to identify the fungal diversity of St. Pious X Degree & PG College, Hyderabad.

II. METHODS AND MATERIALS

Collection of soil samples and locations for the study

Different study sites of St. Pious X Degree & PG College have been selected for the collection sites of soil samples. Soil samples were collected from the sites such as Botanical garden of the campus, chemistry laboratory, Rhizosphere regions of sapota and neem tree, Backside of the college, Cafeteria and Hostel premises. Soil samples were taken with the help of a sterile spatula, in a sterile plastic bag. The samples were brought to the microbiology laboratory and stored at 4^0C for further use.

Isolation of Fungi

The technique, soil dilution plate method (Waksman,1922) was used to isolate the fungal organisms from the soil samples. 1.0 gram of soil sample was suspended in 10ml of distilled water to make microbial suspensions 10^{-3},10^{-4} and 10^{-5} were used and 0.1 ml of microbial suspension of each concentration were added to sterile Petri dishes containing sterile potato dextrose agar media.

Preparation of Lacto-phenol Cotton Blue Slide Mounts

The lacto-phenol cotton blue (LPBS) wet mount preparation is the most widely used method of staining and observing fungi. The stain has three components: Phenol, which will kill any live microorganisms. Lactic acid which preserves the fungal structures, and Cotton blue which stains the chitin in the fungal cell walls. The samples were immersed in a drop of alcohol and it was viewed under the microscope after the addition of two drops of the lacto-phenol/cotton blue/stain. (Jeyanthi Rebecca. L *et al* 2012).

III. RESULTS AND DISCUSSION

RESULTS

Collection of soil samples and locations for the study

Different study sites of St.Pious X Degree & PG College have been selected for the collection sites of soil samples. Soil samples were collected from the sites such as Botanical garden of the campus, chemistry laboratory, Rhizosphere regions of sapota and neem tree, Backside of the college, Cafeteria and Hostel premises. **(Table-1)**. Soil samples were taken with the help of a sterile spatula, in a sterile plastic bag. The samples were brought to the microbiology laboratory and stored at 4^0C for further use.

Isolation of Fungi

Soil dilution plate method (Waksman,1922) was used to isolate the fungal organisms from the soil samples. PDA medium is the most commonly used culture growth by several workers worked with it earlier due to its simple formulation and potential to support wide range of fungal growth. A total of 104 colonies were enumerated (Table-2). 6 genera of fungi were identified. Maximum number of colonies belonged to genera Ascomycetes, Deuteromycetes and Phycomycetes.

Lacto-phenol Cotton Blue Slide Mounts

The Lacto-phenol cotton blue (LPBS) wet mount preparation is the most widely used method of staining and observing fungi. Identification was done by preparing the LPCB mounts. In the present study *Aspergillus sp, Fusarium sp, Alternaria sp, Trichoderma sp, Cladosporium sp, Mucor sp* were observed. (Table-3).

Table 1: Locations taken for the study

S.No	STUDY SITE
1.	Botanical garden Soil (BG)
2.	Backside of the Chemistry Laboratory (BSCL)
3.	Sapota and Neem tree (SANT)
4.	Backside of the college (BC)
5.	Near the Cafeteria (NC)
6.	Near the Hostel Premises (NH)

Table 2: Total number of fungal colonies at different locations

S.No	STUDY SITE	TOTAL NUMBER OF FUNGAL COLONIES
1.	Botanical garden Soil (BG)	29
2.	Backside of the Chemistry Laboratory (BSCL)	13
3.	Sapota and Neem tree (SANT)	13
4.	Backside of the college (BC)	9
5.	Near the Cafeteria (NC)	21
6.	Near the Hostel Premises (NH)	19

Table 3: Number of fungal colonies at different study sites

1.	Botanical garden Soil (BG)	*Aspergillus sps*	26
		Fusarium sps	03
2.	Backside of the Chemistry Laboratory (BSCL)	*Aspergillus sps*	10
		Fusarium	03
3.	Sapota and Neem tree (SANT)	*Trichoderma*	05
		Aspergillus sps	06
		Trichoderma	01
		Fusarium	01
4.	Backside of the College (BC)	*Trichoderma*	02
		Aspergillus	03
		Fusarium	01
		Cladosporium	02
		Alternaria	01
5.	Near the Cafeteria (NC)	*Aspergillus sps*	13
		Fusarium sps	07
		Alternaria	01
6.	Near the Hostel Premises (NH)	*Aspergilllus sps*	07
		Fusarium	07
		Trichodertma	01
		Alternaria	03
		Mucor	01

