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Primary screening, Characterization and Seasonal variations of *Aspergillus* and *Penicillium* species in the Black cotton soils (Vertisols) of Salur Mandal, Viziyanagaram District, Andhra Pradesh, India

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Abstract

Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. This study deals with the primary screening, characterization and seasonal variations of mycoflora, isolated from twelve soil samples. The soil samples were collected from agricultural fields of five different locations at Salur Mandal during three seasons such as Kharif, Rabi and summer respectively. Dilution plate technique was used to isolate soil fungi from various soil samples. Fungal isolates were screened on different culture media namely Potato Dextrose Agar, Czapek's Dox Agar, and Sabouraud's Dextrose Agar supplemented with 1% Streptomycin. Fungal colonies were counted and screened for the occurrence of different fungal species along with Aspergillus and Penicillium presented in soil samples. Identification of the soil isolates were made with help of the relevant literature and standard manuals of soil fungi. Mycoflora including Aspergillus (12 species), Penicillium (6species), Trichoderma (3 species), Cunninghamella (2 species), Curvularia (2 species), Fusarium (2 species), Mucor (2 species) and Rhizopus (1 species) were identified with the help of taxonomic keys. Among the isolates the genera Aspergillus and Penicillium were dominant due to their sporulation ability. The periodicity of occurrence of each species was calculated by using data analysis.

Keywords: Mycoflora, *Aspergillus*, *Penicillium,* Black cotton soils, Salur.

Introduction

Many microbes have their origin in the soil or are closely allied with soil environment. Throughout history these microbes have a substantial impact on humankind. The soil microbes decompose the plant and animal residues 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[1]. Microbes are especially important components of biodiversity. Particularly fungi and bacteria are crucial, as they change and release many nutrients playing important roles in nutrient cycling[2,3] and sustenance of vegetation. The efficiency of fungi in decomposition and their potentiality depend upon their abundance and composition.

Aspergillus and *Penicillium* are economically, ecologically, and medically important and large genera. They are important in view of health hazards. In addition, they are used in industrial and food fermentation processes, and they exist commonly in different types of soils, indoor and outdoor air, food and water[4]. *Aspergillus and Penicillium* are ubiquitous fungi. The species of *Aspergillus* and *Penicillium* are among the most abundant and widely distributed microfungi in nature[5-8]. The decomposition process carried out by these moulds is important in driving natural cycling of chemical elements, particularly in the carbon cycle where they contribute to replacement of the supply of carbon dioxide and other inorganic compounds[9].

Christensen[10] reported that species diversity of soil fungi is a reflection of multiple factors and appears to be reduced by disturbances and manipulation activities. Natural or anthropogenic disturbances can alter the species composition or may have negative effect on species diversity of the decomposer fungi[11]. These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization. During the past four decades, a large number of herbicides have been introduced as pre or post emergent weed killers in many countries of the world. These herbicides could accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man[12].

Black soils, popularly known as black cotton soils, are usually deep to very deep and are dominated by smectitic clays. They are characterized by the presence of either slicken- sides or wedge-shaped peds, ≥ 30% clay and cracks that open and close periodically. Recent findings on shrink–swell soils (generally known as black soils) have shown them to be red in colour which makes the common name of red and black soils[13]. Vertisols are a group of heavy-textured soils which occur extensively in the tropics, subtropics and warm temperate zones and are known as Dark Clays, Black Earths, Black Cotton soils, Dark Cracking soils, Grumusols and Regurs in other classification systems[14]. The major areas of Vertisols are found in Australia (70.5 mha), India (70 mha), Sudan (40 mha), Chad (16.5 mha) and Ethiopia (10 mha); these five countries contain over 80% of the total area of 250 mha of Vertisols in the world[14]. The major factor contributing to the productivity of Vertisols in semi-arid environments is their high water-holding capacity.

The present work was designed to study the fungal diversity and their seasonal variations in the soils of agricultural fields of Salur Mandal. We presented an account on the periodicity of occurrence of *Aspergillus* and *Penicillium* species in agricultural soils which are the major decomposers of soil ecosystem and the impact of management practices on species richness and abundance of microfungi occurring in soil during different seasons. The detail information of edaphic factors which influence the fungal diversity in the soil of study area was described.

Material and Methods

Topography and general features of the study area: Location and site description: Salur or Saluru is a Municipality and Mandal headquarter in Vizianagaram district of Andhra Pradesh state in India. It is bounded by Makkuva, Bobbili, Ramabhadrapuram and Pachipenta mandals of Andhra Pradesh state and Koraput district of Odisha. Salur is located on the River bank of Vegavathi at 18.5333^0 N 83.2167^0 E. The climate of the town is generally characterized by high humidity almost all round the year, oppressive summer and seasonal rainfall. The temperature varies between 17 and 40°C and average annual rainfall is 1074 mm. The nature of the soil is generally black cotton soil. Paddy, Maize, Ground nut, Cotton are main Kharif crops while Sugarcane, Maize, Pulses and Tobacco are cultivated in Rabi season.

