

Seasonal Distribution of Fungi and Physiochemical Properties of Soil in Different Agricultural Fields of Tiruvarur District, Rice Bowl of Tamil Nadu

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Abstract

Rice plants have direct contact with soil via their roots. Root-dwelling microbes in soil associated with rice live in three various regions that include the endosphere (inside the root), rhizoplane (root surface), and rhizosphere (soil surrounding the root surface), which play crucial roles in plant health and productivity. There is only limited information about the composition of the microbiome during different seasons of rice cultivation. Therefore, the goal of our study was to analyze, compare, and correlate the fungal population observed during different seasons across various places in Tiruvarur district along with their physiochemical parameters. This study addressed 33 species that belong to four major phyla, six classes, five orders, eight families, and 12 genera. In the tiruvarur rice field, fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Rhizopus*, *Verticillium*, *Helminthosporium*, *Microsporum*, *Sclerotium*, *Alternaria*, *Curvularia* and *Choanephora* were found. The soil physiochemical characteristics like pH, electrical conductivity, organic carbon, available nitrogen, phosphorus, potassium, zinc, copper, iron, and manganese were studied in five places in the Tiruvarur rice field. The current study found that macronutrients (N, P, and K) and micronutrients (Zn, Cu, Fe, and Mn) were more abundant during the monsoon and post monsoon seasons. Moreover, the Pearson correlation coefficient was performed on the obtained databases, and the level of significance was seen at $P < 0.05$. The results of the present study demonstrate that a unique group of fungi inhabit the rice soil during different seasons, which may help to improve crop health and productivity.

Key words: Rhizosphere, Rice field, Microbiome, Physiochemical Parameters, Fungal population, Monsoon

Rice is a major cereal food crop and feeds over 50% of the world population [1]. Rice consumption is very high in developing countries and nations in Asia [2]. Rice is an annual monocot grass belongs to the family Poaceae. It contains abundant carbohydrates and lot of vitamins, minerals and polyphenols. Among the twenty-three species of rice, only two major species are cultivated extensively. They are *Oryza sativa* or the Asian rice and *Oryza glaberrima* or the African rice. *Oryza sativa* is cultivated worldwide, whereas *Oryza glaberrima* cultivation is confined to Africa only. There are two main subspecies of *Oryza sativa*: the indica which has long grains, and the Japonica, which has round grains. Indica rice varieties are grown widely in Asian continent whereas Japonica rice is mainly grown and consumed in Australia, China, Taiwan, Korea, Europe, Japan, Russia, Turkey and the United States of America [3].

Rice is the important staple food in India, which provides roughly 60% of daily energy needs or 41% of total food grain production from 35% of the country's food grain area, making it essential for national food security. India is the second largest rice producer and the largest exporter of rice worldwide. Rice Production in India expanded from 53.6 million tons in FY 1980 to 120 million tons in FY 2020-21. The area for rice cultivation in India comprises about 43,388,000 hectares of land and rice contributes to 689 kcal/capita/day of the food supply in India [4-5]. In India rice is grown under widely varying conditions of altitude and climate. Rice cultivation in India extends from 8 to 35 °N latitude and from sea level to as high as 3000 meters. Rice crop needs a hot and humid climate. It is best suited to regions which have high humidity, prolonged sunshine and an assured supply of water. The average temperature required throughout the life period of the crop

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ranges from 21 to 37 °C. Maximum temp which the crop can tolerate 40 °C to 42 °C.

Soil microbiota are important in suppressing soil-borne plant pathogens and improving the natural suppressiveness of soil [6-7]. Microbiota plays a key role in soil health-regulating dynamics of soil organic matter (SOM) and plant nutrient availability in agro ecosystems [8-9]. Among the soil microbiome, Fungi are very successful inhabitants of soil due to their high capacity to adopt various forms in response to unfavorable conditions [10]. Soil fungi can be classified into three functional groups including: (1) biological controllers, (2) ecosystem regulators, and (3) species participating in organic matter decomposition and compound transformations [6]. Many fungal species possess the ability to break down all kinds of organic matter, decomposing soil components and thereby regulating carbon and nutrients [12]. In addition, some fungi possess the ability to act as an effective absorbent of toxic metals such as copper, lead, mercury, cadmium and zinc by accumulating them in their fruit bodies [11]. The diversity and activity of fungi is regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) factors. This work reports and discusses the most reliable findings in relation to a comprehensive understanding of soil fungi pertaining to rice crop and their correlation with various physiochemical parameters.

MATERIALS AND METHODS

Study site and sample collection

Paddy fields of the Kaveri delta region Tiruvarur District, Tamilnadu, India well known to be the Rice bowl of Tamil Nadu (Fig 1). The district lies between 10° 16' and 11° 50' North latitude and 79° 27' and 79° 50' East longitude. The total area of the district is 2377 km². The district is bounded by Nagapattinam district on the east, Mayiladuthurai district on the north, Thanjavur district on the west; Palk Strait on the south. The district has a hot tropical climate with average temperature ranges from 26.39 °C to 35.19 °C.

