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Studies on physicochemical and fungal diversity from rhizosphere soils of different Chilli Field

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Abstract
Rhizosphere soil samples were collected from different chilli field of various Districts, Tamil Nadu. A soil dilution plate method was used for isolation of fungi on Potato Dextrose Agar (PDA) media. Identification and characterization was done with the help of authentic manuals of fungi. Soil samples were also characterized for physicochemical properties such as pH, moisture content, N, P, K, Organic Carbon and Sulphur. Overall, the microbial and physicochemical indicators showed that the chilli field soil needs to be supplemented with soil nutrients. A total 15 species belonging to 10 genera were isolated and identified which are parasitic, saprophytic and free-living fungi including pathogenic species. *Alternaria, Aspergillus, Penicillium, Rhizoctonia* and *Fusarium* were predominant genera. *Curvularia, Mucor species, Rhizopus, Pythium and Phytophthora* were the most frequently isolated genera. Otherwise, *Rhizoctonia solani* may cause damping off disease and reduce the production of chilli significantly.

Keywords: physico-chemical, rhizosphere soil, parasitic, pathogenic species, damping off

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) [1]. 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 complementary medium for biological reactions and life support in the soil environment (Olson *et.al.*, 2000) [2]. The physicochemical study of parameters is important to agricultural chemists for plants growth and soil management (Kanimozhi and Paneerselvam, 2011) [3]. 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 (Ainsworth and Bisby, 1995) [4]. The pathogenic micro-fungal floras of field soils cause root rots, seedling damping-off and vascular wilts diseases in plants. Soil borne fungal pathogens infect number of plant. The most important genera include *Alternaria, Armillaria, Aspergillus, Chaetomium, Cylindrocladium, Fusarium, Geotrichum, Penicillium, Phyto phthora, Pythium, Rhizoctonia* and *Sclerotinia* (Azaz, 2003) [5]. The species of *Armillaria, Cylindrocladium, Phytophthora, Pythium, Rhizoctonia* cause root rot diseases characterized by decay of true root system. Stem, collar and head rots are caused by species of *Fusarium, Rhizoctonia, Sclerotina, Sclerotium, Phytophthora* and occasionally *Aspergillus niger*, all produce symptoms of wilting and death of leaves as well as whole plant. Species of *Fusarium, Pythium, Phytophthora, Rhizoctonia* and *Sclerotium* fungi affect the seeds during germination, pre-emergence or post-emergence phases of seedling establishment. The aim of present investigation has made to isolate, characterize and enumerate the fungal strains from the rhizosphere soil samples of different Chilli field to observe the occurrence of soil fungi.

Materials and Methods
Collection of soil samples
Rhizosphere soil samples were collected from different chilli field of various Districts in Tamil Nadu (S1, S2, S3, S4, S5, S6, S7 and S8). In each field 1kg of soil sample was collected from the surface area reaching about 10 – 15 cm depth and near the rhizosphere region of plants. Soil were collected in sterile polythene bags and sealed on the spot. Samples were stored in laboratory at 4^0C until further analysis.

Isolation of Mycoflora
Dilution plate technique described by Warcup (1955) [6] 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, 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^{-2} to 10^{-4}. The volume of 1 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 majority of fungi. Each culture media was prepared in a liter of distilled water and autoclaved at 120ºC at 15 psi for 20 min. 1% Streptomycin was used as an antibiotic for the inhibition 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 fungus

The fungal species were identified and characterized based on their morphological characters and microscopic observation by using standard procedures and relevant literature (Gilman, 2001 and Nagamani *et.al*, 2006) [7, 8].

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 in aseptically at laboratory for characterization of physico-chemical properties. The physico-chemical parameters of the soil samples were analyzed at Dr. M.S. Swaminathan Research Foundation, Thiruvaiyaru, Thanjavur District, Tamil Nadu.

Results and discussion

The physiochemical properties of soil used for isolation of microbial species were analyzed in the present study. The color of soil samples was brown to black, with variation in pH (7.82 - 8.65). The temperature of the soil was high (30.2–33.2ºC) with great variation in percent moisture content (0.35 – 0.95), organic carbon (0.2568 – 0.4125) and percent organic nitrogen (0.2213 – 0.3555). The results were shown in table 1.

