

Isolation and Identification of soil Mycoflora in Crop Fields at Chintalapudi Mandal, West Godavari District

Ratna Kumar P.K¹, Manimala*. N², Samuel. K. Kolli³

Professor, Department of Botany, Andhra University, Visakhapatnam, India¹

Part time Research scholar, Department of Botany, Andhra University, Visakhapatnam, India²

Assistant Professor, Department of Botany, Adikavi Nannaya University, Rajamahendravaram, India³

ABSTRACT: Soil samples were collected from various crop fields within the course of the months of January to November 2015, at Chintalapudi region of West Godavari District. An overall of 14 species belonging 7 genera of fungi were isolated throughout this period in 3 intermissions. The mycoflora were isolated by using soil dilution technique and soil plate technique on PDA and CZA medium appended with appropriate antibiotics like penicillin and streptomycin. Identification and characterization of the mycoflora were made with the assistance of authentic handbooks of fungi. The present investigation was conducted to seek out the plant life diversity in 3 completely different crop fields like Rice, Maize, and Ground nut. *Aspergillus* and *Penicillium* were predominant altogether 3 soil samples. Amongst the isolates, 5 *Aspergillus* species (*A.niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A.terreus*) 3 *Penicillium* species (*P.Chrysogenum*, *P. frequentans*, *P. funiculosum*), 2 *Trichoderma* species (*T.viride* and *T.harzianum*) and 1 species each from *Curvularia clavata*, *Fusarium oxysporum*, *Mucor recemosus*, *Rhizopus stolonifer* were characterized and percentage occurrence of the mycoflora was statistically evaluated.

KEYWORDS: Chintalapudi; Mycoflora; Diversity; Deuteromycotina; Zygomycotina; Crop fields.

I.INTRODUCTION

Soils are terribly composite systems with several elements taking part in various functions principally because of the activity of soil organisms (Chiang and Saudi, 1994). Microorganisms within the soil are helpful in growing soil fertility and plant growth, as they're concerned in many biochemical transformations and mineralization activities. Fungi are a crucial part of soil microbiota additional in abundance than bacterium, contingent on soil depth and nutrient conditions (P. Ganasekaran et al., 2015).

The soil is a reservoir for several microorganism communities of plant and herbs which might be manufacturing CO₂, Nitrogen cycle. The microorganisms plays major role in soil ecosystem (Stefanis et al., 2013). Nevertheless, microbes are the smallest amount unuttered mechanism of soil by each agronomists and soil practitioners. On the farm many soil organisms supply advantages to crop growing in an ecosystem however aren't well understood (Olson et al., 2000). The soil microbes decompose the plant and animal residues coming into the soil and convert them into soil organic matter that influences soil physical, chemical and biological properties and on making a complimentary medium for biological reactions and life support within the soil setting. Adametz, 1886 made attempts to isolate fungi from the soil and to give them, names and descriptions. A systematic study of the occurrence of fungi was provided by Oudemans & Koning, for the first time in 1902. Tresner et. al., (1954) reported that the composition of fungal spp. in communities is affected by aerial vegetation. Thornton (1960), Christensen et. al., (1962), Gochenaur and Whittingham (1967) and Kamal & Bhargav (1973) have reported that the prevailing climate and above ground vegetation are known to influence the abundance and quality of fungi in a particular soil. R.K. Saksena et al., (1966) reported two spp. of *Penicillia* from Jhansi soils while five in case of Mirzapur soil. The most common amongst them were *P.frequentans* and

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

P.funiculosum and the remaining were of restricted distribution. In Mirzapur soils, genera of the class Phycomycetes were dominant. Rai et al.,(1966), isolated *F.oxysporum* from mangrove mud in Kagh Islands, India. Anusuya and Jayarajan (1998) identified *Trichoderma viride* fungal culture isolated from rhizosphere soil. Azaz (2003) isolated 109 species and varieties plus 16 sterile microfungi from a total of 1690 isolates from the analysis of 105 soilsamples, taken from 89 farm field soils irrigated in Harran Plain in Sanliurfa within the GAP irrigated area. Nieves-Rivera, (2003) isolated fungi from damp and musty caves in Puerto-Rico. The aim of the current study is to isolate mycoflora from completely altered crop fields and to look at their proportion contribution.

