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ANTIMICROBIAL ASSAY OF SOIL FUNGI ISOLATED FROM RHIZOSPHERE AND NON-RHIZOSPHERE AREA OF *PLECTRANTHUS ROTUNDIFOLIUS* POIR.

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ABSTRACT

Soil fungi are generally very reachable to different sources and their involvement in providing the basic needs for bioprospecting. Rhizosphere micro fungi are considered as significant decomposers in the root ecosystems, ensuring the assimilation of dead plants and animals into smaller molecules that can be used by other organisms of the soil ecosystem. During the present study, rhizosphere and non-rhizosphere soils of the plant, *Plectranthus rotundifolius* Poir. were studied to record the prevalence of fungal communities. Rhizosphere soil was dominated (81%) with the fungal flora than Non-rhizosphere (19%) in our study. *Aspergillus niger* was found as the dominant one in Rhizosphere soil but *Penicillium chrysogenum* was the dominant one in Non-rhizosphere soil. The results obtained clearly indicated that *Aspergillus awamori*, *A. flavus*, *A. terreus*, *Aspergillus niger*, White sterile mycelia, Gray sterile mycelia and *Penicillium citrinum* were recorded at different concentration in both the soils. Among the isolates *Aspergillus* and white sterile mycelia were dominant in all the soil conditions due to high sporulation capacity. Quantitatively, the rhizosphere and non-rhizosphere soils contributed 3000 and 1000 fungal spores respectively in their environments. Antimicrobial properties of the active grown agar plug of the isolated dominant fungi were found very good against all the pathogens but *Klebsiella* sp. and *Pseudomonas* sp. were found as more susceptible towards the fungal extracts in comparison to other bacteria and *Candida albicans*, the later one was the third susceptible microbe among all.

KEYWORDS: Soil fungi, Rhizosphere and Non-rhizosphere, *Plectranthus rotundifolius* Poir, Antimicrobial assay, *Aspergillus niger*, *Penicillium chrysogenum.*

INTRODUCTION

The rhizosphere is generally considered as the volume of soil surrounding the plant root is influenced by root activities such as exudation of reactive carbon compounds and uptake of moveable nutrients and water.[1] Roots are being evolved to adapt to their surrounding environments by optimizing their functional construction to use resources in heterogeneous soils.[2,3] Therefore, the co-evolution of rhizosphere and plant roots play a major role in soil physical, chemical, and biological processes which sustain the biodiversity, provide soil carbon confiscation and cycle nutrients in both natural and agricultural systems.[4] The symbiotic plant-rhizosphere system also affects the biomass and activity of soil microbes that is generally enhanced due to root exudates.[5] Different soil types anchorage particular indigenous microorganisms that control the effect of plant root activity on rhizosphere microbial communities.[6] Different plant species releasing root exudates are thought to select of rhizosphere microbial populations that respond with chemotaxis and fast growth.[7] Further plant growth also affects the composition of the rhizosphere microbial community, as root exudates change during the plant's life cycle and seasonal environment responses.[8] Though, most of the studies have focused on the bacterial communities in rhizosphere and only a few works have focused on fungal communities in the plant rhizosphere. The present study is an attempt to isolate, enumerate and identify different fungal species from rhizosphere and non-rhizosphere soil of the Chinese potato plant; *Plectranthus rotundifolius* Poir as well as their antimicrobial potential against pathogenic bacteria and *Candida albicans*.

MATERIALS AND METHODS

During the present study period, isolation and identification of fungi from Rhizosphere and Non-rhizosphere soils of the Chinese potato plant; *Plectranthus rotundifolius* Poir. was done at Microbiology Laboratory, Department of Botany. K. M. Govt. Institute for Postgraduate Studies (Autonomous), Puducherry-605008, India during the year, 2020.

Method for collection of soil samples

The rhizosphere and non-rhizosphere soil samples were collected from Chinese potato plant; *Plectranthus rotundifolius* Poir. grown at Sellipet, Puducherry. Soil samples of both the sites were collected during January 2020 to March 2020 at different intervals. The soil samples were collected form the soil of the plant viz., one sample from the vicinity of the roots of the plant and other sample 5ft away from the roots of the plant by small sterilized polythene bags and brought to the Microbiology laboratory, Department of Botany, K. M. Govt. Institute for Postgraduate Studies and Research (Autonomous), Pondicherry-605008 with utmost care and stored at 4^{o} C in the refrigerator for further studies. The collection of soil samples is given in Plate I and II, which shows the types of soil collection and the morphology of the plant respectively.

