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Pelagia Research Library

Der Chemica Sinica, 2011, 2 (2): 9-19

Der Chemica Sinica
Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Ecology of soil fungi in paddy field of Tamilnadu-Thanjavur District

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ABSTRACT

Soil is a complex ecosystem, delimited by physicochemical parameters that hold enormous number of living organisms. This study deals with the seasonal and depthwise variations in soil fungal population in relation to the soil nutrient variablility in one of the least studied traditional Paddy field in Thanjavur district, TamilNadu viz Nadur, Orathanadu, Punnainallur and Tholkappiyar Square. About 30 different species belonging to Ascomycetes and Phycomycetes were isolated by using PDA medium and identified by using Standard Manual. Nonetheless, Fungal counts were greater in the surface (SS-10cm) layer of the soil as compared to others. During rainy season, maximum fungal count was, recorded in the subsoil layer (8-10cm). The dominant species were Aspergillus niger, Cunninghamella sp. followed by T.viride, T.harzianum Penicillium janthinellium, P.claviforme, A.terreus and Aspergillus conecium from the paddy field soils of Nadur soils in various seasons whereas, in Orathanadu soils the dominant species were A.niger, Curvularia sp. followed by Aspergillus conecium, A.oryzae, F.oxysporam, P.janthinellium and Trichoderma koeningii. In Tholkapiyar sadhukam, the dominant species were A.niger, T.viride, T.harzianum followed by P.janthinellium ,Penicillium citrinum and Rhizopus sps. whereas in Punnainallur, the dominant species were A.niger, T.harzianum and Cunninghamella sp. followed by F.oxysporum, P.claviforme, P.janthinellium, Trichoderma koeningii and T.viride respectively

Key words: Phycomycetes, Ascomycetes, Fungal population, Paddy field.

INTRODUCTION

Soil is a complex ecosystem, delimited by physicochemical parameters that hold enormous number of living organisms. Nevertheless, microbes are the least unstated mechanism of soil by both agronomists and soil practitioners. On the farm several soil organisms offer benefits to crop growing in an ecosystem, but are not well understood. The soil microbes decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influences

on soil physical, chemical and biological properties and on creating a complimentary medium for biological reactions and life support in the soil environment. Nonetheless, enhanced site-specific diversity typically results in higher levels of below ground microbial diversity and production (Olson *et al*., 2000).

Large quantities of readily decomposable organic matter are added to agricultural soils every year as crop residues or animal wastes and have a significant outcome on soil microbial commotion. The plant species growing on the soil also equally influence the population and species composition of the soil fungi. (Hackle *et al*., 2000)

Microfungi play a focal role in nutrient cycling by regulating soil biological activity (Arunachalam *et al*.,1997). However, the rate at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions. There have been a number of studies on the distribution of soil microfungi in Agricultural field. Some studies dealt with the influence of plant community (Chung *et al*., 2007., Carney and Matson., 2006). Some with depth effects (Arunachalam *et al*.,1997) and others attempted to examine seasonal trends (Kennedy *et al*.,2005).

This study deals with the seasonal and depthwise variations in soil fungal population in relation to the soil nutrient variability in one of the least studied traditional Agricultural field in South India.

Collection of soil sample

About Twelve Soil samples were collected from the Thanjavur district -Tamilnadu in four villages, viz Nadur, Orathanadu, Punainallur, Tholkappiyar square, the soil samples were taken during the three seasons in Paddy field.

Soil analysis

The mechanical and chemical analyses of the soils were made with the help of Lamottes'soil testing outfit, Nitrogen and organic, etc., were estimated as outlined in Piper's book (1950).

Isolation of microfungi

These soils were studied for the soil fungi with the help of the following four methods.

1. Direct method
2. Dilution method
3. Soil plate and
4. Hyphal isolation method

Dilution plate technique described by (Warcup, 1955) was used for the isolation of fungi from soil sample. 10g of soil from each sample was weighed separately and then dissolved in 100ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil. The soil suspensions were diluted in 10 fold increment from 10^{-2} to 10^{-4}. One ml of the diluted sample was plated onto sterilized Potato Dextrose Agar medium supplemented with 1%streptomycine (1gram of streptomycin was mixed throughly in 100ml of sterilized distilled water). The plates were incubated at room temperature 28ºC for 3-4 days. Three replicates were maintained for each sample.

