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**RESEARCH ARTICLE**

# ISOLATION AND IDENTIFICATION OF SOIL MICROFLORA IN THE RICE FIELDS OF NARASANNAPETA MANDAL IN SRIKAKULAM DISTRICT

**Ratna Kumar, P.K., *Shiny Niharika P., Hemanth, G. and Samuel K. Kolli**

Department of Botany, Andhra University, Visakhapatnam – 530003, Andhra Pradesh, India

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**ABSTRACT**

Soil mycoflora were isolated from the rice fields of Narasanapeta during a period of one year ie., from January 2013 to December 2013. Isolation of the mycoflora was done by serial Dilution Plate Method. During the investigation period a total of 118 fungal colonies of 27 fungal species were observed. Identification was done by using standard manuals and literature. The maximum percentage contribution was that of Aspergillus niger (13.55%), followed by Aspergillus flavus (11.01%) and the minimum percentage contribution was that of Aspergillus restrictus and Trichoderma koeningii (0.84%) and also of unidentified species of Mucor and Penicillium. Among the isolates were *Aspergillus candidus, A.clavatus, A.flaviceps, A.flavus, A.fumigatus, A.nidulans, A.niger, A.oryzae, A.restrictus, A.tamarii, A.terreus, Bispora species, Curvularia clavata, C.lunata, Fusarium oxysporum, F.solani, Mucor recimosus, Mucor species,Penicillium chrysogenum, P.frequentens, P.funiculosum, P.species, Rhizopus nigricans*, *R.stolonifer, Trichoderma harzianum, T.koeningii and T.viride* were authentically characterized. Most of the fungal species belonged to anamorphic fungi ie., Deuteromycotina (94 colonies), Zygomycotina (17 colonies) and the remaining to that of unidentified white mycelia (7 colonies) were observed. The percentage frequency of the fungal species was statistically analyzed.



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## INTRODUCTION

The physical, chemical and biological properties of the soils are influenced by the decomposition of plant and animal residues for their conversion into soil organic matter by the soil microbes. Soil helps the plants in anchoring, supplying water and mineral salts, thus taking part in primary biomass production. The distribution of the micro-organisms is inclined by the abundance and nature of the organic content of the soil, as well as by other soil and climatic environment, surface vegetation and soil texture (Marschner *et al*., 2003; Waksman 1944). Micro-organisms are of assistance in increasing the soil fertility and plant growth as they are concerned in several biochemical conversions and mineralization actions in soils. The soil micro-organisms ensure the permanence of element cycles in nature due to the array of their metabolic behavior. The consequence of their activities is not only mineralization of organic compounds but also the changes of mineral compounds, which have an immense impact on the development of the plants. Micro fungi play an imperative role in nutrient cycling by regulating soil biological activity. The quantity of different micro-organisms present in the soil depends upon the soil moisture, aeration, ph, temperature and nutrients available etc. Specific requirements of certain specific elements for growth and reproduction of fungi helps in their distribution on diverse habitats in nature. Hence fungi are major components of the soil micro-biota characteristically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby 1995). The composition and quality of microbes in the soil influence the ability of plants to obtain nitrogen and other nutrients. Fungi benefit most plants by suppressing plant root diseases and promoting healthier plants by attacking plant pathogens with fungal enzymes (James J.Hoorman 2011). Large quantities of decomposable organic matter are added to agricultural soils as crop residues or animal wastes and have substantial out come on soil microbial commotion (Prince *et al*., 2011). The plant species growing on the soil also similarly influence the population and species composition of the soil fungi (Hackel *et al*., 2000). The pace at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions. Fungi can efficiently grope around for N and P

***Corresponding author: Shiny Niharika, P.***
*Department of Botany, Andhra University, Visakhapatnam – 530003, Andhra Pradesh, India.*

better than the plant root hairs and wholly increase the plant root nutrient extraction efficiency. Fungi get dominated over other micro-organisms by secreting enzymes. They also have the ability to survive and propagate in various extreme conditions of the environment. They also use antagonisms to reduce competition by producing antibodies, which suppress other micro-organisms from growing (James J.Hoorman 2011).

Fungi are one of the principal groups present in the soil than that of other micro-organisms and play essential role in the composition of the soil. The present investigation aims to isolate mycoflora from the agricultural rice fields of Narasannapeta and to observe the percentage contribution and diversity of different fungal species.

**Site and Location Description**

Narasannapeta is a town and mandal in Srikakulam District in the state of Andhra Pradesh in India. It is located at 18.4167$^{o}$N and 84.0500$^{o}$E and has an average elevation of 18 meters (62 feet). The annual rainfall in Narasannapeta was recorded as 1037mm. The major rain fed and irrigated field crops were paddy, sugarcane, ground nut, green gram, black gram, sesame, maize and sunflower.