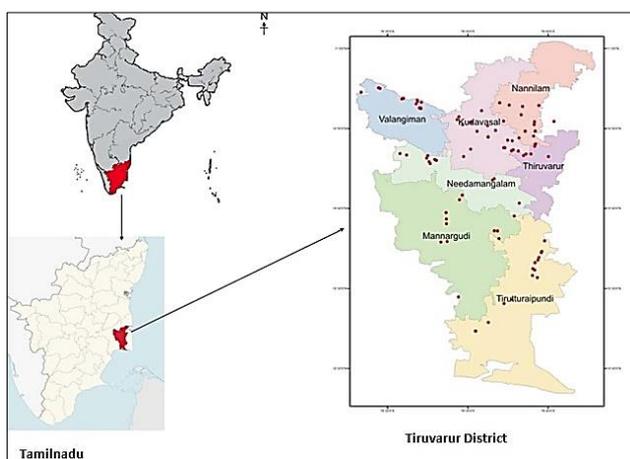


Fig 1 Geographical location of the study area

The soil samples were taken during Pre monsoon (Aug-Sep, 2021), Monsoon (October- December, 2021) and Post monsoon (Jan- Feb, 2022) periods in various rice fields of Tiruvarur district i. e., Thiruthuraiipoondi, Tiruvarur, Mannargudi, Needamangalam and Nannilam. Three subsamples of 200-250g soil were collected from around the root at a depth of 15cm to form a composite sample (n=45). The samples were placed in Ziplock bags in such a way it does not allow air to pass through them. Then, the soil samples were stored at -80°C until further analysis.

Physiochemical analysis

The soil samples were suspended in distilled water and the particles were allowed to settle down. The pH values, electrical conductivity, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed. Using a pH meter (Duralab, India), the suspension pH was determined. The electrical conductivity of the soil was measured using a conductivity meter in the water extract filtrate. The macro nutrients such as Nitrogen by Alkaline permanganate method [17], phosphorous by Olsen method [18], potassium (neutral normal ammonium acetate method), organic carbon by Walkley and Block method [19] and micro nutrients such as copper, iron, manganese and zinc were analyzed by DTPA extract method using atomic absorption spectrophotometer [16].

Isolation and identification of soil mycoflora

The soil micro fungi were enumerated by two methods, namely, Soil dilution and Soil plate method on potato dextrose agar media at pH 6.5. All the Petri dishes were incubated at room temperature 27 ± 3°C for a period of 4 – 7 days and then examined. The first set of observations were made at the end of two days to make sure that the fast-growing flocculent types, such as Rhizopus, Trichoderma, etc., had grown excessively to interfere with the growth of other species. When these had progressed to the point where they could be identified, a second observation was made. Finally, slow growing organisms must be subcultured in different media plates for further growth in order to avoid being overrun by more aggressive types. Nevertheless, pure culture plates of obtained mycobiota were cultured and stained by Lactophenol cotton blue for microscopic analysis. Identification of the fungal organisms was made by using Nikon optiphot microscope. The Fungal organisms were confirmed using standard manual of soil fungi [12], Dematiaceous Hyphomycetes [13], A manual of penicillia [14] and Compendium of soil fungi [15].

Statistical analysis

Using SPSS software, the Pearson correlation matrix was generated. For level of significance, correlation coefficients between physicochemical parameters and fungus populations were recorded.

RESULTS AND DISCUSSION

Taxonomic analysis of fungal community abundance on different rice field soils of Tiruvarur district

The pure culture plates and microphotography were shown in (Fig 2-3). The abundance of fungal communities was examined in different seasons, including monsoon, pre monsoon, and post monsoon, for all taxonomic levels among different areas of Tiruvarur district. The results were displayed as graphs (Fig 4-8).

Phyla and class analysis

A total of four fungal phyla were discovered in different seasons such as pre monsoon, monsoon, and post monsoon. Ascomycota (92.45%) is the most abundant phylum found in all five sites of Tiruvarur district. Zygomycota (4.5%), Basidiomycota (1.6%), and Deuteromycetes (1.3%) are the next three abundant phyla found in the rice field soil. A total of six fungal classes were observed in rice field soil. They are Dothideomycetes, Eurotiomycetes, Sordariomycetes, Agaricomycetes, Incertae sedis, and Mucoromycotina. Eurotiomycetes (74.5%) was the most abundant class, while Agaricomycetes (1.66%) was the least abundant in all seasons

of Tiruvarur rice field soil. Next to Eurotiomycetes, Sordariomycetes (12.4%) and Mucormycotina (4.54%) ranked

as the second and third most abundant classes in rice field soil, respectively.

