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Diversity of Soil Fungi from the Campus of Government Arts College, Nandanam, Chennai, Tamil Nadu, India

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ABSTRACT

Keywords

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Soil is a major natural resource in the biosphere in which the fungi are the important component which role play in controlling various physiochemical and nutrient parameters that reflects environment. Therefore, the present investigation was planned to find out the fungal diversity in soil samples collected from the college campus of Government Arts College, Nandanam, Chennai-35, Tamil Nadu, India. Totally, 18 samples were collected randomly from 18 different locations of the college campus, 3 -15 cm depth of surface soils removed and collected sample to analyze the moisture content and the presence of fungi. The moisture content of the soil samples was determined by standard hot-air oven drying method and the analysis of fungi was carried out by standard serial dilution technique with Potato Dextrose Agar (PDA) as a nutrient medium. The results showed that the moisture content of soil samples varied from 10% to 28% with an average of 16%. Further, the analysis of fungi, it was observed that 20 species of fungi in which *Aspergillus niger*, *A. terreus*, *A. flavus*, *A. glaucus*, *A. nidulans*, *A. fumigatus*, *Penicillium citrinum*, *Fusarium oxysporum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Helminthosporium sp.*, *Trichoderma sp.*, and nonsporulating fungi were significantly present. Among the species of fungi enumerated in the study, in point of quantitative view, *Aspergillus niger* showed highest percentage occurrence in soil samples followed by *A. terreus*, *A. flavus*, *Penicillium sp.* and *Fusarium sp.* and the details were discussed. Based on the present analysis, indicates that the soil is the potent source for various fungi which are responsible for the effect of soil parameters and influence the ecosystem.

Introduction

The quality of Soil including physiochemical and biological characteristic features is considered as one of the most important characteristics of soil ecosystems. The integrated approach to soil health and its features depends on the interaction between different processes and properties, with a strong

effect on the activity of soil microbiota such as bacteria, fungi, and protozoa (Magdalena Fraç *et al.*, 2018). Most type of soils can be described using physical, chemical, and biological properties, but adaptation to environmental changes mainly due to the biological parameters. Among the biological organisms present in soil especially the microbes, the fungi are the major component which are active

role in regulate the soil parameters and quality. Fungi determines the good or defective condition of the soil health as it acts as primary decomposer and some of the functions of soil fungi were discovered by Christensen (1989). These microorganisms are essential for the purpose of recycling of dead organic matter and thus making them available for the next generation to maintain the ecological balance in the environment. Recent evidence suggests that out of 1.5 million fungi, about one third of the fungal population exist in India. Out of these fungi present in India, many species of fungi are yet to be identified.

Fungi are very productive inhabitants in soil, due to their high flexibility and their capacity to adopt various forms in response to unfavorable conditions (Sun *et al.*, 2005). Due to their ability to produce a wide variety of extracellular enzymes, they can break down all kinds of complex organic matter, decomposing soil components and thereby regulating the balance of carbon and nutrients. Many species of fungi retain the ability to act as an effective bio sorbent of toxic metals such as cadmium, copper, mercury, lead, and zinc, by accumulating them in their vegetative as well as fruiting bodies, though these elements may inhibit their growth and affect their reproduction (Baldrian, 2003). The diversity and various activity of fungi is regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) factors have been reported by López-Bucio *et al.*, 2015; Roupheal *et al.*, 2015. It is also reported that the fungi can be found in almost every environment and can live in wide range of physiochemical parameters such as pH and temperature (Frąc *et al.*, 2015). Christensen (1989) studied that the fungi define the good or defective condition of the soil health as it acts as primary decomposer and some functions of soil fungi. These microorganisms are essential for the purpose of recycling of dead organic matter and thus making them available for the next generation to maintain the ecological balance in the environment. The scientific reports different parts of the world on fungal diversity concluded that different habitat