After three to four days of incubation, the colonies growing on Potato Dextrose Agar plates, with different Morphology, were counted and purified on medium separately.

Presentation of data
Population of fungi

$$\text{g-1 dry wt. of the soil} = \frac{\text{Mean no. of propagules in dilution plate}}{\text{Weight of the dry soil}} \times \text{Dilution factor}$$

Pure culture and identification
After the isolation of the microfungi their pure cultures were made by single-spore culture method. A portion of the growing edge of each colony was picked up with the help of a pair of needles and mounted on a clean slide with Lactophenol cotton blue. The slide was gently heated over the flame so as to remove air bubbles. The excess stain was wiped off with help of tissue paper and then the cover slip was sealed with transparent Nail Polish/DPX mountant. The slide was observed under Microscope and Microphotographs of the individual fungal species were also taken using Nikon Microphotograph Microscope (Japan).

Identifications of the organisms were made with help of the relevant literature (Thom and Raper 1945., Raper and Thom 1949., Gillman 1957).

RESULT

Fungal counts were greater in the surface (SS-10cm) layer of the soil as compared to others. During rainy season, maximum fungal count was, recorded in the subsoil layer (8-10cm).

Altogether, 30 forms of fungi were isolated in the four sites. List of isolated fungi during the study is shown in Table III.

A critical review of Table I shows Microfungi in different seasons at various depths from various soils and Table II shows that dominant species were *Aspergillus niger, Cunninghamella* sp. followed by *T.viride, T.harzianum Penicillium janthinellium, P.claviforme, A.terreus* and *Aspergillus conecium* from the paddy field soils of Nadur soils in various seasons whereas, in Orathanadu soils the dominant species were *A.niger, Curvularia* sp. followed by *Aspergillus conecium, A.oryzae, F.oxysporam, P.janthinellium* and *Trichoderma koeningii.*

In Tholkappiyar square paddy field soils the dominant species were *A.niger, T.viride, T.harzianum* followed by *P.janthinellum ,Penicillium citrinum* and *Rhizopus* sp. whereas in Punnainallur paddy field soils the dominant species were *A.niger, T.harzianum* and *Cunninghamella* sp. followed by *F.oxysporam P.claviforme, P.janthinellium, Trichoderma koeningii and T.viride.*

Table 1 Comparison of Mechanical and Chemical analysis of the Nadur, Orathanadu, Punainallur and Tholkappiyar square soils in Paddy field (per hectar)

S	Season	Moisture content in %								pH	EC	N	P	K
		ss*	2”	4”	6”	8”	10”	12”	14”					
Nadur	Winter	28.1	30.2	31.9	32.2	32.5	33.8	34.2	34.6	6.9-7.1	0.25	1.7	0.136	1.58
	Summer	18.0	18.9	20.0	21.0	20.0	20.0	19.0	19.2	6.9-7.0	0.66	1.73	0.152	1.58
	Rainy	43.5	43.2	43.2	41.6	41.8	41.6	41.4	41.0	7.1-7.4	0.69	1.75	0.135	1.54
Orathanadu	Winter	31.2	31.6	31.0	32.4	33.2	33.8	34.2	35.8	7.1-7.4	0.35	1.56	0.132	1.46
	S0 ffppummer	17.8	17.8	17.8	17.8	17.8	17.8	17.8	17.8	7.2-7.8	0.19	1.56	0.138	1.47
	Rainy	42.0	40.9	41.0	41.1	40.5	39.5	39.0	38.5	7.0-7.5	0.61	1.54	0.142	1.29
Punnainalur	Winter	16.6	16.8	16.9	16.4	16.0	15.8	15.8	15.0	7.1-7.3	0.35	1.28	0.189	1.44
	Summer	17.0	18.0	18.2	17.8	16.4	16.2	16.0	16.3	7.2-7.5	0.19	1.22	0.219	1.49
	Rainy	42.6	41.9	41.0	40.9	41.5	41.0	40.8	40.8	7-7.5	0.38	1.24	0.175	1.35
Thulkappiyar square sadhukam	Winter	43.1	43.1	43.3	44.2	44.5	44.2	44.2	44.2	7-7.5	0.72	1.38	0.127	1.59
	Summer	17.6	17.0	17.4	18.2	18.8	19.2	19.8	20.6	7.3-7.8	0.68	1.1	0.138	1.76
	Rainy	40.2	40.0	40.3	40.7	40.3	40.2	40.2	40.0	7-7.4	0.67	1.24	0.135	1.30

