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DIVERSITY OF RHIZOPSPHERE MYCOFLORA OF SANTALUM ALBUM L.

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ABSTRACT - A total of 10 species of 5 genera of fungi were isolated from the rhizosphere of Santalum album L. and identified based on their morphological characteristics. Soil dilution method was used to isolate the flora on potato dextrose agar medium, with appropriate antibiotics, such as streptomycin. Among them, the most common fungi were Aspergillus sp. Aspergillus niger, Aspergillus ustus, Fusarium moniliforme, Fusarium solani, Fusarium verticillium, Mucor hiemalis, Penicillium implicatum, Pseudomonas citrinum and Talaromyces funiculosum. Talaromyces funiculosus was a dominant fungal species, showing the highest number of fungal colonies (15) with a percentage incidence (20.83%). F. verticillioides showed the lowest number of fungal colonies (3) and percentage incidence (4.16%) compared with other rhizosphere fungi tested in composite rhizosphere samples of medicinal plants.
KEYWORDS - Rhizosphere mycoflora (RM), Santalum album L., Talaromyces funiculosus, F. verticillioides.

INTRODUCTION

Soil microbial communities play an important role in assessing soil conditions and stimulating plant growth. Microorganisms help improve soil fertility and plant growth because they participate in a variety of biochemical transformation and mineralization activities in the soil. The amount of organic and inorganic materials in the soil has a direct effect on the number of fungi in the soil. In addition to chemical fertilizers, various pesticides have an adverse effect on the flora, which is very useful for maintaining soil fertility and the ecological balance in the soil atmosphere. Fungi are essential to the function of soil ecosystems. Fungi are an important part of the soil. They play an important role in nutrient cycling by regulating soil biological activity.

They promote plant health and growth by attacking plant pathogens with enzyme secretions. They also use antagonism by producing antibodies and inhibit the growth of other microorganisms. They also form protective nets around the roots and protect plants [1]. A microbial community composed of several species. They are more likely to be responsible for the biodegradation of pesticides in the soil and rhizosphere than a single species. The fungicide applied to the soil during planting will continue to exist during the development of the plant's root system. Therefore, some pesticides

interact with microorganisms in the soil and rhizosphere[2]. The rhizosphere is a narrow soil area surrounding the roots, and root activity stimulates the microbial population. As we all know, the rhizosphere is the hot spot of microbial activity [3]. The term rhizosphere was proposed by Hiltner (1904) and Brimecombe et. al.(2001) for the first time. The rhizosphere microbial community varies with plant species [4]. Soil microbiota plays a key role in assessing soil conditions and stimulating plant growth. Microorganisms in the soil and rhizosphere are beneficial to improve soil fertility and plant growth because they participate in a variety of biochemical transformation and mineralization activities in the soil. However, the structure of the microbial community also varies with soil characteristics, including pH, available water and nutrient limitations. The possible association of plants and microorganisms with certain soil characteristics contributes to the relationship between plants and microorganisms [5]. The stimulating effect of the rhizosphere on microorganisms is called the rhizosphere effect. It is nothing but the interaction between soil and rhizosphere microorganisms and their ratio. The chemical and physical properties of the rhizosphere are very different from those of soil far away from the root zone. Soil fungi play a very important role in maintaining soil fertility. However, agrochemicals can harm soil flora [6].

MATERIALS AND METHODS

Selection of Medicinal Plants for Rhizosphere:

The medicinal plants were selected and collected from Siddhanath, a village on the Godavari River, 5 kilometres from the city of Nanded. Used identification keywords, flora and related literature to describe and identify medicinal plants. The flora of Marathwada (Naik, 1998) was extensively used.