Plate I: Soil samples collected from Rhizosphere and Non-Rhizosphere soils.

Sl. No.	Soil	Plant
1	Rhizosphere	*Plectranthus rotundifolius* Poir.
2	Non-Rhizosphere	*Plectranthus rotundifolius* Poir.

Plate II: Soil samples collected from *Plectranthus rotundifolius* Poir.

Isolation of fungi from the soil samples

The soil microfungi were isolated and enumerated by two methods, namely Soil Dilution[9] (Waksman, 1922) and soil plate method[10] on different media such as Potato Dextrose Agar and Sabouraud Dextrose Agar.

Soil dilution plate method

1gram of each soil sample was suspended in 10ml of double distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-2} and 10^{-3} were standardized in order to get countable number of colony forming units (CFUs) and 10^{-3} dilution was employed to isolate fungi in the present study.[9] 1 ml of microbial suspension of each concentration were added to sterile Petridishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar and Sabouraud Dextrose Agar. 50mg/1l of streptomycin was added to the medium before pouring into petriplates for preventing the bacterial growth. The petridishes were then incubated at 25 ± 3^{0}c in dark condition. The plates were observed after second day up to seventh day with routine check-up. On third day onwards, the plates were counted for the total number of colony forming units (CFUs) and followed by identification of fungi up to species level.

Identification of the soil fungi

Fungal morphology was studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lactophenol cotton blue and observed under compound microscope for the conidia, conidiophores and arrangement of spores. The fungi were identified with the help of available literature and monographs present in the laboratory and with the expertise of the research scholars.[11,12,13,14,15,16]

Physico-chemical analysis of soil

The collected soil was characterized for its physico-chemical properties. The physico-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH, salinity, organic carbon, nitrogen, phosphorous and potassium were analyzed. The physico-chemical parameters of the soil samples were analysed at KVK, Pondicherry.

Statistical analysis

The number of colonies per plate in 1gram of soil was calculated.

The percent contribution of each isolate was calculated by using the following formula:

$$\% \text{ contribution} = \frac{\text{Total no. of CFU of an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

Antimicrobial analysis by well diffusion method using fungal agar plugs

Agar plugs of 6mm size from the purified fungal mycelia of the respective PDA culture plates of the active grown soil fungi studied for antimicrobial activity against pathogenic bacteria and *Candida albicans* using nutrient agar well diffusion assay method (Bauer et al., 1966) separately. The MTCC culture of the test organisms used were *Staphylococcus aureus, Vibrio parahaemolyticus, Escherichia coli, Shewanella putrefaciens* and *Candida albicans.* The pathogenic microbes were grown in nutrient broth for 24h. Lawns of pathogenic bacteria and the fungus were prepared on nutrient agar plates using sterile cotton swabs. Agar plugs of 6mm size were carefully taken by sterile cork borer from the active grown sterile mycelia from the PDA agar pates and placed on the wells prepared on the nutrient agar plates over the precultured bacterial and fungal lawns. The plates containing bacteria and agar plugs were incubated at37^{o}C for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs, which appeared as clear are surrounding the plugs. Inhibition zone diameter was measured in mm by the HI-Media scale.

RESULTS AND DISCUSSION

Growth of soil fungi (CFUs) on agar plates isolated from rhizosphere and non-rhizosphere soil of *Plectranthus rotundifolius* Poir. by serial dilution method[9] is given in Fig 1a & 1b respectively. In concentration and composition, rhizosphere soil was recorded as good contributor of soil fungi per gram soil than non-rhizosphere soil. It was found that Rhizosphere soil contributed more than 3 thousand fungal spores in comparison to non-rhizosphere soil (thousand fungal spores) per gram soil. Ziting[17] explained in their study that differences in nutrient resources between rhizosphere and bulk soils can select for different types of fungi thereby increasing community variation during plant growth in comparison to non-rhizosphere soil.