Cultivation of crops: Agricultural practices were carried out on the basis of different monsoons like south west monsoon (June-Sep), north east Monsoon (Oct-Dec), winter (Jan- Feb) and summer (March-May). The nature of the soil is generally black cotton and there are 6 types of major soils: 1. Red Sandy loams 2 .Red Sandy clay loams 3. Red Loamy sandy soils 4. Clay loam 5. Sandy soils 6. Clay soils. The Sources of Irrigation were Canals, Tanks, Open wells, Bore wells (tube wells) and other sources. Paddy, Sugarcane, Ground nut, Mesta , Sesame, Maize, Ragi, Green gram, Black gram and Cotton were the major field crops cultivated in distinct seasons and Mango, Cashew were main horticulture crops (fruits) and Brinjal is main vegetable crop and Areca nut, Oil palm, Coconut were major plantation crops.

Soil sampling: Soil samples were collected based on three different seasons viz., Summer (March 2011 June 2011), Kharif (July 2011-October 2011) and Rabi (November2011 – February2012). The dry season samples were merely to standardize the methodology and not subjected to detailed analysis. From each selected hectare, the soil was collected (between 10:30 am and 4:30pm each day) under sterile conditions with the help of 15 cm iron cores from four symmetrically situated locations near the corners of a square as well as from the centre of the square. The soil samples were collected from five different locations viz., Jeegiram, Kurmaraju peta, Sivarampuram and Neliparthi. Soil samples were collected from the depth of approximately 10-15 cm in sterilized polyethylene bags and stored at 4°C in the laboratory until the examination. The collected soils samples along with locations showed in table-1.

Table- 1
Collection of soil samples from various crops seasonally at four locations of Salur Mandal

S.No	Sample No	Season	vegetation	location
1	Sample-1	Summer	Paddy	Jeegiram
2	Sample-2	Summer	Maize	Kurmaraju peta
3	Sample-3	Summer	Ground nut	Sivarampuram
4	Sample-4	Summer	Cotton	Neliparthi
5	Sample-5	Kharif	Sugarcane	Jeegiram
6	Sample-6	Kharif	Maize	Kurmaraju peta
7	Sample-7	Kharif	Red gram	Sivarampuram
8	Sample-8	Kharif	Sugarcane	Neliparthi
9	Sample-9	Rabi	Red gram	Jeegiram
10	Sample-10	Rabi	Black gram	Kurmaraju peta
11	Sample-11	Rabi	Sesamum	Sivarampuram
12	Sample-12	Rabi	Sugarcane	Neliparthi

Analysis of soil samples: The collected soil samples ware dried in aseptically at departmental laboratory for characterization of physico-chemical properties. The physico-chemical parameters of the soil samples were analyzed at Mobile Soil Testing Laboratory (MSTL), Pothinamallayapalem, Visakhapatnam, Department of Agriculture, Andhra Pradesh. The physico-chemical properties of soils were showed in table -2 and 3.

Isolation of Soil mycoflora: Dilution plate technique described by Warcup[15] was used for the isolation of fungi from various soil samples collected in agricultural crop fields at Salur Mandal. Ten grams of soil samples were suspended in 90 mL of distilled water (in Erlenmeyer glass flask), then mix by using wrist action shaker for one hour at 120 rpm. 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^{-3} to 10^{-5}. The Volume of 1 mL of soil sample suspension from each serial dilution was pipetted onto different melted, cooled culture media namely Potato Dextrose Agar(PDA) [Potatoes(peeled) 200 g; Dextrose 20 g; Agar 20 g; Distilled water 1L][16] Czapek's Dox Agar(CZA) [Sucrose 30g; $NaNO_3$ 2 g; K_2HPO_4 1g; $MgSO_4$+$7H_2O$ 0.5 g; KCl 0.5 g; $FeSO_4$+$7H_2O$ 0.01g; Agar 15 g; Distilled water 1L][17] and Sabouraud's Dextrose Agar (SDA)[Glucose 40g; Peptone 10g; Agar 15g; Distilled water 1L][18] supplemented with 1% Streptomycin (1gram of streptomycin was mixed thoroughly in 100ml of sterilized distilled water). The pH of the culture media was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. Each culture media was prepared in a liter of distilled water and autoclaved at 120°C at 15 psi for 20 min.1% Streptomycin was used as an antibiotic for the restrain of bacterial growth. Fungi were maintained on half-strength PDA slants in test tubes as stock cultures[19].

Table -2
Analyses of soil physical characters (The dry season samples were used to standardize the methodology)

Soil Sample	Soil type	Soil color	Soil texture (%)		
			Sand	clay	Dust
1	Sandy Clay loam	Dark brown	17.23	42.19	40.55
2	Red sandy loam	red	11.35	37.58	21.11
3	Red loamy sandy	Brown Reddish	20.11	57.25	42.21
4	Clay loam	Black	12.78	62.45	24.77
5	Sandy Clay loam	Dark brown	16.98	40.26	42.25
6	Red sandy loam	red	06.85	53.01	40.02
7	Red loamy sandy	Brown Reddish	20.51	19.32	62.21
8	Clay loam	Black	13.26	65.23	25.06
9	Sandy Clay loam	Dark brown	24.68	18.56	56.28
10	Red sandy loam	red	08.36	54.81	35.26
11	Red loamy sandy	Brown Reddish	29.14	15.69	54.24
12	Clay loam	Black	17.58	42.56	39.69

Table-3
Analyses of soil chemical characters (The dry season samples were used to standardize the methodology)

