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Isolation and identification of soil mycoflora in different crop fields at Salur Mandal

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ABSTRACT

A total of 15 species belonging to 6 genera of fungi were isolated from agricultural fields at Salur Mandal during March 2011 to November 2011 in three intervals. The mycoflora were isolated by using soil dilution technique and soil plate technique on Potato Dextrose Agar and Czapek's Dox Agar medium supplemented by suitable antibiotics such as penicillin and streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. The most common among them viz; Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium funiculosum, Trichoderma viride, Trichoderma harzianum, Fusarium oxysporum, Fusarium solani, Curvularia clavata, Curvularia lunata, and Rhizopus stolanifer were isolated and characterized. The seasonal variation and percentage frequency of the mycoflora were statistically analyzed.

Key Words: Salur, Agricultural Fields, Mycoflora, Diversity, Deuteromycotina, Zygomycotina.

INTRODUCTION

Soils are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms [6].Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth [10]. Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soils. Type of cultivation and crop management practices found to have greater influence on the activity of soil microflora [11].Continuous use of chemical fertilizers over a long period may cause imbalance in soil microflora and there by indirectly affect biological properties of soil leading to soil degradation [12].

Fungi are fundamental for soil ecosystem functioning [18]. Especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization [7]. Fungi are an important component of the soil micro biota [1].Micro fungi play a focal role in nutrient cycling by regulating soil biological activity [4].The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. In addition to chemical fertilizers and wide range of pesticides shows adverse effect on mycoflora which are much useful to maintain soil fertility and eco-balance in the soil atmosphere. The members and kinds of micro organisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration, pH and temperature etc. The aim of the present investigation is to isolate mycoflora from different crop fields, and to observe the percentage contribution of different fungal species.

MATERIALS AND METHODS

Study site and location:
Salur or Saluru is a Municipality and Mandal headquarters in Vizianagaram district. 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 Average annual rainfall is 1074 mm. The nature of the soil is generally black cotton soil. Paddy, Gingelly, Maize, Ground nut, Cotton are main Kharif crops while Sugarcane, Maize, Pulses and Tobacco are cultivated in Rabi season.

Method for collection of soil samples:
The soil samples were collected from six different crop fields in various locations of salur Mandal. Soil samples of six crop fields at Salur Mandal were collected during march 2011 to November 2011 in three intervals .The soil samples were collected from different crop fields (up to 15cm depth) into a small sterilized polythene bags and brought to laboratory for further studies. (Table:1).

Table 1: Agricultural soil samples collected from different places in Salur Mandal

Sample No	Agricultural field	Place
1	Paddy	Bangaramma peta
2	Corn	Jeegiram
3	Ragi	PN boddavalasa
4	Red gram	Kotthavalasa
5	cotton	Kurmarajupeta
6	Sugarcane	Salur

Isolation of fungi from the soil samples:
The soil micro fungi were enumerated by two methods, namely Soil Dilution [16] and soil plate method [17] on different media such as Potato Dextrose Agar and Czapek's Dox Agar.

Soil dilution plate method (Waksman, 1922): 1gr of soil sample was suspended in 10ml of double distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi. 1 ml of microbial suspension of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar and Czapek's Dox Agar. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. The Petri dishes were then incubated at 28 ± 2^0 C in dark. The plates were observed everyday up to three days.

Soil plate method (Warcup, 1950): About 0.005g of soil was scattered on the bottom of a sterile petri dish and molten cooled (40-45°C) agar medium (PDA)&(CZA) was added, which was then rotated gently to disperse the soil particles in the medium. The Petri dishes were then incubated at 28 ± 2^0 C in dark for three days.

Identification of the soil fungi :
Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores [3].The fungi were identified with the help of literature [9, 13].