exhibited variation in different plant systems and environment and edaphic factors which greatly influence the growth and development of microorganisms (Gentry, 1998; Bohera *et al.*, 1991; Nilima, *et al.*, 2007). More number of research papers is published in recent days regarding agriculture soil fungal diversity and polluted soil including industrial environment than the natural soil. So, in the view of the above information, there is a need to study the diversity of soil fungi in unexplored area. Fungi are not only wonderful organisms in nature, but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural nutrient cycling and many other ways. The exploration of fungi through fungal biotechnology has become an integral part of the human welfare such as food, pharma, textiles, leather industries have been reported (Karthikeyan *et al.*, 2014; Saraswathy *et al.*, 2020). Fungus benefits most plants by suppressing plant diseases and promote nutrient uptake and utilization for plant health by attacking plants pathogens with fungal enzymes. Fungi also use antagonism to reduce competition by producing antibodies, which suppress other microorganisms from growing. They produce many vitamins which promote plant growth. Beneficial fungi also form protective webs and nets around roots and leaves to protect the host plants (Lowenfels and Lewis, 2006). Fungus also protects plants by supplying a protective health to supply both water and phosphorus to the plant roots during droughts (Magdoff and VanEs, 2009). During this Covid19 situation there are other fungal diseases including mucor mycosis (Black fungi), candidiasis (White fungi). Aspergillosis (Yellow fungi) also have been threatening to the human society which needs investigation on these fungal physiology and biochemistry.

With reference to the above scientific literature and background study, the present analysis was carried out to study the diversity of fungi present in the soil atmosphere of the college campus for both qualitative and quantitatively. Where large number of students, teachers and various other people visit

and expose to this soil environment and possible source of contamination also the fungal biodiversity is believed to be more useful on taxonomy and diversity nature of fungi.

Materials and Methods

Collection of soil samples

Soil samples were collected from 18 different locations of the College campus of Government Arts College for Men (Autonomous), Nandanam, Chennai, Tamil Nadu, India for the present study during March 2018. About 100 g of Soil samples from each location were collected in polythene bags pre-cleaned with alcohol and dried by using sterile spatula. The collected samples were tied tightly with rubber bands and labelled properly and then brought to the laboratory for further analysis.

Determination of moisture content(Mishra, 1968)

Exactly 5g of freshly collected soil sample was weighed in a pre-weighed empty glass Petri-dish and the initial weight was noted. The petri-dish with soil sample was placed in a hot air oven for 2 hours at 105 o C. After drying, the soil sample of the petri-dish was cooled to room temperature in a vacuum desiccator and weighed for loss of weight i.e final weight. The loss of weight of the Petri-dish with soil sample was calculated and the percentage of moisture content was determined. The following formula was used for the calculation of moisture content percentage.

$$\begin{aligned} & \text{\% of soil moisture} \\ & \frac{\text{Initial weight (Petridish with soil)} - \text{Final weight}}{\text{Weight of soil sample taken (5)}} \times 100 \end{aligned}$$

Analysis of fungi from the soil samples

In a systematic screening process for isolation of fungus on 18 soil samples. The soil samples examined fungal diversity by serial dilution plating method using Potato Dextrose Agar (PDA) is a nutrient medium (Warcup, 1950). The saline

containing Sodium chloride solution (0.9% w/v) was used as a sterile saline solution for preparation of soil dilution. For the serial dilution method, 1gm of the composite soil sample was dispersed thoroughly in 10ml of sterile saline. From this sample solution, 1ml was transferred to 9 ml sterile saline solution by using micropipette. The resulting solution was mixed well and from this 1ml was pipetted out into a test tube containing 9 ml sterile saline. Likewise, the sample dilutions were made up to the 10^{-5} and the dilutions of 10^{-2} , 10^{-3} and 10^{-4} were used for plating on PDA medium. About 1ml of soil suspension of each concentration was added and speeded on sterile petri-dish containing antibiotic amended PDA medium by spread plate method. Replicates were maintained for each dilution of the samples. The inoculated PDA agar plates were incubated at $30 \pm 1^\circ \text{C}$ in an Incubator for 3 to 7 days till the growth and sporulation of individual species of fungi. Then the developed culture plates were taken for observation of both qualitative and quantitative pattern of fungi and the data were recorded for interpretation of the results.