**Physicochemical Analysis of Soil**

The physicochemical properties of the collected soil samples were analyzed. The physical and chemical parameters of the soil such as the pH values, salinity, organic carbon percentage, nitrogen, phosphorus and potassium were analyzed. The color and type of soil were also determined. The parameters of the soil sample were analyzed at Mobile Soil Testing laboratory (MSTL), Pothinamallayapalem, Visakhapatnam, Department of Agriculture, Andhra Pradesh (Table 1).

**Table 1. Physico-chemical properties of soil sample collected from rice fields of Nimmada**

| Crop Field | Rice |
|---|---|
| Place | Narasannapeta |
| Soil Type | Sandy Loam |
| Soil color | Brown |
| pH | 6.6 |
| Salinity | 0.21 |
| Organic Carbon % | Medium |
| Nitrogen(Kg/h) | 65 |
| Phosphorus(Kg/h) | 56 high |
| Potassium(Kg/h) | 37 low |

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

**Methods of Collection of Soil Samples**

The soil samples were collected from January 2013 to December 2013. Four samples were collected from different sites. Main mass of fungi is found in upper 20-30 layers. Hence the samples were collected with the help of a sterilized cork-borer pushed horizontally into the ground at a depth of 20 cms at four different points within the area, and the sample caught was emptied into the sterilized polyethylene bags. The bags were labeled and named and brought to the laboratory for further experimentation.

**Isolation**

The fungal species were isolated by serial soil dilution plate method on Potato Dextrose Agar Medium (extract from 250gr of potato boiled and filtered, dextrose 20gr, agar 15gr and distilled water 1000ml).

**Sterilization of the Medium**

The media, distilled water, pipettes, petri dishes were all kept in an autoclave for sterilization. The sterilization is done at 121$^{o}$C for 15 min at 15lbs pressure.

**Soil Dilution Plate Method**

1gr of soil sample was suspended in 10ml of double distilled water to make microbial suspensions of concentrations ($10^{-1}$ to $10^{-5}$). Triplicate of each dilution of $10^{-3}$, $10^{-4}$ and $10^{-5}$ were used to isolate fungi. 1ml of microbial suspension of each concentration was added to sterile petri dishes containing sterile PDA medium at pH 6.6. The bacterial growth was prevented by the addition of 1% of streptomycin solution to the medium before pouring into petri plates. The petri dishes were then incubated for 4-6 days in a dust free cupboard at room temperature 28$\pm$2$^{o}$C and were then examined. Fungi growing on the agar plates were selected for sub-culturing. Each isolate of fungal species were sub-cultured by transferring onto fresh agar slants.

**Identification**

After the incubation the identification of the fungal organisms was made by microscopic analysis using taxonomic guides, manuals and relevant literature (Gilman 2001; Nagamani *et al*., 2006). The frequency distribution of the fungal species was investigated. The colony color, morphology, spore size, shape and spore bearing structures were analyzed. The colonies growing on PDA plates with different morphology were counted. With the help of needles a small portion of the fungal growth from the culture plates was transferred on to a clean slide containing a drop of lactophenol cotton blue stain. The specimen was teased carefully to avoid overcrowding of the fungal mycelium on the slide. It was later observed under the microscope and the micro-morphological characters were identified using standard manuals.

The percentage contribution of each isolate was calculated by using the formula

$$\% \text{ Contribution} = \frac{\text{Total No. of CFU of an individual Sps}}{\text{Total No. of CFU of all sps}} \times 100$$

*CFU – Colony Forming Unit

## RESULTS AND DISCUSSION

In the current research work during a period of one year a total of 118 fungal colonies of 27 fungal species belonging to

Deuteromycotina (94 colonies) and Zygomycotina (17 colonies) and sterile white mycelia (7 colonies) were observed (Table 2). According to the aim of the present study soil samples were collected from different sites of paddy fields in Narasannapeta mandal. The identification of most groups of the fungal species continued to be based on their morphology. The environmental factors such as pH, moisture, temperature, organic carbon and nitrogen play an important role in affecting the fungal population and diversity (Gaddeyya *et al*., 2012).

The investigation resulted the presence of 27 species of fungi in all of them 4 species belonging to 2 genera were Zygomycetes and the remaining 23 species belonging to 6 genera were assigned to Deuteromycetes. Among the 27 fungal species identified, the genus Asergillus was considered by more number of species (11 species) followed by Penicillium (4 species), Trichoderma (3 species), Curvularia, Fusarium, Rhizopus and Mucor (2 each) were identified but Bisporis was identified only upto genus level.