II. MATERIALS AND METHODS

A. Study site and location:

Chintalapudi could be a major Panchayati and Mandal in West Godavari District of the Indian state of Andhra Pradesh. The Mandal is finite by T. Narasapuram Mandal, Lingapalem Mandal, khammam district. Chintalapudi is found at 17.06⁰N 80.98⁰E. The temperature varies between 16⁰C – 35⁰C. Average annual precipitation is 1081.7mm. The character of the soil is usually red soils. The Mandal has 34.393 hectares of geographic area, 6581 hectares of forest space and 20.735 hectares of net space underneath cultivation. Main crops grown up within the Mandal is Paddy, Maize, Sugarcane and Mango gardens.

B. Technique for collection of soil samples:

The earth specimens were collected from 3 completely different crop fields in varied locations of Chintalapudi Mandal throughout Jan 2015 to November 2015 in three intervals. The examples were collected up to 15 cm depth, into little sterilized polyethylene baggage and brought to laboratory for further studies. (Table1).

Sample No	Agricultural field	Place
1	Rice	Raghavapuram
2	Maize	Endapalli
3	Groundnut	Recharla

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

C. Isolation of fungi from the soil samples:

The soil micro fungi were enumerated by two ways, particularly Soil Dilution (Waksman, 1922) and soil plate technique(Warcup, 1950) on totally different media like Potato Dextrose Agar and Czapek's Dox Agar.

D. Soil dilution plate method(Waksman, 1922): Take 1 gram of soil and suspended in 10 ml of double distill water for making microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-3} , 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid overcrowding of the fungal colonies. 1 ml of suspension from each respective dilution was added to sterile petri plates containing 15ml of PDA and CZA medium. For preventing bacterial contamination medium supplemented with 1% streptomycin. Finally plates were incubated at 28±2⁰C for 4-7 days. Organisms were easily isolated because they formed surface colonies that were well dispersed, particularly at higher dilutions.

E. Soil plate method(Warcup, 1950):

Approximately 0.005g of loam was scattered on the underside of a sterile Petri Dish and melted cooled (40-45⁰C) agar medium i.e. PDA and CZA was added, that was then revolved gently to disperse the soil particles within the medium. The Petri Plates were then incubated at 28± 20⁰C in dark for three days.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

F. Identification of the soil fungi:

Fungal morphology was measured macroscopically by perceptive colony options (Colour and Texture) and microscopically by stain with lactophenol cotton blue and uncovered below light microscope for the conidia, conidiophores and arrangement of spores (Stefanis, Christos 2013)⁷. The fungi were identified with the help of literature (Gilman, J.C 2001, A, Nagamani et al., 2006)

G. Statistical analysis:

The number of colonies per plate in 1g of soil was calculated. The percentage 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

III. RESULTS AND DISCUSSION

In the current study, a dilution plating methodology was used for isolating fungi from wholly different field soils placed in Chintalapudi mandal. Many authors used dilution plating methodology for isolation of fungi (Ishaq and Khan, 2011; Nilima Wahegaonkar *et al.*, 2011; Shiny Niharika *et al.*, 2013; Rakesh Sharma and Raju, 2013; Ibrahim and Shehu, 2014; Pandey *et al.*, 2014; Chandrashekar *et al.*, 2014).

Throughout the research period 112 fungal colonies of that maximum species belonged to Deuteromycotina, followed by Zygomycotina and few of unknown were determined. While incubation period unlike colonies of soil fungi were detected in inoculated petri plates. Amid the isolates, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium chrysogenum*, *Penicillium frequentans*, *Penicillium funiculosum*, *Fusarium oxysporum*, *Curvularia clavata*, *Trichoderma viride*, *Trichoderma harzianum*, *Mucor racemosus*, *Rhizopus stolonifer* and some unknown colonies were saw (Table 2). Microscopic observations of the fungi were done by staining with lactophenol cotton blue (figure-2). Identification of the isolates was done using legitimate taxonomic guides, standard procedures and relevant literature.

112 fungal colonies representing 7 genera were isolated and identified during this study from different field areas located in Chintalapudi mandal (Table 2). Apart from the above, maximum number of mycoflora belonged to the class Deuteromycotina followed by Zygomycotina and unknown were recorded.

