

Original Research Article

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Comparative Studies on Fungal Biodiversity of agricultural field soil from Thiruvannamalai District, Tamil Nadu, India

P. Jayaraman^{1*}, S. Shalini², K. Saraswathi¹, K. Vadamalai¹ and R. Logambal¹

¹Research Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai 600 035, Tamil Nadu, India

²Research Department of Botany, Government Arts College, Thiruvannamalai 606 603, Tamil Nadu, India

*Corresponding author

ABSTRACT

Soil microorganisms such as bacteria and fungi play an important role in soil fertility and promoting the plant growth. Sixty soil samples are collected of different agricultural crop fields such as groundnut field, sugarcane field, Plantain field, turmeric field, tapioca field and wasteland with *Parthenium* field in and around Thiruvannamalai, Tamil Nadu were investigated soil moisture content by oven drying method and diversity of fungi by using serial dilution plating method with standard Potato Dextrose Agar (PDA) for qualitative and quantitative pattern. The moisture content of different soil samples collected from the above fields were found from 11.0% to 26.3%. Among the soil samples, the groundnut and turmeric field show higher moisture as 26.0% and 26.3% and the lowest level was observed in sugarcane and tapioca soil with 11.0% and 17.0% respectively. For analysis of fungi, 22 species were enumerated from the above soil samples 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 were remarkably present. Regarding the distribution of occurrence, Plantain field show highest as 28% followed by groundnut field with 23%, Sugarcane field 17%, Tapioca and waste land soil each with 14% and the turmeric field soil has the lowest occurrence as 4% of fungal population. It is concluded that from the present study as the turmeric field soil has an antimicrobial effect which shows very low population of fungi in their soil environment when compared with other soil samples.

Keywords

Soil fungi, Fungal biodiversity, Agricultural soil, Rhizosphere mycoflora, Antifungal effect

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Introduction

The relationship between biodiversity of soil fungi and function of ecosystem is an issue of paramount importance, particularly in the face of global climate change and human alteration ecosystem processes. Fungi are an important component of the soil micro- biota typically of

soil biomass than bacteria depending on soil depth and nutrient condition such as micro and macro nutrients. The saprophytic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses and lignin thus contributing carbon and

nitrogen source to increase the fertility of soil (Breure 2004). Mostly the distribution of fungi in agricultural soils is prominent (Carney *et al.*, 2006) and less is reported about the occurrence of fungi under natural soil condition. Soil microorganisms including fungi can have both positive and negative effects on plant growth and facilitate nutrient absorption by plants, promote plant growth or stimulate seedling development by producing hormone-like substances. Therefore, the soil is the most suitable environment and serves as a good culture medium for the growth of fungi. Generally, soils contain 10^5 to 10^6 cfu/g and 100 to 1500 g biomass per meter square. Some of the fungi have the ability to make rapid growth on organic substrates in dry, acid, coarse textured soil and thus to reach nutrients available to unicellular microorganisms by diffusion (Trinci, 1969; Ambikapathy *et al.*, 1994).

There are many reports on biodiversity and influence of different species of fungi on various soil locations were available at global level which indicates the soil fungi are ubiquitous and inhabit different types of soils. From various reports, the fungi commonly present in different type of soils found as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. terreus*, *Curvularia lunata*, *Penicillium janthinellum*, *P. citrinum*, *Rhizopus nigricans*, *Trichoderma viride*, *T. harzianum*, *Fusarium oxysporium*, *F. moniliforme*, *Mucor*, *Cladosporium*, *Verticillium*, *Acremonium*, *Chaetomium globosum*, *Corynespora cassicola*, *Eurotium chevelari*, *Botrytis cinea*, *Helminthosporium sp. etc.* (Breure (2004); Mahmood Khalid *et al.*, (2006); Falih (1997); (El-Said, 2001). Among the species of fungi reported in soil by various authors, the *Aspergillus* is the dominant genus followed by *Penicillium* species, several species of hyphomycetes and Zygomycetes are frequently occurring in various soil samples Falih (1997); Moallaei *et al.*, 2006; Gauri

Rane *et al.*, (2006); Gangawane and Despande (1972), Reddy *et al.*, (1987), Saksena *et. al.* (1967), Manoharachary *et al.*, (1990); (Guleri *et. al.* (2010); Suderma *et al.*, (2011); Madhan Raj *et al.*, (2010).

The study of population determinations of soil fungi from two different groundnut fields for three years period suggested that planting peanuts did not result in an increased *A.flavus* which may be able to colonize peanut fruits from low inoculum density levels (Gray *et al.*, 1974). The investigation on the paddy field, the population of soil mycoflora was studied by Ambikapathy *et al.*, (2001) found with 26 individual fungal species of fungi. Among the species of fungi isolated from peanut field, most of them were belonged to euteromycetes. *Aspergillus*, *Penicillium* and *Fusarium* were comparatively more than the other species in most of the soil samples. In Sudan, the investigation of soil mycoflora of sugarcane field at Kenana sugar Estate was carried out and there are 23 species were isolated which are belonging to 16 genera and the richest taxa in abundance were *Aspergillus spp.*, *Penicillium sp.*, *Rhizopus sp.*, *Alternaria sp.*, *Curvularia sp.* and *Fusarium sp.* (Al-Nur El-Amin *et al.*, (2007). During monsoon season, maximum fungal count was recorded in 2009-2010 and found dominant with species of *Aspergillus niger*, *A. flavus*, *A. clavatus*, *Curvularia lunata*, *Bipolaris oryzae*, *Penicillium janthinellum*, *P. citrinum* followed by *Rhizopus nigricans*, *Trichoderma viride*, *T. harzianum* from the paddy field were recorded (Manimegalai *et al.*, (2011). Jayalakshmi and Valluva paridasan (2010) were investigated the fungus from Parthenium weed infested soil. There are 13 fungal species were isolated, that are *A. niger*, *A. flavus*, *A. fumigatus*, *Chaetomium globosum*, *Corynespora cassicola*, *Curvularia lunata*, *Curvularia sp.*, *Eurotium chavelari*, *Fusarium oxysporum*, *F. moniliforme*, *Monilia sp.*, *Rhizopus Stolonifer* and *Trichoderma harzianum*. Among this, *A.*