Soil sample	Soil moisture	Soil pH	Electrical conductivity (Dsm^{-1})	Nitrogen (Kg/ac)	Organic carbon (%)	P_2O_2 (Kg/ac)	K_2O (Kg/ac)	Iron (ppm conc.)	Copper (ppm Conc.)	Mn (ppm Conc.)	Zn (ppm Conc.)
1	18.05	7.4	0.25	131.4	1.54	10.95	37.84	16.79	0.69	5.56	0.52
2	14.21	6.7	0.66	169.5	1.85	13.14	49.28	18.95	0.66	6.01	0.51
3	15.68	7.6	0.69	120.1	1.35	15.12	35.29	15.26	0.52	6.02	0.56
4	24.08	7.7	0.35	135.2	1.62	14.28	45.32	16.23	0.46	6.32	0.46
5	20.52	6.8	0.19	142.5	1.45	15.28	36.28	15.26	0.53	5.86	0.65
6	18.25	7.3	0.61	132.5	1.24	11.35	26.18	14.65	0.64	5.23	0.54
7	21.84	7.1	0.35	143.2	1.24	12.35	45.32	18.23	0.45	6.54	0.53
8	24.36	7.3	0.35	156.6	1.54	10.35	45.36	16.25	0.53	5.86	0.52
9	15.02	6.8	0.19	169.6	1.64	10.56	35.36	15.32	0.66	6.25	0.53
10	13.21	6.9	0.38	132.1	1.84	13.25	38.47	16.25	0.64	5.32	0.45
11	17.54	7.2	0.72	142.6	1.24	14.25	42.35	13.26	0.54	5.24	0.62
12	16.25	7.4	0.64	135.8	1.48	10.22	32.28	15.65	0.65	6.06	0.56

Incubation of Soil mycoflora: The plates were incubated at room temperature 28±2°C in an inverted position for 5-7 days. Three replicates were maintained for each sample**.** During the incubation period pure cultures of soil mycoflora were prepared on PDA slants under aseptic condition. Individual colonies of *Aspergillus* and *Penicillium* were incubated on potato dextrose agar medium for seven days to measure their diameter.

Identification of Soil mycoflora: Identification of the soil isolates were made with help of the relevant literature[20,21]. Fungal morphology was studied macroscopically by observing colony features (colony diameter, colour texture, and colony colour) and microscopically by staining with lacto phenol cotton blue called as mounting fluid and observed under compound microscope for the observation of the conidia, conidiophores and arrangement of spores.

Data analysis: Number of species is referred as species diversity. Population density is expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. The percentage contribution of each isolate was calculated by using the following formula.

$$\frac{\text{Total no. of CFU of an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

**CFU-Colony Forming Unit.*

The periodicity of occurrence was calculated for fungi are arbitrarily classified as follows: Common - recorded in 10 - 12 samplings, Frequent - recorded in 7 - 9 samplings, Occasional - recorded in 4 - 6 samplings, Rare - recorded in 1 - 3 samplings

Results and Discussion

A total of 30 fungal species were isolated from twelve soil samples. The identification of these isolates resulted in 8 genera of soil fungi including *Aspergillus* (12 species), *Penicillium* (6 species), *Trichoderma* (3 species), *Cunninghamella* (2 species), *Curvularia* (2 species), *Fusarium* (2 species), *Mucor* (2 species) and *Rhizopus* (1 species). The soil mycoflora of twelve samples were screened and the associated mycoflora with *Aspergillus* and *Penicillium* species were identified. The fungal isolates of *Cunninghamella blakesleeana, Cunninghamella elegans, Curvularia clavata, Curvularia lunata, Fusarium oxysporum, Fusarium solani, Mucor hiemalis, Mucor racemosus, Rhizopus stolanifer, Trichoderma virens, Trichoderma viride,* and *Trichoderma harzianum* species were identified along with *Aspergillus* and *Penicillium* species by the standard macro and microscopic observations. The Characterization of mycoflora was made with the help of relevant literature and standard manuals. Among the isolates a total of 327,252 and 716 colonies of *Aspergillus* and a total of 132,135 and 219 colonies of *Penicillium* were screened seasonally in three different seasons such as Summer, Kharif and Rabi, respectively. Different isolates of Aspergillus species including *Aspergillus awamori, A. candidus, A. deflectus , A. flavus , A. fumigatus , A. nidulans, A. niger , A. repens , A. tamari , A. terreus , A. ustus and A. versicolor* were identified by using macro and microscopic methods and standard manuals. On the other hand different species of *Penicillium* species including *Penicillium aurantiogriseum, P. chrysogenum, P. citreonigrum, P. citrinum, P. digitatum* and *P. purpurogenum* has been isolated (table-4 and 5).

The average number of different species of *Aspergillus* and *Penicillium* mycoflora was studied to estimate the dominant species in agricultural fields of Salur Mandal. Specises wise percentage of *Aspergillus* and *Penicillum* was showed in table 4 and 5.The frequency of all isolates was studied by using data analysis and the genus *Aspergillus* and *Penicillium* were dominant in all soil samples. The results showed that the species of *Aspergillus niger* (*8.0 %, 1.06 %,* 9.9%) *Aspergillus flavus* (10.4%, 9.8%, 8.9%) and *Aspergillus terreus* (8.0%, 9.0%, 8.3%) were dominant among the isolates of Aspergillus, whereas species of *Penicillium chrysogenum* (5.0%, 4.8%, 4.4%) and *Penicillium citrinum* (4.3%, 3.4%, 7.1%) were the most dominant among the isolates of *Penicillium* in particular three seasons viz., Kharif, Rabi, and summer respectively. The periodicity of occurrence is significantly different among the three seasons due to different climatic conditions and agricultural practices. When compared with the summer seasons, the number of colonies per gram of soil shows a decline by about 50% in the dry season. Rainfall is the most important factor for microbial diversity. The low count in the March (dry) collection is also likely to have been a consequence of the annual fires that occur between January and March.