Qualitative and Quantitative study of fungi from soil samples

Qualitative analysis of fungi

After the incubation, occurrence of sufficient growth of individual species of fungi presents in various soil samples plated on the nutrient agar medium were observed and identified for the qualitative pattern. The fungal colonies present in the soil sample inoculated culture plates were isolated by pure culture method and observed for the colony morphology and microscopic characters. The characteristic features such as colony colour, morphology, growth, and appearance of the colony in PDA and CDA medium; microscopic characters include hyphae structure, conidiophore structure, structure, and shape of conidiosporangium, number of sterigmata, conidia shape and structure of the individual fungal species were observed for further identification. Then the individual species of fungi were identified by using standard identification

manuals for *Aspergillus*, *Penicillium* and *Hyphomycetes* fungi (Raper and Fennel, 1965; Raper, Fennel and Thom, 1949; Barnett and Hunter, 1948; Ellis, 1976).

Quantitative estimation of fungi

Different species of fungi grown on the culture plates were observed after the complete growth of fungi from individual sample plated on agar plates. For the quantitative pattern, the individual colonies of fungi present in each culture plate plated with 1 ml of soil sample were counted and the total number of colonies of fungi were calculated with reference to the dilution made.

The overall population or total number of fungi present in each soil sample is expressed as colony forming units per gram (cfu/g) of soil sample. The total number of fungi and number of individual species of fungi present in 1 g of soil sample were recorded and calculated for the percentage occurrence. The details of percentage distribution of individual species of fungi in individual soil samples were calculated. The following methods were used for the calculation of individual species of fungi and percentage occurrence in each species of fungi.

Occurrence of individual species = No. of individual fungal colonies X dilution factor

$$\frac{\text{Percentage occurrence of fungi}}{\text{Number of individual species}} = \frac{\text{Total Number of fungi}}{\text{Total Number of fungi}} \times 100$$

Maintenance of fungal cultures

The frequently occurring species of fungi such as *Mucor sp.*, *Aspergillus terreus*, *A. niger*, *A. flavus*, *A. glaucus*, *Curvularia lunata* and *Penicillium sp.* occurred from soil samples were isolated and cultured in the agar slants containing PDA by subculture method. The sub cultured agar slants were incubated for the complete growth of fungi.

Then the pure culture of fungi was labeled properly and stored in a refrigerator for future reference in the laboratory and an in vitro growth experiment.

Statistical analysis

To avoid experimental error in the present research work, sufficient duplicates were made for all analysis. The mean values of the experimental results were calculated, and SD values were expressed.

Results and Discussion

Soil samples collection

The details of samples collected for analysis of the present investigation were presented in the Table 1.

Details of moisture content of soil samples

The moisture content of soil samples from above sources was determined and found in the range of 10.8% to 20.4% with an average of 13.07% for 18 soil samples. The highest moisture was observed in sample No.18 collected from the playground near main roadside which is 20.4% and the lowest moisture is 10.8% in the sample No. 11 which is collected near Arts block.

The frequency of moisture range is as 5 samples with 10% to 11.0%, 8 samples with 11.0 to 13%, 3 samples with 13.0% to 15.0% and 2 sample showed above 15% of moisture. The moisture content of different soil samples collected from various locations of the college campus were found from 10.0% to 20.4% which is due to the water usage in the area and the shade of trees covering the soil area.

Previous research reports on soil moisture determined in the agricultural land such as groundnut and turmeric field show higher moisture as 26.0% and 26.3% and the lowest level observed in sugarcane and tapioca soil with 11.0% and 17.0% respectively which may be due to irrigation of soil (Jayaraman *et al.*, 2018). However, in the present

study, the lower to higher moisture level in soil found is quite natural because the location is educational institution where the required level of water is used for necessary purpose. The pattern of the moisture content of the individual soil samples were presented in Table 2 and Graph 1.

Analysis of fungi in soil sample

Qualitative pattern of Fungi

Based on the identification of fungi isolated from different soil samples in the present study by using standard manuals and mycology experts, the following individual species of fungi were encountered.

Aspergillus niger,

A. terreus,

A. flavus,

A. glaucus,

A. nidulans,

A. fumigatus,

A. candidus,

Penicillium citrinum,

Fusarium oxysporum,

Cladosporium cladosporioides,

Alternaria sp.,

Curvularia lunata,

Trichoderma sp.,

Mucor sp. and

Nonsporulating fungi

Characteristic features of individual species of fungi.