niger and *R. Stolonifer* were found to be present throughout Tamil Nadu. Both *A.flavus* and *T. harizianum* ranked next with an Equal distribution. *Monilia sp.* and *Corynospora cassicola* represented poor percentage distribution in Tamilnadu. However, the studies of fungal biodiversity in the rhizosphere of crop plants in agricultural soil and its interaction with micro-organisms is not sufficient to understand the mechanism of soil microbes. Suderma *et al.*, (2011) have reported the fungi inhabited in Banana field of the soil collected from Bali i.e. Karangasem, Klungkung and Jembrana which are main banana growing areas in which *Streptomyces spp.*, *Trichoderma spp.*, *Aspergillus spp.*, *Penicillium spp* and *Glocladium spp* are higher in the soil.

Therefore, the present study was undertaken to analyze the soil mycoflora of various agricultural field soil samples which are collected during the crop cultivation to know the seasonal relationship, effect of crop plants on the soil mycoflora and interaction between the soil ecosystem and other physiochemical parameters. The results are discussed in detail in the present study.

Materials and Methods

Collection of soil samples

The soil samples from groundnut field, sugarcane field, Plantain field, turmeric field, tapioca field and wasteland with Parthenium field were collected from Thiruvannamalai, Tamil Nadu at different locations within 15 km radius. In each field, 200g soil samples were collected from 10 different places of the same field at 5 - 15 cm depth and pooled together to obtain composite sample. The soil samples were collected in pre-cleaned polythene bags cleaned with alcohol and labeled after collection. The samples were kept in the laboratory for further analysis.

Analysis of soil moisture

The moisture content of the soil samples were determined by standard oven-drying method (Mishra, 1968). Exactly 1 g of freshly collected sample of soil was weighed in a pre-weighed empty glass Petri-dish. The petri-dish with soil sample was placed in a hot air oven for 2 hours at 105 ° C. After drying, the soil sample of the petri-dish was cooled to room temperature and weighed for loss of weight. The initial and final weight of the Petri-dish with soil sample was determined to calculate the percentage of moisture content. The following standard formula was used for the calculation of moisture content percentage.

$$\% \text{ of soil moisture} = \frac{\text{Initial weight (Petridish with soil)} - \text{Final weight}}{\text{Weight of soil sample taken}} \times 100$$

Isolation of fungi from the soil samples

The soil fungi were enumerated by Soil Dilution plating method (Walksman, 1922) on Potato Dextrose Agar (PDA) media and Johnson *et al.*, (1960).

Serial Dilution Plate method for Isolation of Fungi (Waksman, 1922)

Exactly 10g of soil sample was suspended in 90ml of sterile saline (1%) water and kept in shaker for 15mins and allowed to settle the soil particles. From each suspended sample to make microbial dilutions from 10⁻¹ to 10⁻⁵, dilution of 10⁻², 10⁻³ and 10⁻⁴ were used to isolate fungi. About 1ml of microbial suspension of each concentration was added and spreaded on pre-plated sterile PDA medium by spread plate method. One percent streptomycin solution was used for an antibacterial. Sufficient duplicates in each dilution were maintained to avoid experimental error. The inoculated plates were

incubated at 28°C for 5 – 7 days. The plates were observed periodically everyday up to 7 days. The colony forming units (CFU) of the fungal isolates were calculated. All the results were calculated and statistical analysis was performed.

Identification of Mycoflora in Soil

The fungal colonies formed in the agar plates were observed for qualitative and quantitative pattern. The morphology of fungi was studied macroscopically by observing colony features such as texture and color. The hyphal structure, spore shapes and size, spore bearing structures were observed microscopically by staining with cotton blue under light microscope. The observations were compared with the standard works of Raper and Thom (1949), Van Ark (1974), Raper *et al.*, (1965), Eills (1976) and Barnett and Hunter, (1998) for identification of the fungi at species level.

Quantitative pattern of fungi

The total number of fungal colonies present in the plates by using 1g of soil from each sample was observed and calculated. The percent contribution of each isolate was calculated by using the following formula:

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

*CFU-Colony forming Unit

Results and Discussion

Details of sample collection

The sample collection is an important role for the better analysis, also considered as a crop harvesting period which should cover within the sampling protocol. The details of soil sample collected from various agricultural

field soils during crop cultivation in and around Thiruvannamalai, Tamil Nadu for the present analysis is given in Table 1.

Moisture content of soil samples

Since the moisture content in soil affects the physiological state of microorganisms and plants, it is extremely important to determine correlations between the soil moisture content and soil microbial activity. In view of the above consideration the % of moisture content of the individual soil samples were presented in the following table 2. Among the samples analyzed for the moisture content, the groundnut and turmeric field show higher moisture as 26.0% and 26.3% respectively. The lowest moisture content was observed in sugarcane and tapioca as 11.0% and 17.0% respectively. The waste land soil with parthenium field shows 22.2% moisture content.