Table II Microfungi in different seasons at various depths from various soils (cm)

S.No	Species	NADUR			ORATHANADU			PUNNAINALUR			THOLKAPPIYARSQUARE		
		R	S	W	R	S	W	R	S	W	R	S	W
Phycomycetes													
1.	*Rhizopus* sp.	-	-	-	-	-	-	-	-	-	**8”**	**8”**	-
Ascomycetes													
2.	*Neurospora crassa*	**SS**	-	-	-	-	-	-	-	-	-	-	-
3.	*Sclerotium* sp.	-	-	**4”**	**4”**	-	-	-	-	-	**2”**	-	-
4.	*Acrocylindrium oryzae*	**2”**	-	-	**4”**	-	-	-	-	-	**2”**	-	-
5.	*Aspergillus conecium*	**4”**	**SS**	-	-	**SS**	**SS**	**2”**	-	-	-	-	-
6.	*A. flavus*	-	-	**SS**	-	-	-	-	-	**SS**	-	-	**SS**
7.	*A.fumigatus*	-	**2”**	-	-	-	-	**SS**	-	-	-	-	**4”**
8.	*A.luchensis*	**2”**	-	-	**4”**	-	-	-	**SS**	-	-	-	-
9.	*A.nidulans*	-	-	**2”**	-	**SS**	-	-	-	**6”**	-	-	-
10.	*A.niger*	**2”**	**4”**	**6”**	**8”**	**4”**	**2”**	**SS**	**2”**	**4”**	**SS**	**2”**	**4”**
11.	*A.ochraceous*	**SS**	-	**6”**	-	-	-	-	**SS**	-	**4”**	-	**SS**
12.	*A.oryzae*	-	-	-	**SS**	-	**4”**	-	-	-	**2”**	**SS**	-
13.	*A.sydowi*	**SS**	-	-	-	-	**SS**	-	**6”**	-	-	**2”**	-
14.	*A.terreus*	**2”**	**2”**	-	-	-	-	-	-	**4”**	-	-	-
15.	*Fusarium moniliformi*	-	-	**4”**	**SS**	-	-	**4”**	-	-	-	-	**6”**
16.	*F.oxysporam*	**SS**	-	-	**10”**	**2”**	-	**6”**	**2”**	-	**SS**	-	-
17.	*Helminthosporium oryzae*	-	**2”**	-	-	-	-	-	-	**2”**	-	-	**2”**
18.	*Penicillium citrinum*	**4”**	-	-	**8”**	-	-	**4”**	-	-	**2”**	-	**SS**
19.	*P.claviforme*	**SS**	-	**4”**	-	**2”**	-	-	**2”**	**SS**	-	-	-
20.	*P.herquei*	-	**4”**	-	-	-	**2”**	-	-	**2”**	-	-	**SS**
21.	*P.janthinellium*	**SS**	**2”**	-	**2”**	-	**SS**	**8”**	-	**SS**	**2”**	**4”**	**SS**
22.	*P.lanosum*	-	-	**SS**	-	**2”**	-	-	**SS**	-	-	**2”**	-
23.	*P.bioforme*	-	-	-	**SS**	-	-	-	**SS**	-	-	-	-

24.	*P.bovis*	**2”**	-	-	-	-	**4”**	-	-	**2”**	-	-	**SS**
25.	*Trichoderma koeningii*	-	**SS**	-	**2”**	**4”**	-	**8”**	**6”**	-	-	-	-
26.	*T.harzianum*	4”	SS	-	-	SS	-	4”	2”	SS	-	4”	SS
27.	*T.viride*	**SS**	-	**8”**	**2”**	-	**4”**	**SS**	-	**4”**	**SS**	**4”**	**6”**
28.	*Circinella* sp.	-	-	**SS**	-	-	-	-	-	-	-	-	-
29.	*Cunninghamella* sp.	**4”**	**4”**	**6”**	-	-	-	**8”**	**2”**	**4”**	-	-	-
30.	*Curvularia* sp.	-	-	-	**8”**	**6”**	**SS**	**SS**	-	-	**4”**	-	-

