*International Research Journal of Environment Sciences*_____ ISSN 2319–1414
Vol. **2(9),** 38-44, September **(2013)** *Int. Res. J. Environment Sci.*

An Investigation on Soil Mycoflora of Different Crop Fields at Narasannapeta Mandal, Srikakulam District

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Available online at: www.isca.in
Received 4th August 2013, revised 11th August 2013, accepted 19th August 2013

Abstract

Soil samples were collected based on different crop fields, during the month of January 2011 to November 2011 at Narasannapeta region of Srikakulam District. During the investigation period 232 fungal colonies were observed. The maximum fungal species belongs to Deuteromycotina (200 colonies) and Zygomycotina (11 colonies) and 21 colonies of unknown were observed. Culture media namely, Potato Dextrose Agar (PDA) Czapek's Dox Agar(CZA) and Sabouraud's Dextrose Agar (SA) supplemented with 1% Streptomycin was used as nutrient media for the growth and sporulation of soil fungi. The Present investigation was conducted to find out the fungal diversity in eight different crop fields such as Sunflower, Sesame, Capsicum, Rice, Green gram, Sugarcane, Ground nut and Black gram. The colonies of Aspergillus and Penicillium were predominant in all soil samples of crop fields. Among the isolates, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium Funiculosum, Alternaria alternata, Curvularia lunata, Trichoderma viride, Rhizopus stolonifer were authentically characteraized and the percentile contribution of these isolates was statically analyzed.

Keywords: Soil mycoflora**,** Deuteromycotina, Zygomycotina, fungal diversity, Crop fields, Narasannapeta.

Introduction

Soil represents a favorable habitat for microorganisms and inhabited by a wide range of microorganisms. Microorganisms are found in large numbers in soil, usually one to ten million microorganisms were present per gram of soil with a dominant number of bacteria and fungi. The contribution of soil organisms was very significant in many soil functions such as supporting the growth of plants, absorbing, neutralizing and transforming compounds that might otherwise become pollutants in the environment. Soil is a complex habitat for microbial growth and these microbes generally exist as micro colonies or biofilms on mineral particles, organic matter, and roots. Soil organisms are both numerous and highly diverse and the competition exists among enormous variety of organisms for nutrients, space, and moisture. 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[1].

All organisms in the biosphere depend on microbial activity[2]. Soil microorganisms are vital for the continuing of nutrients and for driving above-ground ecosystems[3-6]. Soil bacteria and fungi play pivotal roles in various biochemical cycles (BGC)[7-9] and are responsible for the cycling of organic compounds. Fungi are an important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrition conditions[10]. 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. Fungi are fundamental for soil ecosystem functioning[11]. Especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition, elemental release by mineralization, and protection against leaching by elemental storage in biomass[12] and their mycelia contribute to soil aggregate stability, thereby avoiding erosion. Soil mycoflora plays a pivotal role in evaluation of soil conditions and in simulating plant growth. Microfungi play a focal role in nutrient cycling by regulating soil biological activity[13].

The quality and quantity of organic materials present in the soil have a direct effect on the fungal population of the soil. The distribution of these organisms is influenced by the abundance and nature of the organic context of the soil, as well as by other soil and climatic conditions, surface vegetation and soil texture[14,15]. The numbers and kinds of micro-organisms present in soil depend on many environmental factors such as amount and type of nutrients available, available moisture, degree of aeration, pH, temperature etc. Continuous use of chemical fertilizers over a long period may cause imbalance in soil microflora and thereby indirectly affect biological properties of soil leading to soil degradation. Some studies dealt with the influence of plant community and others attempted to examine seasonal trends on soil microorganisms.

The study deals with the percentile contribution of soil mycoflora of various crop fields and their characterization authentically. The investigation on soil mycoflora becomes significant in the view of conservation of soil ecosystem and soil microbial diversity and sustainable agriculture.