During our soil mycoflora study, altogether 37 fungal colony forming units (CFUs) were isolated from the soil samples of rhizosphere and non-rhizosphere soil of *Plectranthus rotundifolius* Poir cultivated fields. In fungal composition, a total of 9 species under 4 genera were recorded from both of the soils. Total number of isolated fungal colony forming units (CFUs) with their percentage contribution from soil samples of rhizosphere and non-rhizosphere zones is given in Table 1. Rhizosphere soil contributed the maximum (81%) of fungal population followed by Non-rhizosphere soil (19%) (Fig 2). Fungal diversity of the soil depends on a large number of factors of the soil such as pH, organic content and the relative humidity prevailing in the soil environment.[18] Gaddeyya[19] and his coworkers[17] described in their work that the physicochemical parameters like, soil pH and their textures are also determining the fungal population in agricultural fields of different places. There work was in agreement with our report since they also isolated same types of fungi from soil.

The maximum fungal species were belonged to Deuteromycotina followed by Zygomycotina and a few were under ascomycotina, but no fungi were recorded from basidiomycotina group. Among the fungal isolates, aspergilli were dominant followed by sterile mycelia. It may be attributed that the chemical fertilizers used in the field, which prevented the growth of fungi in the field or acted as killer of fungi in the soil.

Aspergillus were isolated with five species like, *Aspergillus awamori, A. flavus, A. niger* and *A. terreus. Penicillium* spp like *P. citrinum, P. chrysogenum* and Gray sterile mycelia were recorded from Rhizosphere soil but *A. niger, Penicillium chrysogenum* and White sterile mycelia were recorded from Non-rhizosphere soil only. White sterile mycelia were recorded in more numbers from both the soils. Other Dematiaceous fungi were isolated sporadically from the soil samples.

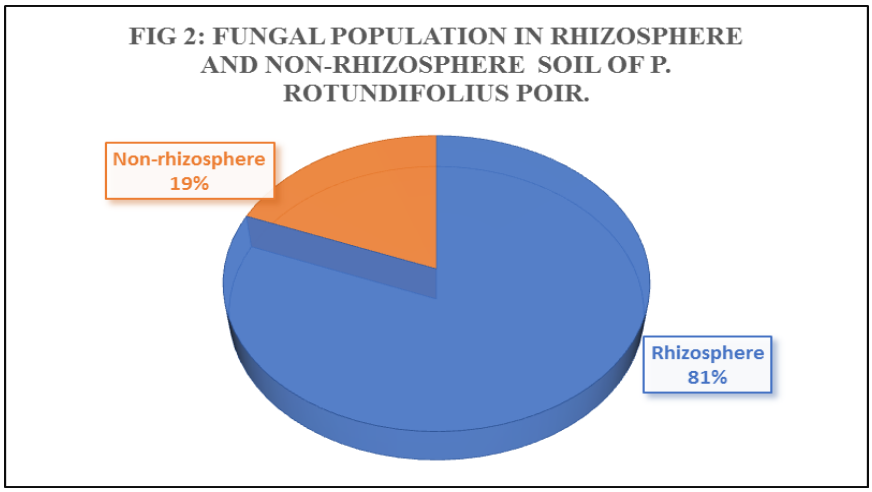
Fig 1a: Growth of fungi (CFUs) on agar plates isolated from Rhizosphere soil.

Fig 1b: Growth of fungi (CFUs) on agar plates isolated from Non-rhizosphere soil.