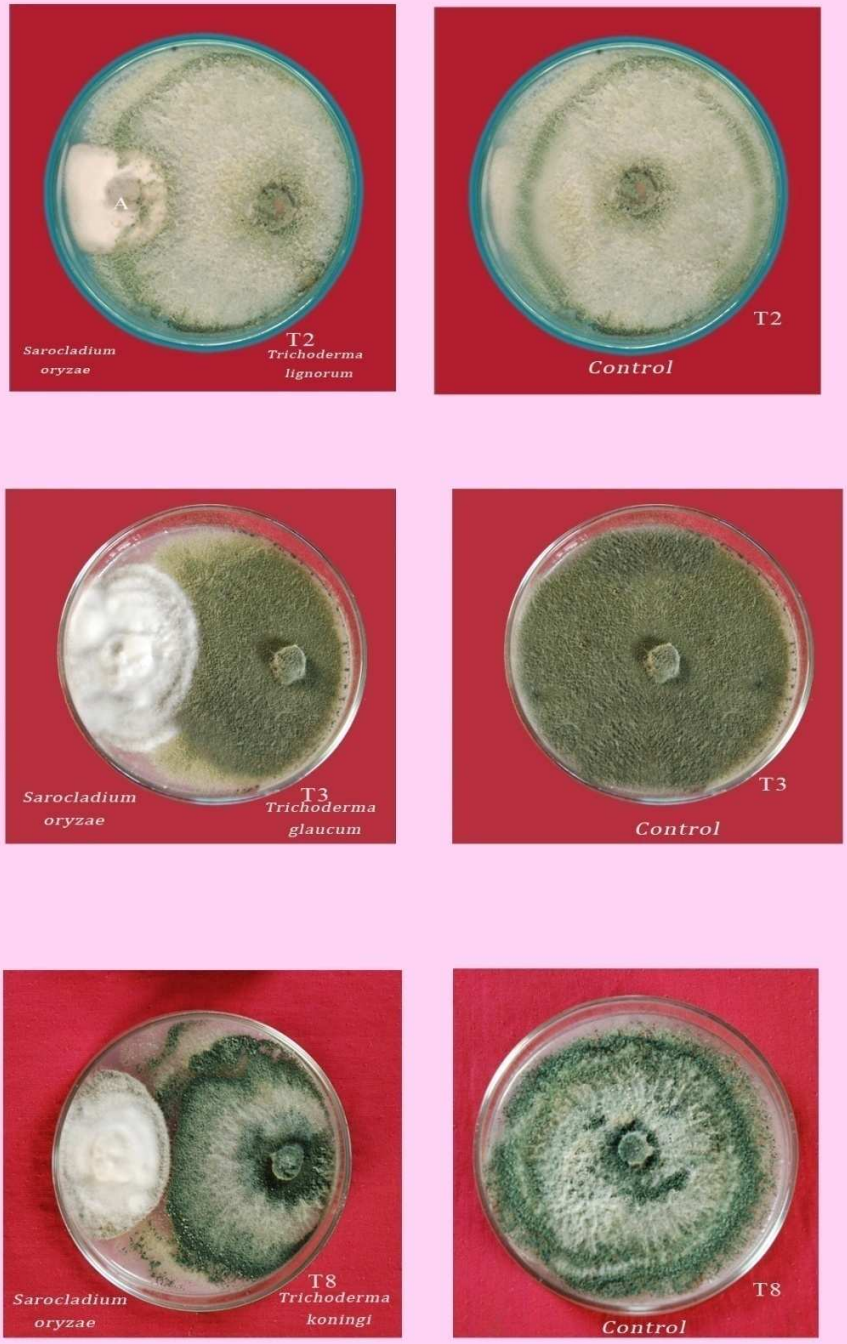
Table III Cultural characteristic on PDA of the isolated genera