The total of 12 strains of *Aspergillus* and 6 strains of *Penicillium* were isolated by using Potato Dextrose Agar (PDA), Czapek's Dox Agar(CZA) and Sabouraud's Dextrose Agar (SDA) media. The nutritional composition was main factor for the growth and sporulation of soil fungi. However the various species of soil fungi were isolated on Potato Dextrose Agar which was favorable for most soil fungi. The various observations of *Aspergillus* and *Penicillium* mycoflora were made by using macroscopic and microscopic methods. Macroscopic features including the colony diameter, colony texture and colony colour (upper and lower surfaces), and exudates of mycelium were examined for the identification of soil mycoflora (figure-2 and 3). We also examined the microscopic features of mycelium including conidial heads, conidiophores, conidia and the vesicles for the identification of isolates of *Aspergillus* and *Penicillium* mycoflora. The exudates of isolates were examined and appeared in pale, red, yellow and orange colour. The systematic study for characterization of Aspergillus and Penicillium species was done by using the standard manuals and relevant literature of soil fungi (table-6 and 7).

Table- 4
Mycoflora of agricultural soils and their percentage of occurrence in three distinct seasons

Name of the fungal species	Summer		Kharif		Rabi	
	Ave.no.ofCFU per gram of dry soil	Occurrence (%)	Ave.no.ofCFU per gram of dry soil	Occurrence (%)	Ave.no.ofCFU per gram of dry soil	Occurrence (%)
Aspergillus awamori	22	3.7	26	3.4	42	4.0
Aspergillus candidus	23	3.8	23	3.0	36	3.4
Aspergillus deflectus	40	6.7	45	6.0	56	5.4
Aspergillus flavus	62	10.4	74	9.8	92	8.9
Aspergillus fumigatus	34	5.7	60	8.0	84	8.1
Aspergillus nidulans	18	3.0	22	2.9	44	4.2
Aspergillus niger	48	8.0	80	10.6	102	9.9
Aspergillus repens	10	1.6	10	1.3	20	1.9
Aspergillus tamarii	28	4.7	44	5.8	56	5.4
Aspergillus terreus	48	8.0	68	9.0	86	8.3
Aspergillus ustus	10	1.6	14	1.8	26	2.5
Aspergillus versicolor	34	5.7	59	7.8	72	6.9
Penicillium aurantiogriseum	14	2.3	15	2.0	30	2.9
Penicillium chrysogenum	30	5.0	36	4.8	46	4.4
Penicillium citreonigrum	18	3.0	20	2.6	42	4.0
Penicillium citrinum	26	4.3	26	3.4	43	4.1
Penicillium digitatum	20	3.3	22	2.9	36	3.4
Penicillium purpurogenum	24	4.0	16	2.1	22	2.1
Cunninghamella blakesleeana	5	0.8	7	0.9	4	0.3
Cunninghamella elegans	1	0.1	1	0.1	2	0.1
Curvularia clavata	8	1.3	10	1.3	15	1.4
Curvularia lunata	7	1.1	12	1.6	14	1.3
Fusarium oxysporum	10	1.6	12	1.6	14	1.3
Fusarium solani	8	1.3	10	1.3	12	0.1
Mucor hiemalis	4	0.6	5	0.6	4	0.3
Mucor racemosus	4	0.6	5	0.6	4	0.3
Rhizopus stolanifer	5	0.8	5	0.6	6	0.5
Trichoderma virens	12	2.0	14	1.8	15	1.4
Trichoderma viride	10	1.6	15	2.0	18	1.7
Trichoderma harzianum	10	1.6	12	1.6	14	1.3
Total	593	-	750	-	1030	-

CFU-Colony Forming Unit (per gram of dry soil x 10^5)

Table- 5
Periodicity of occurrence of mycoflora in various samples collected from agricultural soils at Salur Mandal.

