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RESEARCH ARTICLE

STUDIES ON SOIL MYCOFLORA IN DIFFERENT AGRICULTURAL FIELD OF BULDHANA DISTRICT(MS).

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Abstract

Total of six Rhizosphere soil samples of different agricultural fields form Buldhana district were investigated for diversity of fungi. The mycoflora were isolated by using soil dilution Plate technique on Potato Dextrose agar medium supplemented with 1% Streptomycin. Identification and characterization of fungi with physicochemical parameters were done. A total of 13 species belonging to 5 genera of fungi were isolated and their percent contribution were studied.The most common among them viz; *Aspergillus and Penicillium* were predominant genera. *Rhizopus, mucor and Trichoderma* was isolated and characterized.

Introduction:-

Soil is the major component of earth's ecosystem which comprises of organic matter, minerals, gases and large numbers of macro and microorganisms. The soil ecosystem is supported by several interactions among its physical, chemical and biological components (Buscot 2005).Soils are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms (Chiang *et al.*, 1994). Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Kiran Singh *et al.*,1999). 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 agricultural management practice found to have greater influence on the activity of soil microflora (Mc. Gill *et al.*, 1980). Fungi are fundamental for soil ecosystem functioning, 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 (Christensen, 1989). The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. The rhizosphere micro-organisms predominantly help in metabolizing the root exudates. Micro-organisms in the Rhizosphere complete both chemical and physical modifications to the soil profile in and around the rhizosphere that affect plants. They can be beneficial to the plant (by pathogen suppression) or detrimental (by competition for nutrients) (Sylvia *et al*., 2005). Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bishy, 1995). The role of fungi in the soil is an extremely complex one and it is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits. Microfungi play a focal role in nutrient cycling by regulating soil biological activity (Arunachalam *et al*., 1997). The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil.

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The members and kinds of microorganisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, aeration, pH and temperature etc. The aim of the present investigation is to isolate mycoflora from different agricultural fields and to observe the percentage contribution of different fungal species, by soil dilution method and soil plate method.

Materials and Methods:-

Collection of soil samples:-

Rhizosphere soil samples were collected from different agricultural field of Buldhana district (S1, S2, S3, S4, S5, and S6) Table-1. In each field soil sample was collected from the surface area reaching about 10 – 15 cm depth and near the rhizosphere region of plants. Soils were collected in sterile polythene bags and sealed on the spot. Samples were stored in laboratory until further analysis.

Isolation of Mycoflora:-

Dilution plate technique described by Warcup (1955) was used for the isolation of fungi from various rhizosphere soil samples. 10 grams of soil samples were suspended in 90 ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil particles. The soil suspensions were diluted from 10^{-2} to 10^{-4}. The Volume of 10 ml of soil sample suspension from each serial dilution was pipetted onto different melted, cooled culture media Potato Dextrose Agar (PDA) supplemented with 1% Streptomycin. The pH of the culture media was maintained at 5.5 being optimal for the growth and sporulation in a fungi. Each culture media was prepared in distilled water and autoclaved at 120^{o}C at 15 psi for 20 min. 1% Streptomycin was used as an antibiotic to inhibit 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.

Pure Culture and identification:-

Purification of the fungi was made by single spore culture method. A portion of the growing edge of each colony was picked up with the help of a pair of needles and mounted on a clean slide with lactophenol cotton blue. The slide was gently heated over the flame so as to remove air bubbles. The excess stain was wiped off with the help of tissue paper and then the cover slip was sealed with grease. The slide was observed under microscope. Identification of the organisms were made with the help of Manual of soil fungi (Gillman, 2001).

Statistical analysis:-

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

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

(*CFU-Colony forming Unit)

Physicochemical analysis of soil samples:-

The collected soil samples ware dried aseptically at laboratory for characterization of physico-chemical properties. Physical and chemical parameters of soil such as pH, salinity, soil texture, organic carbon, nitrogen, phosphorus, potassium were analyzed. The physico-chemical parameters of the soil samples were analyzed.

Results and Discussion:-

Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. The saprophytic fungi represent the largest proportion of fungal species in soil and they perform a major role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses and lignin, thus contributing to the maintenance of global carbon cycle. In the present study 112 fungal colonies of 13 fungal species were isolated from different agricultural fields of Buldhana district. The maximum fungal species belonging to Ascomycets (96 colonies) and Zygomycetes (16 colonies) were observed. *Apergillus*, *Penicillium* and *Mucor species* were the dominant fungal species found among the isolates. (Table-2).

