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RHIZOSPHERE MYCOFLORA ASSOCIATED WITH LEGUMINOUS TREES AROUND KARACHI UNIVERSITY CAMPUS

Marium Tariq, Kanwal Tayyab, Shahnaz Dawar and M. Javed Zaki

Department of Botany, University of Karachi, Karachi-75270 Pakistan
Email: mariumtariq02@gmail.com

ABSTRACT

Using serial dilution and direct plate methods for the detection of rhizosphere mycoflora of 22 soil samples of 3 different leguminous trees (*Prosopis juliflora, Leucaena leucocephala* and *Adenanthera pavonina*) were collected from Karachi University Campus. By direct plating method, total number of 27 species belonging to 19 genera were isolated from all leguminous trees rhizosphere soils, where 26 species belonging to 19 genera from *P. juliflora*, 22 species belonging to 16 genera from *A. pavonina* and 21 species belonging to 15 genera were isolated from *L. leucocephala*. Out of these fungal species *Alternaria alternata, Aspergillus flavus, A. niger, A. wentii, Chaetomium indicum, Cunninghamella elegans, Curvularia lunata, Drechslera maydis, Fusarium solani, F. oxysporum, Mucor* sp., *Paecilomyces variotii, Phoma eupyrena, Rhizopus* sp., and *Trichoderma viride* were observed on all three leguminous trees rhizosphere soils. Serial dilution method elicited 14 species belonging to 12 genera where *A. flavus, A. niger, A. wentii, P. eupyrena, F. oxysporum, A. alternata, P. variotii, C. elegans, C. lunata, D. maydis, Chaetomium indicum, Mucor* sp., and *Rhizopus* sp., were common in the soils of all leguminous trees.

Key Words: Direct plating, Serial dilution method, Leguminous trees, Rhizosphere soil

INTRODUCTION

Soil is a rich habitat for the microorganism's growth as compared to other microbial habitats and among these microorganisms, fungi are one of the dominant groups present in soil. Fungi live, multiply and die or disintegrate in the soil and thus they provide rich organic matter, which could be recycled as plant nutrition (Guleri *et al.*, 2010). Leguminous tree develop a double symbiosis with both nitrogen fixing bacteria and Vesicular Arbuscular Mycorrhizae, VAM (Qadri *et al.*, 2004). VAM are advantageous to woody legume because they increase the uptake of low mobile nutrients in the soil especially Phosphorus (Manjunath *et al.*, 1984). The combined effect of two symbionts, the *Rhizobia* and VAM help legume species to absorbs nitrogen and Phosphorus which are two of the most important nutritional deficiencies (Barea *et al.*, 1988).

Leucaena is a genus of flowering plants in the sub family Mimosoideae of the legume family Fabaceae which contains about 24 species of trees and shrubs. *Leucaena leucocephala* is a fast growing woody mimosoid legume which is well known for its adaptability in arid and semi arid lands of the world including Pakistan. The tree has gained much importance due to its uses to mankind. Roots of *Leucaena* usually carry nitrogen fixing nodules (Iqbal and Mahmood, 1992).

Prosopis juliflora (Mimosaceae) commonly known as mesquite is a shrub or small tree, grows to a height of upto 12 meters (39 ft) and has a trunk with a diameter of upto 1.2 meters (3.9 ft). Its uses include forage, wood and environmental management. Research on *P. juliflora* leaves showed that it has antibacterial, antifungal, hemolytic and anti-inflammatory activities (Ahmed *et al.*, 1986). *Adenanthera pavonina* (L.) (family Leguminosae, subfamily Mimosoideae) has long been an important tree in Southeast Asia and the Pacific Islands and this useful tree provides quality fuel wood, wood for furniture (Clark and Thaman, 1993).

The purpose of this study was to investigate the soil mycoflora of leguminous trees which promotes beneficial relation between biotic and abiotic factors.

