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Research Article

FUNGAL DIVERSITY IN THE RHIZOSPHERE OF *ACACIA AURICULIFORMIS* A. CUNN. EX BENTH AND *BAMBUSA BALCOOA* ROXB. GROWING IN SURYAMANINAGAR, TRIPURA, NORTHEAST INDIA

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ABSTRACT

Investigation was done for fungal population and fungal species in the rhizosphere of *Bambusa balcooa* and *Acacia auriculiformis.* The CFU range was high in the rhizosphere of *B. balcooa* than *A. auriculiformis*. A total of 25 species was isolated from the rhizosphere of both the plant species. 21 species and 13 species were isolated from the *B. balcooa* and *A. auriculiformis*, respectively. Sterile hyphae, *Gliocladium viride, Pythium ultimum, Cladosporium cladosporioides* and *Trichoderma viride* were the most frequent among the isolated fungal species. Shannon diversity index was high in the month of May in both the species. Evenness was high in the month of March in *A. auriculiformis* and in the month of May for *B. balcooa*.

Key Words: *Acacia auriculiformis, Bambusa balcooa, Fungal Population, Fungal Diversity*

INTRODUCTION

Soil is a most precious natural resource and contains the most diverse assemblages of living organisms (Swer *et al.,* 2011). Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural soil and managed agricultural soils (O'Donnell *et al*., 1994 and Doran and Zeiss, 2000) and their involvement in soil structure formation, organic matter decomposition, nutrient cycling and toxic removal is well recognized (Van Elsas and Trevors, 1997 and Doran and Zeiss, 2000). Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems (Swer *et al.,* 2011). However, fungi play an important role in terrestrial ecosystems or soil ecosystem as major decomposers of plant residues, releasing nutrients that sustain and stimulate plant growth in the process (Smit *et al.,* 1999). In the study of soil fungi, particular attention is given to the rhizosphere (Alabouvette, 1990), where they mediate many ecological processes, and are influential for plant growth and soil health (Zachow *et al.,* 2009) as a reason soil fungus are abundant in the rhizosphere than the other parts of plants. Some fungi were found to possess properties antagonistic towards plant pathogens also (Lumsden, 1981). Due to the ubiquity of mycorrhizal symbioses in terrestrial ecosystems, most of the actively absorbing rootlets are connected with the surrounding soil through an interface called the mycorrhizosphere (Johansson *et al.*, 2004).The diversity of soil fungus and rhizosphere fungus is much higher than previously thought (Vandenkoornhuyse *et al*., 2002 and Gams, 2007). Furthermore, microorganisms comprise much of the Earth's biodiversity, but our knowledge about their biodiversity scaling relationships relative to that of plants and animals is limited (Green and Bohannan, 2006). It has been estimated that 1.5 million fungal species are present in natural ecosystems, but only 5 –10% have been described formally (Hawksworth, 2001). Schmit and Mueller (2007) estimated that there is a minimum of 7, 12,000 fungal species worldwide. The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been known to be described (Wang *et al*., 2008). There is a close link between plant species and microbial community structure in the rhizosphere, and the hypothesis is that bacteria and fungi have also developed a unique diversity pattern over time, which may give insights into microbial evolution (Zachow *et al.,* 2009).

However, there are no studies related to the fungal diversity in the rhizosphere of plant species in Tripura, northeast India. In this connection, we have selected *Acacia auriculiformis* A. Cunn. ex Benth and *Bambusa balcooa Roxb.* Moreover, *A. auriculiformis* is a plant from Leguminosae which grows like an invasive species abundantly. In addition, bamboo is an important species which grows abundantly in Tripura. In paucity of this kind of studies, it is our interest to analyse microbial diversity particular fungi from the rhizosphere of *A. auriculiformis* and *B. balcooa* from Tripura University Campus, Suryamaninagar, Tripura, orth east India.

MATERIALS AND METHODS

Site Description

The soil samples were taken from the rhizosphere of the individual tree to study the rhizosphere mycoflora. For this purpose, soil samples were collected from the rhizosphere of *A. auriculiformis* and *B. balcooa* from the Tripura University campus, Suryamaninagar, Tripura west. It is situated between 22°57′ and 24°32′ N and 91°10′ and 92°20′ E.

Sampling

The soil samples were collected from the rhizosphere of *A. auriculiformis* and *B. balcooa* from 4 different sampling points from 4 different sides of the same tree. At first the sampling area was cleaned by removing dry leaves and other unwanted materials. Samples were collected from 10 cm below the soil surface by digging it and measured it with a scale. Then the samples of the four different areas were mixed well and transferred to the laboratory.

Preparation of Media and Inoculation

The culture media used was Rose Bengal agar media. The glass wares and the prepared media were sterilized in the autoclave and transferred to the laminar air flow. 0.05 g of Rose Bengal is added to the media. Then antibiotic (Streptomycin) was added for preventing it from the contamination of bacteria. The media is then poured in the petri plates and allowed to cool at room temperature. After inoculation, the plates were kept for minimum incubation for 4-6 days at 25^0C till the colonies grow well in dust free chamber. After the incubation period, the colony forming units were counted and expressed as CFU/g of soil. Isolates which did not sporulate were placed under near UV light (black light for 12h dark: 12h light) in an attempt to stimulate sporulation. Isolates which did not produce spores were treated as sterile mycelium (Lacap *et al*., 2003).

Lacto Phenol Cotton Blue Mounting

A portion of the mycelium of the representative colonies was picked up with the help of a pair of needles and semi permanent slides were prepared using Lactophenol cotton blue. The slide was gently heated in a spirit lamp so as to release the air bubbles, if any present inside the cover glass. The excess stain was removed using tissue paper and the cover glass was sealed using white nail polish.