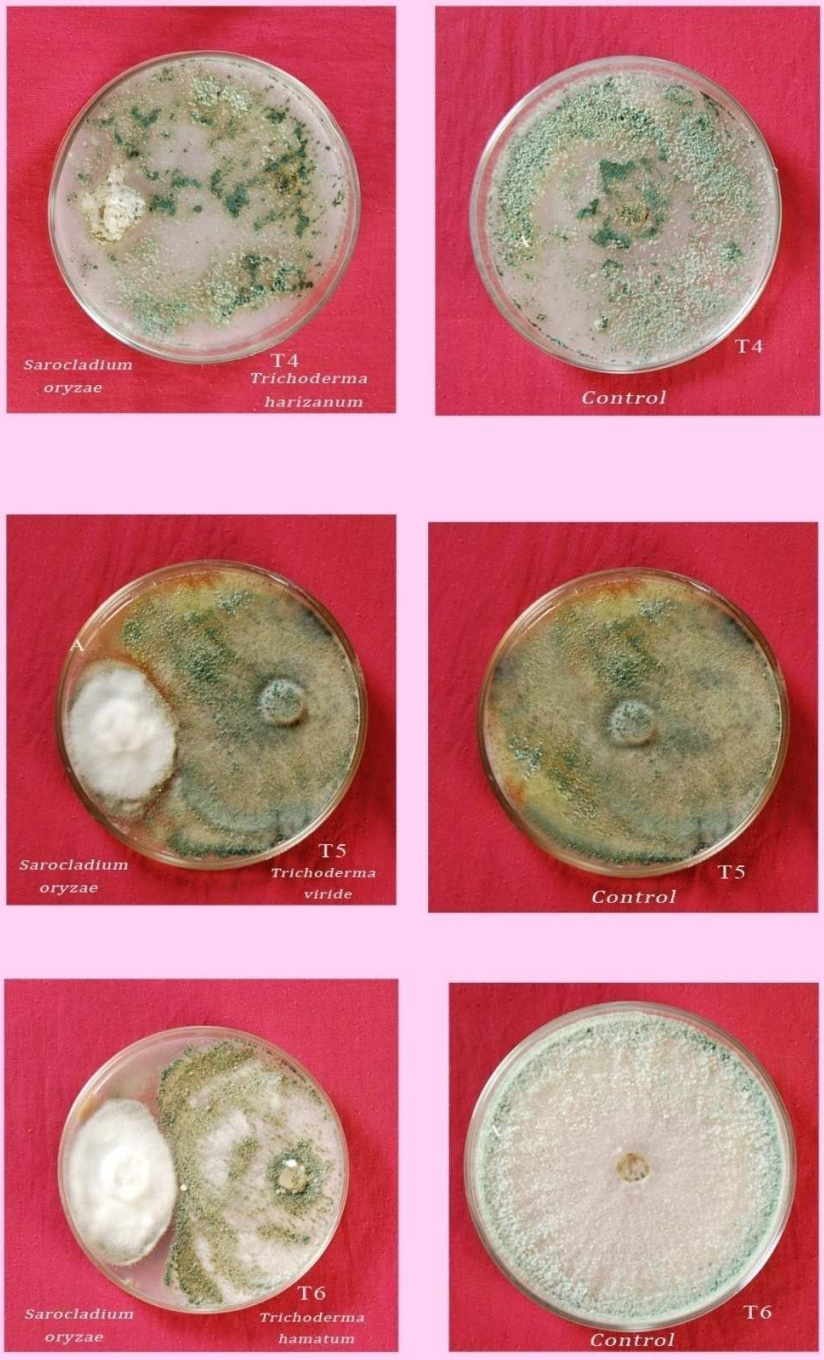
Species	**Upper surface**			**Lower surface**	**Observations**
	Cultural aspect	**Density**	**Color**		
Rhizopus sp.	Effuse, cotton like	light	White at first, become bluish black at maturity	Idem to upper face	Rhizoids rare; Sporangiophores and stolons branched.
Neurospora crassa	Effuse, floccose	High	White	Idem to upper face(maturity yellowish orange)	Curved like ascospore
Sclerotium sp.	Effuse, globose	Medium	Dark olive-gray granules, Philades	Idem to upper face	Filamentous hyphae, with conidiophore
Acrocylindrium oryzae	Effuse, floccose	High	White	Idem to upper face	Filamentous hyphae, with conidiophore
Aspergillus conecium	Effuse, globose	High	Orange to vinaceous or purple sclerotia; two Phialides	Idem to upper face	Hyphae, septate with Conidiophore
A. flavus	Effuse, floccose	Medium	Conidial heads yellow to green	Idem to upper face	Hyphae, septate with Conidiophore
A.fumigatus	Effuse, globose	High	Green to dark green, becoming black in age	Idem to upper face except the color(yellowish)	Hyphae, septate with Conidiophore
A.luchensis	Effuse, powdery	High	Conidial heads dark brown to black; Phialides	Idem to upper face except the color(yellowish)	Hyphae, septate with Conidiophore
A.nidulans	Effuse, globose	High	Drak cress-green	Idem to upper face except the color(Purplish - red)	Hyphae, septate with ascospore
A.niger	Effuse, globose	High	Blackish brown; Phialides	Idem to upper face	Hyphae, septate with Conidiophore
A.ochraceous	Effuse, globose	High	Orange to vinaceous or purple sclerotia; two Phialides	Idem to upper face	Hyphae, septate with Conidiophore
A.oryzae	Effuse, globose	High	Orange to vinaceous or purple sclerotia; two Phialides	Idem to upper face	Hyphae, septate with Conidiophore
A.sydowi	Effuse, floccose	Medium	Velvety, Bluish-green	Idem to upper face except the color (orange to red later black)	Hyphae, septate with Conidiophore
A.terreus	Effuse, floccose	High	Pinkish-Cinnamon, deeper brown shades	Idem to upper face except the color (Pale or bright yellow to deep brown)	Hyphae, septate with Conidiophore
Fusarium moniliformi	Effuse, floccose	High	White	Idem to upper face	Cushion-shaped,conidiophores branched, conida terminal sickle shaped with septate
F.oxysporam	Effuse, globose	High	Brownish white to violet	Idem to upper face except the color	Oval to reniform chlamydospores