They are dependent on the nature of substrate and temporal region that favors the colonization, growth and substrate possession of the fungi (Rani *et al.*, 2010). The soil mycoflora in different agricultural fields viz; S1, S2, S3, S4, S5 and S6, were observed. The highest contribution among them Viz; *Aspergillus flavus* (28.6%), *Aspergillus sydowii* (33.33%) *Aspergillus niger* (25%), *Penicillum citrinum* (20.83%) and *Rhizopus stolanifer (*19.04%) were isolated and identified (Table-3). The frequency of mycoflora in different 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.(Vinay K. *et al.*,2015)

Table 1:- Collection of soil samples.

Soil Sample	District	Places
S1	Buldhana	Janori
S2		Sangawa
S3		Deulgaon mali
S4		Pimpri mali
S5		Misal wadi
S6		Shelgaon

Table 2:- Occurrence of soil mycoflora in different agricultural field.

District	Study area	Sample	Average No. of Individual Colonies													
			Aspergillus Species							*Penicillium Species*			*Rhizopus stolanifer*	*Mucor sp.*	*Trichoderma viridi*	Total no. of colonies
			A. flavus	*A. sydowi*	*A. niger*	*A. versicolor*	*A. candidus*	*A. sulphureus*	*A. terreus*	*P. chrysogenum*	*P. citrinum*	*P. notatum*				
Buldhana	Janori	S1	2	5	5	1	1	1	1	2	5	1	2	2	2	30
	Sangawa	S2	2	-	2	-	1	1	1	3	5	1	3	2	3	24
	Deulgaon mali	S3	1	3	3	-	-	2	1	1	-	-	4	2	4	21
	Pimpri mali	S4	4	1	1	2	1	2	2	-	-	1	-	-	-	14
	Misal wadi	S5	3	4	3	1	-	-	1	-	-	-	-	-	-	12
	Shelgaon	S6	1	2	2	-	2	2	1	-	-	1	-	-	-	11
Total			13	15	16	4	5	8	7	6	10	4	9	6	9	112
% contribution			11.6	13.4	14.3	3.5	4.5	7.14	6.25	5.35	8.92	3.57	8.03	5.35	8.03	

Table 3:- Percent contribution of fungal species in different agricultural field.

Sr,no.	Fungal isolates	% contribution					
		S1	**S2**	**S3**	**S4**	**S5**	**S6**
1	*Aspergillus flavus*	6.7	8.33	4.8	28.6	25	9.1
2	*Aspergillus sydowii*	16.7	---	14.3	7.14	33.33	18.2
3	*Aspergillus niger*	16.7	8.33	14.28	7.14	25	18.2
4	*Aspergilllus versicolor*	3.33	---	---	14.3	8.33	---
5	*Aspergillus candidus*	3.33	4.2	---	7.14	---	18.2
6	*Aspergillus sulphureus*	3.33	4.2	9.52	14.3	---	18.2
7	*Aspegillus terreus*	3.33	4.2	4.8	14.3	8.33	9.1
8	*Penicillum chrysogenum*	6.7	12.5	4.8	---	---	---
9	*Penicillum citrinum*	16.7	20.83	---	---	---	---
10	*Penicillium notatum*	3.33	4.2	---	7.14	---	9.1
11	*Rhizopus stolanifer*	6.7	12.5	19.04	---	---	---
12	*Mucor sp.*	6.7	8.33	9.52	---	---	---
13	*Trichoderma viridi*	6.7	12.5	19.04	---	---	---