DISCUSSION

There is a vast microbial flora inhabiting the earth and they are found in all types of soils. These microbes may interact with the plants resulting sometimes in useful effect and other times in harmful consequences. Fungi are an important component of the soil microbiota and are present as mycelial bits, rhizomorphs or as spores. they play significant role in soils and plants nutrition (Ratna kumar P.K *et.al* 2015*).* The number and kinds of microorganisms found in soil depend upon the nature of soil, depth, season of the year, state of cultivation, reaction, organic matter, temperature, moisture, aeration etc,. Fungi are fundamental for soil ecosystem functioning. Especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization. The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. The members and kinds of microorganisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeratiuon, pH and temperature etc. Microfungi play a focal role in nutrient cycling by regulating the soil biological activity. (Gaddeyya.G *et.al* 2012).

The soil samples were analysed for the presence of different types of fungi. The most common fungi viz., *Aspergillus, Fusarium sps* were found in (Botanical garden soil). *Aspergillus and Fusarium sps* were found in (Backside of the Chemistry Laboratory),*Aspergillus,*

Trichoderma, Fusarium sps were found in (Near Sapota and Neem tree),*Aspergillus, Trichoderma, Alternaria, Fusarium, Cladosporium sps* were found in (Backside of the College),*Aspergillus sps, Alternaria, Fusarium sps* were found in (Near the Cafeteria), *Aspergillus sps, Trichoderma, Alternaria, Mucor* were found in (Near the Hostel premises).

Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture. (Alexander, M., 1977, Rangaswami, G and Bagyaraj,D.J.,1998). In our present study fungal isolates of genera *Aspergillus sp, Fusarium sp* were dominant, found in large numbers and showed profuse growth.

The most important component of soil fungi is their biomass, with biomass being directly proportional to hyphal length (Joergensen and Wichern, 2008). The amount of fungal biomass in a soil represents a significant pool of available nutrients, with its turnover having important consequences for carbon and nutrient cycling. (Langle and Hungate, 2003)

The studies on fungal diversity an percentile contribution and periodic occurence of soil mycoflora are useful for farmers, agronomists, researchers and microbiologists for future conservation of soil microbial activity and sustainable agriculture. (Ratna Kumar P.K *et,al* 2015).

The quantities of 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 effects on mycoflora the members and kinds of microorganisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration pH and temperature.

Aspergillus sp was found in to be present in almost all the six soil samples taken for the experimental study. *Aspergillus species* are one of the most fast-growing fungal organisms which can grow in varied environments irrespective of various factors governing the growth of organisms. In the present context it was found that these species outnumber the other fungal organisms in terms of growth and number two in all of the six soil samples taken for the analysis.

Botanical garden soils were exposed to constant human and mechanical activity with temperature fluctuations and heterogenous nutrient source and the most possible effect in terms of timely nutrient addition in the form of organic manure support luxuriant growth of more number of mycoflora.

The soils of backside of Chemistry laboratory and near sapota and Neem tree showed moderate colonies number due to the spillage of various chemical waste near the trees and backside of laboratory. These soils were exposed to chemical pollution which may be the reason for the limited growth.

The soils near the Cafeteria and Hostel premises supported growth and the number was found to be moderate among all other soil samples, as the major factor governing the growth of fungal organisms is nutrient availability, there by supporting the growth of different types of fungal organisms.

Aspergillus, Trichoderma, Alternaria, Fusarium, Cladosporium sps were isolated from the soil of rear or back side of the College. Due to the continuous anthropogenic activities the soil encounters the minimum required nutrition and in addition, the available carbon source by-product in the rhizosphere may support the growth of wide range of fungal organisms.

During the course of study, the diversity of mycoflora in different soils may be attributed to the various components like rhizosphere environment, nutrient availability, seasonal variation and the physical and chemical factors varies from genus to genus and is specific to the plant environment and had direct impact on the growth of fungal organisms.

From the above discussion the significant observations focus on the applied aspects of mycoflora which hold immense importance in the allied fields.

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