Soil properties like the soil pH, organic matter and moisture content are the main factors affecting the fungal population and diversity (Yu *et.al.,* 2007, Dong *et.al.,* 2004, Zhang *et.al.,* 2001 and Jha *et.al.,* 1992) [9, 10, 11, 12]. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. The moisture content in soil acts as solvent and is essential for microbial functioning. A certain minimum level of organic matter and moisture content is essential to ensure the presence of an active microbial population in the soil. In the present study, the important physicochemical properties of the soils, used for the evaluation of natural fungal density were determined.

In the present study 182 fungal colonies of 10 fungal species were isolated from different chilli fields of various Districts in Tamil Nadu. (Table 2). Percentage contribution of fungal species in chilli field of different districts were calculated (Table 3). The maximum fungal species belonging to Deuteromycotina (155 colonies) and Zygomycotina (27 colonies) were observed (Figure-1). *Apergillus*, *Penicillium* and *Mucor* species were the dominant fungal species found among the isolates. They are dependent on the nature of substrate and temporal region that favours the colonization, growth and substrate possession of the fungi (Rani and Paneerselvam, 2010) [13]. The percentage contribution of each fungal species in different fields was statistically analyzed (Table 3). *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Mucor species* were dominant in all agricultural fields, due to high sporulation capacity. Graphical representation of percent contribution of fungal species in various crop fields was showed in Figure-2.

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.

Table 1: Physico-chemical parameters of soil samples

S. No.	Samples	S1	S2	S3	S4	S5	S6	S7	S8
1	pH	7.0	7.9	6.3	7.3	7.3	7.5	6.8	7.3
2	Soil Salinity	0.45	0.73	0.39	0.40	0.83	0.20	0.77	0.81
3	Soil Colour	Red Soil	Gray	Black Soil	Red Soil	Gray	Gray	Red Soil	Gray
4	Soil texture	Sandy-loam	Sandy-clay	Sandy-clay	Sandy	Sandy-clay	Sandy	Sandy-clay	Sandy
5	Organic Carbon (OC%)	0.71	0.59	0.81	0.96	0.68	0.08	0.39	0.18
6	Nitrogen (kg/h)	78.8	67.2	85.2	94.6	75.6	37.2	56.4	43.6
7	Phosphorus (Kg/h)	14.2	16.2	12.2	16.2	14.2	10.2	16.2	6.2
8	Potash (Kg/h)	275	353	136	255	443	97.5	225	97.5

Table 2: Occurrence of soil mycoflora in different chilli field from various Districts of Tamil Nadu

Districts	Study Area	*A. fl*	*A. co*	*A. fu*	*A. ni*	*A. sy*	*A. te*	*A. ve*	*A. sul*	*A. ca*	*Rh. so*	*P. ch*	*P. ci*	*P. fre*	*P. no*	*P. ru*	*C. lu*	*R. st*	*M. sp*	*T. vi*	Total no. of colonies
Ariyalur	S1	1	-	4	2	2	1	-	1	-	1	-	2	1	3	1	-	1	1	-	21
	S2	3	-	-	3	1	2	-	1	-	1	1	2	1	2	-	1	2	1	1	22
Pudukkottai	S3	2	-	1	3	2	1	1	1	-	-	2	1	1	4	-	2	2	-	1	24
	S4	1	1	-	2	1	2	-	-	-	1	2	1	2	-	1	2	4	1	2	23
Thanjavur	S5	2	1	1	4	1	2	-	1	1	2	2	-	2	1	-	1	2	-	1	24
	S6	2	-	2	2	2	4	1	1	-	-	2	3	-	2	-	-	1	1	-	23
Thiruvarur	S7	2	1	2	4	1	3	1	1	-	2	1	-	-	3	2	1	1	-	2	27
	S8	1	1	-	2	2	2	1	1	-	2	-	2	-	2	1	1	3	4	-	25
Total		14	4	10	22	12	17	4	7	1	9	10	11	7	17	5	8	16	8	7	189
% contribution		7.4	2.1	5.2	11.6	6.3	8.9	2.1	3.7	0.5	4.7	5.2	5.8	3.7	8.9	2.6	4.2	8.4	4.2	3.7	