Material and Methods

Study Site and Location: Narasannapeta is a town and a Mandal in Srikakulam District in the state of Andhra Pradesh in India. Narasannapeta Mandal is bordered by Jalumuru, Sarubujjili, Srikakulam and Polaki Mandals of Srikakulam district. Narsannapeta is located at 18.4167°N 84.0500°E. It has an average elevation of 18 meters (62 feet) and annual rainfall of 1037mm. Paddy, Sugarcane, Groundnut, Mesta , Sesame, Maize, Green gram, Black gram and Sunflower were cultivated as irrigated and rain fed major field crops. Soils were classified into five major types: Red soils (344 ha), Brown forest soils (85 ha), Alluvial soils (61 ha), Black soils (30 ha) and sandy soils (13 ha).

Collection of Soil Samples: Soil samples were collected based on different crop fields, during the month of January 2011 to November 2011 at Narasannapeta region of Srikakulam District. 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 three different locations/sites Devadi, Makivalasa, Karagham. 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.

Isolation of Soil mycoflora: Dilution plate technique described by Warcup[16] was used for the isolation of fungi from various soil samples. 10 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 particles. 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) Czapek's Dox Agar(CZA) and Sabouraud's Dextrose Agar (SDA) supplemented with 1% Streptomycin. 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^{o}C at 15 psi for 20 min. 1% Streptomycin was used as an antibiotic for the restrain of bacterial growth. Each colony was sub cultured and maintained on potato dextrose agar slants. The inoculated plates were incubated at room temperature 28±2°C in an inverted position for 5-7 days. Three replicates were maintained for each sample**.** Identification of the organisms was made by microscopic observation by using taxonomic guides, standard procedures and relevant literature[17,18].

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 1.

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.

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

**CFU-Colony Forming Unit.*

Results and Discussion

During the investigation period 232 fungal colonies were observed. The maximum fungal species belongs to Deuteromycotina (200 colonies) and Zygomycotina (11 colonies) and 21 colonies of unknown were observed. After incubation period different colonies of soil fungi were observed in inoculated petriplates containing fungal growth media, Potato Dextrose Agar (PDA), Czapek's Dox Agar (CZA) and Sabouraud's Dextrose Agar (SDA) supplemented with 1% Streptomycin. Fungal colonies of various soil samples collected from different crop fields were isolated using surface –sterilized needles on to PDA slants for microscopic observation. Characterization of isolates up to species level was made by using taxonomic tools and authentic manuals of soil fungi. Among the isolates, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus, Penicillium chrysogenum*, *Penicillium frequentans, Penicillium funiculosum, Alternaria alternate, Curvularia lunata, Trichoderma viride, Rhizopus stolonifer* and some unknown colonies also observed (table-2). Identification of the organisms was made by using microscopic observations by staining with lacto-phenol cotton blue (figure-2). Isolates were authenticated using taxonomic guides, standard procedures and relevant literature.

The Present investigation was conducted to find out the fungal diversity in eight different crop fields such as Sunflower, Sesame, Capsicum, Rice, Green gram, Sugarcane, Ground nut and Black gram. The colonies of *Aspergillus and Penicillium* were predominant in all soil samples of crop fields. The abundance of fungal colonies was high in the fields of sugar cane (34 colonies), Sesame (33 colonies) and groundnut (31 colonies). Fungal isolates of *Aspergillus niger* (15.3), *Aspergillus fumigatus* (15.3) and *Penicillium frequentans* (15.3)

were dominant in the soil of Sunflower field. *Aspergillus niger* (18.8), *Aspergillus fumigatus* (19.2) and *Aspergillus terreus* (12.1) were dominant in the soil of Sesame field. *Aspergillus niger* (23.07), *Aspergillus flavus* (19.2) and *Aspergillus terreus* (11.5) were dominant in the soil of Capsicum field. *Aspergillus niger* (14.2), *Aspergillus nidulans* (17.8), *Aspergillus terreus* (17.8) *and Trichoderma viride* (14.2) were dominant in the soil of Black gram field. *Aspergillus flavus* (15.3), *Aspergillus fumigatus* (11.5) and *Penicillium frequentans* (11.5) were dominant in the soil of Green gram field. *Aspergillus niger* (11.7) and *Aspergillus terreus* (14.7) were dominant in the soil of Sugarcane field. *Aspergillus flavus* (16.1), *Aspergillus terreus* (16.1) and *Penicillium frequentans* (12.9) were dominant in the soil of Ground nut field. *Aspergillus flavus* (17.8), *Aspergillus fumigatus* (14.2) and *Penicillium frequentans* (14.2) were dominant in the soil of Rice field. The percentile contribution of different soil mycoflora of crop fields was showed in table-3. Graphical representation of percent contribution of fungal species in various crop fields was showed in figure-1.