				(Carmine to yellow)	
Helminthosporium oryzae	Effuse, floccose	High	Velvety Green-brown to black	Idem to upper face	Conidia terminal, cylindrical, clavate with four cross walls(septate)
Penicillium citrinum	Effuse, globose	High	Bluish green to clear green	Idem to upper face(Yellow)	Conidiophore, compact vertical of Phialides,brush like head
P.claviforme	Effuse,floccose	High	Yellow-green	Idem to upper face(yellow)	Conidiophore, compact vertical of Phialides.
P.herquei	Effuse, floccose	High	Yellow-green	Idem to upper face(yellow)	Conidiophore, compact vertical of Phialides.
P.janthinellium	Effuse, globose	High	Gray -green	Idem to upper face(yellow to ochraceous odor)	Conidiophore, compact vertical of Phialides.
P.lanosum	Effuse, floccose	High	Lanose, white with centre and form gray-green	Idem to upper face(slightly yellow)	Conidiophore, compact vertical of Phialides.
P.bioforme	Effuse, floccose	High	Gray-green to brownish	Idem to upper face(cream color)	Conidiophore, compact vertical of Phialides.
P.bovis	Effuse, floccose	High	Gray-green to brownish	Idem to upper face(cream color)	Conidiophore, compact vertical of Phialides.
Trichoderma koeningii	Effuse, globose	Medium	Light green	Idem to upper face	Conidia elliptical,septate, smooth, hyaline.
T.harzianum	Effuse, globose	Medium	Green-yellowish with white to grey flakes	Idem to upper face	Conidiophores,hyphae,chlamydospores and phialides, flsk like pins-shaped.
T.viride	Effuse, globose	High	Green-white to grey flakes	Idem to upper face	Conidiophores,hyphae,chlamydospores and phialides, flsk like pins-shaped.
Circinella sp.	Effuse, High White	High	Turf white, then gray	Idem to upper face	Mycelium strongly branched,nonseptate with sporangiophores.
Cunninghamella sp.	Effuse, floccose	Light thick	Turf white to silver	Idem to upper face	Septate,conidiophores straight branched in spherical heads.
Curvularia sp.	Effuse, floccose	Light thick	Dark olive-gray	Idem to upper face(Bluish black)	Curved three or four Septate, thread-like conidiophore