Physico-chemical analysis of soil:
The collected soil was characterized for its physico-chemical properties. The physico-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH, salinity, organic carbon, nitrogen, phosphorous and potassium were analyzed. The physico-chemical parameters of the soil samples were analyzed at Mobile Soil Testing Laboratory (MSTL), Pothinamallayapalem, Visakhapatnam, Department of Agriculture, Andhra Pradesh . (Table:2)

Table-2: Physico-chemical properties of soil samples collected from different agricultural fields of Salur Mandal

S.NO	Crop field	Place	Soil colour	Soil type	pH	salinity	Organic carbon%	N Kg/h	P Kg/h	K Kg/h
1	Paddy	Bangaramma peta	Grey	SCL	7.5	0.24	Medium	68	56	265
2	Corn	Jeegiram	Brown	SL	6.2	0.22	Medium	72	39	85
3	Ragi	PN boddavalasa	Brown	SCL	6.3	0.15	Low	66	27	42
4	Red gram	Kotthavalasa	Brown	SL	5.1	0.37	Medium	95	36	104
5	cotton	Kurmarajupeta	Light grey	SICL	6.8	0.21	Low	84	46	196
6	Sugarcane	Salur	Dark Grey	SCL	6.9	0.48	Low	78	25	64

SL - Sandy Loam
SCL - Sandy Clay Loam
SICL - Silt Clay Loam

Organic carbon %
0.3 very low
0.3-0.5 low
0.5-0.75 medium
0.75-1 high
>1 very high

Statistical analysis :
The number of colonies per plate in 1g of soil was calculated. The percent contribution of each isolate was calculated by using the following formula:

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

**CFU-Colony Forming Unit*

RESULTS AND DISCUSSION

Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture [2, 14]. Physico- chemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures were determined the fungal population and their diversity in agricultural fields of Salur. During the investigation period 173 fungal colonies of 15 fungal species were observed (Table:3). The maximum fungal species belongs to Deuteromycotina (169 colonies) and Zygomycotina (4 colonies) were observed. Among the isolates the genera *Aspergillus* and *Penicillium* were dominant (Table:3) .

Table-3: Frequency of mycoflora in different crop fields at Salur Mandal

S.No	Crop field	Average no of total colonies	Average no of individual colonies														
			Aspergillus					Penicillium			Fusarium		Curvularia		Trichoderma		Rhizopus
			An	Afl	Afu	Ani	At	Pch	Pf	Pfu	Fo	Fs	Ccl	Clu	Tv	Th	Rs
1	Paddy	34	3	4	4	-	2	3	2	2	2	1	2	3	3	2	1
2	Corn	32	4	3	5	2	3	2	3	2	1	1	1	2	3	2	-
3	Ragi	21	2	2	2	2	2	2	2	-	2	1	1	-	1	2	-
4	Red gram	30	2	4	3	2	4	3	2	-	1	-	2	3	2	1	-
5	Cotton	28	3	2	-	3	2	2	2	2	1	3	1	1	1	2	2
6	Sugarcane	28	4	4	3	2	3	-	2	-	2	2	-	1	2	2	1
Total		173	18	19	17	11	16	12	13	6	9	8	7	10	12	11	4
% Contribution			**10.4**	**10.9**	**9.8**	**6.3**	**9.2**	**6.9**	**7.5**	**3.4**	**5.2**	**4.6**	**4.0**	**5.7**	**6.9**	**6.3**	**2.3**

01 An - Aspergillus niger
Afl- *Aspergillus flavus*
Afu- *Aspergillus fumigatus*
Ani- Aspergillus nidulans
At- *Aspergillus terreus*