Collection of Composite Rhizosphere Sample:

In this study, the rhizosphere samples of the tested medicinal plants were collected separately. The four different points in the four directions two meters away from the trunk of the test medicinal plant were first marked and used as sampling points. The sampling site was then cleaned by removing dry leaves and other plant debris. In order to expose the roots of medicinal plants, 2×2 small pits were made at each sampling point. Then collected the soil 5 cm around the roots and the soil attached to the roots, and treat them as the rhizosphere. The rhizosphere of all four sampling points was mixed and collected together to make a composite rhizosphere sample of about 500gm, which was used as the composite rhizosphere sample of the tested medicinal plants. The composite rhizosphere samples of all tested medicinal plants were individually packaged in fresh polyethylene bags, labelled, and taken to the laboratory and stored at room temperature for further study. As described by Waksman (1961) and Aneja (2003), the rhizosphere flora (RM) of test medicinal plants was isolated by serial dilution and agar plate method [7-8]. For this, sterile potato dextrose agar (PDA) plate inoculated by applying 1ml serially diluted compound rhizosphere samples of test medicinal plants. The inoculated PDA plates were incubated at room temperature. Then observed the appearance of the rhizosphere flora of the plate every day. The rhizosphere

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fungal flora was sub-cultured separately and made into a pure form, and they were regarded as rhizosphere fungi isolates of medicinal plants. The rhizosphere fungi isolates were stored on PDA slants and plates for further study.

Preparation of Serial Dilutions:

The 10gm composite rhizosphere samples were respectively dissolved in 90ml 0.85% NaCl and treated as a 10-1 dilution of the composite rhizosphere samples. Then transferred 1 ml of 10-1 composite rhizosphere sample suspension to a test tube containing 9ml of 0.85% NaCl, and treated it as a 10-2 dilution of the composite rhizosphere sample. Again transferred 1 ml of 10-2 composite rhizosphere sample suspension to 9 ml of 0.85% NaCl, and used it as the 10-3 dilution of the composite rhizosphere sample. Similarly the composite rhizosphere sample further diluted to make 10-4, and 10-5 dilutions.

Staining Technique for Fungi:

The mycelium and conidia were stained with lactophenol and cotton blue. Cotton blue stains the cytoplasm and results in a light blue background. Lactophenol was used as a cleaning agent. The specimens were observed and identified under the Olympus digital cam microscope, and micrographs were taken at 10X×40X magnification.

Identification of Fungi:

According to the asexual and sexual spores and fruit structure, the colony of the rhizosphere flora of the tested medicinal plants was preliminarily identified. Detailed inspection of fungal properties carried out under a compound microscope. Observation was initially performed by fixing the mycelium in water, and then by fixing it in Lactophenol and cotton blue [9]. Photography was performed under a Trinocular Labomed microscope. The identity was confirmed with the help of manuals, keys, the internet, and different sources[10-17].

Percent contribution of rhizosphere mycoflora:

The number of fungal colonies per plate in 1 gram of soil was calculated. Following formula used to calculate the contribution percentage of each isolated colony.

RESULTS AND DISCUSSION

In the current study, Santalum album L. was selected as their rhizosphere flora. The rhizosphere of Santalum album L. was collected and the rhizosphere flora was screened. According to the morphological characteristics, 10 species of rhizosphere fungi were isolated and identified. Talaromyces funiculosus was a dominant fungal species, showing the highest number of fungal colonies (15) and a percentage incidence (20.83%). F.verticillium had the lowest fungal colony count (3) and morbidity percentage (4.16%) compared to other rhizosphere fungi from composite

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rhizosphere samples of the tested medicinal plants. Similar to this work, El-Amin and Saadabi (2007) used serial dilution plate method to isolate 20 fungal flora from Sugarcane rhizosphere, including Aspergillus (2), Altemaria (1), Penicillium Genus (1 species) has the most species, Rhizopus (1 species), Curvularia (1 species) and Fusarium (1 species)[18]. Duroward, et. al. (2008) isolated 34 Ascomycota species at 6 different selected sites by serial dilution. Among these Penicillium genera, Fusarium and Aspergillus predominated. The physicochemical properties of soil samples affect populations and distribution[19]. Sarvanakumar and Kaviyarasan (2010) found that Alternaria, Rhizopus and Aspergillus were dominant in soil. Soil microbes play an important role in biogeochemical processes that determine plant productivity, the successful function of introduced microbial inoculants and their impact on soil health. Exhaustive efforts have been made to explore the soil microbial diversity of indigenous communities, their distribution and behaviour in soil habitats[20]. Shivanna and Vasanthkumari (2011) isolated A. flavious, Aspergillus sp., A. niger, A. flavus and A. ustus from the rhizosphere soil of wild plants[21].