Among the 112 colonies recorded, the genus *Aspergillus* (48 colonies) was dominant followed by *Penicillium* (21 colonies), *Trichoderma* (14 colonies), *Rhizopus* (8 colonies), *Fusarium* (5 colonies), *Mucor* (5 colonies) *Curvularia* (3 colonies) and unknown (8 colonies). Accordingly in the existing examination the *Aspergillus* was prevailing followed by *Penicillium* and *Trichoderma*.

Percentage contribution of the individual species to the total fungal population showed variation. The maximum percentage contribution was of *Trichoderma viride* 32.14% in groundnut field. The minimum of 2.94% was of *Aspergillus flavus*, *Fusarium oxysporum*, *Trichoderma harzianum* and *Mucor racemosus* (Table3). *Aspergillus niger* was the common one in all the three crop fields followed by *Penicillium chrysogenum*, *Trichoderma viride*, *Aspergillus terreus* and *Rhizopus stolonifer*.

Microbes are particularly necessary parts of diverseness. Significantly fungi and bacterium are crucial, as they modify and unleash several nutrients taking part in necessary roles in nutrient cycle and sustenance of vegetation (Parrotta J.A 1999, Sall S.N ET AL., 2003). The potency of fungi in decomposition and their potentiality rely upon their abundance and composition. Large quantities of readily decomposable organic matter are added to agricultural soils each year as crop residues or animal wastes and have a noteworthy outcome on soil microbial commotion. The plant species growing on the soil likewise equally influence the population and species composition of the soil fungi (Hackl E ET AL., 2000).

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

The soil pH, organic content and water are the most factors moving the plant population and variety (Yu C et al., 2007, Dong A.R et al., 2004, Zhang C.B et al., 2001). The organic carbon, nitrogen, phosphorus, potassium are necessary for fungi. Within the absence of any of those the growth and asexual reproduction of moulds moreover as alternative microorganisms are hampered lots (Marschner P et al., 2003)²³. The fungi were principally ascertained in months of June to September because of appropriate temperature and wetness. It's been reportable that the density of flora inhabitants befell throughout the monsoon (rainy) term once the soil wetness remained considerably high (Deka, H.K and Mishra R.R 1984, Bissett, J. and Parkinson, D 1979).

S.NO	Crop	Total colonies	Avg No. of Individual colonies														
			An	Afl	Afu	Ani	At	Pch	Pf	Pfu	Fo	Ccl	Tv	Th	Mre	Rst	Un
1	Rice	50	7	3	5	2	2	4	3	3	2	2	4	4	2	3	4
2	Maize	34	5	1	-	4	5	4	-	2	2	1	3	1	1	2	3
3	Ground nut	28	4	3	3	2	2	2	1	2	1	-	2	-	2	3	1
	Total	112	16	7	8	8	9	10	4	7	5	3	9	5	5	8	8
	%contribution		14.28	6.25	7.14	7.14	8.03	8.92	3.57	6.25	4.46	2.67	8.03	4.46	4.46	8.03	8.03

Un-Unknown colonies, Rst – *Rhizopus stolonifer*, Mre – *Mucor racemosus*, Th- *Trichoderma harzianum*, Tv- *Trichoderma viride*, Ccl-*Curvularia clavata*, Fo- *Fusarium oxysporum*, Pfu- *Penicillium funiculosum*, Pf- *Penicillium frequentans*, Pch- *Penicillium chrysogenum*, At- *Aspergillus terreus*, Ani- *Aspergillus nidulans*, Afu- *Aspergillus fumigatus*, Afl- *Aspergillus flavus*, An - *Aspergillus niger*.