02 Pch- Penicillium chrysogenum
Pf- *Penicillium frequentans*
Pfu- *Penicillium funiculosum*

03 Fo- Fusarium oxysporum
Fs- *Fusarium solani.*

04 Ccl- Curvularia clavata
Clu- *Curvularia lunata.*

05 Tv- Trichoderma viride
Th- *Trichoderma harzianum*

06 Rs- Rhizopus stolanifer.

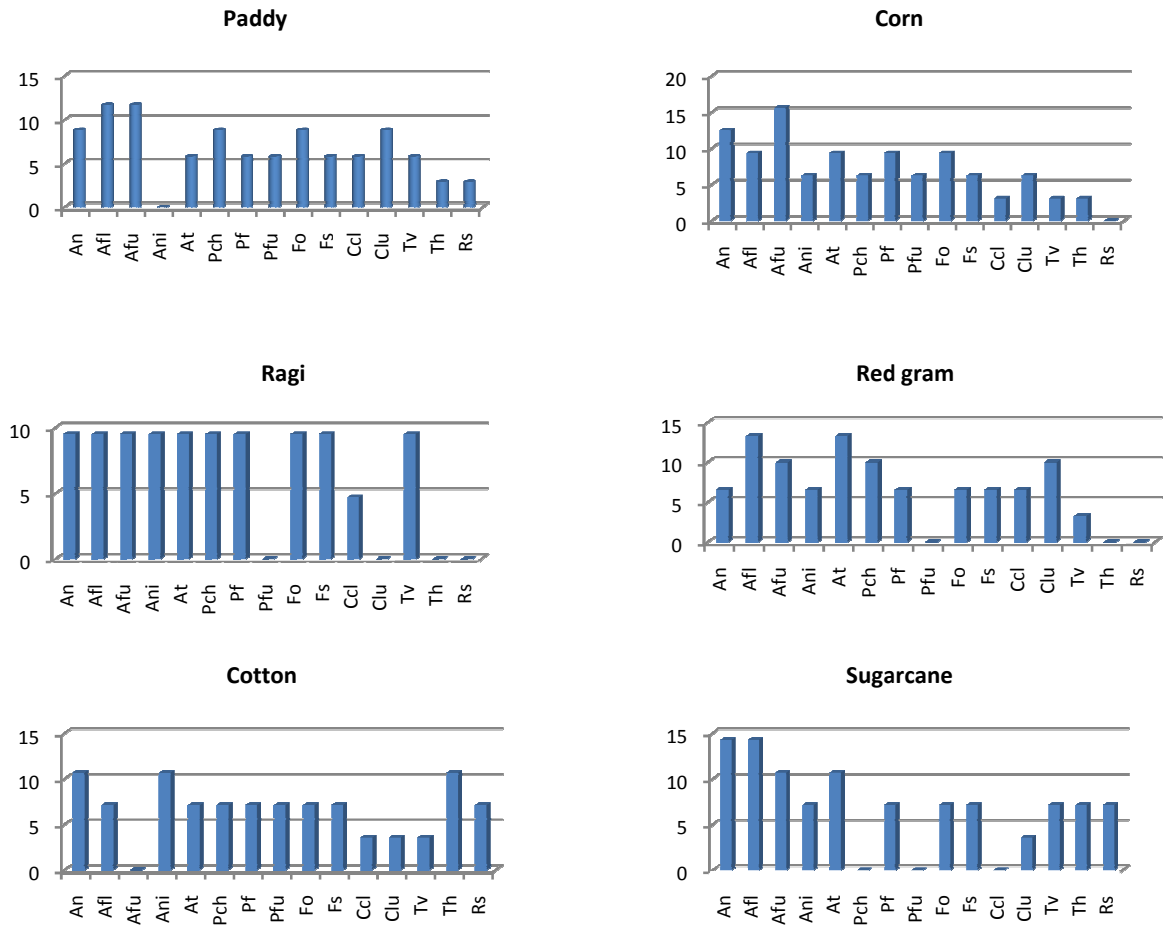

Fig.1 **Percent contribution of mycoflora in six crop fields at Salur**. **An** -*Aspergillus niger*, **Afl**-*Aspergillus flavus* , **Afu**- *Aspergillus fumigatus* , **Ani**- *Aspergillus nidulans* , **At**- *Aspergillus terreus*, **Pch**- *Penicillium chrysogenum* , **Pf**-*Penicillium frequentans*, **Pfu**- *Penicillium funiculosum*, **Fo**- *Fusarium oxysporum* ,**Fs**-*Fusarium solani*, **Ccl**-*Curvularia clavata* ,**Clu**- *Curvularia lunata*, **Tv**-*Trichoderma viride* ,**Th**- *Trichoderma harzianum*, **Rs**- *Rhizopus stolanifer*