**Table 2. Frequency of mycoflora in rice fields at Narasannapeta**

| S.No | Name of the Fungal Species | Total No. of Colonies | Mean Density | Percentage Contribution |
|---|---|---|---|---|
| 1 | Aspergillus candidus | 02 | 0.66 | 1.69 |
| 2 | A.clavatus | 03 | 1 | 2.54 |
| 3 | A.flaviceps | 05 | 1.66 | 4.23 |
| 4 | A.flavus | 13 | 4.33 | 11.01 |
| 5 | A.fumigatus | 10 | 3.33 | 8.47 |
| 6 | A.nidulans | 03 | 1 | 2.54 |
| 7 | A.niger | 16 | 5.33 | 13.55 |
| 8 | A.oryzae | 03 | 1 | 2.54 |
| 9 | A.restrictus | 01 | 0.33 | 0.84 |
| 10 | A.tamarii | 02 | 0.66 | 1.69 |
| 11 | A.terreus | 07 | 2.33 | 5.93 |
| 12 | Bispora species | 02 | 0.66 | 1.69 |
| 13 | Curvularia clavata | 02 | 0.66 | 1.69 |
| 14 | C.lunata | 03 | 1 | 2.54 |
| 15 | Fusarium oxysporum | 03 | 1 | 2.54 |
| 16 | F.solani | 02 | 0.66 | 1.69 |
| 17 | Mucor recemosus | 03 | 1 | 2.54 |
| 18 | Mucor species | 01 | 0.33 | 0.84 |
| 19 | Penicillium chrysogenum | 03 | 1 | 2.54 |
| 20 | P.frequentens | 04 | 1.33 | 3.38 |
| 21 | P.funiculosum | 03 | 1 | 2.54 |
| 22 | Penicillium species | 01 | 0.33 | 0.84 |
| 23 | Rhizopus nigricans | 03 | 1 | 2.54 |
| 24 | R.stolonifer | 10 | 3.33 | 8.47 |
| 25 | Trichoderma harzianum | 03 | 1 | 2.54 |
| 26 | T.koeningii | 01 | 0.33 | 0.84 |
| 27 | T.viride | 02 | 0.66 | 1.69 |
| 28 | Unknown white mycelium | 07 | 2.33 | 5.93 |
| | Total | | 118 | |

**Fig.1. Soil serial dilution $10^3$-$10^5$ in PDA**

**Fig.2. Choose individual petri dish from serial dilution**

**Fig.3. Pure culture of one selected fungal colony**

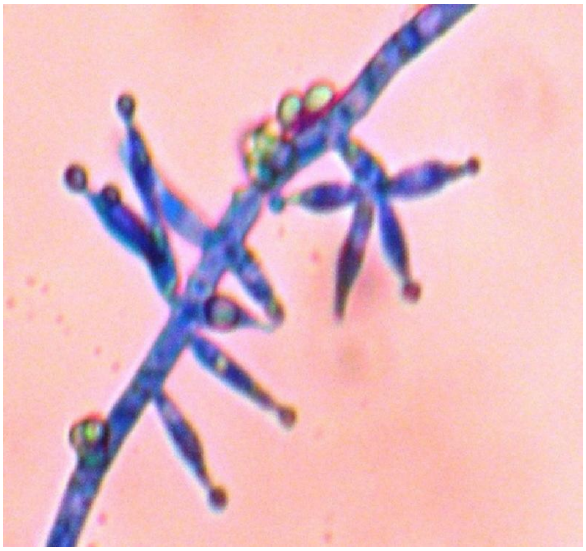


**Fig.4. Microscopic observation of Trichoderma**

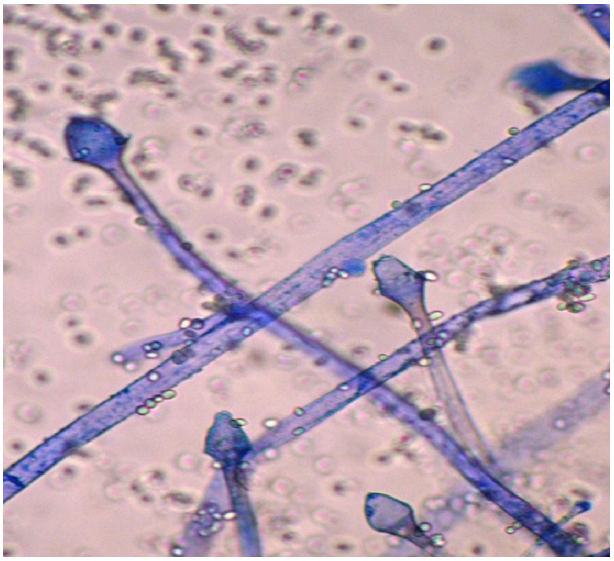
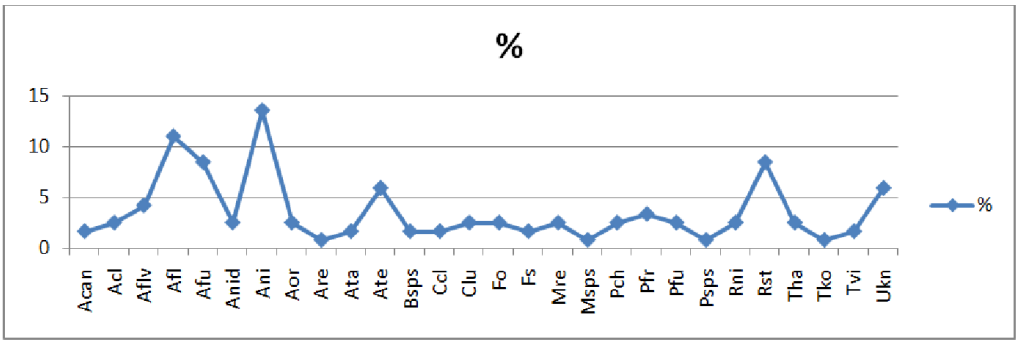