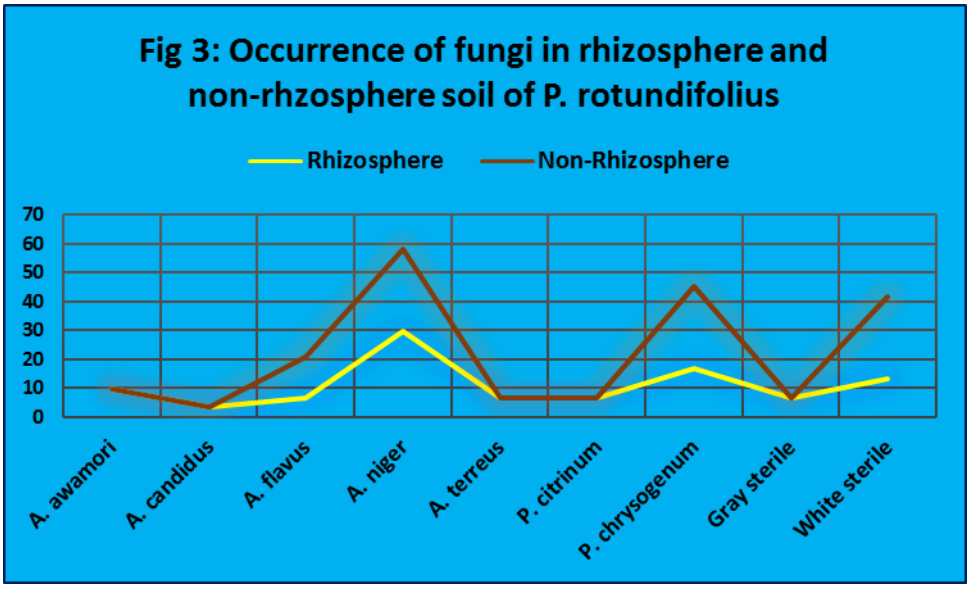
Table 1: Number of fungal CFUs isolated from rhizosphere and non-rhizosphere soil of the plant, *Plectranthus rotundifolius* Poir and their percentage occurrence.

Sl. No.	Fungi	Rhizosphere	Non-Rhizosphere
1.	*Aspergillus awamori*	3/9.6	0
2.	*A. candidus*	1/3.3	0
3.	*A. flavus*	2/6.6	1/14.3
4.	*A. niger*	9/29.6	2/28.5
5.	*A. terreus*	3/9.6	0
6.	*Penicillium citrinum*	2/6.6	0
7	*Penicillium chrysogenum*	5/16.6	2/28.5
8	Gray sterile mycelia	2/6.6	0
9.	White sterile mycelia	4/13.3	2/28.5

In total, 30 fungi were isolated only from rhizosphere soil but 7 fungi were recorded from Non-rhizosphere soil. *Aspergillus niger* was the dominant fungi among all and have (29.6%) contribution in rhizosphere soil but *Aspergillus niger* was the dominant (28.5%) one in Non-rhizosphere soil (Table 1). Table 1 showed the percentage contribution of fungal isolates from the two soils. The most common among them viz, *Aspergillus awamori* (9.6%), *Aspergillus candidus* (3.3%), *Aspergillus niger* (29.6%), *Aspergillus terreus* (9.6%), *Aspergillus flavus* (6.6%), *Penicillium citrinum* (6.6%), *Penicillium chrysogenum* (16.6%) and white sterile mycelia (13.3%) were isolated and characterized from rhizosphere soil. Relative occurrence of soil fungi isolated from the two soils are given in Fig 2 and distribution and composition of fungal isolates are given in Fig 3, which showed the different pattern of their distribution in the soil.



Diversity of soil fungi was found to be higher in the rhizosphere soil as compared to the non-rhizosphere soil, where the mycorrhizal association as well as the nutrients secreted from roots aggregated with soil particles might have enhanced the mycoflora. The fungal variation and percentage frequency of the mycoflora were analysed and given in Table 2.