Qualitative analysis of Mycoflora

The following individual species of fungi were isolated from various samples of soil collected in the present study.

The morphological appearance of different species of fungi growing in Potato Dextrose Agar (PDA) plates were shown in the figures 1, 2, 3, 4, 5 and 6.. Among the soil samples plated in agar plates, the Fig. 3 show only two white non-sporulating fungi which indicates the turmeric soil environment has inhibitory effect of fungi.

Quantitative distribution of individual fungi

Groundnut field

The occurrence of individual species of fungi present in soil sample of Groundnut field is presented in Table 3 and Chart 1.

Fig.1 The growth of fungi from the soil collected from sugarcane field

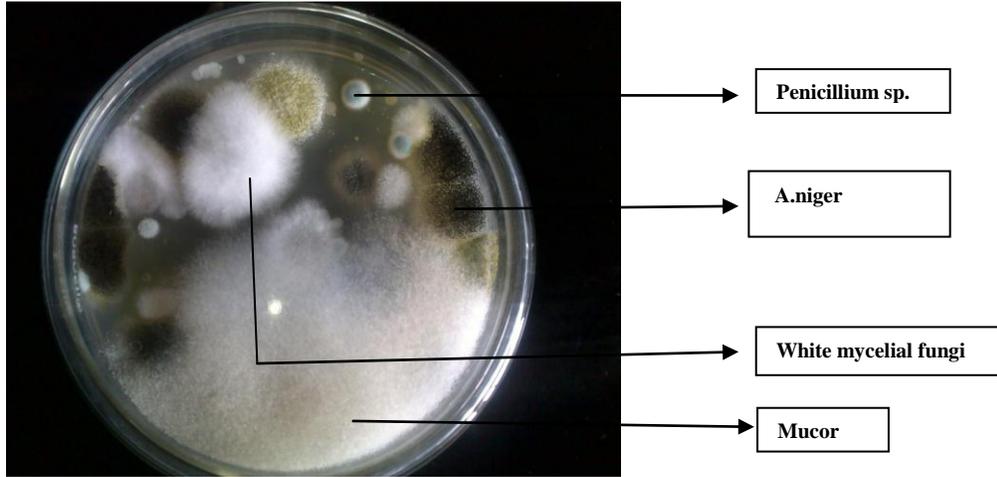


Fig.2 Growth of fungi in soil sample of Tapioca field

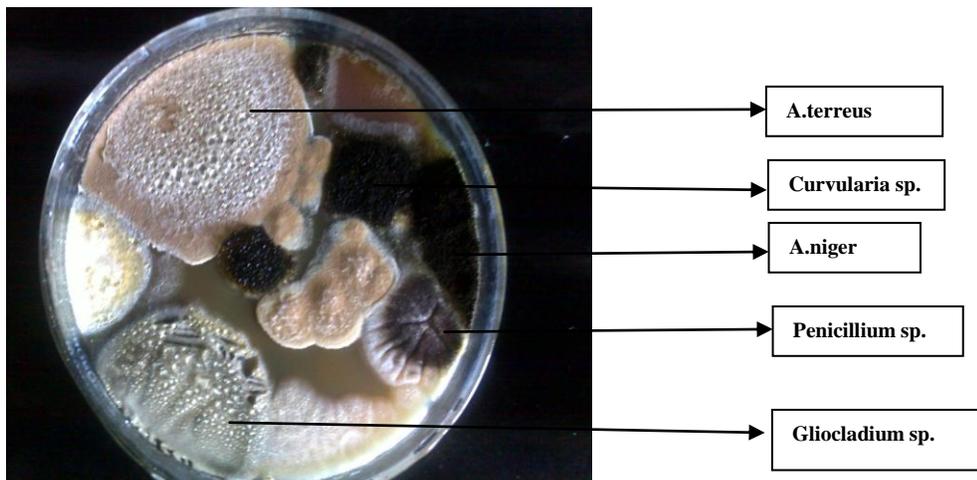


Fig.3 Growth of fungi in soil sample of Turmeric field

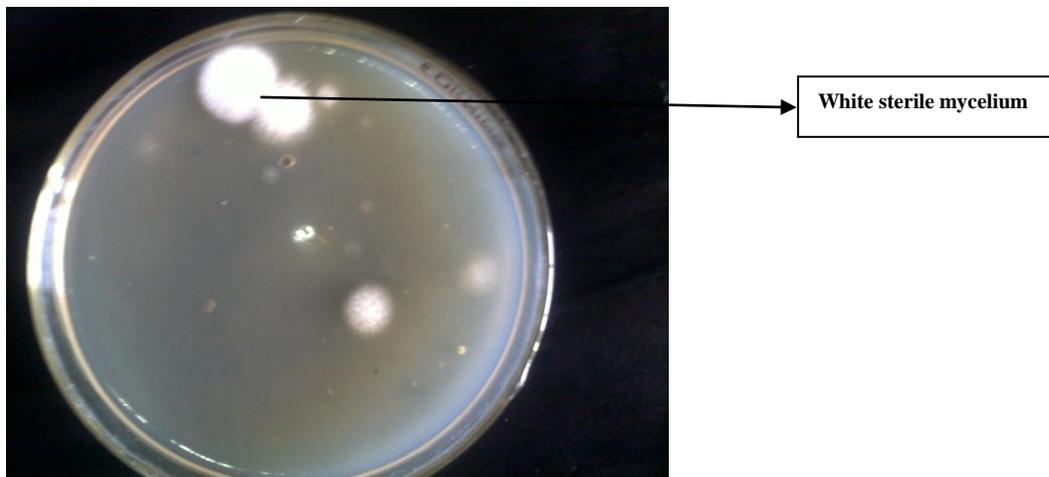


Fig.4 Growth of fungi in soil sample of Plantain field

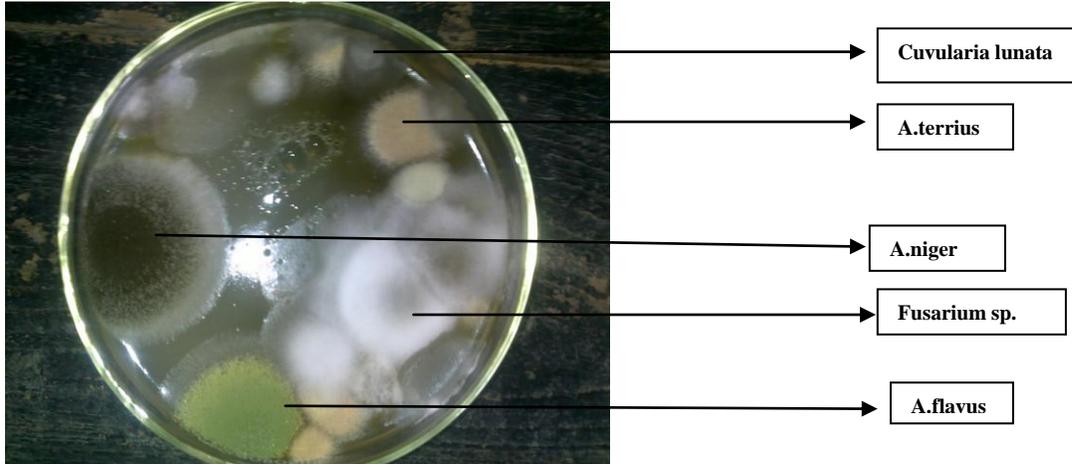


Fig.5 Growth of fungi in soil sample of Groundnut field

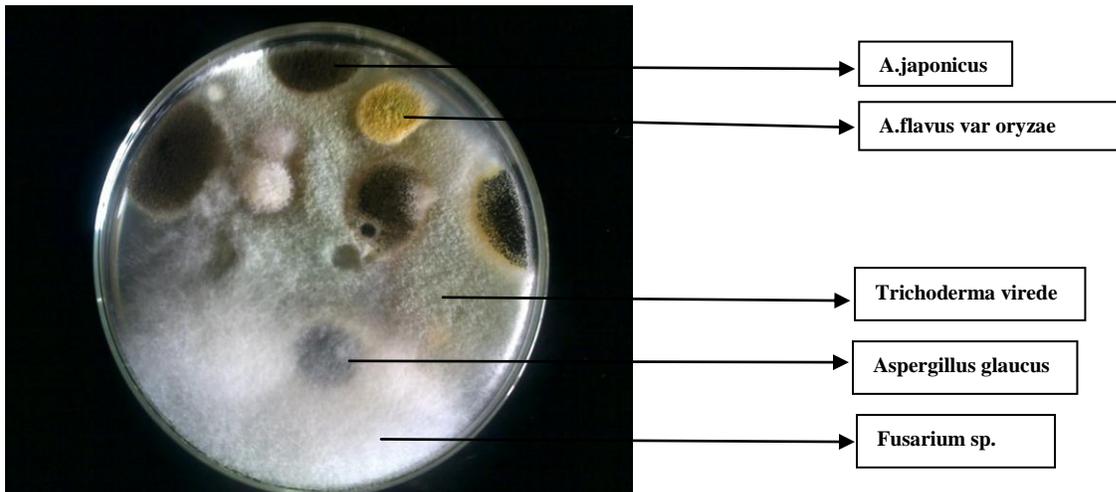


Fig.6 Growth of fungi in soil sample of Parthenium field

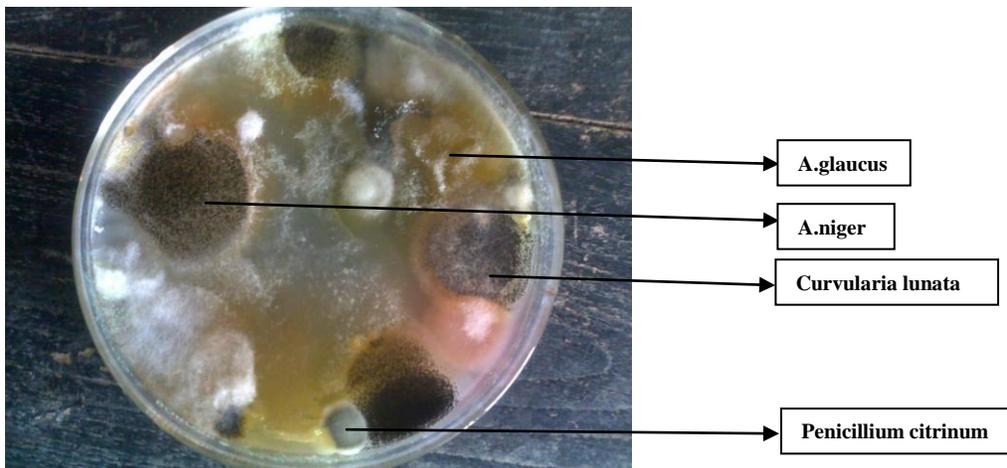


Chart.1 The quantitative occurrence of individual fungi present in soil sample from Groundnut filed