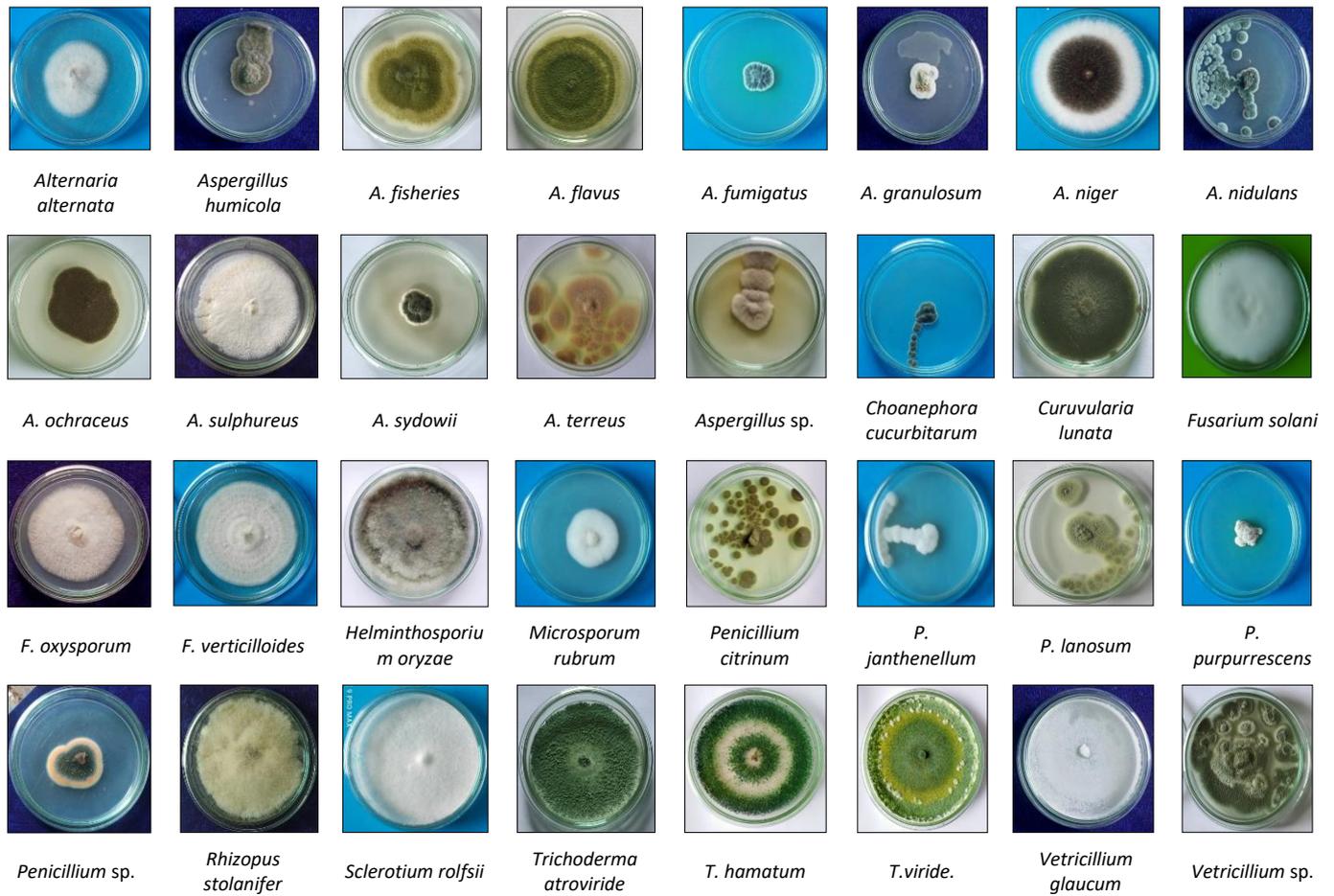
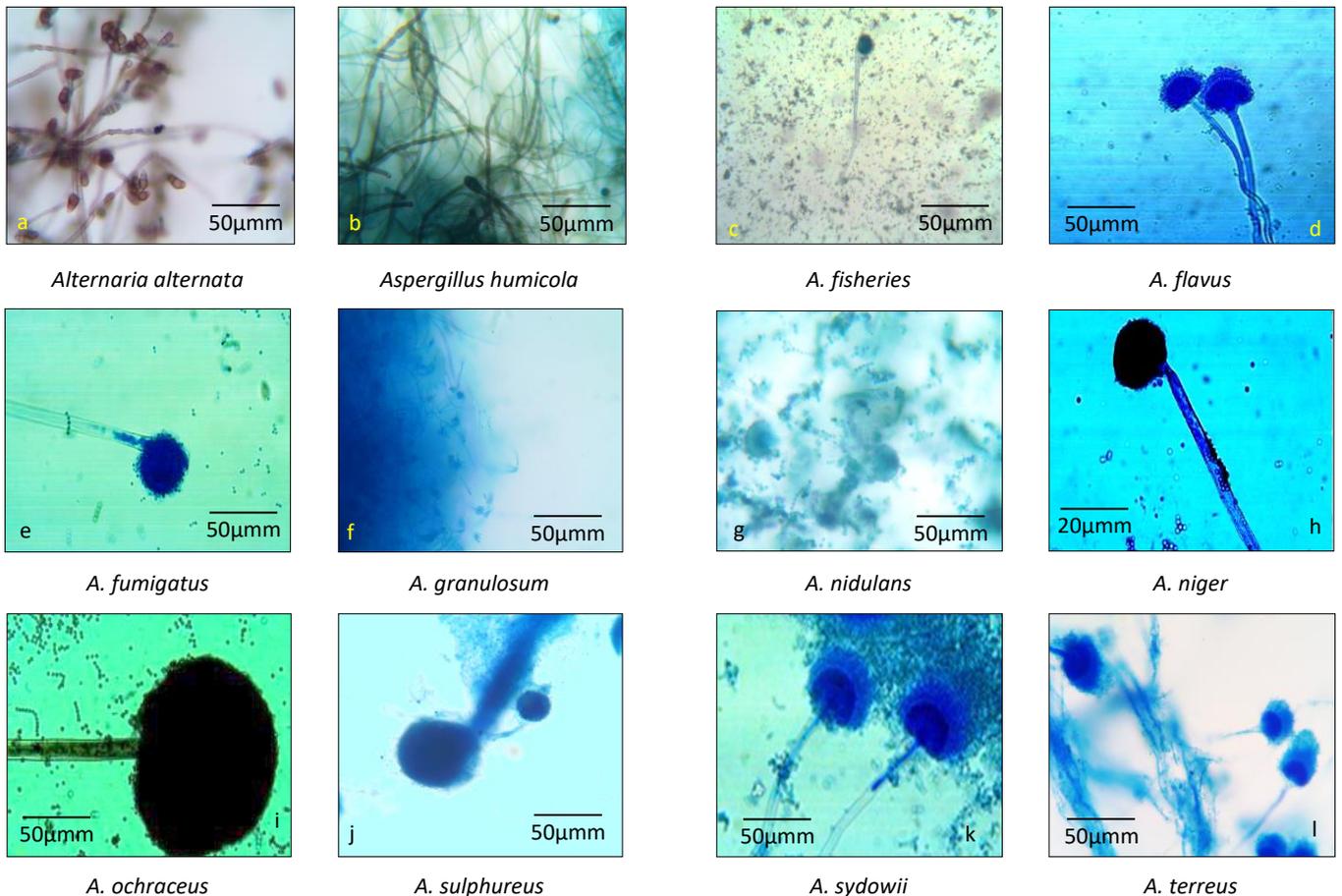