S. No	Name of the Fungus	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	Periodicity of occurrence
1	*Aspergillus awamori*	+	-	+	+	-	-	+	+	-	-	-	-	O
2	*Aspergillus candidus*	-	+	+	-	-	+	+	-	-	+	-	+	O
3	*Aspergillus deflectus*	+	+	+	+	+	-	-	+	+	+	+	-	F
4	*Aspergillus flavus*	+	+	+	+	+	+	+	+	+	+	+	+	C
5	*Aspergillus fumigatus*	+	-	+	+	+	+	-	-	+	+	+	-	F
6	*Aspergillus nidulans*	+	+	+	-	-	-	+	+	-	-	-	+	O
7	*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	+	C
8	*Aspergillus repens*	-	+	+	-	-	+	+	-	+	-	+	-	O
9	*Aspergillus tamarii*	+	+	-	-	+	-	+	+	+	+	+	+	F
10	*Aspergillus terreus*	+	+	+	+	+	+	+	+	+	+	+	+	C
11	*Aspergillus ustus*	-	-	+	-	+	-	-	-	+	-	-	-	R
12	*Aspergillus versicolor*	+	+	-	-	+	-	+	+	+	+	+	+	F
13	*Penicillium aurantiogriseum*	-	+	+	+	-	-	+	+	+	-	-	-	O
14	*Penicillium chrysogenum*	+	+	+	+	+	+	+	+	+	+	+	+	C
15	*Penicillium citreonigrum*	-	+	-	+	-	-	+	-	-	-	+	+	O
16	*Penicillium citrinum*	+	-	+	+	+	-	+	+	-	+	+	-	F
17	*Penicillium digitatum*	+	+	-	-	-	+	+	+	+	+	+	+	F
18	*Penicillium purpurogenum*	+	-	-	-	+	+	+	+	+	-	-	-	O
19	*Cunninghamella blakesleeana*	-	+	+	+	-	+	-	+	-	-	-	-	O
20	*Cunninghamella elegans*	-	+	-	+	-	-	+	-	-	-	-	-	R
21	*Curvularia clavata*	-	+	-	+	+	+	-	+	+	-	+	+	F
22	*Curvularia lunata*	+	+	+	-	-	+	+	+	+	-	+	+	F
23	*Fusarium oxysporum*	+	+	+	+	-	+	+	-	+	+	+	-	F
24	*Fusarium solani*	+		+	-	+	-	+	+	+	+	+	-	F
25	*Mucor hiemalis*	+	+	-	-	-	+	-	-	-	-	-	-	R
26	*Mucor racemosus*	-	-	+	-	-		+	-	--	+	-	-	R
27	*Rhizopus stolanifer*	-	+	-	+	-	+	-	+	+	-	+	-	O
28	*Trichoderma virens*	+	+	+	-	+	-	-	+	+	+	+	-	F
29	*Trichoderma viride*	+	+	-	+	+	+	-	-	+	+	+	+	F
30	*Trichoderma harzianum*	+	+	-	-	-	+	+	+	+	+	+	+	F

C- Common; F- Frequent; O-Occasional; R-Rare.

Table -6
Macro and microscopic features of *Aspergillus* species isolated from various agricultural fields at Salur

S.No	Name of the species	Morphological features				Microscopic observations
		Diam (cm)	Texture	Upper surface	Lower surface	Conidiophores/ conidia/vesicles
1	*A.awamori*	5	Radially furrowed, tough	Greenish brown to dark chocolate brown	Yellow to reddish brown in age	Conidial heads globose loosely radiate. Conidiophores coloured in terminal areas. Phialides biseriate. Conidia globose. Vesicles globose, light brown in colour.
2	*A. candidus*	3	Effuse, globose	Persistently white or cream with age.	Colorless or pale gray brown	Conidial heads white, globose, often splitting in age. Conidiophores colorless, smooth, thick walled. Vesicles globose to subglobose.
3	*A. deflectus*	4	Compact tough	Deep mouse grey, margins pink	Dull orange, brown with age	Conidial heads broadly columnar, Conidiophores smooth, reddish brown. Vesicles round, flask shaped.
4	*A. flavus*	6	Velvety	Yellow to dark green in age	Colourless to pale yellow brown	Conidial heads yellow in young, green in age. Phialides uniseriate or biseriate. Conidia globose to subglobose, yellowish green. Exudates inconspicuous.
5	*A.fumigatus*	6	Velvety to floccose	Dull blue-green	colourless	Conidial heads columnar, compact, often densely crowded. Conidiophores short, smooth, light green. Phialides uniseriate. Conidia globose .
6	*A. nidulans*	5	Effuse, globose	Dark cress green	Purplish red to very dark in age	Conidial heads slightly large. Conidial heads loosely radiate when young later short columnar. Phialides biseriate. Vesicles hemispherical, brown. Conidia globose, green. Exudates lacking.
7	*A. niger*	6	Effuse, globose	Carbon black or deep brownish black	Colourless to pale yellow	Conidial heads large and black, at first globose then radiate or splitting in well defined columns in age. Phialides biseriate, brownish .Vesicles globose. Conidia globose, spinulose, black in colour.
8	*A. repens*	4	Wrinkled	Yellow green to grayish green.	Yellow- orange to deep maroon	Conidial heads abundant radiate, often splitting into columns with diverging chains of conidia. Conidiophores smooth, colorless.
9	*A. tamarii*	4	Effuse, floccose	Brownish green	colourless	Conidial heads globose to loosely radiate. Conidiophores smooth, colorless. Vesicles globose to sub globose.
10	*A. terreus*	5	Velvety to floccose	Pinkish cinnamon, deep brown shades	Dull brown	Conidial heads long columnar, compact with uniform diameter. Conidiophores smooth and colorless. Exudates amber coloured. Conidia globose to sub globose, smooth.
11	*A. ustus*	5	Floccose radially zonate	White to grayish or olive-grey	yellow	Conidial heads radiate, broadly columnar at maturity. Conidiophores sparsely septate,light brown, thin walled. Vesicles hemispherical to sub globose. Exudates colourless.
12	*A.versicolor*	3	Floccose radially zonate	Yellow to pea green.	Pale yellow	Conidial heads roughly hemi-spherical, radiate Conidiophores smooth and colorless. Vesicles semi spherical to elliptical, phialides in two series. Conidia globose.