Naveen Kumar, et. al. (2011) proposed that the serial dilution method is superior to the direct plating method in isolating soil fungi[22]. Rebecca, et. al. (2012) investigated the isolation and identification of the rhizosphere flora of Barleria cristata. A total of 6 fungi were isolated, of which 4 were identified as Ascomycota (Arthrinium sp., Aspergillus sp., Fusarium sp., Sporothrix sp.), and 2 samples were unknown[23]. Saler (2012) studied the rhizosphere micro fungi of peanut (Arachis hypogea L.) var CVSB-11 from Nashik District, Maharashtra. He recorded a total of 21 fungi by serial dilution[24-26]. Srivastava and Kumar (2013) identified a total of 37 fungi from different plant rhizospheres such as Abutilon indicum (11 sp.) followed by Aloe vera (9 sp.), Achyranthes aspera (9 sp.), Amaranthus polygamus (8 sp.), and Argemone mexicana (7 sp.). He reported that Stachybotrys atra., Chaetomium globosum and C. spirale were dominant[27].

Sr. No.	**RM of CRS of *Santalum album* L.**	**Number of RM colonies.**	**% Incidence of RM colonies**
1.	*Aspergillus* sp.	6	8.33
2.	*Aspergillus niger* Van. Tiegh	7	9.72
3.	*A. ustus* (Bainier) Thom and Church	8	11.1
4.	*Fusarium moniliforme* Link.	5	6.94
5.	*Fusarium solani* (Mart.) Sacc.	10	13.8
6.	*F. verticillioides* (Sacc.) Nirenberg	3	4.16
7.	*Mucor hiemalis* Wehmer.	4	5.55
8.	*Penicillium implicatum* Biourge.	9	12.5
9.	*P. citrinum* Thom.	5	6.94
10.	*Talaromyces funiculosus* (Thom.)	15	20.83
11.	Total no. of colonies	72	100
	Total no. of species	**10**	

Table 1: Percent incidence of RM colonies of CRS of Santalum album L. by agar plate method.

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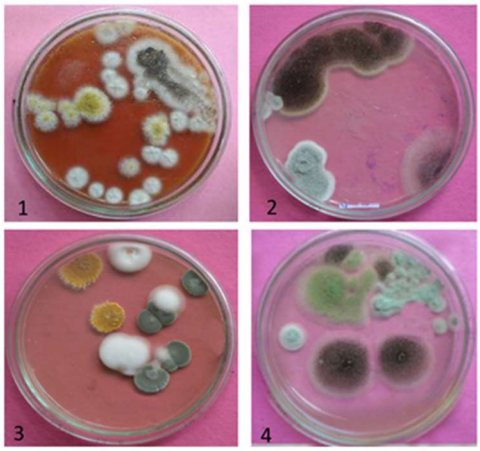

Figure 1. Fungal culture plates of RM of Santalum album L.

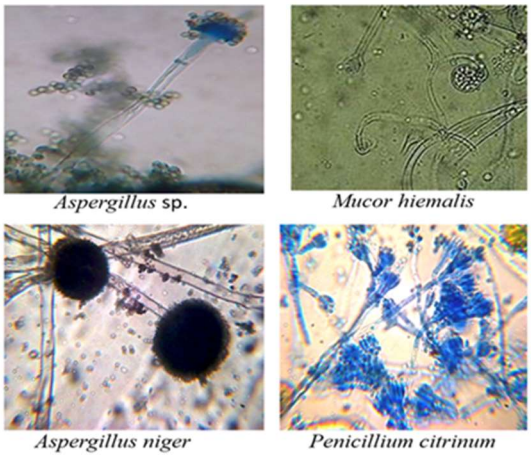

Figure 2. Microscopic photographs of RM of Santalum album L.