Fig 2 Pure plates of fungi isolated from paddy fields in different seasons



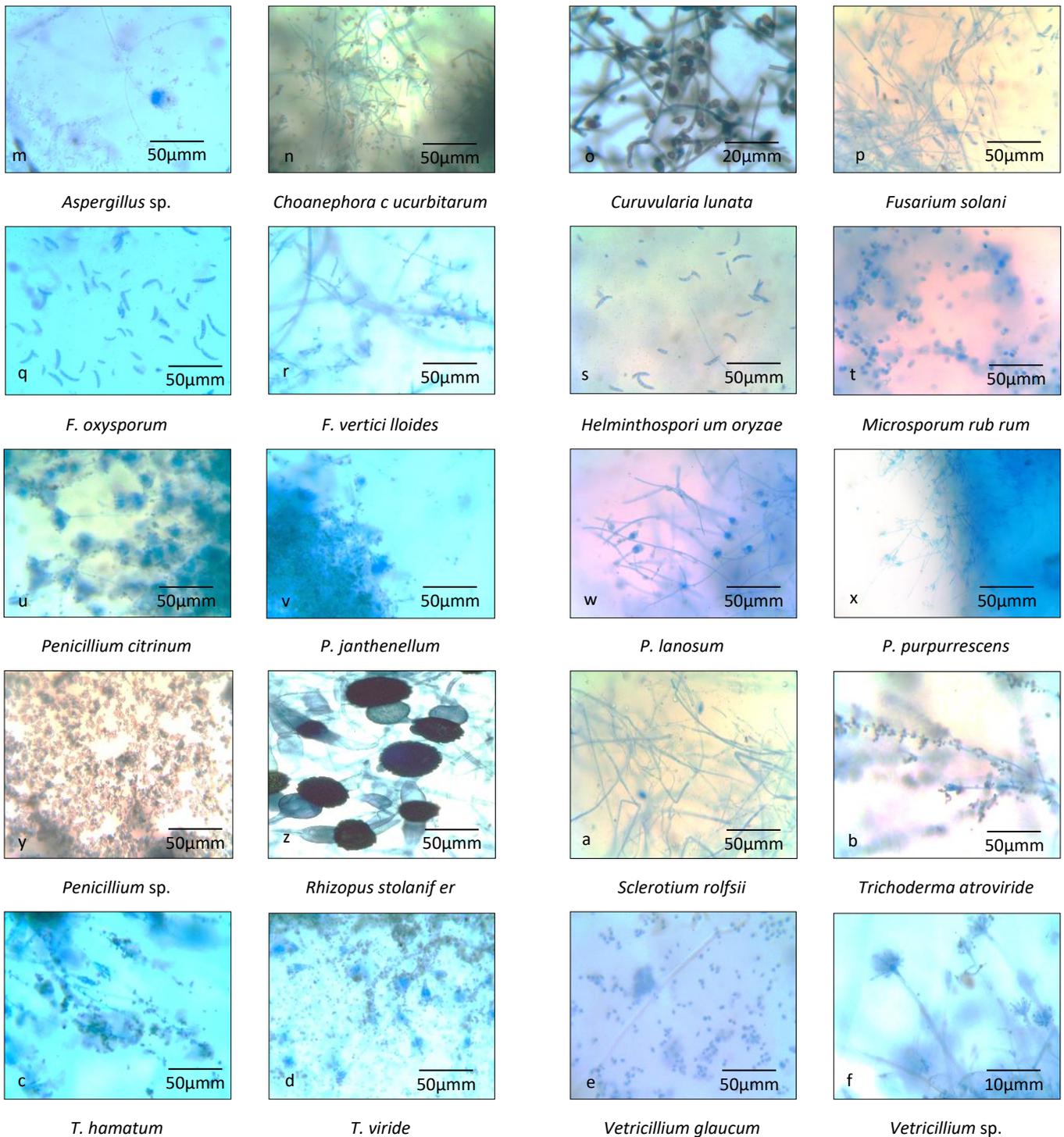


Fig 3 Microphotography of isolated fungi from paddy fields in different seasons

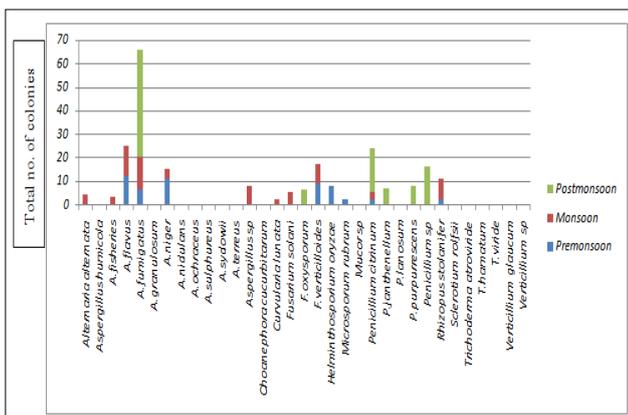


Fig 4 Mycoflora of paddy fields in Thiruthurai region of Tiruvarur district during different seasons

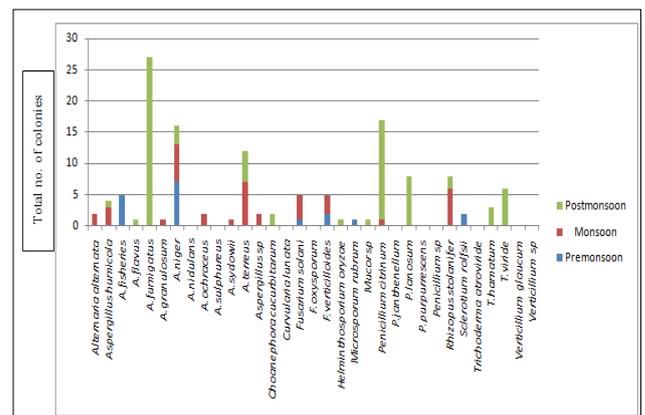


Fig 5 Mycoflora of paddy fields in Mannargudi region of Tiruvarur district during different seasons