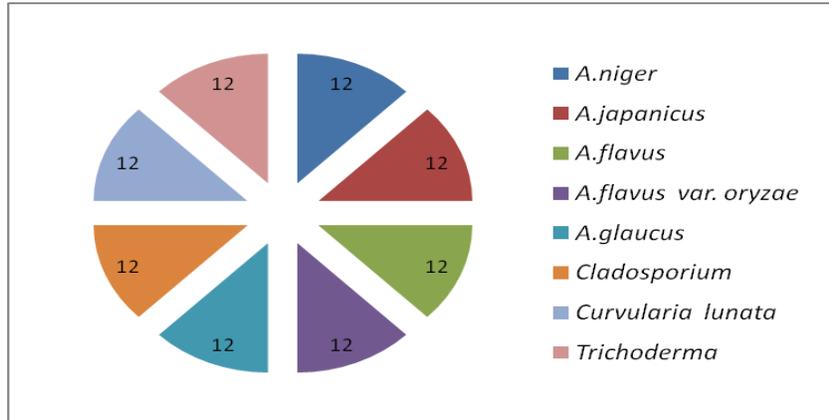


Chart.2 The quantitative occurrence of individual fungi present in soil sample from Sugarcane filed

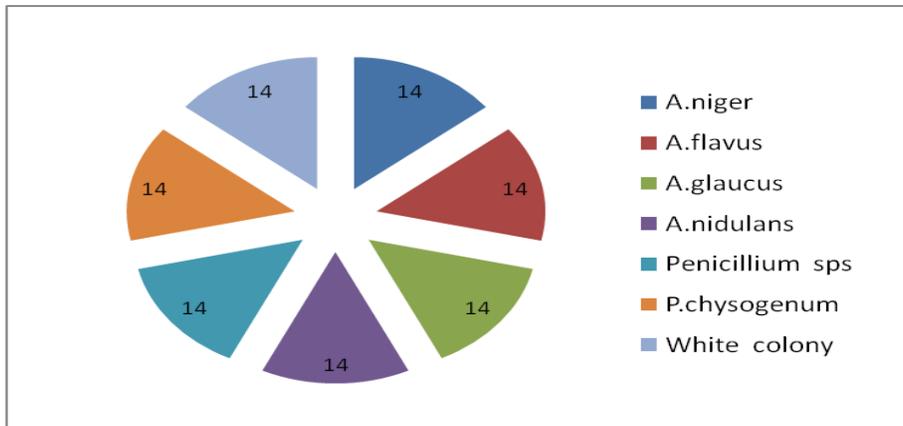


Chart.3 The quantitative occurrence of individual fungi present in soil sample from Turmeric filed

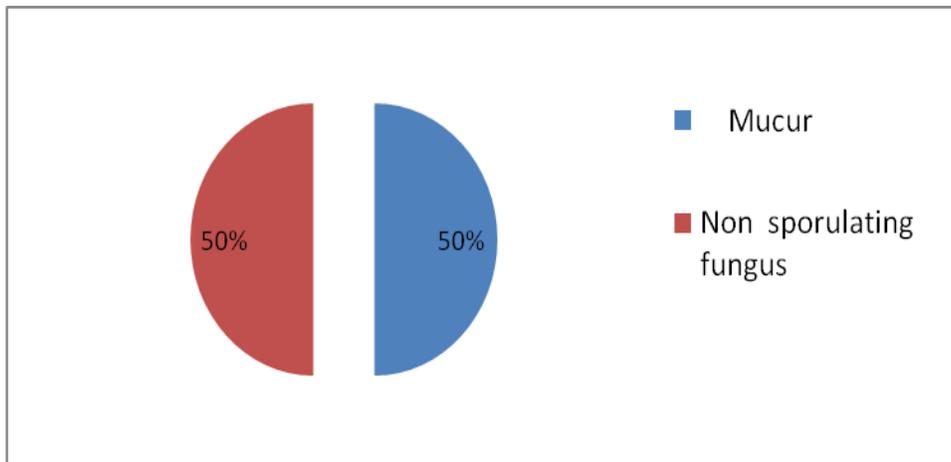


Chart.4 The quantitative occurrence of individual fungi present in soil sample from Plantain field

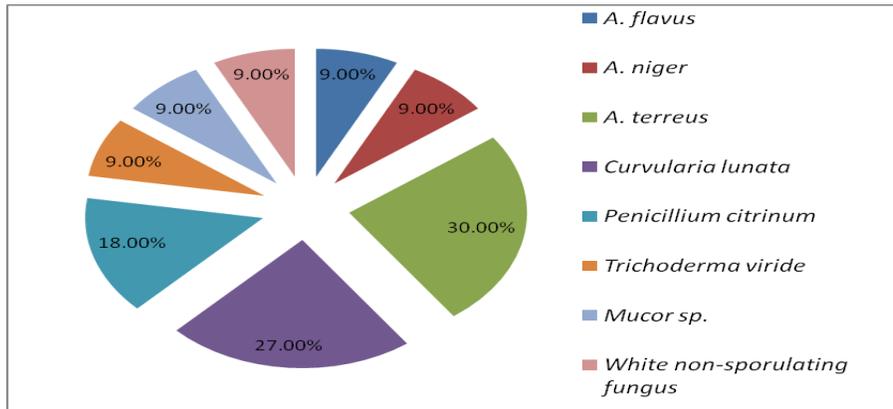


Chart.5 The quantitative occurrence of individual fungi present in soil sample from Tapioca field

