



ISOLATION AND IDENTIFICATION OF SOIL MYCOFLORA IN AGRICULTURAL FIELD OF SADAK ARJUNI OF GONDIA DISTRICT (MS)

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ABSTRACT:

Soil samples were collected from different locations of Sadak Arjuni of Gondia District during the months of February 2014 to January 2015 in three intervals. The samples collected in two zones viz. rhizoplane and rhizosphere. The collected samples were inoculated in Potato Dextrose Agar (PDA) and CzapekDox Agar (CDA) medium supplemented by antibiotics such as penicillin and Streptomycin by using Serial dilution method and soil plate method. A total of 230 colonies were isolated. About 19 species belonging to 7 genera of fungi were isolated and identified while 21 strains were unidentified. Identification and characterization of the soil mycoflora were made with the help of authentic manuals of soil fungi. Maximum number of fungal colonies belonged to Ascomycotina and *Deutero mycotina* (191) and few to *zygomycotina* (18). Among the isolates *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Rhizopusstolonifer* and *Trichoderma viridae* were predominant. The percentile contribution of the mycoflora was graphically and statistically analyzed.

Keywords: *Microfungi, Culture Media, Isolation, Fungal Diversity.*

INTRODUCTION:

Soils are extremely complex structures with many constituents playing diverse functions mainly due to the activity of soil organisms (Chiang and Soudi, 1994). Soil microflora plays a essential role in evaluation of soil conditions and in stimulating plant growth (Kiran *et al.*, 1999). Microorganisms are helpful in increasing the soil fertility and plant development as they are included in several biochemical transformation and mineralization activities in soils. Method of cultivation and crop management practices found to have greater influence on the activity of soil microflora (Mc.Gill *et al.*, 1980). Continuous use of chemical fertilizers over a long period affecting soil microflora and thereby indirectly affect biological properties of soil leading to soil degradation (Manickam *et al.*, 1972). There is a massive microbial flora present in the earth and they are found in all types of soils (Mukherji *et al.*, 2006). These microbes may interact with the plants resulting sometimes in useful effect and other times in harmful consequences. Fungi are an important component of the soil microflora and are present in the form of mycelium, rhizomorphs or as spores. They play important role in soils and plant nutrition. Saprophytic fungi are able to live on dead and decaying organic matter. They secrete a varied number of enzymes that attacks effectively any organic material and converting it into simple soluble forms, which are readily available to higher plants. Due the degradative activities fungi play important role in recycling organic waste in

environment. Unfortunately their degradative ability also results in the undesirable growth of fungi that destroy useful materials (Aina *et al.*, 2011). Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examinations and biochemical and physiological characterization (Aina *et al.*, 2011).

The species richness of a fungal community and relative abundance of individual species have been considered as measures of functional activities of the group in the particular habitat (Kjoller and Struwe, 1982). Fungi, bacteria and actinomycetes colonize diverse habitats and substrates and play substantial role in plant health and productivity besides producing diseases. The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem.

Rhizosphere

The rhizosphere is a micro-ecological zone in direct closeness of plant roots. It is functionally defined as the particulate matter and microorganisms that adhere to roots after being moderately shaken in water. The theoretical extent of the rhizosphere is dependent on the zone of influence of the plant roots and associated microorganisms. The rhizosphere is a metabolically busier, faster moving, more competitive environment than the surrounding soil.

Rhizoplane

The rhizoplane is the region around the root epidermis and outer cortex where soil particles, bacterial and fungal hyphae adhere. The functional definition is that after the roots have been shaken rapidly in water the remaining microorganisms and soil particles left are considered as belonging to the region of rhizoplane.

There are more numbers of microbes present in the rhizoplane than in the rhizosphere. The diversity of the fungal population is determined by counting the number of colony forming units (CFUs). By spreading the extracted soil microorganisms across agar and counting the number of independent clusters of microorganisms the CFUs were determined. Micro-organisms are abundant where the reliability of the root is compromised. Hence rhizoplane microorganisms tend to be found on older ones rather than younger roots.

STUDY SITE AND LOCATION:

Sadak Arjuni of Gondia district (MS) located at 21.10°N 80.15°E. It has an average elevation of 256 metres (843 feet). It is located near the Maharashtra Chhattisgarh border on Mumbai-Kolkata National Highway 6. The temperature ranges from 15 - 42°C. Red Sandy soils and Laterite soils are the major soil types existing.