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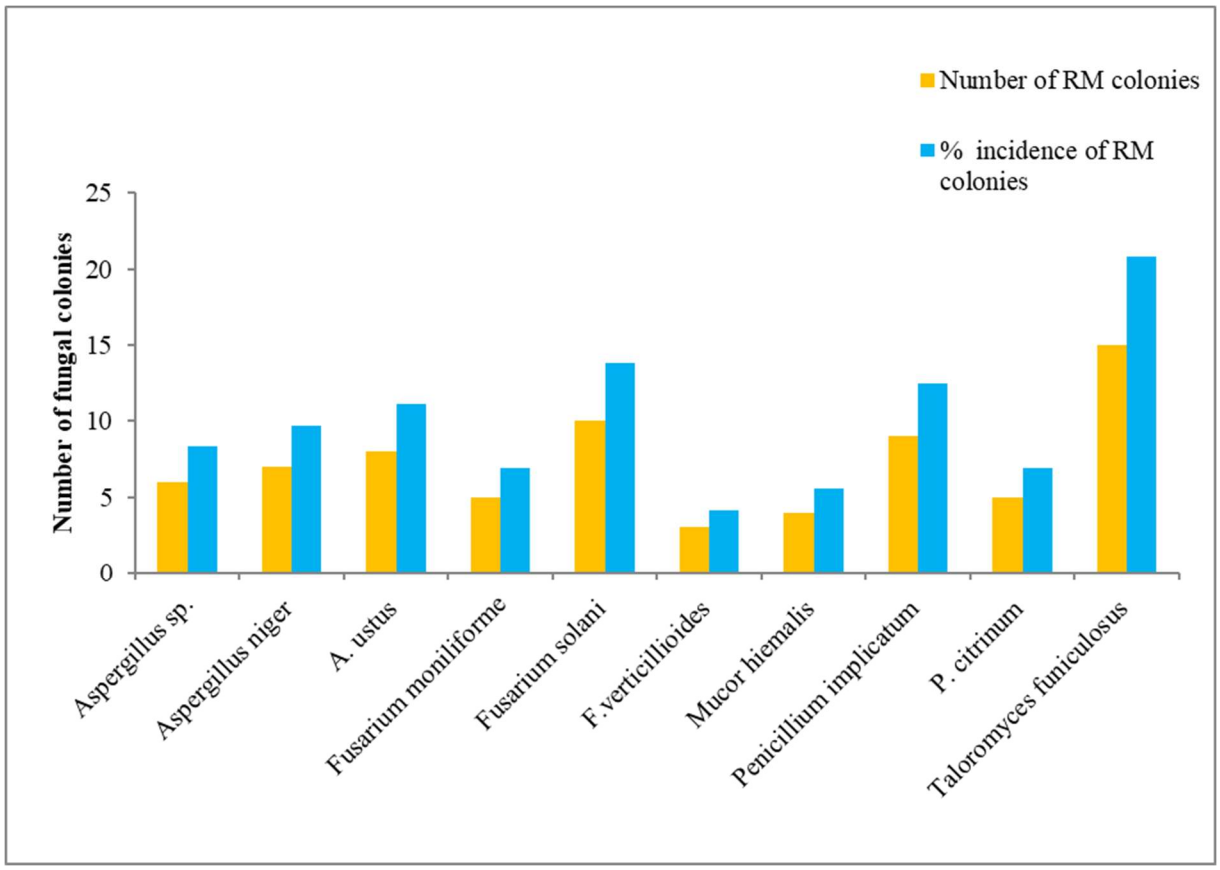

Figure 3. Percent incidence of RM colonies on CRS of Santalum album L.

CONCLUSION

This is the first report of fungal diversity in the rhizosphere of Santalum album L. which seen too similar as in the case of rhizosphere of other plants.

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