Aspergillus niger

Initially The colony appear with black heads after sporulation, rapid in growth, cottony in texture, reverse off-white to light yellow in colour, mycelium septate, conidiophore arise from fertile branch and with terminal globose shaped sporangium, sporangium bearing with phialides or sterigmata in biseriate. Conidiospores are globoid and rich in spore production.

Aspergillus flavus

Colony appears in sparse green to normal green-coloured heads with conidophores, rapid growth, radial pattern, cottony texture, reverse off-white to light yellow colour, exudate produced in some conditions on the colony, septate hyphae. Sporangial heads looks in globose appearance, sterigmata biseriate, spores are globoidal.

Aspergillus glaucus

Colony grow rapidly and spread on substrate, appears in gray green to yellow colour after sporulation, reverse yellow to orange colour, exudate appear sometimes, globose heads or sporangium, sterigmata uniseriate and globose conidiospores.

Aspergillus nidulans

Mycelium appears white with dark green-coloured conidiophores, restricted growth or slow growing, often white batches of mycelium appear in morphology, exudate appeared, smaller heads, globose to sub globose heads, biseriate sterigmata, globose to elliptical spores appear.

Aspergillus terreus

Colony initially looks in white mycelium and light brown to chocolate brown after sporulation, velvets

appearance, restricted growth to rapid growth, center of the colony with dense conidiophores with mycelium in the margin, conidiophores small, sporangium clavate to subclavate shaped, sterigmata biseriate, dense and appear as flame from the vesicle, conidia small in size, ovate to globose.

Penicillium sp.

Colony appears in grey green to bluish green with white mycelium in the margin, yellow to off-white in colour, mycelium dense and septate, short branches of conidiophore, small sporangial heads, Phialide long with 2 to 3 seriate, conidia elliptical to sub globose.

Trichoderma viride

The colony appears to be a bit granular on PDA, with green conidia distributed throughout the culture plate with white mycelium. An irregular yellow zone without conidia was present around the inoculum. Some white pustules were also found growing on the green mat of conidia, The conidia of *T. viride* were appear in clusters, globose, light green in colour, phialides were arranged in divergent groups of 2-4,

White non-sporulating fungus

The species appear in White bushy mycelial colony, sometime dense and spread through the culture medium, septate to non-septate mycelium without any fertile branches. Hence, it is sterile colony.

The morphological appearance of different species of fungi cultured from PDA plates were shown in the plate 1.

Quantitative pattern of fungi

In the quantitative pattern of fungi. Out of 18 soil samples analyzed, 7 samples such as Sample No.3, 6, 11, 13, 16, 17 and 18 were found with higher population (21000 cfu/g to 43,100 cfu/g) with an individual species of fungi, 6 samples such as

Sample No. 1, 5, 7, 10, 12 and 14 occurred with moderate number (7,000 cfu/g to 12,000 cfu/g) and remaining 5 samples as Sample No. 2, 4, 8, 9 and 15 showed lower level (3,000 cfu/g to 7,000 cfu/g) population of fungi. Out of 18 soil samples analyzed in the present study, the sample No. 1 from backside of the Arts block which contain more organic contents in soil showed higher number of fungi i.e., 41,100 cfu/g followed by sample No. 14 (29,600 cfu/g) and sample No. 18 (28,900 cfu/g).

Among the soil samples analyzed for the occurrence of fungi, it was observed that *Aspergillus niger*, *A. flavus*, *A. terreus*, *penicillium sp.*, White non-sporulating fungus, *Trichoderma viride*, *A. glaucus* and *Cladosporium sp.* were occurred in the order of dominance.

Other species of fungi were present in lower number. Our earlier investigations on the analysis of fungi from the soil samples of agricultural field shows enumeration of 22 species of fungi, in which *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus*, *Trichoderma spp.*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Gliocladium sp.*, *Fusarium sp.* and white non sporulating fungus (Jayaraman *et al.*, 2018) which resemble the results of the present study.