MATERIALS AND METHODS

Collection of samples

Twenty two rhizospheric soil samples with three replicates of each leguminous trees (*Prosopis juliflora, Adenanthera pavonina, Leucaena leucocephala*) were collected from Karachi University Campus. The soil samples were brought to the laboratory in sterile polythene bags.

Detection of rhizosphere soil mycoflora
a. Direct plate method

Soil sample (0.01g) was dispersed in 1 ml sterile distilled water in a sterilized Petri dish and molten cooled Potato Dextrose Agar (PDA) was poured containing Benzyl Penicillin Potassium Salt (0.1 g^{-1}) and Streptomycin Sulphate (0.2 g^{-1}). The Petri dishes were slightly rotate for evenly distribution of soil particle throughout medium and left to solidify. The Petri dishes were incubated for 5-7 days at temperature of 30 ± 1 °C (Naveenkumar *et al.*, 2011).

b. Serial dilution method

Serial dilution method of Aneja (2001) was followed where soil sample (2.0 g) was suspended in 18 ml sterilized distilled water and mixed well and appropriate serial dilutions of 1.0 ml aliquots was poured onto sterilized PDA Petri dishes containing Benzyl Penicillin Potassium Salt (0.1 g^{-1}) and Streptomycin Sulphate (0.2 g^{-1}). Each dilution was replicated three times and the dishes were incubated at 30 ± 1ºC. After one week of incubation, the total fungal colonies forming unit (CFU)/g soil were recorded.

Literature for identification

Growing fungi on plates were identified by standard mycological literatures (Ellis, 1971; Booth, 1971; Domsch *et al*., 1980; Nelson *et al*., 1983; Raper and Fennell, 1965; Barnett, 1960; Thom and Raper, 1945). The data were represented as percentage mean ± standard error.

RESULTS AND DISCUSSION

Detection of fungi by direct plate method

Of 22 rhizosphere soil samples tested from Karachi University campus, 27 species belonging to 19 genera were isolated from all the leguminous trees soils collected. Total number of 26 species were isolated from *P. juliflora* including *Absidia* sp., *Acremonium* sp., *Alternaria alternata* (Fr.) Keissler, syn, *Aspergillus flavus* (Link ex Gray), *A. flavipes, A. niger* Van Tieghem, *A. japonicus, A. wentii* Wehmer, *Chaetomium globosum* (Kunz ex Staud), *C. indicum* (Corda), *Cunninghamella elegans* Matr., *Curvularia lunata* (Wakker), *Drechslera maydis* (M.B. Ellis), *Fusarium oxysporum* (Schlect. & Hans), *F. solani* (Mart.) Appel & Wollenw, *Geotrichum candidum, Macrophomina phaseolina* (Tassi) Goid, *Monoascus ruber, Monilia* sp., *Mucor* sp., *Myrothecium roridum* (Tode ex Steudel), *Phoma eupyrena, Paecilomyces variotii, Rhizopus* sp., *Trichoderma viride* Pers. ex Gray and *T. americana. A. pavonina* showed 22 species which was followed by *L. leucocephala* soil (21 species). *A. flavus* and *A. niger* were the predominant species of the genus *Aspergillus* which were isolated from all the 22 samples collected. Cotty (2001) recorded the same result in desert leguminous trees in uncultivated areas. *Aspergilli* are the most frequently occurring in soil caused rapidly colonization of roots and degrade organic matter (Austwick, 1965). It was recorded that *Monoascus rubber* and *Acremonium* sp., was absent from the rhizosphere soil of *A. pavonina* and *L. leucocephala* (Table 1).