The climate is characterized by a hot summer, well distributed rainfall during the south-west monsoon season and generally dry weather during the rest of the year. Farmers take up first crop of Paddy with monsoon rainfall and a second of Wheat, Gram, Linseed, Sunflower and many more crops with irrigation in Rabi Season.

METHOD AND MATERIAL:

Nutrient Medium Used:

Potato Dextrose Agar (PDA) and CzapekDox Agar (CDA) medium used for isolation of fungi. The pH of the medium was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi.

Collection of Soil Samples:

The soil samples were collected from Six different crop fields from Six different locations of Sadak Arjuni. The samples were collected between the months of February 2014 to January 2015 in three intervals. Majority of fungi are microscopic and show huge variation in different sites of collection and at different depths. Therefore soils were collected from a depth of 15cm and are kept in sterilized Ziploc polyethylene bags. Each sample bag was labeled properly by indicating the site of collection, time, date and place of collection. The

collected soil samples along with locations showed in Table:1.

Isolation of fungi from the soil samples:

The soil microfungi were isolated by two methods, Soil Dilution and Soil Plate method on Potato Dextrose Agar and Czapek, sDox Agar media.

Soil Dilution Plate Method (Waksman, 1922):

1gm 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 or Czapek, sDox Agar medium. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth and incubated at $28 \pm 2^\circ\text{C}$ in dark. The plates were observed everyday up to 4-7 days. Fungi were easily isolated because they formed surface colonies that were well dispersed (Fig: 2), particularly at higher dilutions.

Soil Plate Method (Warcup, 1950):

Almost 0.005g of soil was dispersed on the bottom of a sterile petri dish and molten cooled ($40-45^\circ\text{C}$) agar medium (PDA) and (CZA) was added, which was then rotated gently to scatter the soil particles in the medium. The Petri dishes were then incubated at $28 \pm 2^\circ\text{C}$ in dark for 4-5 days. One isolate of each fungal genus from each soil sample were selected at random for further subculturing and experiments. The subcultures were maintained on Potato Dextrose Agar.

Inoculating Techniques:

The working benches in the laboratory were thoroughly sterilized by swabbed with 70% alcohol, and also a burning blue flame was allowed to sterilize the surrounding air before the inoculation proper. The conical flasks were corked tightly with cotton wool and the Petri dishes were fully autoclaved (Aina *et al.*, 2011).

Identification of the Soil Fungi:

Generally identification of the fungal species is based on morphological characteristics of the colony and microscopic examinations (Diba *et al.*, 2007). The colony growth which includes length and width of the colony, the presence or absence of aerial mycelium, the color, wrinkles furrows and any other pigment production were the macro morphological characters evaluated. Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly

used and essential tools for identification of fungal species (Diba *et al.*, 2007). The fungi were identified with the help of standard procedure and relevant literature (Gilman, 2001; Nagamani *et al.*, 2006).

Staining Technique for Fungi:

Inoculating needles were flamed over the burning Bunsen burner. Then using the needle, a small portion of the growth on the culture plate was transferred into the drop of lacto phenol in cotton blue on the slide. The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium (Aina *et al.*, 2011). The specimen is observed under the microscope for microscopic identification (Fig: 4).

Statistical Analysis:

Population density expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. The CFU and Percent Contribution of each isolate was calculated by –

$$\text{CFU/gm of Soil} = \frac{\text{Average number of fungal colonies}}{\text{Dry weight of sample}} \times \text{Dilution Factor}$$

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

RESULT AND DISCUSSION:

Soil microorganisms act as vital determinants of plant community variety and productivity (Wardle *et al.*, 2004). The environmental factors such as the soil pH, moisture, temperature, organic carbon and nitrogen play an important role in the distribution of mycoflora (Gaddeyya *et al.*, 2012). These are the main factors affecting the fungal population and diversity. The soil mycoflora in six different rice fields viz., Sadak Arjuni, Wadegaon, Kohmara, Sawangi, Saundad and Tidka were observed. Soil Dilution Plate and Soil Plate methods were used for the isolation of fungi during the present investigation.

During a period of 12 months the total number of fungal colonies isolated on Petri plates containing PDA medium were 229. As stated earlier Soil dilution plate and Soil plate method were employed for the isolation of fungi during the present investigation. A greater number of species and colonies were isolated on soil plates than on dilution plates and further the total number of species isolated decreased with increased dilutions of the samples. The purification of the culture (Fig: 3) was

done either by single spore isolation or by culturing of the hyphal tips and was transferred to fresh agar slants of CDA medium. Most of the fungal forms which sporulate heavily were abundant on dilution plates.