The soil pH, organic content and water are the main factors affecting the fungal population and diversity[19-22]. 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 .It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high.

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[23,24] and sustenance of vegetation. The efficiency of fungi in decomposition and their potentiality depend upon their abundance and composition. 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[25].

Christensen[26] 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[27]. These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization and may result in the disturbance of the balance between the rate of substrate input and the rate of mineralization.

Soil fungi have significant impact on the several activities of soil ecosystem. Some studies on soil fungi of agricultural fields of Tamilnadu[28,29], Andhra Pradesh[30], Odisha[31] and other remaining states of India enlightened the importance of soil mycoflora in agricultural fields. The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture. The studies on fungal diversity and percentile contributions and periodic occurrence of soil mycoflora are useful for Formers, Agronomists, Researchers and Microbiologists for future activities in the view of conservation of soil ecosystem, conservation of soil microbial diversity and sustainable agriculture.

Table-1
Various soil samples collected from agricultural fields and their analysis

S.No	Crop and Location	Soil type	pH	Salt	OC%	P	K	No. of colonies isolated
1	**Sunflower** Karagham	SCL	6	0.38	0.5-0.75	25- high	50-low	26
2	**Sesame** Makivalasa	SCL	6.6	0.19	0.5-0.75	52-high	98-medium	33
3	**Capsicum** Makivalasa	SCL	5.8	0.16	0.3-0.5	31-high	98-medium	26
4	**Black gram** Karagham	SICL	5.8	0.16	0.3-0.5	38-high	64-medium	28
5	**Green gram** Karagham	SICL	6	0.27	0.5-0.75	30-high	64-medium	26
6	**Sugarcane** – Devadi	SCL	7.6	0.52	0.5-0.75	35-high	148 -high	34
7	**Ground nut** – Karagham	SCL	6	0.34	0.5-0.75	29-high	77-medium	31
8	**Rice** – Karagham	SL	6.7	0.21	0.5-0.75	56-high	37-low	28
Total number of colonies								**232**

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

Table-2
Occurrence of soil mycoflora in different crop fields at Narasannapeta Mandal.

S. No	Crop	Avg No. of Individual colonies													
		Total colonies	An	Afl	Afu	Ani	At	Pch	Pfre	Pfu	Al	Clu	Tvi	Rst	Un
1	Sunflower	26	4	6	4	-	2	-	4	-	1	1	-	1	3
2	Sesame	33	6	3	4	-	4	-	2	2	2	2	2	2	4
3	Capsicum	26	6	5	2	-	3	-	2	-	2	1	-	2	3
4	Blackgram	28	4	2	-	5	5	-	-	3	3	-	4	-	2
5	Greengram	26	2	4	3	2	2	2	3	-	2	2	-	1	3
6	Sugarcane	34	4	3	3	2	5	2	2	-	3	2	3	3	2
7	Groundnut	31	3	5	-	-	5	3	4	-	2	2	4	1	2
8	Rice	28	3	5	4	-	2	3	4	-	2	2	-	1	2
	Total	**232**	**32**	**33**	**20**	**9**	**28**	**10**	**21**	**5**	**17**	**12**	**13**	**11**	**21**