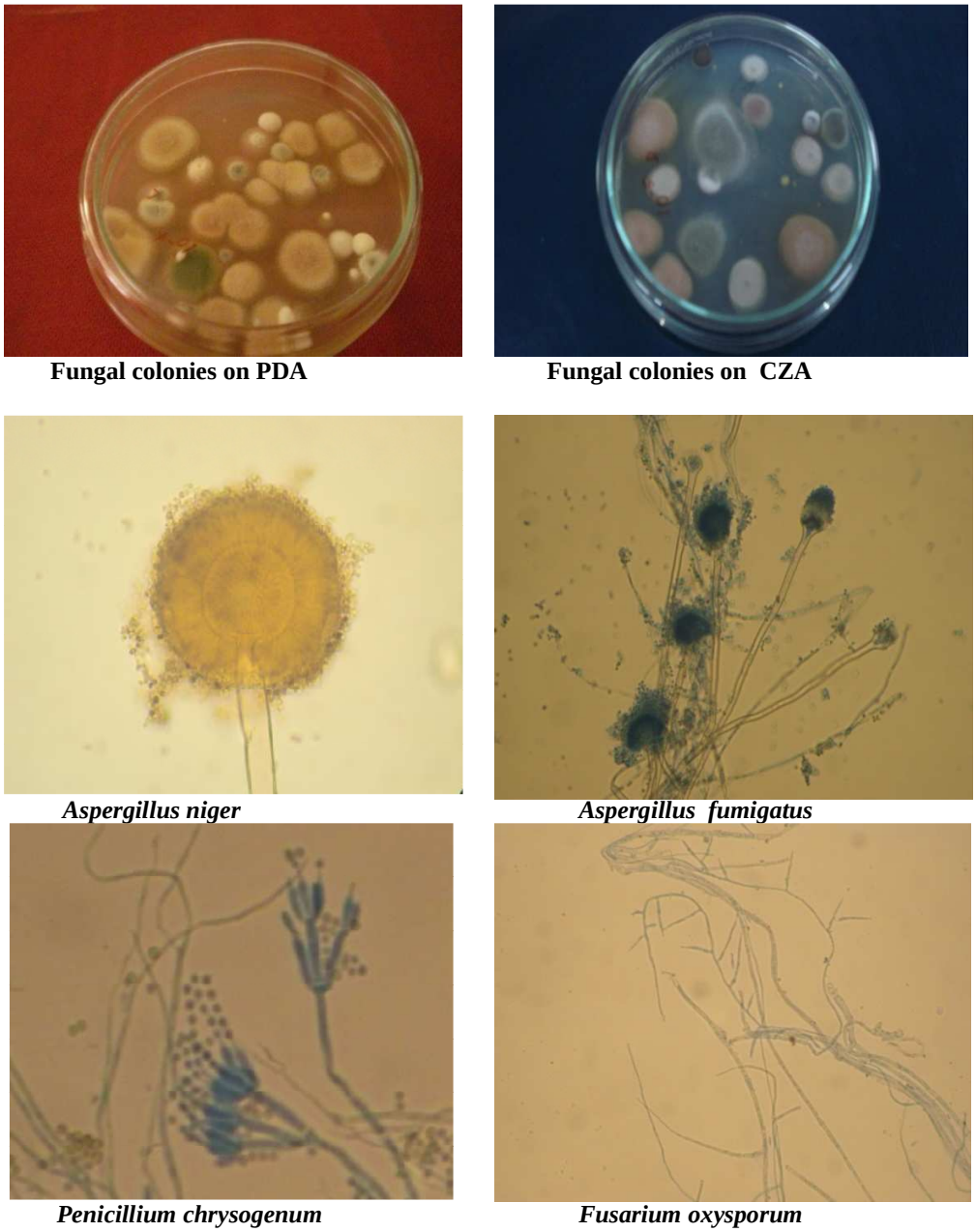

Fig:2 Microscopic observation of some soil mycoflora

The soil mycoflora in different crop fields viz; Paddy, Corn, Ragi, Red gram, Cotton and Sugarcane were observed. The most common among them viz; *Aspergillus flavus*(10.9%) *Aspergillus niger* (10.4%), *Aspergillus fumigatus*(9.8%), *Aspergillus terreus*(9.2%), *Aspergillus nidulans* (6.3%), *Penicillium frequentans*(7.5%), *Penicillium chrysogenum*(6.9%), *Penicillium funiculosum*(3.4%), *Trichoderma viride*(6.9%), *Trichoderma harzianum*(6.3%), *Fusarium oxysporum*(5.2%), *Fusarium solani*(4.6%), *Curvularia lunata* (5.7%) *Curvularia clavata* (4.0%) *and Rhizopus stolanifer* (2.3%) were isolated and characterized. Diversity was found to be higher in the agricultural fields of paddy, corn , red gram and sugarcane as compared to the other agricultural fields where the mycorrhizal association aggregated with soil particles. The seasonal variation and percentage frequency of the mycoflora were statistically analyzed (Table-4).

Table – 4: Percent contribution of fungal species in different crop fields at Salur Mandal

S.No	Fungal species	% contribution					
		Paddy	Corn	Ragi	Red gram	Cotton	Sugarcane
1	*Aspergillus niger*	8.82	12.5	9.52	6.6	**10.7**	**14.28**
2	*A .flavus*	**11.7**	9.37	9.52	**13.3**	7.14	**14.28**
3	*A .fumigatus*	**11.7**	**15.62**	9.52	10.0	-	10.7
4	*A. nidulans*	-	6.25	9.52	6.6	10.7	7.14
5	*A. terreus*	5.8	9.37	9.52	**13.3**	7.14	10.7
6	*Penicillium chrysogenum*	8.82	6.25	9.52	10.0	7.14	-
7	*P. frequentans*	5.8	9.37	9.52	6.6	7.14	7.14
8	*P. funiculosum*	5.8	6.25	-	-	7.14	-
9	*Trichoderma viride*	8.82	9.37	9.52	6.6	7.14	7.14
10	*T. harzianum*	5.8	6.25	9.52	6.6	7.14	7.14
11	*Curvularia clavata*	5.8	3.12	4.7	6.6	3.57	-