Table -7
Macro and microscopic features of *Aspergillus* species isolated from various agricultural fields at Salur

S. No	Name of the species	Morphological features				Microscopic observations
		Diam. (mm)	Texture	Upper surface	Lower surface	Conidiophores/ conidia/vesicles
1	*P.aurantiogriseum*	35	Radially sulcate, smooth to granular	Mycelium white to dull green	Pale light to brilliant orange	Conidiation moderate to heavy, dull green to glaucous blue –green. Exudates typically conspicuous near the margins and brown in colour.conidia subspheroidal to ellipsoidal. Phialides 5-8 per metula.
2	*P. chrysogenum*	45	Radially sulcate to plicate/floccose	Margins white, centers yellowish white.	Brilliant yellow or yellow brown	Conidiation moderate to heavy, dull green to glaucous blue –green or yellow –green. Exudates pale to bright yellow. Conidia subspheroidal to ellipsoidal. Phialides 4-7 per metula.
3	*P. citreonigrum*	25	Radially sulcate Centrally convolute	White to bright yellow	Brilliant yellow or yellow brown	Conidiation sparse to moderate, greenish grey or pale olivaceous grey. Exudates rarely present. Phialides5-8.conidia spheroidal to subspheroidal
4	*P. citrinum*	30	Radially sulcate centrally floccose.	Mycelium white in peripheral areas	Yellow or yellow brown	Conidiation moderate, turquoise grey to glaucous sky blue. Exudates pale yellow to radish brown.Phialides 8-12,conidia spheroidal to subspheroidal
5	*P. digitatum*	40	velutinous to deeply floccose	Mycelium white to olive green	Pale to brownish	Conidiation moderate to heavy, grayish green to olive. Exudates absent. Phialides 3-5, conidia borne as cylinders, later ellipsoidal to cylindroidal, smooth,borne in distorted chains.
6	*P.purpurogenum*	25	velutinous to floccose	Mycelium white to bright yellow	Very dark red or purple	Conidiation moderate to heavy, grayish green to dark green. Exudates orange to red coloured. Phialides 5-8 per metula. Conidia ellipsoidal to subspheroidal.

The saprobic 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 to the maintenance of global carbon cycle. The distribution of these organisms is influenced by the abundance and nature of the organic content of the soil, as well as by other soil texture[22,23]. Identification is complicated by the fact that fungal life cycles in the soil and in the laboratory can be quite different. Fungi are so nutritionally diverse that there is no one medium that can isolate all of them. The technique used in the present study is a standard one[15]. Through our observations, the media used in our study appeared to favor of most soil fungi. *Aspergillus* and *Penicillium* usually appeared abundantly in collections because of their prolific sporulating capacity.

A distinct pattern of fungal community structure was observed in all the samples during the study period. The percentage composition and rank abundances of different fungal species fluctuated. The majority was from the genus *Aspergillus* and the next two in order of dominance were *Penicillium* and *Trichoderma*. Earlier reports have indicated that, these genera appeared abundantly in soils [24]. This may be due to the faster growth rate of these fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate. Considering the dominant species, it is clear that fungal succession in the plantation site greatly differed. The species composition in soil showed marked differences with a change in habitat and surface vegetation. Their occurrence might be due to the ability of these groups of fungi to survive in adverse conditions, as well as their ability to the environment. They have frequently been isolated in various soils in India[25-28].

Modern pesticides are almost all completely new synthetic chemicals, previously unknown in nature. The herbicide causes adverse effect on microbial populations and their activities[29]. Metolachlor is often used in combination with other broadleaved herbicides like atrazine, metobromuron and propazine was highly influences the microbial diversity in the present study area, the black soils. The soil pH, organic content and water are the main factors affecting the fungal population and diversity[30-33]. Organic carbon largely controls microbial growth and it is a key factor governing Nitrogen, Phosphorus, and Sulphur cycles. The microbial activity and species compositions are generally influenced by the physical characteristics and soil chemical properties, climate and vegetation[34]. The seasonal variations of the fungal diversity of various soil samples showed in figure-1.

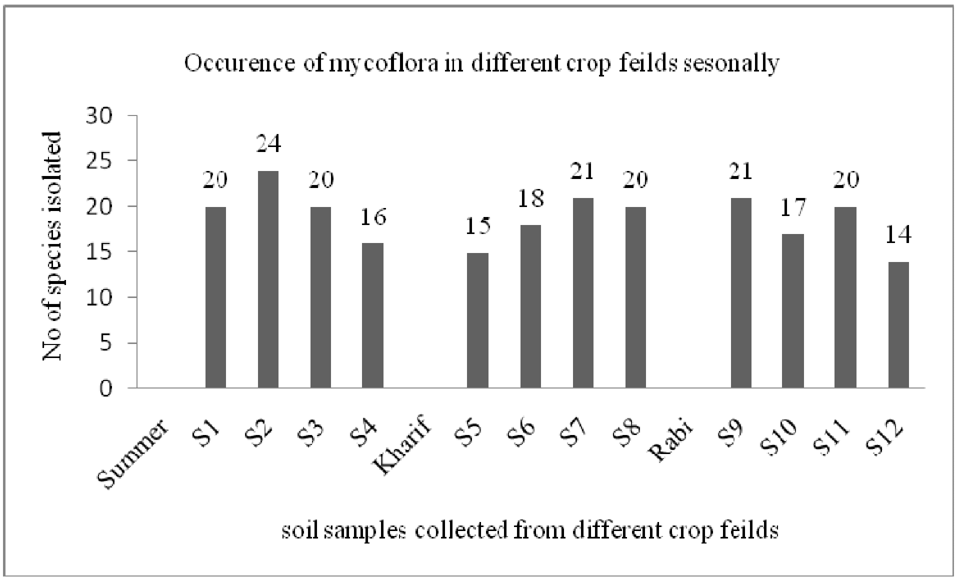
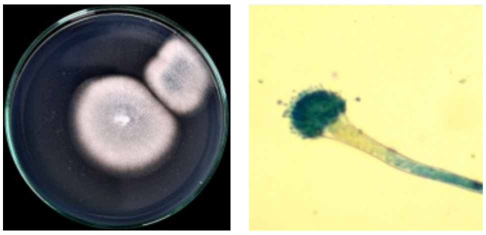
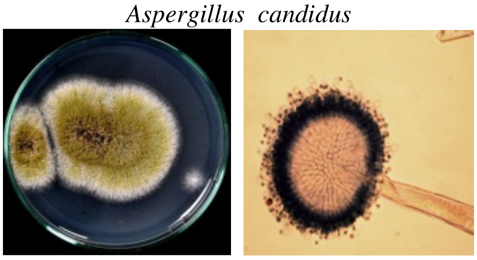