Fungi act as major decomposers of dead organic matter and contribute significantly in recycling of nutrients in natural and modified ecosystems (Gadd, 2004). Altogether six soil samples from six different locations were examined for fungal diversity. The study resulted the presence of 19 species of fungi were identified and characterized from PDA plates (Table: 2). the maximum fungal species belonged to Ascomycotina and Deuteromycotina (191 colonies) and Zygomycotina (18 colonies) and 21 colonies were left unknown on the plates containing PDA medium (Table: 2)

PDA medium is the most frequently used culture media and was stated to be the best media for mycelia growth by several workers worked with it earlier (Maheshwari *et al.*, 1999; Saha *et al.*, 2008), due to its simple formulation and potential to support wide range of fungal growth. CDA medium preferred for pure culture to minimize the contamination of *Rhizopusstolonifer*. Characterization of the isolates up to genus level and to the species level was made based on the macromorphological (Colony characters) and micro-morphological characters by using authentic manuals of soil fungi.

Our findings are in accordance with the results of Noor Zaman *et al.*, (2012) as the microbial analysis of different samples in rainfed areas of Punjab, Pakistan. They isolated genera like *Aspergillus*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus*. Similar genera were isolated during our investigation. These findings were similar to those isolated by Rasheed *et al.*, (2004). *Aspergillus* species particularly like *A. flavus* and *A. niger*, *Penicillium* and *Rhizopus* were isolated only from the soil where as *Alternaria alternata*, *Curvularia lunata* and *Fusarium* species were obtained from both soil and plant parts. Hence it is considered that isolation of soil samples yielded more fungal species than from plants (Noor Zaman *et al.*, 2012).

In our investigation among the obtained fungal isolates the genera *Aspergillus*, *Fusarium*, *Rhizopus* and *Penicillium* were dominant on media used (Tables: 2). The most common isolates among them viz., *A. candidus*, *A. flavus*, *A. fumigatus*, *A. granulosis*, *A. nidulans*, *A. niger*, *A. ochrasius*, *A. terreus*, *Curvularia clavata*, *C. lunata*, *C. lunata*,

Fusarium oxysporium, *F. solani*, *Penicillium chrysogenum*, *P. digitatum*, *Rhizopus stolonifer*, *R. oryzae*, *Trichoderma hamatum*, *T. viride* were isolated and characterized.

The percent contribution of different soil mycoflora of all the six Rice fields was evaluated. The fungi were mostly observed in the months of June to October as it has been reported that the diversity of fungal population occurred during the monsoon season where the soil moisture was significantly high (Bissett & Parkinson, 1979; Deka & Mishra, 1984).

Isolation of fungal species from soil samples by repeated screening and plating on starch agar medium by Ratnasri *et al.*, 2014 yielded *Aspergillus fumigatus* and *A. niger* along with other fungal species. *Fusarium solani* was isolated from the soils of infected fields and showed 100% frequency (Javed *et al.*, 2008). Recent study on Soil Microflora in National Parks in Gujarat yielded fungal species like *Aspergillus niger* and *Fusarium* species (Megha *et al.*, 2015). The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture (Gaddeyya *et al.*, 2012). Natural and anthropogenic disturbances can alter the species composition or may have negative effect on species diversity of the decomposer fungi (Dong *et al.*, 2004). These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization and may result in disturbances. Graphical representation of percent contribution of fungal species diversity in various paddy fields on the media PDA is represented in (Graph:1).

The studies on fungal diversity and percentile contribution and periodic occurrence of soil mycoflora are useful for farmers, agronomists, researchers and microbiologists for future activities in the view of conservation of soil ecosystem, conservation of soil microbial diversity and sustainable agriculture (Gaddeyya *et al.*, 2012).

CONCLUSION

In the present study the soil sample of six different paddy fields of viz; Sadak Arjuni, Wadegaon, Kohmara, Sawangi, Saundad and Tidka studied for detecting the fungal diversity. The greater fungal population was observed mostly in the monsoon season as the soil moisture was high. Among the isolates *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma* were dominant in all agricultural fields of all areas mentioned due to high sporulation and production of bacterial antibiotics from the

Penicillium species and production of different types of toxins from the *Aspergillus* species may prevent the growth of other fungal species. This study is an effort to understand the soil microbial diversity in the agricultural fields of Sadak Arjuni as soil microflora not only plays an important role in decomposition and contribute to biogeochemical cycling but also are responsible for the prevalence of diseases in the crop fields.