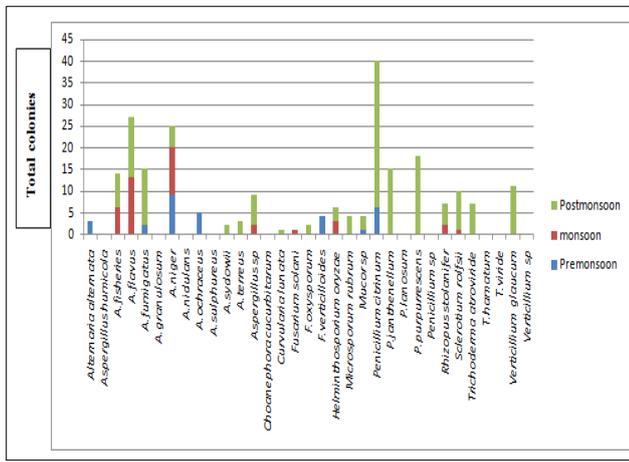


Fig 6 Mycoflora of paddy fields in Tiruvur region of Tiruvur district during different seasons

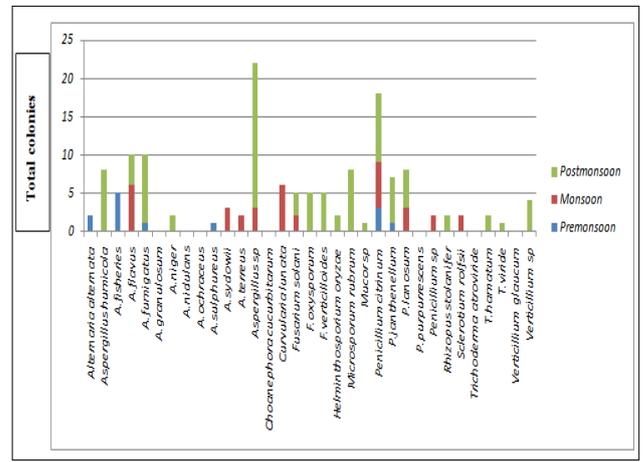


Fig 7 Mycoflora of paddy fields in Nannilam region of Tiruvur district during different seasons

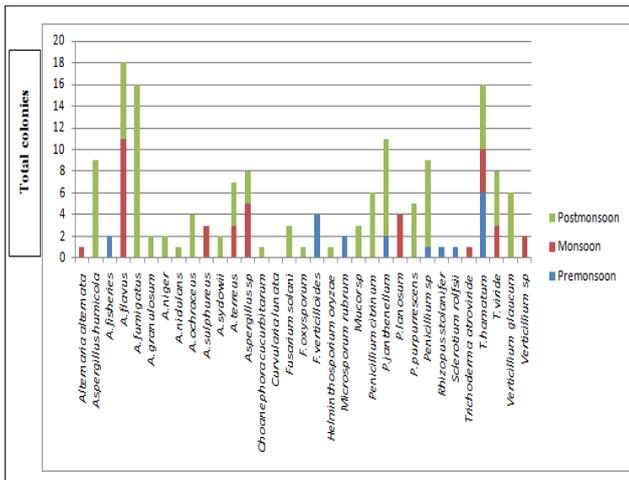


Fig 8 Mycoflora of paddy fields in Needamangalam region of Tiruvur district during different seasons

(1.66%), *Alternaria* (1.32%), *Curvularia* (0.9%), and *Choanephora* (0.33%).

Physiochemical parameters

The current study includes the observation of physiochemical factors with respect to the monsoon, pre monsoon, and postmonsoon seasons. Physiochemical parameters such as pH, electrical conductivity, organic carbon, nitrogen, phosphorus, potassium, iron, manganese, zinc, and copper are shown as graphs (Fig 9-18).

Physiochemical parameters

Almost all sites in Tiruvur, such as Tiruthuraiipoondi, Mannargudi, Tiruvur, Nannilam, and Needamangalam, show a pH value above 7. Among them, the Nannilam and Needamangalam sites possess a high pH value comparable to all other sites in Tiruvur district (Fig 9). Thiruthuraiipoondi and Nannilam sites had high electrical conductivity (0.8–1.2 dsm^{-1}), whereas the Tiruvur site had a low conductivity of 0.15 dsm^{-1} . In all seasons studied, Nannilam and Needamangalam sites have a higher percentage of organic carbon, while Mannargudi and Thiruthuraiipoondi sites have a lower percentage. The Nannilam location has the maximum mass of phosphorus during the monsoon season, whereas Mannargudi and Needamangalam have the lowest mass during the pre-monsoon season. Nannilam and Needamangalam sites have high potassium levels during the monsoon, but Mannargudi sites have lower levels during the pre-monsoon season. Needamangalam has a higher mass of nitrogen than Thiruthuraiipoondi, which has a lower mass of nitrogen throughout the seasons studied. During the monsoon and post monsoon seasons, the levels of iron and manganese are higher in the Nannilam rice field and lower in the Thiruthuraiipoondi location. In a similar way, copper and zinc concentrations are higher in the mannargudi rice field. Further the Pearson correlation coefficient of physiochemical parameters and population density of fungal Isolates are performed as shown in (Table 1) [20].