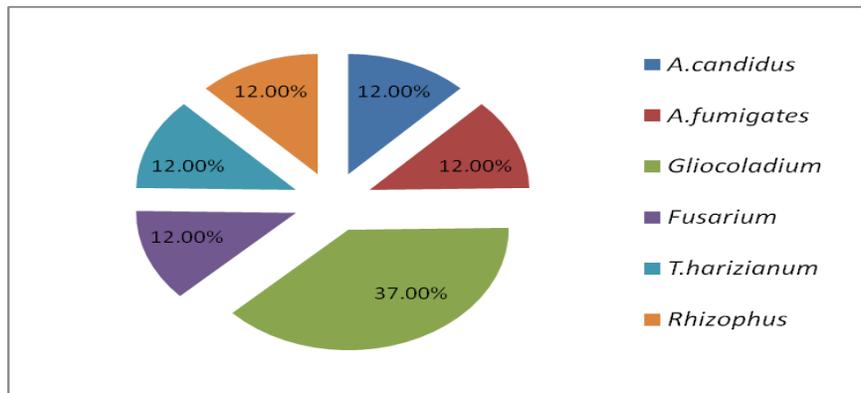


Chart.6 The quantitative occurrence of individual fungi present in soil sample from Parthenium field

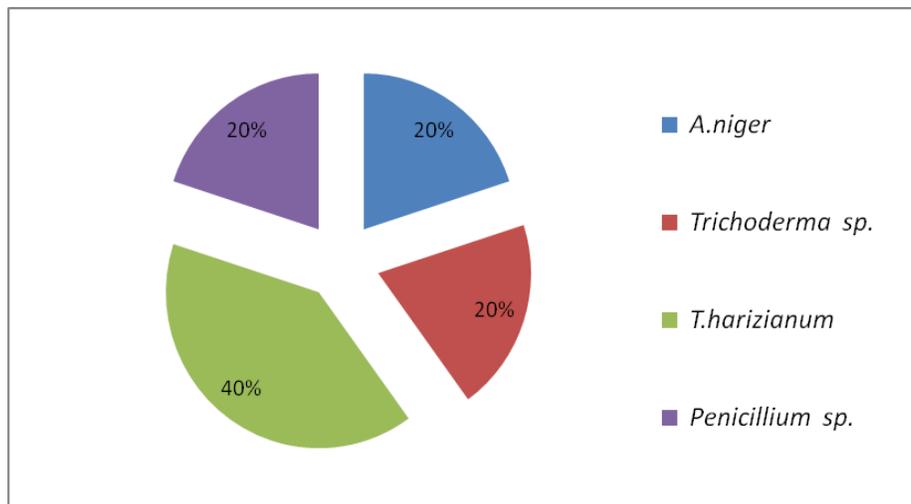


Chart.7 Comparative pattern of Distribution of fungal species in different soil samples

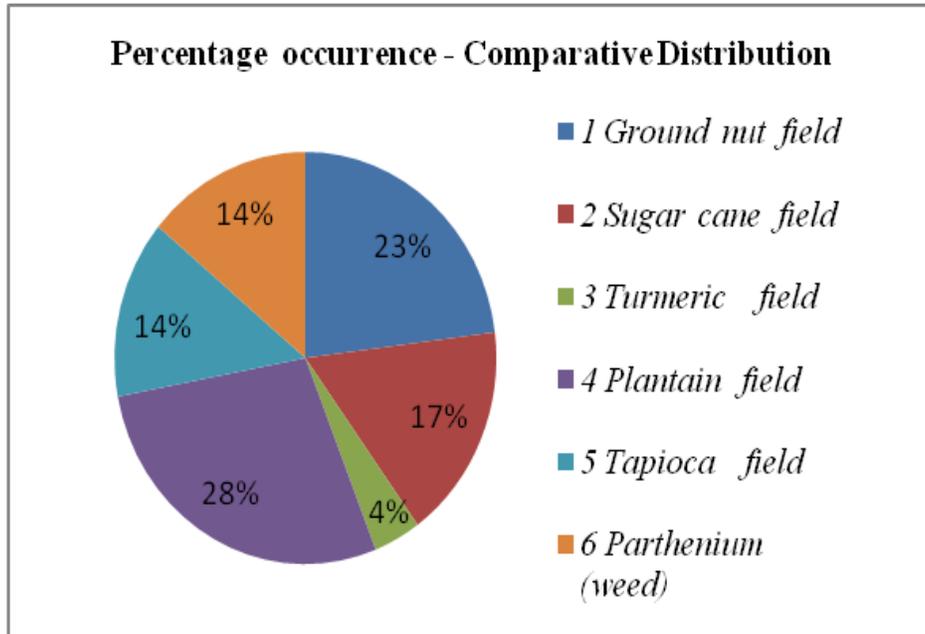


Table.1 Details of soil samples collected from different places of Thiruvannamalai

S. No	Name of the soil sample	Harvesting period of the crop	Time of collection (at crop growth)
1	Ground nut field	3 months	2 nd month
2	Sugar cane field	12 months	9 th month
3	Turmeric field	6-8 months	6 th month
4	Plantain field	10-12 months	10 th month
5	Tapioca field	5-6 months	5 th month
6	Parthenium (weed)	3 months	3 rd month

Table.2 The details of moisture content of various soil samples

S. No	Name of the field soil	Moisture content (%)
1	Ground nut	26.0
2	Sugarcane	11.0
3	Turmeric	26.3
4	Plantain	20.2
5	Tapioca	17.0
6	Parthenium	22.2