12	*C. lunata*	8.82	6.25	-	10.0	3.57	3.57
13	*Fusarium oxysporum*	5.8	3.12	9.52	3.3	3.57	7.14
14	*F. solani*	2.9	3.12	-	-	**10.7**	7.14
15	*Rhizopus stolanifer*	2.9	-	-	-	7.14	7.14

The Soil pH, organic content and water are the main factors affecting the fungal population and diversity [19].The organic carbon, nitrogen, phosphorus, potassium are important for fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot [15]. It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high [8, 5] has reported that environmental factors such as pH, moisture, temperature, organic carbon ,organic nitrogen play an important role in the distribution of mycoflora.

CONCLUSION

In the present study soil samples of six crop fields viz., Paddy, Corn, Ragi, Red gram, Cotton and Sugarcane were studied for screening and detection of fungal diversity. The results obtained clearly indicates that *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum* ,*Trichoderma* were of high occurrence in all crop fields and some other fungi like *Fusarium, Curvularia,* and *Rhizopus* were negligible. Among the isolates *Aspergillus* and *Penicillium* were dominant in all agricultural fields due to high sporelation capacity and the *Penicillium* spp were producing fungal and bacterial antibiotics and the *Aspergillus* spp producing different kinds of toxins such as aflotoxins, achrotoxins etc. These toxins may prevent the growth of other fungal species. The frequency of mycoflora in agricultural fields were found to be regulated by many factors like temperature, humidity, vegetation , organic and inorganic materials ,soil type and texture. The fungi were mostly observed in month of June to September due to suitable temperature and humidity.

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