Identification of Fungi

The fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys. The species was then identified by using the identification manual (Ellis, 1993; Domsch *et al*., 1980; Watanabe, 2002).

Data Analysis

All the calculation was done in Microsoft Excel, 2007. Isolation frequency (IF) of the fungi species was calculated using the following formula: Isolation frequency (IF) = Ni/Nt ×100 Where Ni is the number of petri dishes from which a given species was isolated and Nt is the total number of petri dishes used for isolation. Diversity analysis was conducted using past software (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

The colony forming unit (CFU) value in the month of May was the highest and March was the lowest from the rhizosphere of *A. auriculiformis*. The range was 5.5 $x10^5$-15.6$x10^5$ CFU/g of soil. The CFU value in the month of March was the highest and May was the lowest from the rhizosphere of *B. balcooa.*

Table 1: Colony forming unit (CFU) / g of soil from the rhizosphere of plants

Plant species	January	March	May
Acacia auriculiformis	$9x10^5$	$5.5\ x10^5$	$15.6\ x10^5$
Bambusa balcooa	$23\ x10^5$	$33.4\ x10^5$	$8.4\ x10^5$

The range was $8.4\ x10^5$-$33.4\ x10^5$ CFU/g of soil. A total number of 25 fungi were isolated from the rhizosphere of *A. auriculiformis* and *B. balcooa*. There were 21and 13 species isolated from the rhizosphere of *A. auriculiformis* and *B. balcooa*, respectively.

Table 2: Isolation frequency (%) of fungal species from the rhizosphere of plant species

	Acacia auriculiformis			*Bambusa balcooa*		
Name of the organisms	**January**	**March**	**May**	**January**	**March**	**May**
Absidia corymbifera	0	0	0	0	0	20
Absidia glauca	0	0	20	0	0	0
Acremonium murorum	60	20	0	0	0	60
Alternaria alternata	0	20	0	0	0	0
Aspergillus carneus	60	0	0	0	0	0
Aspergillus flavus	0	20	20	0	0	0
Aspergillus niger	60	0	0	20	40	0
Cladosporium cladosporioides	20	40	40	0	0	60
Fusarium poae	0	0	0	0	0	80
Gliocladium roseum	0	0	40	0	0	0
Gliocladium viride	0	20	60	40	20	0
Mortirella exigua	0	20	0	0	0	0
Mortirella polycephala	0	0	20	20	0	20
Mortirella vinacea	0	0	20	0	0	0
Mucor racemosus	0	0	0	40	0	0
Penicilium brevicompactum	0	0	20	0	0	0
Penicillium canescens	0	20	20	0	0	0
Penicillium chrysogenum	0	40	40	20	0	0
Penicillium frequentans	20	0	0	0	0	0
Penicillium jensenii	0	20	20	0	0	0
Penicillium waksmanii	0	0	0	0	0	40
Pythium ultimum	0	20	0	60	40	40
Sterile Hyphae	40	100	100	80	100	60
Trichoderma harzianum	0	0	20	0	0	0
Trichoderma viride	0	0	100	20	60	40

Sterile hyphae were highly isolated. *Alternaria alternata, Mortirella exigua, Mortirella vinacea, Penicilium brevicompactum, Penicillium frequentans, Absidia corymbifera, Absidia glauca and Trichoderma harzianum* were least isolated. The highest number of species diversity was found in the month of May in *A. auriculiformis*. The month of March has the highest dominance. The Shannon diversity is high in the month of May and the highest evenness riches in the second month of March.

The month of May was found to be highest in terms of species richness in *B. balcooa*. Shannon diversity index was maximum in the month of May. Simpson index of dominance was high in the month of January and evenness was high in the month of May (Table 3). According to Zhou and Hyde (2002), seasonality had an effect as more diversity of fungi on bamboo was present during the rainy season. Lamore and Goos (1978) also noted that fungal species richness on naturally occurring wood sample Submerged in a temperate stream was highest following a period of heavy rainfall. Leung (1998) also

suggested that seasonal factors, especially air, temperature and rainfall affected the development of the fungal communities on bamboo baits. According to Swer *et al.,* (2011) also there is a result of maximum fungal population in the soil in rainfall. These works support our study as there was great number of fungal diversity in the month of May when there was rise in temperature and also rain. According to work

Table 3: Diversity indices of fungi

	Acacia auriculiformis			*Bambusa balcooa*		
Diversity indices	**January**	**March**	**May**	**January**	**March**	**May**
Taxa_S	6	11	14	8	5	9
Dominance_D	0.6653	0.8311	0.3409	0.2324	0.7171	0.685
Shannon_H	1.224	2.434	2.52	2.022	0.7495	2.789
Simpson_1-D	0.3347	0.1689	0.6591	0.7676	0.2829	0.315
Evenness_e^H/S	0.5667	1.037	0.8881	0.9446	0.4232	1.807

of Swer *et al.,* (2011) the most common genera isolated from the soil of the plots (organically fertilizer treated plots and untreated plots) include *Penicillium, Aspergillus, Acremonium, Mortierella, Fusarium, Mucor, Paecilomyces, Talaromyces, Trichoderma* and *Verticillium,* which is to some extent similar to our work i.e., species of *Penicillium, Aspergillus, Acremonium, Mortierella* and *Trichoderma* from the rhizosphere of *A. auriculiformis*. Hackl *et al*., (2000) indicated that the plant species also equally influence the population and species composition of the soil fungi. Thus the difference in the fungal species may also be to some extent for the plant species. It has been observed that a number of fungal species were present in the rhizosphere of bamboo. In this study greater fungal population were the species of *Pythium, Mucor,* Sterile etc. and the lower fungal population were the species of *Absidia, Acremonium, Cladosporium,* etc.

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