Fig 1:- Percent contribution of fungal species in different agricultural field

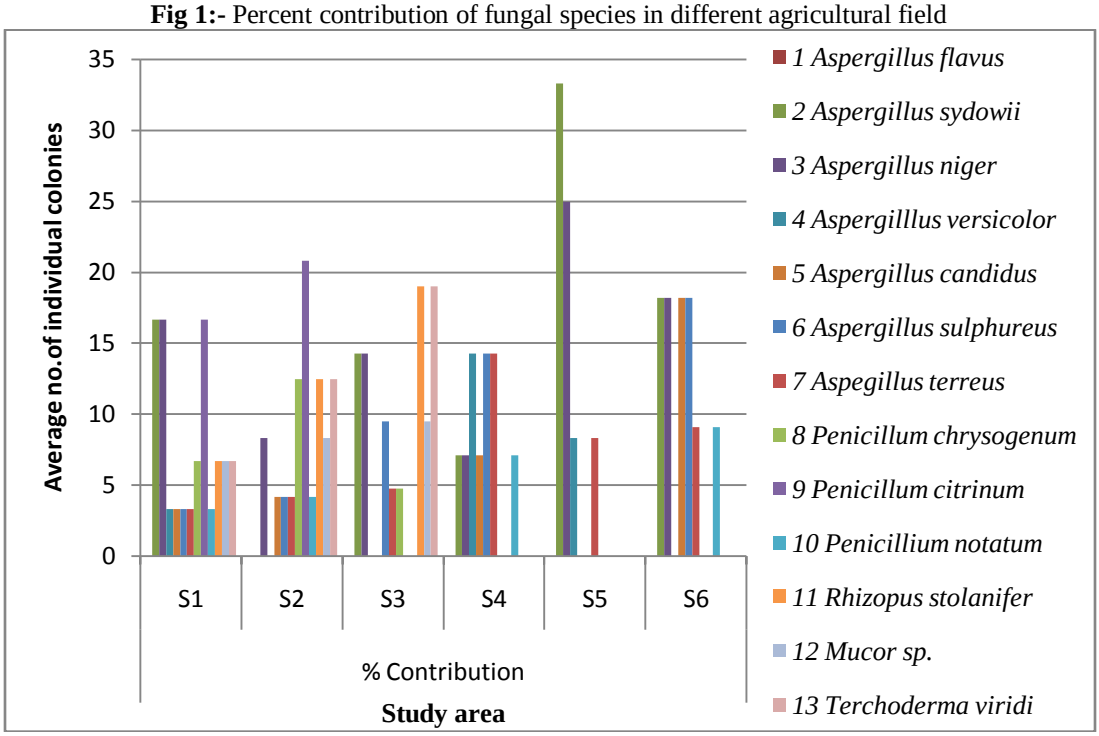
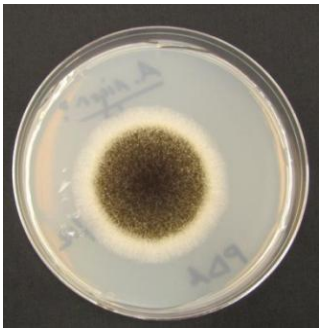
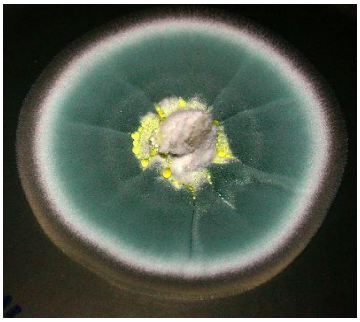


Fig 2:- Isolates of soil mycoflora in different agricultural field.



Aspergillus niger

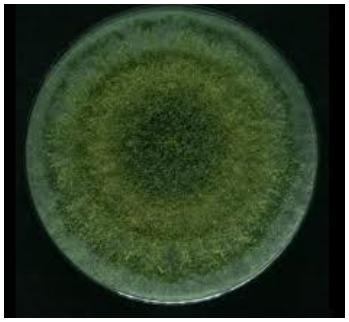
Aspergillus flavus



Penicillum chrysogenum

Penicillum notatum

Rhizopus stolanifer



Trichoderma viridi

Table 4:- Physicochemical parameter of soil samples from different agricultural fields.

Sr.no.	Test	Soil samples					
		S1	S2	S3	S4	S5	S6
1	pH	7.6	7.4	7.9	7.4	7.5	7.4
2	Soil salinity	0.83	0.20	0.87	0.68	0.73	0.59
3	Soil colour	Grey	Black soil	Black soil	Grey	Black soil	Black soil
4	Soil texture	Sandy clay	Sandy clay	Sandy clay	Sandy clay	Sandy clay	Sandy clay
5	Organic carbon%	0.69	0.86	0.32	0.08	0.81	0.62
6	Nitrogen(kg/h)	78.4	85.6	94.2	54.6	43.2	37.7
7	Phosphorus(kg/h)	14.4	16.4	12.5	14.6	16.5	12.4
8	Pottasium(kg/h)	334	423	255	136	225	347

The soil pH, organic content and water are the main factors affecting the fungal population and diversity (Zhang *et al*., 2001). 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. In the present study six different agricultural field soil samples of three districts were studied for screening and detected of fungal diversity. *Aspergillus, Penicillium* and *Mucor* species were dominant in all agricultural fields due to the high sporulation capacity and the *Penicillium* sp. were producing fungal and bacterial antibiotics and the *Aspergillus sp.* producing different kinds of toxins. 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, pH, soil salinity, soil texture, organic carbon and inorganic materials.

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