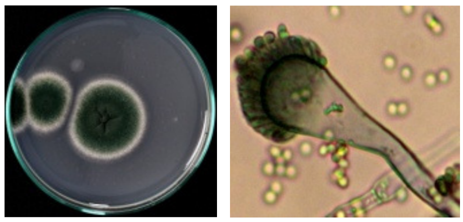
Figure -1

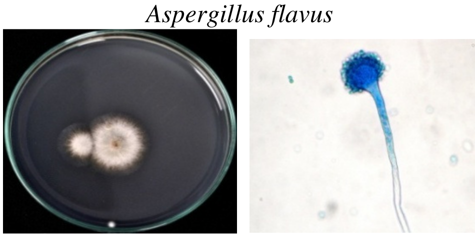
Periodicity of occurrence of mycoflora in various soil samples collected from agricultural soils at Salur Mandal , Vizianagaram District, Andhra Pradesh

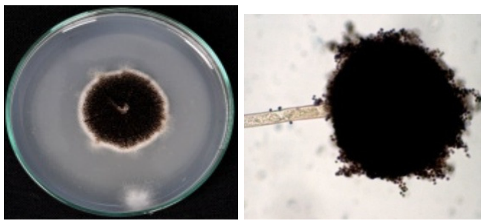

Aspergillus candidus


Aspergillus deflectus


Aspergillus flavus


Aspergillus fumigatus


Aspergillus nidulans


Aspergillus niger

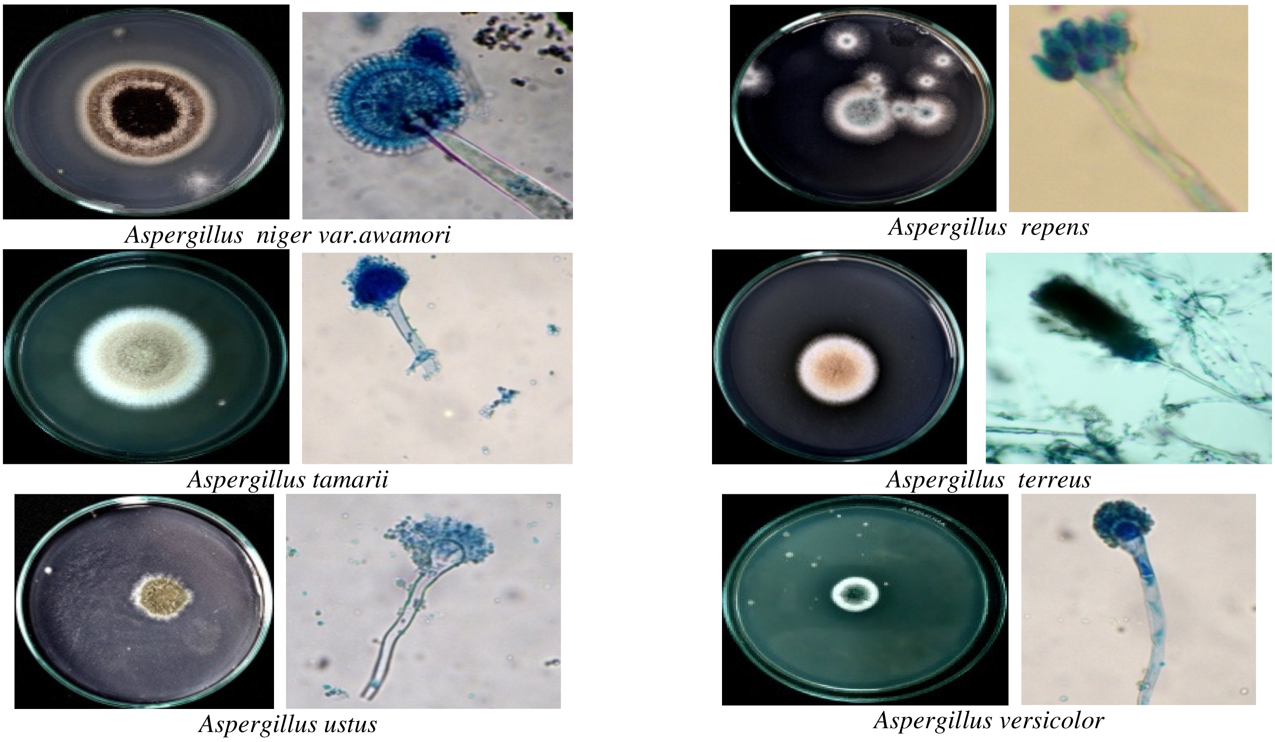


Figure -2
Macro and microscopic observations of *Aspergillus* species isolated from soil samples