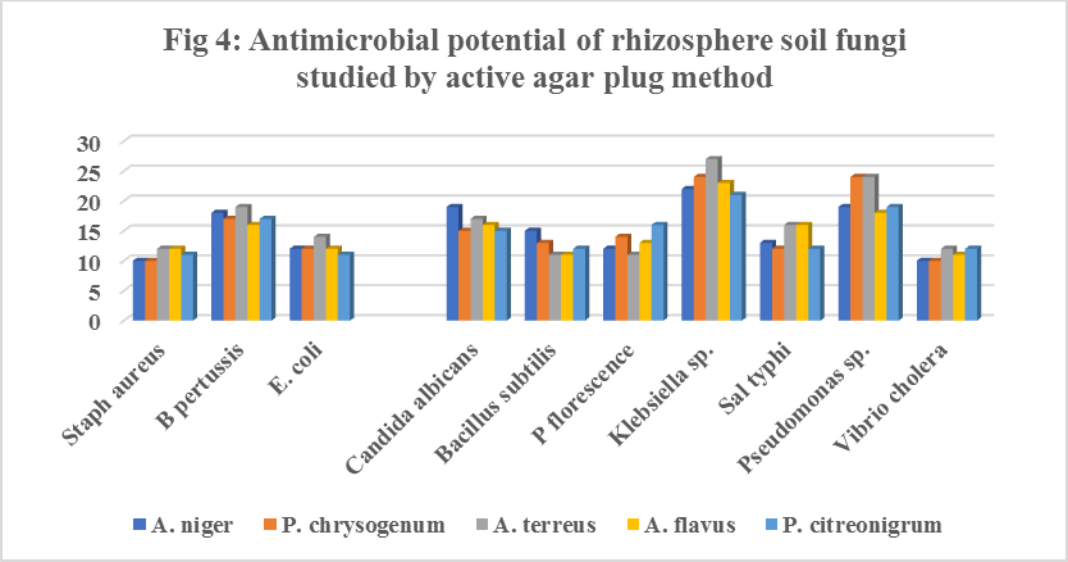


The Soil pH, organic content and water are the main factors affecting the fungal population and diversity.[18] The organic carbon, nitrogen, phosphorus, potassium are to be the important for the growth of soil fungi. In the absence of any of these growth parameters, the growth and sporulation of moulds as well as other microbes are not possible in the soil.[19] It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high[20] has reported that environmental factors such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora. The mycoflora analysis of different soils of all the places should be done to analyse their abundance and distribution.

During the study we found most of the isolated fungi are preventing the growth of pathogenic bacteria studied by well plug method by employing active grown fungal culture (Table 2). *Penicillium chrysogenum* and *Aspergillus flavus* were found to be more effective fungi in order to prevent the growth of all pathogenic bacteria than other fungi isolated and studied in our present study (Table 2). *Klebsiella* sp. and *Pseudomonas* sp. were found as more susceptible towards the fungal extracts in comparison to other bacteria and *Candida albicans*, the later one was third susceptible microbe among all the microbes studied herewith (Fig 4).

Table 2: Antimicrobial potency (inhibition zone) of the active agar plug of several soil fungi against MTCC pathogens studied by well diffusion method.

MTCC strains	Soil fungi and their antimicrobial potency (Zone of inhibition in mm)				
	A. niger	***P. chrysogenum***	***A. terreus***	***A. flavus***	***P. citreonigrum***
Staphylococcus aureus	10	10	12	12	11
Bordetella pertussis	18	17	19	16	17
Escherichia coli	12	12	14	12	11
Candida albicans	19	15	17	16	15
Bacillus subtilis	15	13	11	11	12
Pseudomonas florescence	12	14	11	13	16
Klebsiella sp.	22	24	27	23	21
Salmonella typhi	13	12	16	16	12
Pseudomonas sp.	19	24	24	18	19
Vibrio cholera	10	10	12	11	12



CONCLUSION

In the present study rhizosphere and non-rhizosphere soil of the cultivated land of *P. Rotundifolius* Poir was studied for the isolation and enumeration of fungal concentration and composition based on their availability. Rhizosphere soil was dominated (81%) with the fungal flora than Non-rhizosphere (19%) in our study. *Aspergillus niger* was found as the dominant one in Rhizosphere soil but *Penicillium chrysogenum* was the dominant one in Non-rhizosphere soil. The results obtained clearly indicated that *Aspergillus awamori*, *A. flavus*, *A. terreus*, *Aspergillus niger*, White sterile mycelia, Gray sterile mycelia and *Penicillium citrinum* were recorded at different concentration in both the soils. Among the isolates *Aspergillus* and white sterile mycelia were dominant in all the soil conditions due to high sporulation capacity. Antimicrobial properties of the fungal extracts were found very good against all the pathogens but *Klebsiella* sp. and *Pseudomonas* sp. were found as more susceptible towards the fungal extracts in comparison to other bacteria and *Candida albicans*, the later one was third susceptible microbe among all.

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