Table-2: Frequency of mycoflora in diverse crop fields at Chintalapudi Mandal

S.No	Fungal species	% contribution		
		Paddy	Maize	Ground nut
1	<i>Aspergillus niger</i>	14.0	14.70	14.28
2	<i>Aspergillus flavus</i>	6.0	2.94	10.71
3	<i>Aspergillus fumigatus</i>	10.0	-	10.71
4	<i>Aspergillus nidulans</i>	4.0	11.76	7.14
5	<i>Aspergillus terreus</i>	4.0	14.70	7.14
6	<i>Penicillium chrysogenum</i>	8.0	11.76	7.14
7	<i>Penicillium frequentans</i>	6.0	5.88	3.57
8	<i>Penicillium funiculosum</i>	6.0	5.88	7.14
9	<i>Fusarium oxysporum</i>	4.0	2.94	3.57
10	<i>Curvularia clavata</i>	4.0	-	-
11	<i>Trichoderma viride</i>	8.0	8.82	32.14
12	<i>Trichoderma harzianum</i>	6.0	2.94	17.85
13	<i>Mucor recemosus</i>	4.0	2.94	17.85
14	<i>Rhizopus stolonifer</i>	6.0	5.88	10.71
15	<i>Unknown colonies</i>	8.0	8.82	3.57

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

Table-3

Percent contribution of various mycoflora isolated from soil samples of agricultural fields at Chintalapudi Mandal

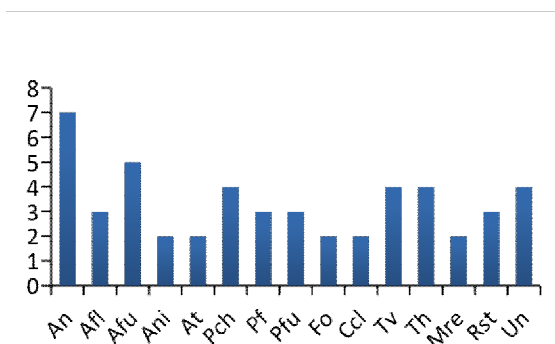


Figure1: Frequency of mycoflora in Rice at Chintalapudi Mandal

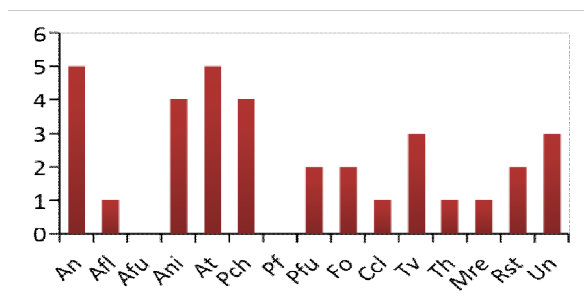


Figure 2: Frequency of mycoflora in Maize at Chintalapudi Mandal

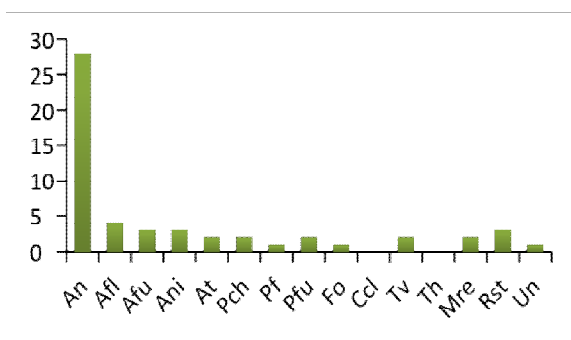
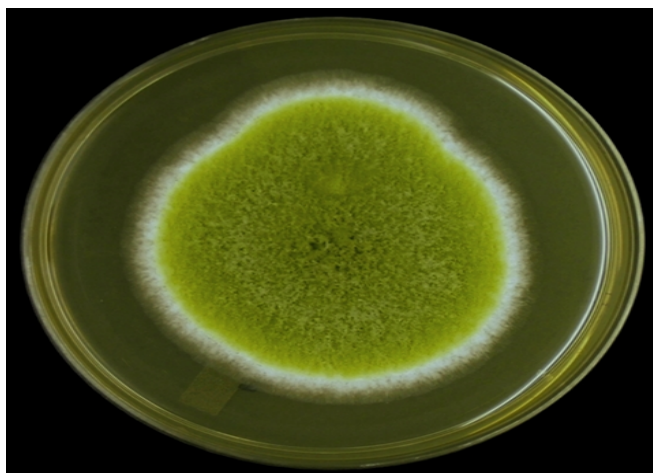
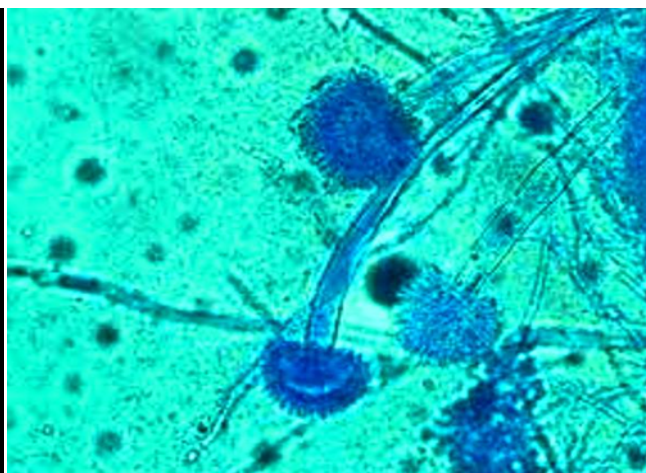