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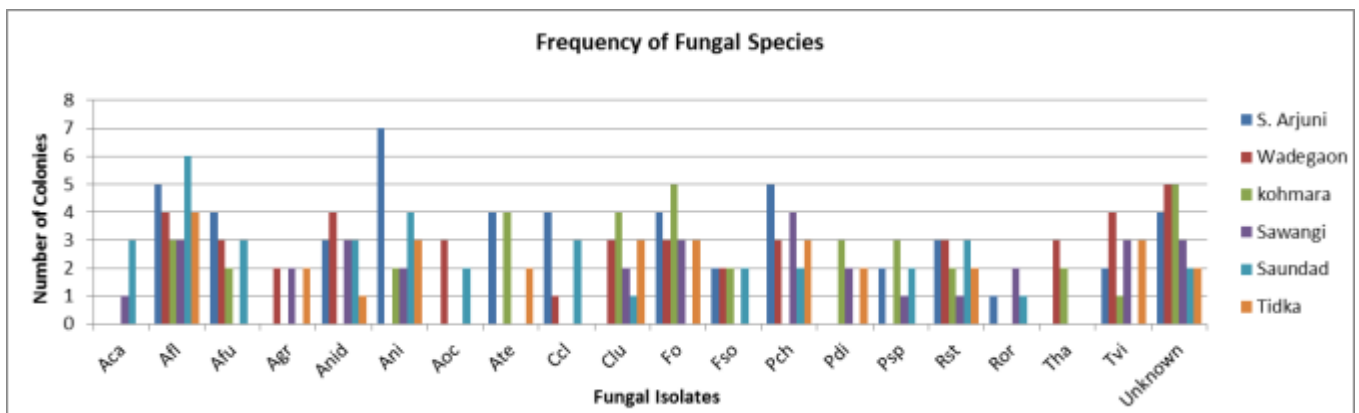
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Sample No.	Agricultural field	Place
1	Paddy	SadakArjuni
2	Paddy	Wadegaon
3	Paddy	Kohmara
4	Paddy	Sawangi
5	Paddy	Saundad
6	Paddy	Tidka

Table 1: Agricultural soil samples collected from different places in SadakArjuni Tehsil

Sr. No.	Rice Fields	Total No. of Colonies	Average Number of Individual Colonies																					
			Aspergillus								Curvularia		Fusarium		Penicillium		Phoma		Rhizopus		Trichoderma		Unknown	
			Aca	Afl	Afu	Agr	Anid	Ani	Aoc	Ate	Ccl	Clu	Fo	Fso	Pch	Pdi	Psp	Rst	Ror	Tha	Tvi			
1	S. Arjuni	50		5	4		3	7		4		4		4	2	5		2	3	1		2	4	
2	Wadegaon	43		4	3	2	4		3		1	3	3	2	3				3			3	4	5
3	Kohmara	38		3	2			2		4		4	5	2		3	3	2			2	1		5
4	Sawangi	32	1	3		2	3	2			2	3		4	2	1	1	2				3		3
5	Saundad	37	3	6	3		3	4	2		3	1		2	2		2	3	1					2
6	Tidka	30		4		2	1	3		2		3	3		3	2		2				3		2
Total		230	4	25	12	6	14	18	5	10	8	13	18	8	17	7	8	14	4	5	13			21
% Contribution			1.7	10.8	5.2	2.6	6.08	7.8	2.1	4.3	3.4	5.6	7.8	3.4	7.3	3.04	3.4	6.08	1.7	2.1	5.6			9.1

Table 2: Frequency of Mycoflora in different Agricultural Fields as on Potato Dextrose Agar Medium