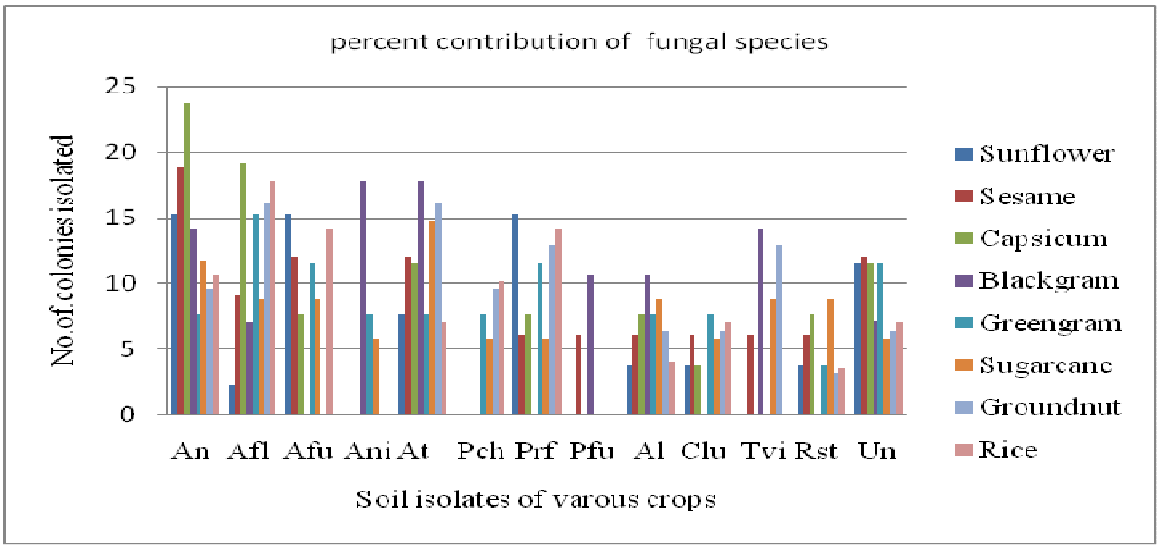
An-*Aspergillus niger*; Afl-*Aspergillus flavus* ; Afu-*Aspergillus fumigatus*; Ani-*Aspergillus nidulans*; At-*Aspergillus terreus;* Pch- *Penicillium chrysogenum*; Pfre- *Penicillium frequentans*; Pfu- *Penicillium funiculosum*; Al-*Alternaria alternata* ; Clu-*Curvularia lunata*; Tvi- *Trichoderma viride* ; Rst- *Rhizopus stolonifer*; Un-Unknown colonies.

Table-3
Percent contribution of different mycoflora isolated from soil samples of agricultural fields at Narasannapeta Mandal.

S.No	Fungal species	Percent contribution (%)							
		Sunflower	Sesame	Capsicum	Black gram	Green gram	Sugarcane	Groundnut	Rice
1	*Aspergillus niger*	15.3	18.8	23.07	14.2	7.6	11.7	9.6	10.7
2	*A.flavus*	2.3	9.09	19.2	7.1	15.3	8.8	16.1	17.8
3	*A.fumigatus*	15.3	12.1	7.6	-	11.5	8.8	-	14.2
4	*A.nidulans*	-	-	-	17.8	7.6	5.8	-	-
5	*A.terreus*	7.6	12.1	11.5	17.8	7.6	14.7	16.1	7.1
6	*Penicillium chrysogenum*	-	-	-	-	7.6	5.8	9.6	10.7
7	*P. frequentans*	15.3	6.06	7.6	-	11.5	5.8	12.9	14.2
8	*P. funiculosum*	-	6.06	-	10.7	-	-	-	-
9	*Alternaria alternate*	3.8	6.06	7.6	10.7	7.6	8.8	6.4	7.1
10	*Curvularia lunata*	3.8	6.06	3.8	-	7.6	5.8	6.4	7.1
11	*Trichoderma viride*	-	6.06	-	14.2	-	8.8	12.9	-
12	*Rhizopus stolonifer*	3.8	6.06	7.6	-	3.8	8.8	3.2	3.5
13	Unknown	11.5	12.1	11.5	7.14	11.5	5.8	6.4	7.1

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 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 periodicity of occurrence of different fungal species fluctuated due to ecological and biological factors of the soil. 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. Our finding demonstrates the differences in fungal species composition of agricultural soils and management practices have greater potential to influence the size and structure of soil fungal community.