The investigations on soil fungi diversity by Selvaraj and Annamalai (2011) highlights the findings of present study in which soil contain a greater number of fungal inoculum which may be due to the presence of organic contents. The highest frequency of occurrence and population of fungi namely *Aspergillus niger* (128.4×10^3 cfu/g) was observed overall in most of the soil samples collected from the present study i.e from the college campus environment. Whereas the lowest number of occurrence of fungus is *Cladosporium* species as 2.6×10^3 cfu/g. The average number of fungal colonies observed in the soil samples collected in the present study shows 15.71×10^3 cfu/g which is moderate in number and less than the fertile soil samples like agricultural soil samples studied by Jayaraman *et al.*, (2018).

Table.1 Details of soil sample collection

S.No.	Sample No.	Name of site
1	SSNME1	Main entrance
2	SSNPO2	Principal room
3	SSNTD3	Tamil Department
4	SSNPH4	Prayer hall side
5	SSNLIB5	Near library
6	SSNBG6	Botany dept. garden
7	SSNBL7	Botany research lab
8	SSNNC8	NCC room opposite
9	SSNPH9	Prayer hall right side
10	SSNBG10	Opposite to Botany Garden
11	SSNAB11	Front of Arts block
12	SSNCS12	Front of Computer science block
13	SSNGP13	Ground near Physics lab
14	SSNPD14	Near physical education dept.
15	SSNSO15	Near Society room
16	SSNGC16	Ground near Chemistry dept.
17	SSNLB17	Library back side
18	SSNGR18	Ground near Main road side

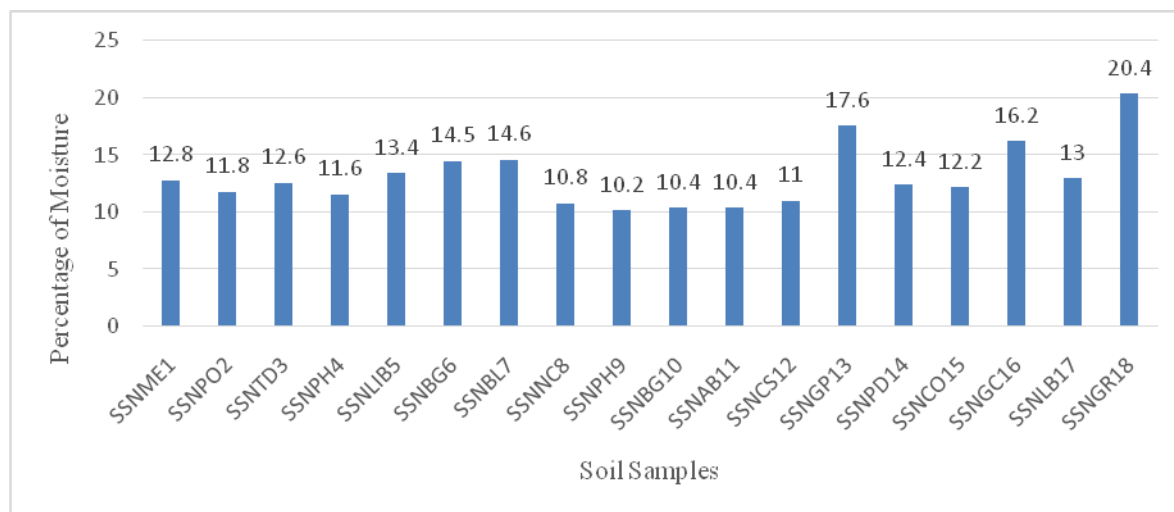
Table.2 Details of moisture content of the soil samples

S.No.	Sample No.	Name of site	Moisture content (%)
1	SSNME1	Main entrance	12.8
2	SSNPO2	Principal room opposite	11.8
3	SSNTD3	Near Tamil Department	12.6
4	SSNPH4	Near Prayer Hall	11.6
5	SSNLIB5	Library backside	13.4
6	SSNBG6	Botany dept. garden	14.5
7	SSNBL7	Near Botany research lab	14.6
8	SSNNC8	NCC room opposite	10.8
9	SSNPH9	Prayer hall right side	10.2
10	SSNBG10	Opposite to Botany Garden	10.4
11	SSNAB11	Arts block backside	10.4
12	SSNCS12	Computer science dept. opposite	11.0
13	SSNGP13	Ground near Physics lab	17.6
14	SSNPD14	Physical education dept. opposite	12.4
15	SSNCO15	Near Co-op Society room	12.2
16	SSNGC16	Ground near Chemistry dept.	16.2
17	SSNLB17	Library back side	13.0
18	SSNGR18	Ground near Main roadside	20.4