Table.3 The quantitative occurrence of individual fungi present in soil sample from Groundnut filed

Sl. No.	Name of the Fungi	No of Colonies (cfu/g)	Percentage occurrence
1	<i>A. niger</i>	1	12
2	<i>A.japanicus</i>	1	12
3	<i>A. flavus</i>	1	12
4	<i>A. flavus var. oryzae</i>	1	12
5	<i>A. glaucus</i>	1	12
6	<i>Cladosporium sp.</i>	1	12
7	<i>Curvularia lunata</i>	1	12
8	<i>Trichoderma sp.</i>	1	12

Table.4 The quantitative occurrence of individual fungi present in soil sample from Sugarcane filed

Sl. No.	Name of the fungi	No of colonies (cfu/g)	Percentage Occurrence
1	<i>A.niger</i>	1	14
2	<i>A.flavus</i>	1	14
3	<i>A.glaucus</i>	1	14
4	<i>A.nidulans</i>	1	14
5	<i>Penicillium sp.</i>	1	14
6	<i>P.chysogenum</i>	1	14
7	White colony	1	14

Table.5 The quantitative occurrence of individual fungi present in soil sample from Turmeric filed

Sl.No	Name of the species	No of colonies(cfu/g)	Percentage Occurrence
1	<i>Mucor</i>	1	50
2	Non sporulating fungus	1	50

Table.6 The quantitative occurrence of individual fungi present in soil sample from Plantain field

Sl. No.	Fungal species	No of colonies (cfu/g)	Percentage occurrence
1	<i>A.flavus</i>	1	9
2	<i>A.niger</i>	1	9
3	<i>A.terreus</i>	3	30
4	<i>Curvularia lunata</i>	1	27
5	<i>Penicillium citrinum</i>	2	18
6	<i>Trichoderma viride</i>	1	9
7	<i>Mucor sp.</i>	1	9
8	White non-sporulating fungus	1	9

Table.7 The quantitative occurrence of individual fungi present in soil sample from Tapioca field

Sl.No.	Fungal species	No of colonies(cfu/g)	Percentage Occurrence
1	<i>A.candidus</i>	1	12
2	<i>A.fumigates</i>	1	12
3	<i>Gliocoladium</i>	3	37
4	<i>Fusarium</i>	1	12
5	<i>T.harizianum</i>	1	12
6	<i>Rhizophus</i>	1	12

Table.8 The quantitative occurrence of individual fungi present in soil sample from Parthenium field

Sl. No.	Fungal species	No of colonies (cfu/g)	Percentage Occurrence
1	<i>A.niger</i>	1	20
2	<i>Trichoderma sp.</i>	1	20
3	<i>T.harizianum</i>	2	40
4	<i>Penicillium sp.</i>	1	20

Qualitative analysis of Mycoflora

1	<i>Aspergillus niger</i>	11	<i>P. chrysogenum</i>
2	<i>A. niger var. japonicas</i>	12	<i>Penicillium sp.</i>
3	<i>A. flavus</i>	13	<i>Mucor sp.</i>
4	<i>A.flavus var oryzae</i>	14	<i>Rhizophus sp.</i>
5	<i>A. glaucus var. amsterdomi</i>	15	<i>Trichoderma virede</i>
6.	<i>A. nidulans</i>	16	<i>T. harzianum</i>
7	<i>A. terreus</i>	17	<i>Cladosporium cladosporioides</i>
8.	<i>A. candidus</i>	18	<i>Curvularia lunata</i>
9.	<i>A. fumigates</i>	19	<i>Gliocoladium sp.</i>
10.	<i>Penicillium citrium</i>	20	<i>Fusarium sp.</i>
		21	White non sporulating fungus

The individual species of fungi such as *A. niger*, *A. japonicas*, *A. flavus*, *A. glaucus*, *Cladosporium sp.*, *Curvulari lunata* were present at equal contribution. The percentage of individual species of fungi is 12%.

Sugarcane field

The occurrence of individual species of fungi and their percentage distribution were presented in the Table 4 and Chart- 2. The

individual species of fungi such as *A. niger*, *A. flavus*, *A. glaucus*, *A. nidulans*, *Pencillium chrysogenum*, *Pencillium sp.*, and non-sporulated white colony were present at 14% level of each fungus.

Turmeric field

In Turmeric field, the occurrence of fungi is very less in numbers. The distribution and percentage occurrence of individual species of

fungi were presented in the Table 5 and Chart 3. Here, the remarkable finding is only 2 colonies as *Mucor sp.* and white non-sporulating fungi present in the culture plate.

Plantain filed

In the plantain field, the soil found with 8 different species of fungi identified as *A. flavus*, *A. niger*, *A. terreus*, *Curvularia lunata*, *Penicillium citrinum*, *Trichoderma virede*, *Mucor sp.*, and White non-sporulating fungus at a different percentage of occurrence. The details of fungi are presented in Table 6 and Chart 4.

Tapioca field

From the study, it was observed that the fungus namely *Gliocladium sp.* is occurred maximum in the Tapioca field as 37% and other species of fungi such as *A. candidus*, *A.fumigatus*, *Fusarium sp.*, *Trichoderma harzianum* and *Rhizopus sp.* at 12%. It indicates, either the soil may contain more population with *Gliocladium sp.* or the rhizosphere of tapioca assumed to be having affinity with the particular fungus. The distribution pattern of individual fungi was shown in Table 7 and Chart 5.