The bacterial community of rice soil microbiota was well studied already but only limited studies reported the fungal community present in rice soil microbiota. Especially, no reports were found in rice bowl of Tamil Nadu, Tiruvur. The distribution of mycoflora is greatly influenced by environmental parameters such as soil pH, moisture,

Order and family analysis

There are five orders found in tiruvur rice field soil observed in all seasons. They are Pleoporales, Eurotiales, Hypocreales, Mucorales and Agaricales. Among them, Eurotiales (73.9%) was the most abundant order whereas Agaricales (1.69%) was the least abundant in all seasons of Tiruvur rice field soil. Next to Eurotiales, Hypocreales (15.3%), Mucorales (4.6%) and Pleoporales (4.39%) ranked as the second, third and fourth most abundant orders in rice field soil, respectively. In total, eight different fungus families were found in the Tiruvur rice field during the year. Pleosporaceae, Trichomaceae, Choanephoraceae, Nectriaceae, Mucoraceae, Typhulaceae, Hypocreaceae, and Plectosphaerellaceae were the eight abundant families found in the rice fields of Tiruvur. Trichomaceae (74% of the total) was the most abundant family, while Choanephoraceae (0.33%) was the least abundant in all seasons of Tiruvur rice field soil. Nectriaceae was the second most abundant, and Pleosporaceae was the third most abundant family in rice field soil.

Genera analysis

There are about 12 genus-level taxa found in various sites of the Tiruvur rice field. Among the genera, *Aspergillus* (47%), *Penicillium* (24.6%), and *Fusarium* (7.5%) appear to be the most abundant during all seasons of rice growth. The other abundant genera are in descending order as follows: *Trichoderma* (4.8%), *Rhizopus* (4.2%), *Verticillium* (2.5%), *Helminthosporium* (2%), *Microsporium* (1.88%), *Sclerotium*

temperature, organic carbon, and nitrogen [24]. In this study, we have collected soil samples of rice and their fungal profile was recorded through classical culturing technique. Our studies showed the abundances of Phylum Ascomycota followed by Zygomycota, Basidiomycota and Deuteromycetes in rice soil

microbiota and are consistent with previous reports [21]. Ascomycota members play a key role as decomposers in agricultural soils, which increase after nitrogen fertilization [25]. Hence the agricultural fields under our study are enriched with nitrogen fertilizers.

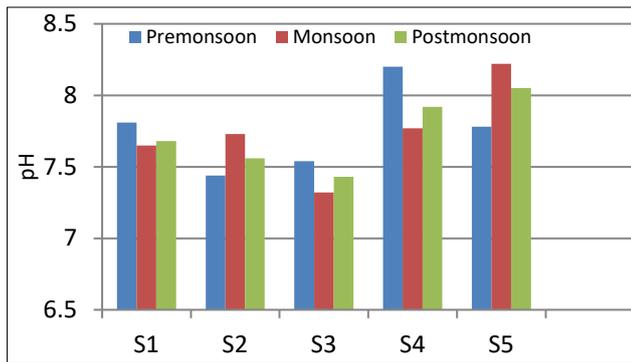


Fig 9 pH value of different regions of Tiruvur with respect to monsoon periods

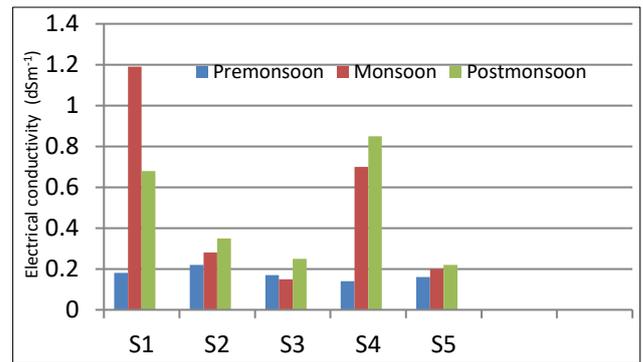


Fig 10 Electrical conductivity (dSm⁻¹) Value of different regions of Tiruvur with respect to monsoon periods

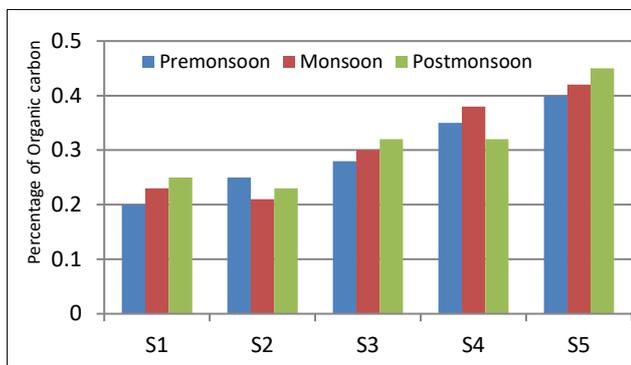


Fig 11 Percentage of organic carbon in soil during different seasons in Tiruvur district

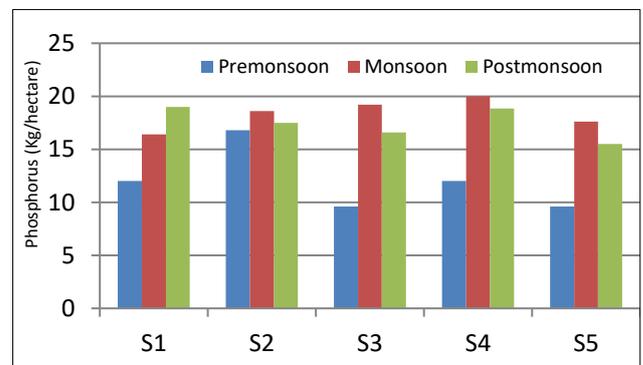


Fig 12 Phosphorus (Kg/hectare) in soil during different seasons in Tiruvur district