Table 3: % contribution of fungal species in chilli field of different districts

S. No.	Fungal species obtained	% Contribution							
		S1	S2	S3	S4	S5	S6	S7	S8
1	*Aspergillus flavus*	4.7	13.6	8.3	4.3	8.3	8.6	7.4	4.0
2	*Aspergillus conicus*	-	-	-	4.3	4.1	-	3.7	4.0
3	*Aspergillus fumigates*	19	-	4.1	-	4.1	8.6	7.4	-
4	*Aspergillus niger*	9.5	13.6	12.5	8.6	16.6	8.6	14.8	8.0
5	*Aspergillus sydowii*	9.5	4.5	8.3	4.3	4.3	8.6	3.7	8.0
6	*Aspergillus terreus*	4.7	9.0	4.1	8.6	8.6	17.3	11.1	8.0
7	*Aspergillus versicolor*	-	-	4.1	-	-	4.3	3.7	4.0
8	*Aspergillus sulphureus*	4.7	4.5	4.1	-	4.3	4.3	3.7	4.0
9	*Aspergillus candidus*	-	-	-	-	4.3	-	-	-
10	*Rhizoctonia solani*	4.7	4.5	-	4.3	8.6	-	7.4	8.0
11	*Penicillium chrysogenum*	-	4.5	8.3	8.6	8.6	8.6	3.7	-
12	*Penicillium citrinum*	9.5	9.0	4.1	4.3	-	13.0	-	8
13	*Penicillium frequentens*	4.7	4.5	4.1	8.6	8.6	-	-	-
14	*Penicillium notatum*	14.2	9.0	16.6	-	4.3	8.6	11.1	8.0
15	*Penicillium rubrum*	4.7	-	-	4.3	-	-	7.4	4.0
16	*Curvularia lunata*	-	4.5	8.3	8.6	4.3	-	3.7	4.0
17	*Rhizopus stolonifer*	4.7	9.0	8.3	17.3	8.6	4.3	3.7	12.0
18	*Mucor sp.*	4.7	4.5	-	4.3	-	4.3	-	16.0
19	*Trichoderma viride*	-	4.5	4.1	8.6	4.3	-	7.4	-