Detection of fungi by serial dilution method

Fourteen fungal species belonging to 12 genera were isolated from rhizosphere soil of the leguminous trees studied. Of these 12 species belonging to 11 genera were isolated from *P. juliflora,* 12 species with 11 genera from *A. pavonina* while 11 species with 9 genera were isolated from *L. leucocephala* rhizosphere soil (Table 2). Fungi which were isolated from leguminous trees namely *Alternaria alternata* (Fr.) Keissler, syn, *Aspergillus flavus* (Link ex Gray), *A. niger* Van Tieghem, *A. japonicus, Chaetomium globosum* (Kunz ex Staud), *Curvularia lunata* (Wakker), *Drechslera maydis* (M.B. Ellis), *Fusarium oxysporum* (Schlect. & Hans), *Mucor* sp., *Myrothecium roridum* (Tode ex Steudel), *Phoma eupyrena, Paecilomyces variotii, Rhizopus* sp., and *Trichoderma viride* Pers. ex Gray. Least number of fungi was recorded from rhizosphere soil of *L. leucocephala* while highest number was isolated from *P. juliflora.* Sahu & Agarwal (2004) detected some important genera of fungi from *L. leucocephala* including *Alternaria, Aspergillus, Fusarium* and *Phoma.*

Comparing between two methods used, direct plate method showed highest number of fungi which were contradiction to the findings of Tariq *et al.* (2008); Naveenkumar *et al.* (2011). Fungi accommodate an important part of ecosystem and play an important role in biomass turnover. However, in future extensive study on the mycoflora of rhzosphere soil of leguminous trees should be carried out.

Table 1. Direct plating technique of rhizosphere soil of leguminous trees.

Organisms	*P. juliflora*	*A. pavonina*	*L. leucocephala*
Absidia sp	+	+	+
Acremonium sp	+	-	-
Alternaria alternata	+	+	+
Aspergillus clavatus	-	+	-
A. flavus	+	+	+
A. flavipes	+	-	+
A.niger	+	+	+
A. japonicus	+	+	+
A.wentii	+	+	+
Chaetomium globossum	+	+	+
C. indicum	+	-	-
Cunninghamella elegans	+	+	+
Curvularia lunata	+	+	+
Drechslera maydis	+	+	+
Fusarium oxysporum	+	+	+
F.solani	+	+	+
Geotrichum candidum	+	+	+
Macrophomina phaseolina	+	+	-
Monascus rubber	+	-	-
Monilia sp.	+	+	-
Mucor sp.	+	+	+
Myrothecium roridum	+	-	+
Phoma eupyrena	+	+	+
Paecilomyces variotii	+	+	+
Rhizopus sp.	+	+	+
Trichoderma viride	+	+	+
T. americana	+	+	+
Total species	26	22	21

+ = fungi observed; – = fungi not observed

Table 2. Number of propagule of rhizosphere soil of leguminous trees.

Organisms	*P. juliflora* Mean ± SE	*A. pavonina* Mean ± SE	*L. leucocephala* Mean ± SE
Alternaria alternata	1.43 ± 1.43	2.92 ± 1.46	1.49 ± 1.49
A. flavus	3.08 ± 0.41	2.92 ± 1.48	4.68 ± 0.05
A.niger	3.09 ± 1.55	3.19 ± 1.59	3.08 ± 1.54
A. japonicus	-	-	1.53 ± 1.53
Chaetomium globossum	2.83 ± 1.42	-	-
Curvularia lunata	1.2 ± 1.19	2.93 ± 1.43	1.49 ± 1.49
Drechslera maydis	1.00 ± 1.73	1.49 ± 1.49	-
Fusarium oxysporum	1.33 ± 1.33	1.49 ± 1.49	2.25 ± 1.12
Mucor sp.	10.0 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
Myrothecium roridum	-	3.30 ± 1.51	-
Phoma eupyrena	1.0 ± 1.33	1.43 ± 1.43	1.00 ± 0.99
Paecilomyces variotii	4.69 ± 0.09	5.52 ± 0.03	4.70 ± 0.14
Rhizopus sp.	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00
Trichoderma viride	1.43 ± 1.43	1.56 ± 1.67	2.96 ± 1.51

SE = standard error

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