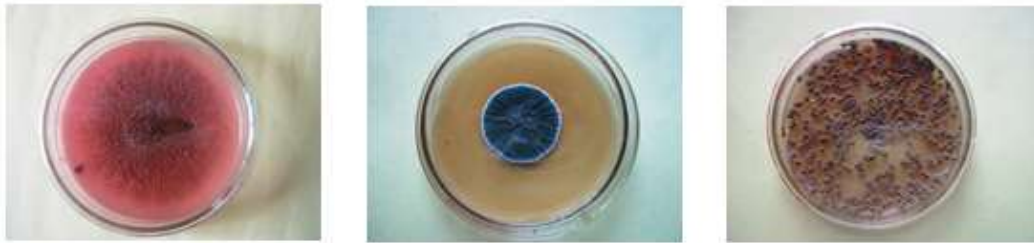


Graph 1: Frequency of Fungal species in different crop fields on PDA

Aca:-*Aspergillus candidus*, Afl:-*A. flavus*, Afu:-*A. fumigatus*, Agr:-*A. granulosis*, Anid:-*A. nidulans*, Ani:-*A. niger*, Ate:-*A. terreus*, Afu:-*Curvularia clavata*, Afu:-*C. lunata*, Afu:-*C. lunata*, Afu:-*Fusarium oxysporium*, Afu:-*F. solani*, Afu:-*Penicillium Chrysogenum*, Pch:-*P. chrysogenum*, Pdi:-*P. digitatum*, Rst:-*Rhizopus stolonifer*, Ror:-*R. oryzae*, Tha:-*Trichoderma hamatum*, Tvi:-*T. viride*



Fungi Isolated by Soil Dilution Method



Pure Culture of Some Fungal species

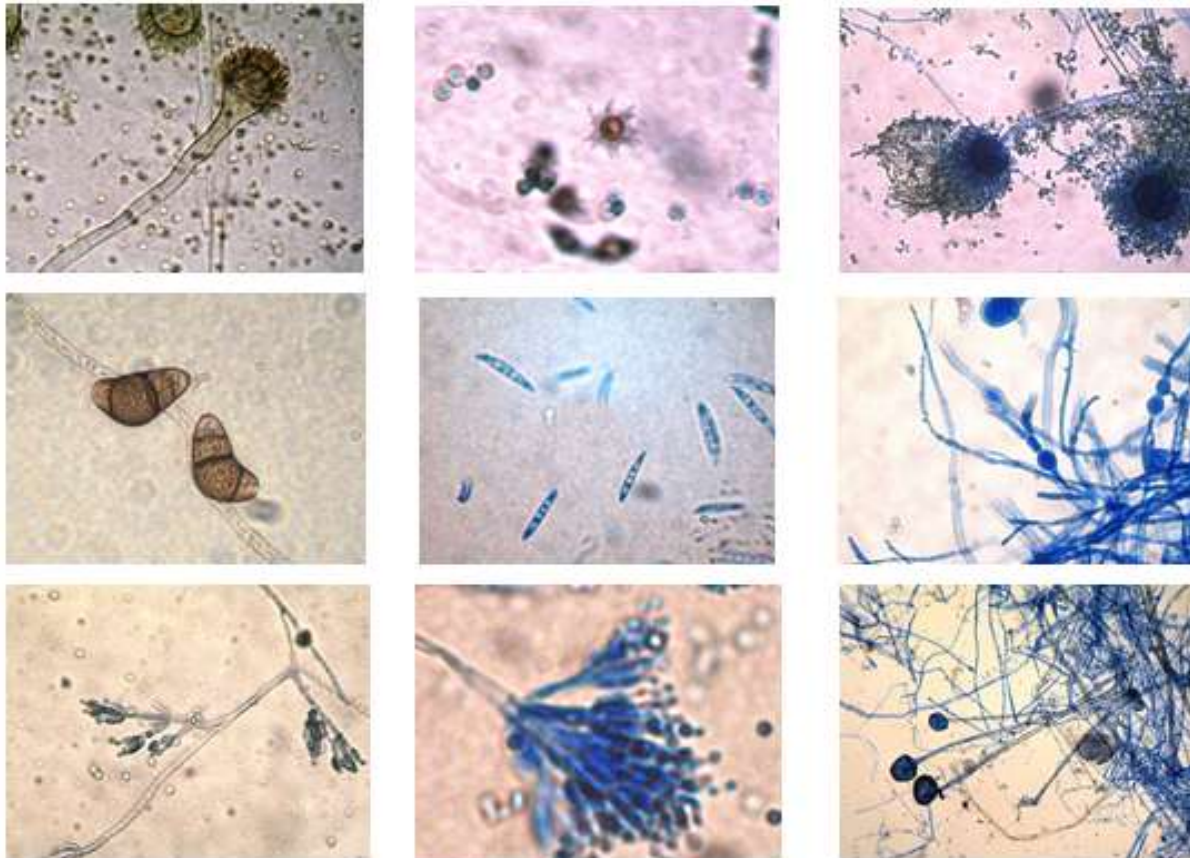


Fig.4: Some of the isolated fungal species