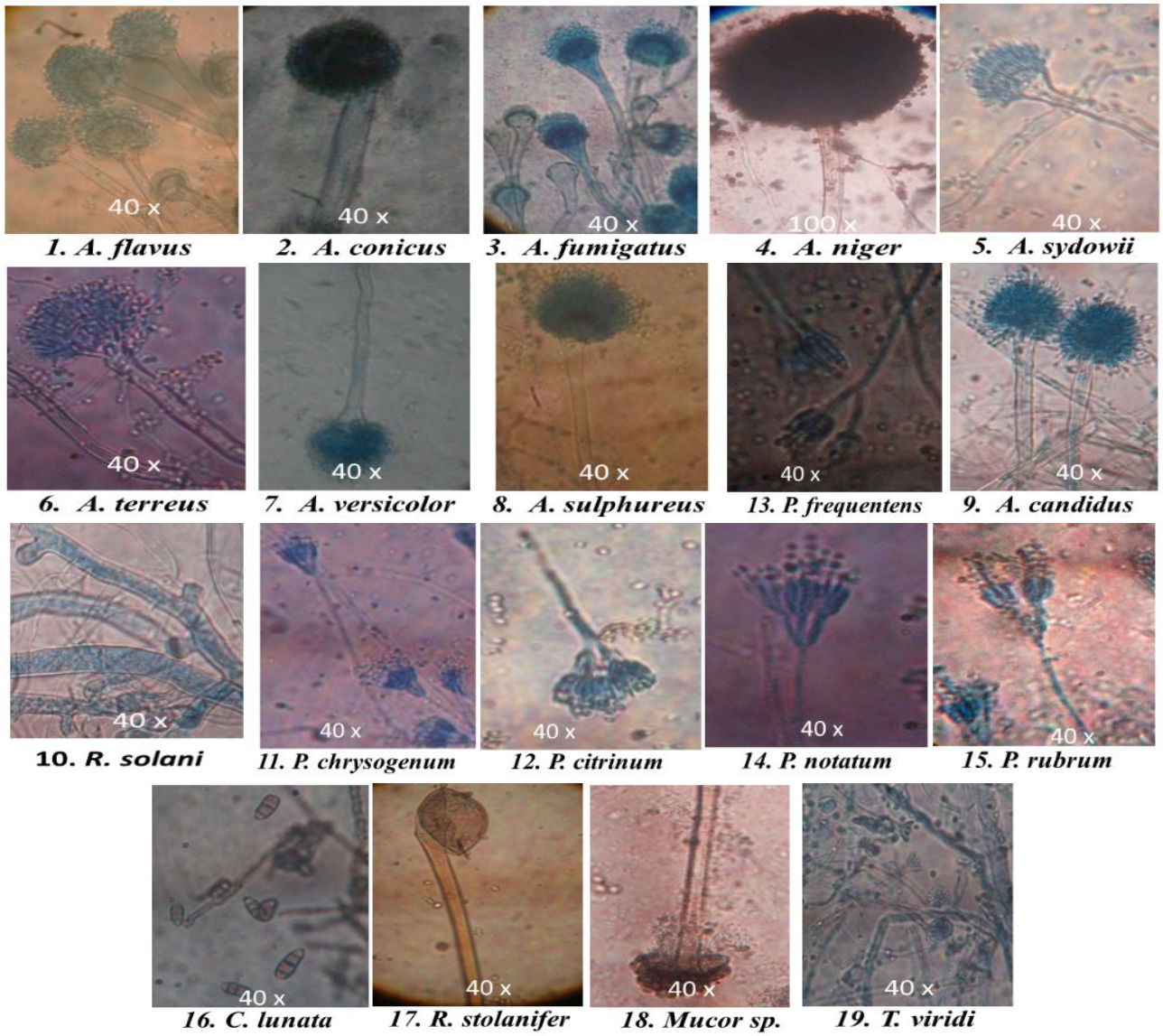

Fig 1: Microscopic observation of some soil fungi isolated from chilli field

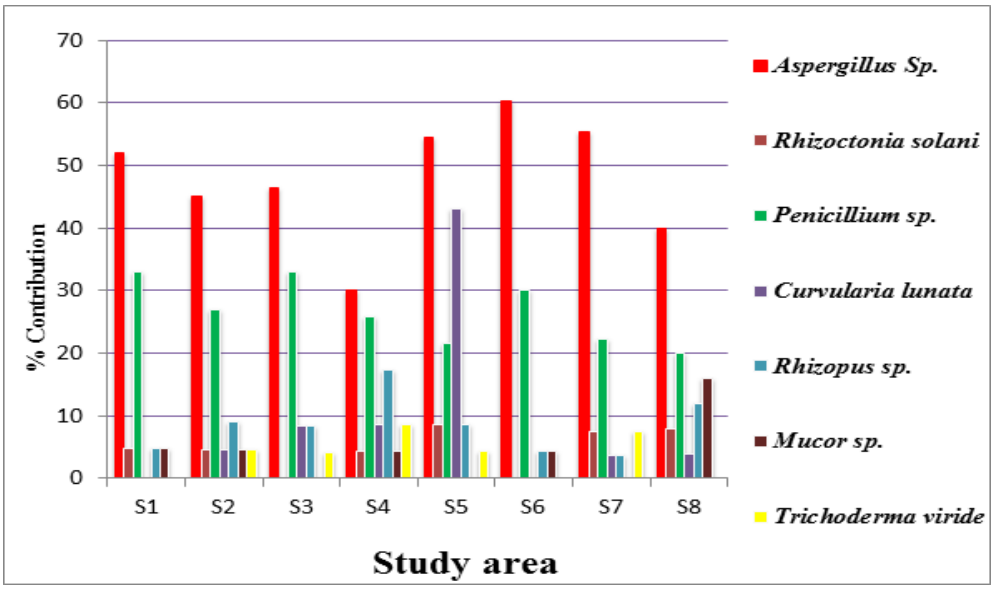

Fig 2: % Contribution of fungal species in different chilli field

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