Figure 3: Frequency of mycoflora in Ground nut at Chintalapudi Mandal

At- *A. terreus*, Ani- *A. nidulans* ,
Afu- *A. fumigatus*, Afl- *A. flavus*,
An – *A. niger*, Pfu- *P. funiculosum*
Pf- *P. frequentans*,
Pch- *P. chrysogenum*,
Fo- *F. oxysporum*, Ccl- *C. clavata*,
Th- *T. harzianum*
Tv- *T. viride*, Mre – *M. recemosus*,
Rst – *R. stolonifer*,
Un-Unknown colonies.



Picture1: Pure culture of *Aspergillus flavus*



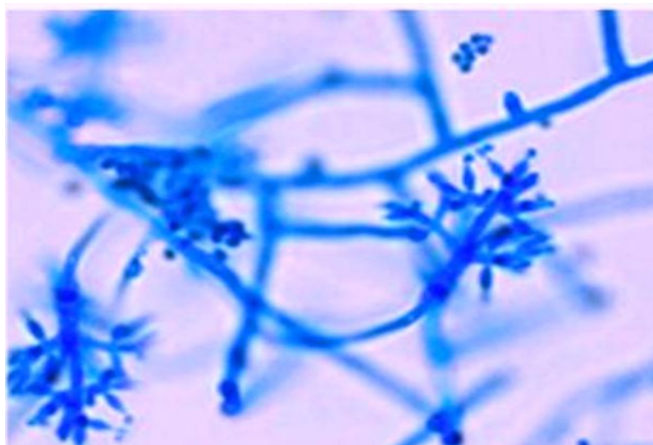
Picture 2: *Aspergillus niger*

International Journal of Innovative Research in Science, Engineering and Technology

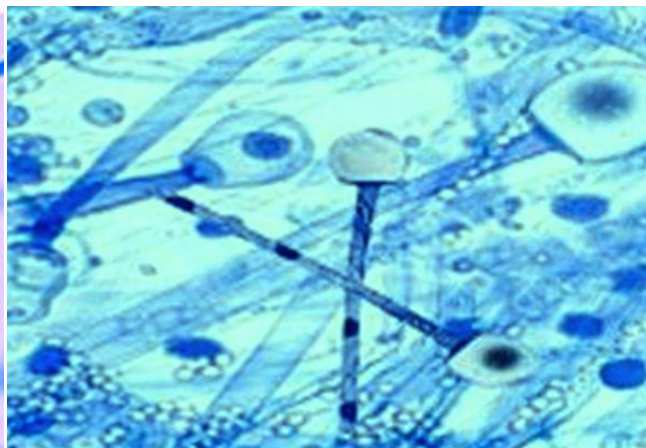
(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019



Picture 3: *Trichoderma harzianum*



Picture 4: *Mucor racemosus*

IV. CONCLUSION

Soil mycoflora plays a significant role in analysis of soil conditions and in stimulating plant growth. Within the extant study soil samples of 3 crop fields i.e. paddy, maize and ground nut were calculated for screening and finding of fungous multiplicity. The outcomes evidently specifies that *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride*, *Aspergillus terreus*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Penicillium funiculosum*, *Aspergillus flavus*, *Fusarium oxysporum*, *Trichoderma harzianum* and *Mucor racemosus* were of high incidence and some other fungi were slight i.e. *Curvularia clavata* and *Penicillium frequentans*. Midst the *Aspergillus* and *Penicillium* were leading genera, and *Aspergillus niger*, *Trichoderma viride*, *Mucor racemosus* and *Aspergillus terreus* were common in all agronomic fields. The rate of mycoflora in farmed fields were found to be regulated by several factors like humidity, temperature, soil sort and texture, vegetation, organic and inorganic materials .