Wasteland with Parthenium (weed) soil

The occurrence of individual species of fungi and their percentage distribution of soil collected from the wasteland mostly grown with *Parthenium sp.* was found with *Trichoderma. hariziamum* at 40% followed by *A. niger*, *Trichoderma vilride.* and *Penicillium sp.* with 20% each. The details were presented in Table 8 and Chart 6.

Comparative pattern of fungi in different field soils

The occurrence of different species of fungi in soil samples collected from different crop

filed is presented in the Chart 7. In order to observe the dominance of individual species of fungi present in different agricultural crop field soil, the percentage contribution was worked out to understand the relationship between the soil environment with and the affinity towards the growth and survival of mycoflora. Here, one can observe that the groundnut filed soil show higher distribution as 23% and lower percentage of distribution in turmeric filed soil sample. This indicates the rhizosphere environment of turmeric field likely affect the growth and development of fungi as the turmeric has significant effect.

Biodiversity of fungi is essential for any one collecting and/or monitoring any fungi from various sources of environment. Fascinating and beautiful fungi are vital components of nearly all ecosystems and impact of human health and our economy in myriad of ways. Soil microorganisms including Bacteria, Fungi, Algae and Protozoa are existing in very large number in the soil as long as there is a carbon source and other nutrient available. Among the microorganisms, fungi are occurring in smaller population, but they dominate the soil biomass when the soil environment is not influenced by other factors. Therefore the soil consist of different type of micro and macro nutrients which provide a natural environment for the survival of microorganisms. The moisture content in soil affects the physiological state of microorganisms and plants and it is worth mentioning that soil microorganisms are generally well-adaptable to changing air and water conditions in a soil pedon (Walker *et al.*, 2003). Therefore, the correlations between the moisture content and mycoflora of the soil were done and found there is no significant level of affinity towards the soil by fungi.

It was observed from the present study as the fungi occurring in different type of soil samples like groundnut soil, sugar cane soil, plantain soil, Tapioca soil, Turmeric soil and

Wasteland with Parthenium soil found with *A. niger* var. *japonicas*, *A. flavus*, *A. flavus* var *oryzae*, *A. glaucus* sp.1, *A. glaucus* sp.2, *A. nidulans*, *A. terreus*, *A. candidus*, *A. fumigates*, *Penicillium citrinum*, *P. chrysogenum*, *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Trichoderma virede*, *T. harzianum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Gliocladium* sp., *Fusarium* sp. and White non sporulating fungus. These results indicates that that the agricultural soil may contain various nutrients which are supporting for the growth and survival of wide variety of fungal species in the soil for prolonged period. The above results are in accordance with various reports, the fungi commonly present in different type of soils found as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. terreus*, *Curvularia lunata*, *Pencillium janthinellum*, *P. citrinum*, *Rhizopus nigricans*, *Trichoderma viride*, *T. harzianum*, *Fusarium. oxysporium*, *F. moniliforme*, *Mucor*, *Cladosporium*, *Verticillium*, *Acremonium*, *Chaetomium globosum*, *Corynespora cassicola*, *Eurotium chevelari*, *Botrycis cineea*, *Helminthosporium* sp. etc. (Chirstensen *et al.*, 1962; Deka and Mishra, 1984; Wahid *et al.*, 1997; Saravanakumar and Kaviarasan, 2010.). Prince *et al.*, 2011 investigated the seasonal variations in soil fungal population of traditional sugarcane field in Thanjavur District, Tamilnadu viz Orathanadu and Pattukottai with 49 different species belonging to Phycomycetes and Deteromycetes with dominant species as *Aspergillus niger*, *A. flavus* followed by *Botrytis cinera*, *Trichoderma viride*, *T. harizianum*, *T. koeningii*, *T. glaucum*, *Penicillium chrysogenum* and *P. citrinum* which is similar to our findings.

On analysis of individual soil samples, in the Groundnut field shows *A. niger*, *A. japonicus*, *A. flavus*, *A. flavua* var *oryzae*, *A. glaucus*, *Cladosporium* sp., *Cuvularia lunata*, and

Trichoderma sp.. are the fungi commonly present in the order of dominance. At the same time the soil samples collected from the sugarcane field shows *A. niger*, *A. flavus*, *A. glaucus amster*, *A. nidulans*, *Penicillium* sp. are present in significant level. But the mycoflora of the soil samples collected from the Turmeric field shows a unexpected results as very low percentage of fungal colonies with one each of *Mucor* and other nonsporulating white colony with poor growth. Surprisingly this absence of fungal load in the turmeric field may be due to the excretion of antimicrobial bioactive compounds from the rhizome in the soil environment. This in accordance with earlier scientific reports that indicates the antimicrobial effect of turmeric on various microorganisms. But in the soil mycoflora of tapioca field shows the sole presence of *Gliocladium* sp. occuring in very high percentage in the culture plates. This may be due versatile nature of fungi present in different crop environment.

In conclusion, the present study shows the versatile nature of fungal biodiversity from different agricultural soil samples with different population density which may be due to there between the soil types, crop cultivation, soil nutrients, soil pH, soil moisture and soil mycoflora. Soil environment of different areas of agricultural field with different crop cultivation was studied to know the relationship of fungal diversity and rhizosphere environment. The interaction between the presence of crop and soil fertility due to manure and pesticide addition and other chemicals present also were interpreted. There is no correlation was found with fungal population and the moisture content of soil samples from different environment. However, there is a antimicrobial effect due to secretion of compounds from plants in the soil environment evidenced from turmeric field

soil from the present study is interesting and pave the way for future work..

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