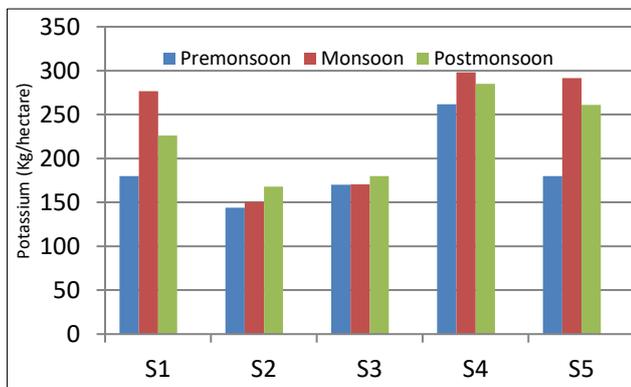


Fig 13 Potassium (Kg/hectare) in soil during different seasons in Tiruvur district

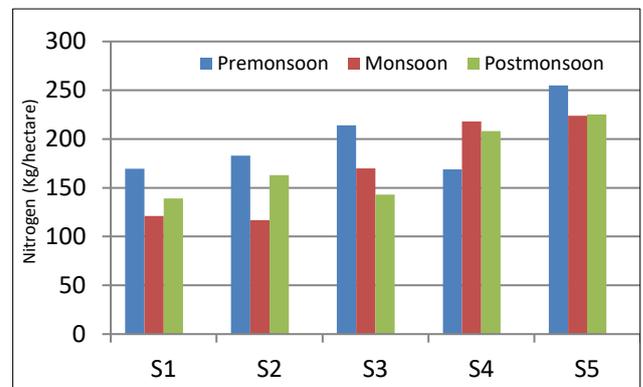


Fig 14 Nitrogen (Kg/hectare) in soil during different seasons in Tiruvur district

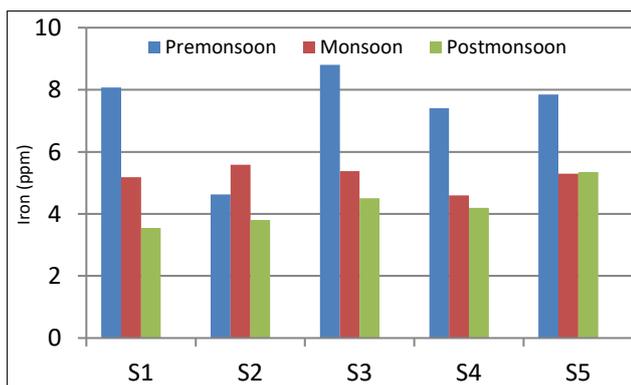


Fig 15 Iron (ppm) in soil during different seasons in Tiruvur district

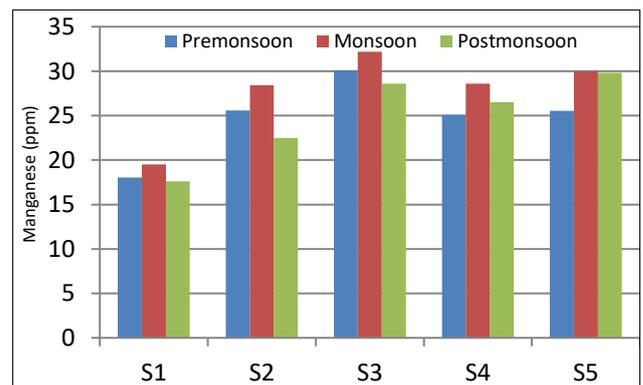


Fig 16 Manganese (ppm) in soil during different seasons in Tiruvur district

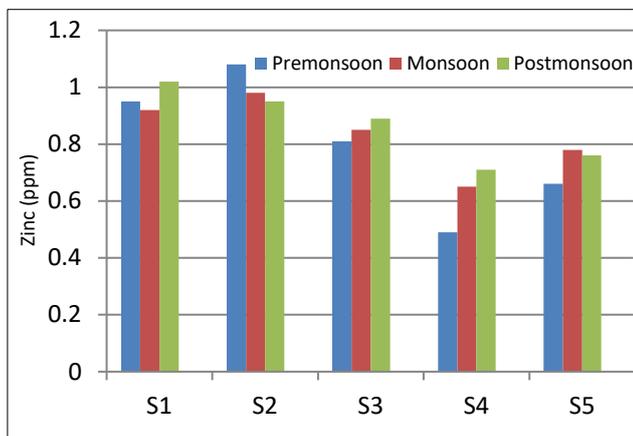


Fig 17 Zinc (ppm) in soil during different seasons in Tiruvarur district

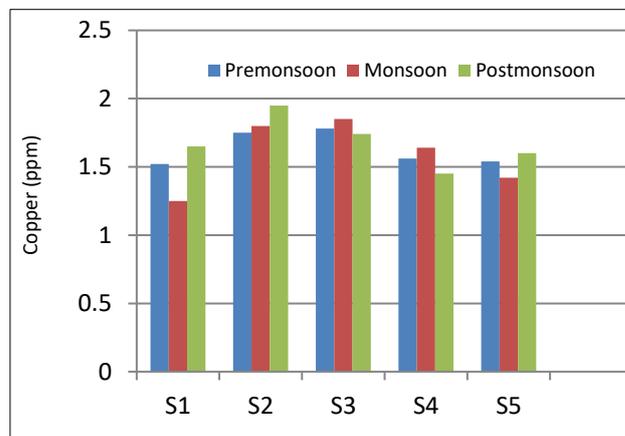


Fig 18 Copper (ppm) in soil during different seasons in Tiruvarur district

S₁: Thiruthuraipoondi site, S₂: Mannargudi site, S₃: Tiruvarur site, S₄: Nannilam site, S₅: Needamangalam site