V. CONFLICTS OF INTEREST

All authors have none to declare.

VI. 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.

REFERENCES

1. Adametz, L. Untersuchungen uber die Pilze der Ackerkrume. Inaug. Diss. Leipzig. 78pp, 1886.
2. Aneja, K.R., Experiments in Microbiology, Plant pathology and Biotechnology, Newage International Publishers, 4; 157-162, 2001.
3. Anusuya, D., and Jayarajan, R., Solubilization of phosphorus by *Trichoderma viride*. Current Science. 74: 464-466, 1988.
4. Azaz, A. D., Isolation and Identification of Soilborne Fungi in Fields Irrigated by GAP in Harran Plain Using Two Isolation Methods. Turk J Bot 27: 83-92, 2003.
5. Bissett, J. and Parkinson, D., Can j. Bot, 57; 1642-59, 1979.
6. Chandrashekar, M. A., Soumya Pai, K. and Raju, N. S., Fungal diversity of rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore District, Karnataka, India. Int. J. Curr. Microbiol. App. Sci, 3(5); 559-566, 2014.
7. Chiang, C.N., and Soudi, B., Biologie du sol et cycles biogéochimiques. In: El Hassani T.A. and Persoon E (Eds), Agronomie Moderne, Bases physiologiques et agronomiques de la production végétale, 85-118, 1994.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