DISCUSSION

Generally, topsoil contains high organic matter, which in the presence of adequate moisture supply is acted upon by the microorganisms to decompose the complex organic residues into simpler forms; hence, microbial counts are generally higher in the surface soil layer (Shamir and Steinberger, 2007) as compared to the lower depths. However, the distribution of soil microbial population is determined by a number of environmental factors like pH, Moisture content and soil organic matter (Kennedy *et al.*, 2005) higher fungal population during rainy and autum supported the findings of other workers (Arunachalam *et al.*,1997), which perhaps is due to prevailing favorable moisture and temperature setting during the period litter and other plant residues are decomposed faster during rainy season and sufficient soil organic matter and humus accumulates that may have enhanced the colonization of the soil microbes in subsequent period.


A
T2
Sarocladium oryzae
Trichoderma lignorum
T2
Control
T3
Sarocladium oryzae
Trichoderma glaucum
T3
Control
T8
Sarocladium oryzae
Trichoderma koningi
T8
Control


Sarocladium oryzae
T4
Trichoderma harizanum
T4
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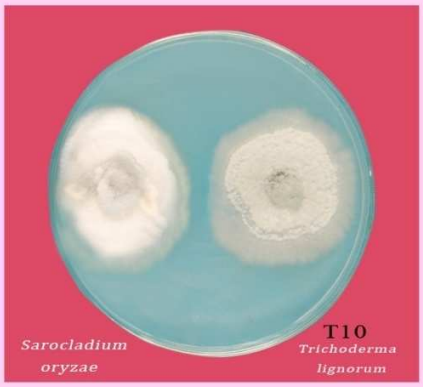
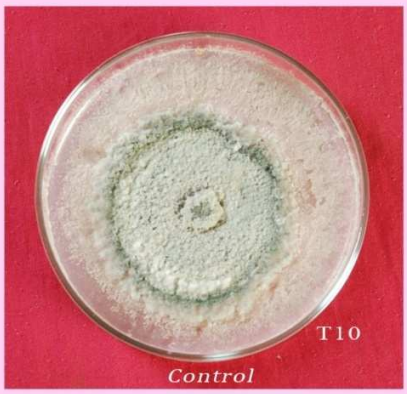
Sarocladium oryzae
T5
Trichoderma viride
T5
Control
Sarocladium oryzae
T6
Trichoderma hamatum
T6
Control


Sarocladium
oryzae
T10
Trichoderma
lignorum


T10
Control

Maximum population in the subsoil layer during rainy season in all the four sites studied corporates to that of Classen *et al*. (2007) who pointed out that during hot summer months, the sub-layer of soil occasionally harbors more fungal populations caused by temperature and moisture regimes than the topsoil layer. However, Shukla *et al. (*1989) found negligible difference in fungal population across depths.

(Rani and Panneerselvam, 2010) reported that the diversity and distribution of different organisms in the marine environment are influenced by the physico-chemical properties of both water and the sediments. Point calimere includes many diverse habitats such as sandy and muddy shores and mangroves, which have various physico-chemical features. A total of 59 fungal species were isolated from all four stations. In our study total of 30 soil microfungi were isolated. However, only a few fungal species were found to be dominant and basically marked variations in the composition of species were noticed in different seasons of the year across the sites. Senthilkumar *et al*. (2009) suggested that 15 soil samples were collected from three different stations along the Muthupet mangroves in Tamilnadu and examined by dilution plating method on PDA medium to access fungal diversity and the population diversity. Of 22 species *Aspergillus, Penicillium* was represented as dominant one of each. Incorporate with our study species like *Aspergillus* sp, *Penicillium* sp. and *Trichoderma* sp. were common to all sites. Some fungal species encountered were rare and restricted to particular site. Dominance of the genus *Aspergillus* sp. and *Penicillium* sp. in the present study sites may be due to their greater rate of spore production and dispersal and partly due to their resistance over extreme environmental conditions (Schimel, 1995).

Soil can be managed to optimize its fertility and health under natural and agricultural land uses, so as to benefit fungal diversity. Due to the dispersed nature of the soil asset, a broad but consistent and economically appealing approach to its protection is needed.

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