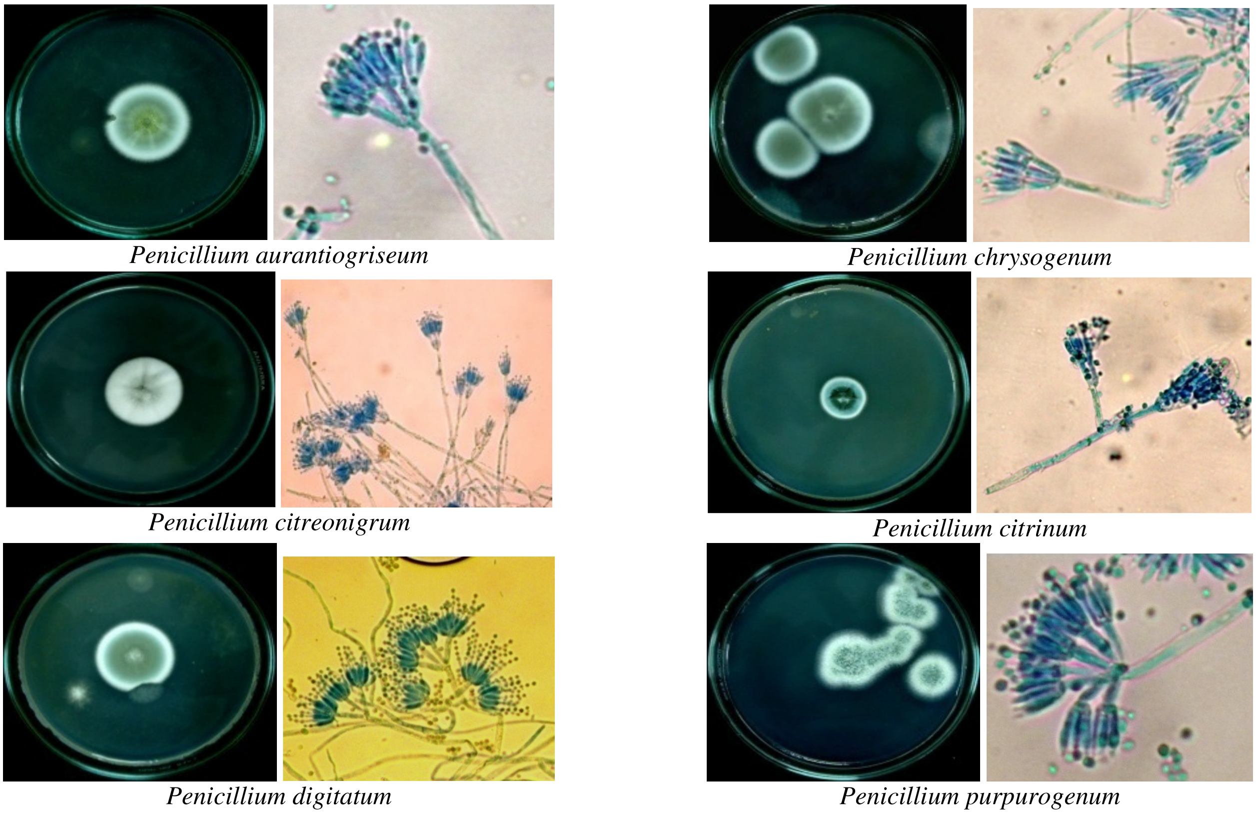


Figure -3
Macro and microscopic observations of *Penicillium* species isolated from soil samples

Conclusion

Fungi are an important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. The saprobic 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 to the maintenance of global carbon cycle. The majority of soil fungi are represented by rapidly growing filamentous fungi of *Aspergillus* and *Penicillium* genus. *Aspergillus* and *Penicillium* are economically, ecologically, and medically important and large genera. They are among the most successful groups of moulds with important roles in natural ecosystems and the human economy. The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the face of global climate change and human alteration of ecosystem processes. The species of *Aspergillus* and *Penicillium* are among the most abundant and widely distributed microfungi in nature. The periodicity of occurrence of different fungal species fluctuated due to ecological and biological factors of the soil.

The microbial activity and species compositions are generally influenced by the physical characteristics and soil chemical properties. Physio chemical analysis showed that the pH range of soil conditions ranging from 6.2 to 7.8 and other factors like organic content, water, the soil textures, and vegetation type can influence the fungal population and diversity of microbes in soil. It has been observed that a change in the habitat can affect species composition and diversity. The above ground vegetation in these agricultural soils is essential, to maintain a productive environment to enhance microbial growth and activity. Pesticides are used in controlling insect pests, diseases and weeds in agriculture. It has been established that pesticides could become a nuisance if they are misused. Some of the negative effects of pesticide misuse include low crop yield, destruction of soil micro-fauna and flora, and undesirable residue accumulation in food crops. The present study should enhance the sufficient knowledge to the formers for the conservation of soil properties, management of soil microbial diversity and the development of sustainable agro system. Further investigations on fertilizer- sensitivity of specific fungal communities will clarify the effect of long term consequences of fertilization on fungal diversity and their ecological significance.

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