**Fig.5. Microscopic observation Mucor**

**Fig.6. Pure culture of Mucor in Petridish**




**Graph 1. Percentage contribution of the fungal species**

Acan – Aspergillus candidus
Acl – Aspergillus clavatus
Aflv- Aspergillus flaviceps
Afl – Aspergillus flavus
Afu – Aspergillus fumigates
Anid – Aspergillus nidulans
Ani – Aspergillus niger
Aor – Aspergillus oryzae
Are – Aspergillus restrictus
Ata – Aspergillus tamari
Ate – Aspergillus terreus
Tha – Trichoderma harzianum
Tko – Trichoderma koeningii
Tvi – Trichoderma viride

Bsps - Bispora sps
Pfr – Penicillium frequentens
Ccl – Curvularia clavata
Clu – Curvularia lunata

Fo – Fusarium oxysporum
Fs – Fusarium solani

Mre – Mucor recemosus
Msps – Mucor species

Ukn - Unknown

Pch – Penicillium chrysogenum

Pfu – penicillium funiculosum
Psps – Penicillium species

Rni – Rhizopus nigricans
Rst – Rhizopus stolonifer

PDA medium is one of the most commonly used culture media because of its simple formulation and its ability to support mycelia growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelia growth (Maheshwar *et al*., 1999; Saha *et al*., 2008; Xu So *et al*., 1984). Totally 27 different species belonging to Deuteromycetes and Zygomycetes were isolated using PDA medium. The dominant species were Aspergillus niger (13.55%) followed by A.flavus (11.01%), A.fumigatus and Rhizopus stolonifer (8.47%). The purification of the culture was done either by single spore culture or by hyphal tip culture. These were transferred to fresh agar slants on PDA medium.

The frequency distribution of individual fungal species was showed in (Table 2). The most common isolates were *Aspergillus candidus, A.clavatus, A.faviceps, A.flavus, A.fumigatus, A.nidulans, A.niger, A.oryzae, A.restrictus, A.tamarii, A.terreus, Bispora species, Curvularia clavata, C.lunata, Fusarium oxysporum, F.solani, Penicillium chrysogenum, P.frequentens, P.funiculosum, Penicillium* species, *Trichoderma harzianum, T.koeningii, T.viride* belonging to Deuteromycetes and *Mucor racemosus*, Mucor species, *Rhizopus nigricans, R.stolonifer* belonging to Zygomycotina were isolated and characterized.

Organic carbon largely controls the microbial growth in the soil (K.Saravanakumar and Kaviyarasan, 2010). Fungi generally have higher carbon content (10:1 C:N ratio) than that of nitrogen in their cells. Hence they help in recycling both N and P to plants. Physical and chemical parameters of the soil such as pH, salinity, macro nutrients like nitrogen, phosphorus potassium and organic carbon were carried out and were represented in (Table 1). The percentage contribution of the soil micro flora were graphically represented in (Graph-1). The results obtained suggest that the Deuteromycotina are the fungal species with higher occurrence in the soil and that Aspergillus and Penicillium species are more frequent relatively compared to other genera of the fungal species occurring in the soil. Molecular methods for identification of fungal species have improved but still microscopy and culture remain commonly used and essential tools for identification of species like Aspergillus and Penicillium.

**Conclusion**

The heterogeneous physical structure of soil affects the spatial allocation of water, oxygen and mineral nutrients which consecutively influence the composition and commotion of the microbial communities. In the *present* study the soil samples from four different sites of rice fields from Narasannapeta mandal were studied for screening and detection of fungal diversity. The results obtained clearly indicate that *Aspergillus* and *Penicillium* were the fungal species which were predominant among the others.

Among the Aspergsillu species *Aspergillus niger* and *A.flavus* showed high occurrence. Since *Aspergillus* produces toxins and *Penicillium* produces antibiotics, these prevent the growth of other fungal species.

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