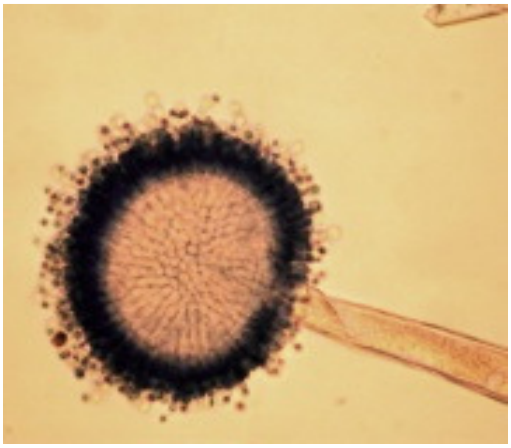
An-*Aspergillus niger*; **Afl-***Aspergillus flavus* ; **Afu-***Aspergillus fumigatus*; **Ani-***Aspergillus nidulans*; **At-***Aspergillus terreus;* **Pch-** *Penicillium chrysogenum*; **Pfre-** *Penicillium frequentans*; **Pfu-** *Penicillium funiculosum*; **Al-***Alternaria alternata* ; **Clu-** *Curvularia lunata*; **Tvi-** *Trichoderma viride* ; **Rst-** *Rhizopus stolonifer* ; **Un-**Unknown colonies.

Figure-1

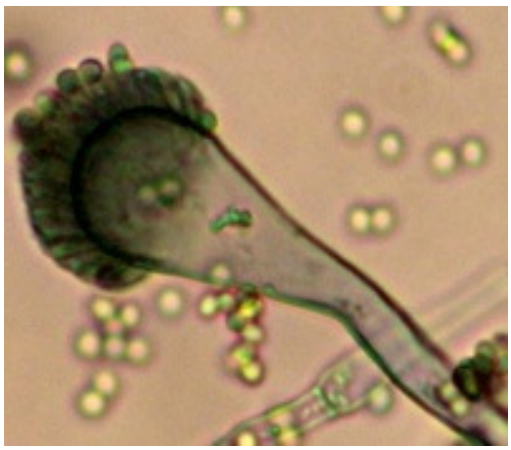
Present contribution of fungal species in various crop fields at Narasannapeta Mandal

Alternaria alternate

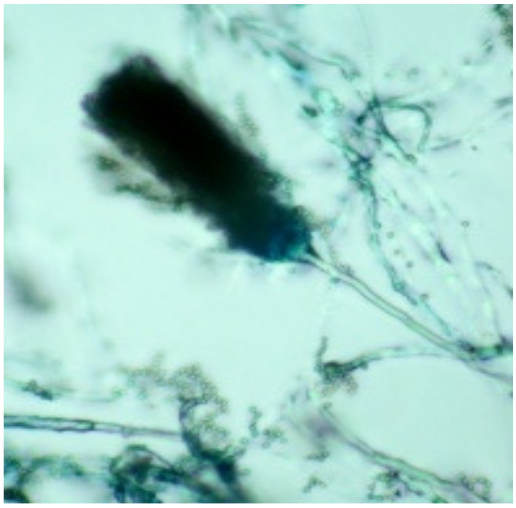
Curvularia lunata



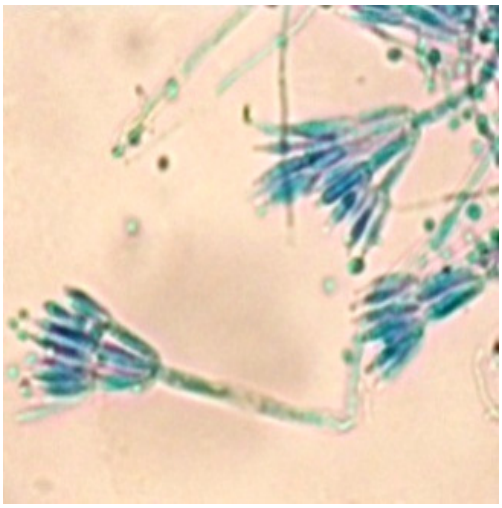
Aspergillus flavus



Aspergillus fumigatus



Aspergillus terreus



Penicillium chrysogenum

Figure -2
Microscopic observations of some mycoflora isolated from agricultural soil samples

Acknowledgments

The authors were grateful to Head and Faculty members, Department of Botany, Centre of Advanced Studies, Andhra University for their the constant encouragement and support. The authors were thankful to University Grants Commission, New Delhi, India for the finance support under UGC-SAP-CAS-I project in the Department of Botany, Centre of Advanced Studies, Andhra University, Visakhapatnam.

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