In the present study, the fungal population isolated from five different sites of rice field in tiruvarur district with respect to three different seasons (Pre monsoon, monsoon and Post monsoon) were recorded. The isolated fungal species in rice field soil include *Alternaria alternata*, *Aspergillus humicola*, *A. fisheries*, *A. flavus*, *A. fumigatus*, *A. granulorum*, *A. niger*, *A. nidulans*, *A. ochraceus*, *A. sulphurii*, *A. sydowii*, *A. terreus*, *Aspergillus sp.*, *Choanephora cucurbitarum*, *Curvularia lunata*, *Fusarium solani*, *F. oxysporum*, *F. verticilloides*, *Helminthosporium oryzae*, *Microsporium rubrum*, *Mucor sp.*, *Penicillium citrinum*, *P. janthinellum*, *P. lanosum*, *P. purpurescens*, *Penicillium sp.*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Trichoderma atroviride*, *T. hamatum*, *T. viride*, *Verticillium glaucum*, *Verticillium sp.* The maximum fungal colonies were screened (233) from Thiruthuraipoondi rice field clay soil whereas minimum colonies were observed (132) from Mannargudi rice field soil. In the rice rhizosphere, fungi-like members of *Aspergillus* genus (within Ascomycota phylum)

can greatly promote phosphorus solubilization and can facilitate phosphorus transport and uptake [22-23]. The genus *Aspergillus* is abundant in all seasons and locations in the Tiruvarur district studied. Furthermore, *Aspergillus* produced toxins such as aflatoxins and ochratoxins, which may inhibit the growth of other fungal species.

The Pearson correlation coefficient of physicochemical parameters (temperature, pH, organic carbon, organic matter, salinity, available nitrogen, phosphorus, potassium, zinc, copper, iron, manganese and sodium, calcium, magnesium, potassium) and population density of fungi were analyzed using SPSS Software. The sign put in front of the coefficient value indicates the direction of the relationship. Relationship values can be between -1 and +1, with +1 signifying an absolutely perfect linear relationship, 0 signifying no linear relationship, and -1 signifying an entirely inverse relationship between the coordinates. The physicochemical parameters were positively correlated at the p 0.05% level of significance [26-27].

Table 1 Pearson correlation coefficient of physicochemical parameters and population density of five different soil samples in Tiruvarur district

Correlation	pH	EC	OC	AP	AK	AN	AI	AM	AZ	AC	PD
pH	1										
EC	-0.29	1									
OC	0.698	-0.454	1								
AP	-0.18	0.164	-0.568	1							
AK	0.876	0.182	0.582	-0.145	1						
AN	0.703	-0.551	0.99**	-0.546	0.533	1					
AI	0.112	0.127	0.576	-0.91**	0.254	0.515	1				
AM	-0.05*	-0.448	0.627	-0.276	-0.133	0.632	0.372	1			
AZ	-0.71	0.043*	-0.84	0.203	-0.802	-0.795	-0.392	-0.473	1		
AC	0.99**	-0.283	0.692	-0.169	0.88	0.696	0.105	-0.052*	-0.713	1	
PD	-0.46	0.629	-0.242	-0.543	-0.159	-0.314	0.649	-0.197	0.276	-0.46	1

OC-Organic Carbon, AP- Available Phosphorus, AK- Available Potassium, AN – Available Nitrogen, AI- Available iron, AM – Available Manganese, AZ- Available Zinc, AC- Available Copper, PD- Population Density

*Strongly presented correlation significance at the (p <0.05%)

**Highly presented correlation significance at the (p <0.05%)

CONCLUSION

Soil microbiota study of rice illustrates a total of 903 fungal colonies observed among 33 species in all seasons of Tiruvarur district. Among them, the Thiruthuraipoondi clay soil possessed the highest number of colonies (227), and the lowest (132) was observed in the Mannargudi soil. These distinct colonies may correspond to specific functional roles to be

performed under various physicochemical conditions. The taxonomic analysis of the rice soil fungal community reported the abundance of Ascomycota, Zygomycota, and Basidiomycota at the phylum level; Eurotiomycetes and Sardariomycetes at the class level; Eurotiales, Hypocreales, and Mucorales at the order level; and Trichocomaceae, Nectriaceae, and Pleosporaceae at the family level taxon. Moreover, the microbiota of rice soil is largely populated by fungi belonged to

genera such as *Aspergillus*, *Penicillium*, and *Fusarium*. This study also addressed the physiochemical parameters such as P^H, electrical conductivity, organic carbon, nitrogen, phosphorus, potassium, iron, manganese, zinc, and copper in seasons such as pre monsoon, monsoon, and post monsoon across all sites of the Tiruvarur district. In addition, more number of fungal colonies observed during post monsoon season when compared to monsoon and pre monsoon seasons. *Aspergillus* and *Penicillium* were dominant in all seasons due to their high sporulation capacities. However, the dominance of *Aspergillus* and *Penicillium* species can be justified by the fact that *Aspergillus* produces aflatoxins and *Penicillium* produces antibiotics that can inhibit other fungal species. With regard to pathogenicity, *Helminthosporium*, *Fusarium*, *Verticillium*, *Alternaria*, and *Curvularia* species were observed in rice field. *Fusarium verticilloides* had the highest percent contribution, followed by *F. solani*. However, the diseases of crop plants can

be controlled by some antagonistic fungi, like *Trichoderma sp.* such as *T. atroviride*, *T. hamatum* and *T. viride* are also present in the Tiruvarur rice soil that helps to suppress the crop pathogens. The discovery of different fungal taxa during different seasons of the year in rice-growing regions may stimulate the development of novel strategies, such as incorporating populations of useful fungi into ecosystems, which could be used to improve plant sustainability, cut down on chemical use, and protect the soil environment.

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