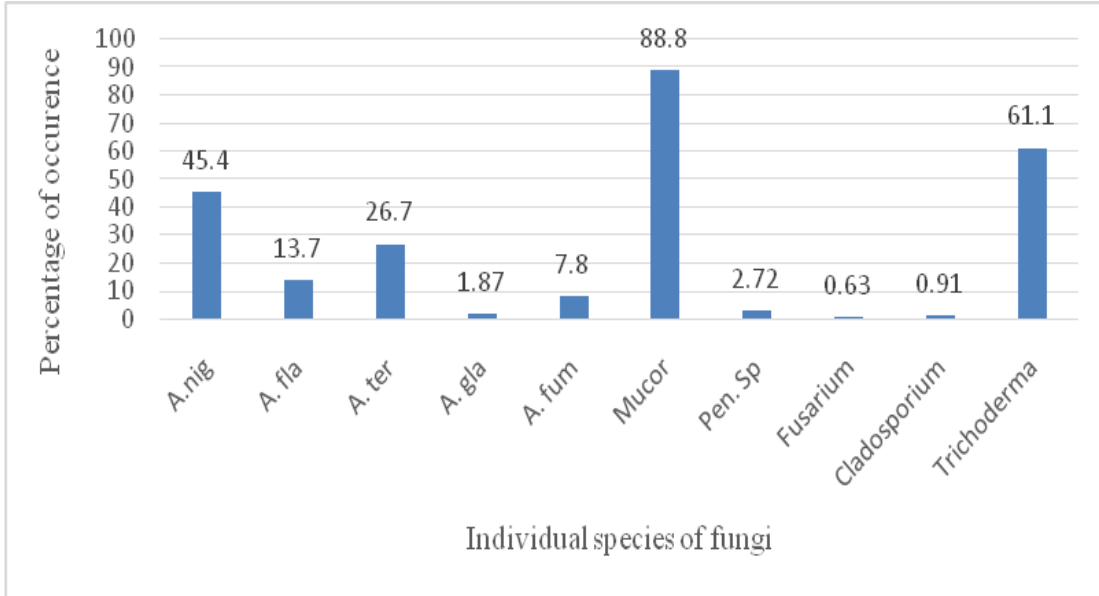
Table.3 The quantitative occurrence of fungi from the soil samples

Sl. No	Sample details	Occurrence of individual species of fungi (CFU/g)											Total	%
		A. nig	A. fla	A. ter	A. gla	A. fu.	Mucor sp.	Penn. sp	Fusa sp.	Clao. Sp.	Trich. sp			
1	SSNME1	7200	500	2800	0	300	P	300	100	0	0	11200	3.96	
2	SSNPO2	1500	800	2200	400	1200	P	0	200	400	P	6700	2.37	
3	SSNTD3	9200	2300	6000	200	800	P	0	0	100	P	18600	6.58	
4	SSNPH4	1200	500	0	0	1000	P	100	400	200	0	3400	1.2	
25	SSNLB5	3000	0	1800	600	1400	P	800	100	0	0	7700	2.72	
6	SSNBG6	12000	8000	1100	1500	0	A	0	0	100	P	22700	8.02	
7	SSNBL7	5400	1800	1200	0	600	P	200	100	0	P	9300	3.28	
8	SSNNC8	3400	600	1000	200	1200	P	0	200	100	P	6700	8.1	
9	SSNPH9	2200	0	1200	800	900	P	200	0	100	P	5400	1.91	
10	SSNBG10	6500	2500	5300	200	0	P	100	200	100	P	14900	5.27	
11	SSNAB11	20700	5800	10400	0	2000	P	0	2200	0	0	41100	14.53	
12	SSNCS12	6000	3400	0	1000	2400	P	0	0	100	P	12900	4.56	
13	SSNGP13	10800	4800	10200	0	2200	P	1600	0	0	0	29600	10.47	
14	SSNPD14	4400	0	2600	100	800	P	0	100	600	P	8600	3.04	
15	SSNCO15	2200	1500	1500	200	1500	P	0	0	100	P	7000	2.47	
16	SSNGC16	9500	2800	8600	0	1200	A	1400	100	200	P	23800	8.41	
17	SSNLB17	10900	2300	8000	100	2000	P	800	100	0	0	24200	8.56	
18	SSNGR18	12300	2400	10700	0	800	P	2200	0	500	P	28900	10.22	
	Total	128400	39000	75600	5300	20300	-	7700	3800	2600	-	282700	-	
	%	45.4	13.7	26.7	1.87	7.18	-	2.72	0.63	0.91	-		-	