8. Christensen, M., Whittingham, W. F., Novak, R. O. The soil micro fungi of wet mesic forest in southern Wisconsin. *Mycologia*, 54: 374-388, 1962.
9. Deka, H.K and Mishra R.R., *Acta Botanica Indica*, 12;180-184, 1984.
10. Dong A.R., Lv G.Z., Wu Q.Y., Song R.Q., Song F.Q., Diversity of soil fungi in Liangshui natural reserve, Xiaoxing'anling forest region, *J. Northeast Forestry University*, 32(1); 8-10, 2004.
11. Gilman, J.C., *A Manual of Soil fungi*, 2nd Indian edition, Biotech Books, Delhi, 2001.
12. Gnanasekaran, P., Mohamed, S, S., and Panneerselvam, A., Isolation and identification of soil mycoflora in banana field at Manachanallur, Tiruchirappalli Dt., Tamil Nadu, India. *Int. J. Curr. Microbiol. App. Sci.*, 4,7; 729-740, 2001
13. Goechenaur, S. E. and Whittingham, W. F. Mycoecology of willow and cottonwood low land communities in southern Wisconsin. I. Soil micro fungi in the cottonwood forests. *Mycopath Mycol Appl*, 33: 125-139, 1967.
14. Hackl E., Bachmann G., Boltstern-Zechmeister S., Soil microbial biomass and rhizosphere effects in natural forest stands *Phyton*, 40; 83-90, 2000.
15. Ibrahim, M. and Shehu, K., Relationship of soilborne mycoflora of cassava growing fields to incidence of postharvest rots of cassava tubers in Sokoto, Nigeria. *Aceh Int. J. Sci. Technol*, 3(3); 168-173, 2014.
16. Ishaq, F. and Khan, A., Isolation, identification and comparative study of fungal and bacterial strains found in organic and inorganic soils of different agricultural fields, *Recent Research in Science and Technology*, 3(11): 30-36, 2011.
17. Kamal, Bhargav, K. S., Studies on soil fungi from teak forest of Gorakhpur X Edaphic factors and distribution of soil microfungi in teak stands of different ages. *Proc Nat Acad Sci India*, 43B: 9-16, 1973.
18. Marschner P., Kandeler E., Marschner B., Structure and function of the soil microbial community in a long-term fertilizer experiment, *Soil Biol. Biochem*, 35; 453-46, 2003.
19. Nagamani, A., Kunwar, I.K., and Manoharachary, C., *Hand book of soil fungi*, I.K. International Pvt.Ltd, 2006.
20. Nieves-Rivera A., Mycological survey of Río Ca-muy Caves Park, Puerto Rico. *J Cave Karst Stud*. 65: 23-28, 2003.
21. Nilima Wahegaonkar, S.M., Salunkhe, P., Palsingankar, L. and Shinde, S.Y., Diversity of fungi from soils of Aurangabad, M.S., India. *Annals of Biological Research* 2 (2);198-205, 2011.
22. Olson, R.K., Schoenherger, M.M., and Aschmann, S.G., *American Society of Agronomy*, Madison, Wisconsin, USA, 2000; 987-1057.
23. Oudemans, C. A. & J. A. & Koning, C. J. *Prodrome d'une flore mycologique obtenue par la culture sur gelatine preparee de la terre humeuse du Spaanderswoud* pres Bussum. *Arch. Neerl. Sci. Nat.*, Ser. 2(7): 286-298, 1902.
24. Pandey, V., Singh, P. K., Mishra, R. K., Srivastva, V. and Shukla, D. N., Studies on soil mycoflora of sugarcane field in central Uttar Pradesh, *Arch. Appl. Sci. Res*, 6 (4);230-237, 2014
25. Parrotta J.A., Productivity, nutrient cycling and succession in single- and mixed-species plantations of *Casuarina equisetifolia*, *Eucalyptus robusta* and *Leucaena leucocephala* in Puerto Rico, *For. Ecol. Manage*, 124(1); 45-77, 1999.
26. Rai, J. N., Tewari, J. P., Mukerji, K. G., Mycoflora of mangrove mud. *Mycopathologia et Mycologia Applicata*. 38: 17-31, 1966.
27. Rakesh Sharma, M.S. and Raju, N.S., Frequency and percentage occurrence of soil mycoflora in different crop fields at H D Kote of Mysore district. *International Journal of Environmental Sciences*, 3(5); 1569- 1576, 2013.
28. Saksena, R. K., Krishna, F. N. I. and Sarbhoy, A. K. Ecology of the soil fungi of Uttar Pradesh. 33(5 & 6): 298-306, 1966.
29. Sall S.N., Masse D., Reversat F.B., Guisse A., Chotte J.L., Microbial activities during the early stage of laboratory decomposition of tropical leaf litters: the effect of interactions between the litter quality and exogenous inorganic nitrogen, *Biol. Fert. Soils*, 39(2); 103-111, 2003.
30. Shiny Niharika, P., Gaddeyya G. and Ratna Kumar P.K., An investigation on soil mycoflora of different crop fields at Narasannapeta Mandal, Srikakulam District. *Int. Res. J. Environment Sci*, 2(9), 38-44, 2013.
31. Stefanis, Christos, Athanasios Alexopoulos, Chrissa Voidarou, Stavros Vavias, and Eugenia Bezirtzoglou. Principal methods for isolation and identification of soil microbial communities. *Folia microbiologica*, 58(1); 61-68, 2013.
32. Thornton, R. H., Growth of fungi in some forests and in grassland soils. In D Parkinson, JS. Waid (Eds.): *The Ecology of Soil Fungi*. Liverpool: Liverpool Univ. Press, pp. 84-91, 1960.
33. Tresner, H. D., Backus, M. P. and Cortis, J. T. Soil microfungi in relation to the hardwood forest continuum in Southern Wisconsin, *Mycologia* 46: 314-333, 1954.
34. Waksman, S.A., *J.Bact*, 7; 339-341, 1922.
35. Warcup, J. H. "On the origin of colonies of fungi developing on soil dilution plates." *Transactions of the British Mycological Society*, 38(3);298-301, 1955.
36. Yu C., Lv D.G, Qin S.J., Du G.D., Liu G.C., Microbial flora in *Cerasus sachalinensis* rhizosphere, *Chinese. J. Appl. Ecol*, 18 (10); 2277-2281, 2007.
37. Zhang C.B., Jin Z.X., Li J.M., Diversity of bacterial physiological groups and microbial flora in the soil of eight forest types of Tiantai Mountain, Zhejiang, *Biodiversity Sci*, 9(4), 382-388, 2001.