Graph.1 Graphical appearance of moisture content of soil samples



Graph.2 Overall Percentage occurrence of individual species of fungi in different soil samples



Graph.3 Overall Percentage occurrence of fungi in different soil samples

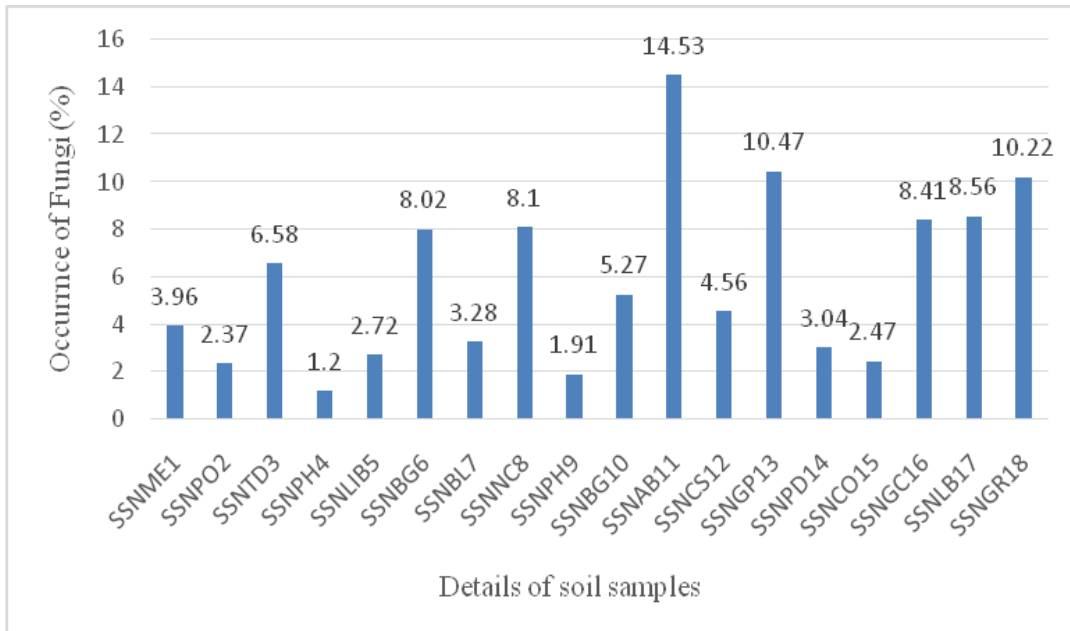
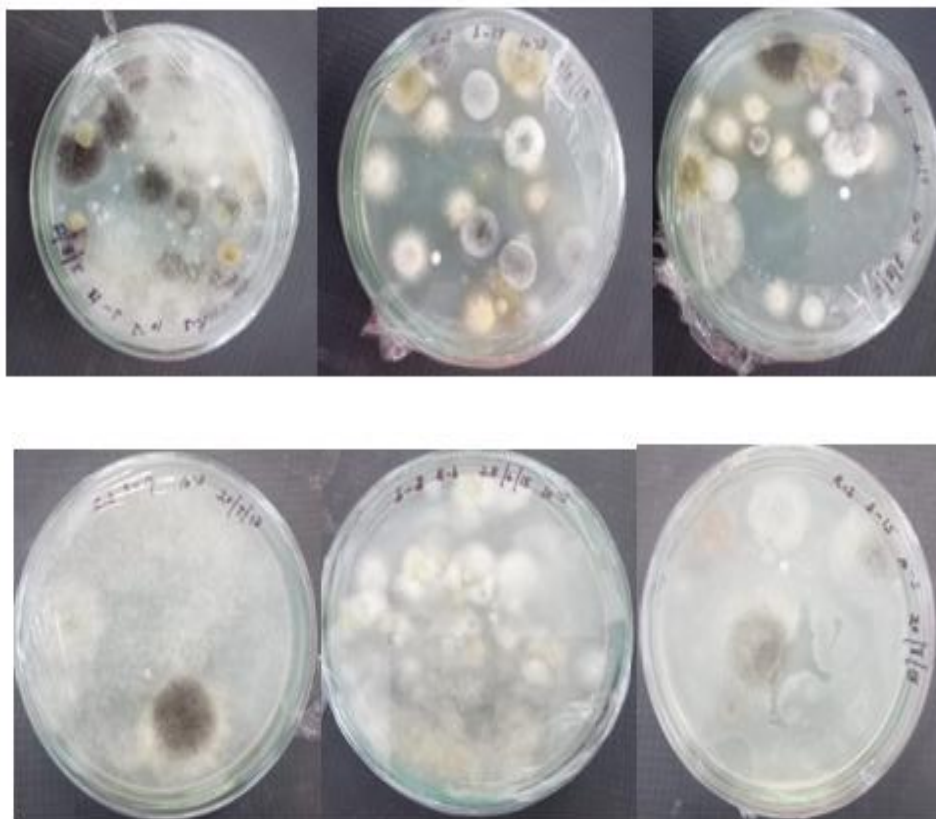


Plate.1 The morphological appearance of fungal colonies



The population of fungi observed in the present study almost resembles the earlier report by Jayaraman *et al.*, (2018) and Saraswathy *et al.*, (2020), in which *A. niger*, *A. terreus*, *A. flavus* and *Trichoderma sp.*, were the significant population in the order of dominance in various agricultural soil samples such as plantain field, groundnut field, Sugarcane field, Tapioca field, Turmeric field and waste land soil as well as Paddy field soil.

The analysis of soil samples from paddy field and leather industry environment showed *Aspergillus niger* was the dominant fungus followed by *A. terreus*, *A. flavus* and other species also were in accordance with our present study (Saraswathy *et al.*, 2020). The details of occurrence of total number of fungi were presented in Table 3, and the percentage occurrence of individual species of fungi from the soil samples were presented in graph 2 and 3. The present study is chosen because it is an effort

to understand the soil fungal diversity in the College Campus where large number of people are assembled. The environmental factors such as pH, temperature, moisture, organic carbon, and nitrogen play an important role in distribution of microflora. The soil moisture has a direct effect on the population of fungi positively hence, at higher moisture the tolerance and colonization are badly affected (Adams *et al.*, 1999) the environment. The identified soil fungi (Table 2) namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium*, *Trichoderma*, *Penicillium* are also some time belongs to Keratinophilic fungi.

From the present study it is concluded that the soil samples of different locations of the college campus environment contain versatile group of fungi which mainly comprised of *Aspergillus* and *Penicillium species*. Among the species of fungi encountered in both the soil environment, *Aspergillus niger* was the

dominant species followed by *A. terreus*, *Aspergillus flavus* and *Muco*. However, it is also concluded that from the observations of various research reports as the species *A. niger* found to be a rich growth and occurrence in various soil samples including agricultural, wasteland and industrially polluted soil samples which indicates *A. niger* is a versatile fungus able to grow and tolerate environment. Since the constant presence of *Mucor* in the soil samples in the campus environment may also be possible to be a harmful allergic organism to human. Therefore, it is concluded from the present study, as the species of *A. niger* and *Mucor* may be a harmful fungi which are present in the soil samples of campus where the students having contact with the soil stressed to follow the cleaning and personal hygiene procedure to avoid infection.

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