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MYCOFLORA IN THE RHIZOSPHERE OF SOME WILD PLANTS AROUND KARACHI UNIVERSITY CAMPUS

SHAHNAZ DAWAR*, MASOOMA BATOOL, MARIUM TARIQ AND M. JAVED ZAKI

Department of Botany, University of Karachi, Karachi-75270, Pakistan
**Corresponding author's email: shahnaz_dawar@yahoo.com*

Abstract

This paper deals with data regarding rhizosphere mycoflora of wild plants including *Amaranthus viridis* L., *Chloris barbata* (L.) Swartz and *Tridax procumbens* L. which were collected from different location around Karachi University campus. Result recorded for the properties of rhizosphere showed texture of soil as sandy loam soil, moisture content ranged between 15-31% and basic pH of *C. barbata* while acidic in case of *A. viridis* and *T. procumbens.* 21 fungal species belonging to 16 genera were isolated from rhizospheric soil of wild plants by direct plate method while 28 species belonging to 20 genera were isolated from rhizosphere soil of wild plants by serial dilution method. The result showed that greatest number of fungi were isolated by serial dilution method. Maximum number of fungi were obtained from rhizosphere soil of *C. barbata* whereas lowest number of fungi were recorded from rhizosphere soil of *A. viridis*.

Introduction

Soil is a complex heterogeneous habitat for a wide variety of organisms including bacteria, fungi, protozoan, nematodes and earthworms in which organisms interact with each other and with their physical environment contributing to plant nutrition, soil structure, soil fertility, decomposition of organic matter, cycling of nutrients, suppression of soil borne pathogens and removal of toxins (Prescott *et al.*, 2005; Kirk *et al.*, 2004; Kozdroj & Van Elsas, 2000). Involvement of soil microorganisms are in a wide variety of metabolic and physiological activities that influence the microhabitat. The plant's root with zone of intense microbial metabolic activity occurring where there is a high concentration of carbon is called the rhizosphere. The rhizosphere can be categorized into three portions: endorhizosphere (interior of the root); the rhizoplane (root surface) and the soil directly adjacent and adhering to the root surface (Barea *et al.*, 2005). Rhizosphere soil is said to have a lower pH, lower water potential, lower oxygen pressure and high levels of carbon dioxide than the bulk soil (Suresh & Bagyaraj, 2002). A range of interactions are present in the rhizosphere from beneficial symbiotic relationships to detrimental pathogenic interactions (Sylvia *et al.,* 2005). The role of fungi in soil is extremely complex and is fundamental to the soil ecosystem (Bridge & Spooner, 2001). Soil fungi play an important role in nutrient cycling, plant health and development (Thorn, 1997). Soil also contains carbon, nitrogen and phosphorous in which values are highest at early stage of crops and become decline at the crop maturity (Asghar *et al.*, 2013). Despite of macronutrients, soil also contains heavy metals like Pb, Cd, Cr, and Ni (Khan *et al.*, 2012).

Amaranthus viridis L. (Amaranthaceae) is cosmopolitan weed in the tropical and subtropical regions of the world. It is erect or ascending annual or short-lived perennial herb upto 1 m tall where flowers unisexual, subsessile, green male and female intermixed but female ones more numerous. The leaves of *Amaranthus* species contains tannin, reducing sugar and resin while Amasterol (24-methylene-20-hydroxycholest-5, 7-en -3β-ol) has been observed from the roots. This compound has observed to have allelopathic effects on lettuce seed germination (Jansen, 2004). Shabbir *et al*., (2006) recorded 8 fungal species belonging to 7 genera on seeds of *A. viridis* namely *Alternaria, Aspergillus, Mucor, Helminthosporium, Phoma, Rhizopus* and *Fusarium*.

Chloris barbata (L.) Swartz (Poaceae) is a tufted annual grass up to 90 cm tall which is common in dry, coastal areas (Whistler, 1988). The plant is used in treating Rheumatism and various skin disorders and possesses antidiabetic, antimicrobial properties (Algesaboopathi, 2009). The fungal community structure varied to some extent depending on the season (Shivanna & Vasanthakumari, 2011).

Tridax procumbens Linn (family Compositae or Asteraceae) is a common grass, widespread in the tropical and subtropical parts of the world (Jahangir, 2001). Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhoea, high blood pressure and to check haemorrhage from cuts, bruises and wounds and to prevent falling of hair. It possesses antiseptic, insecticidal, parasiticidal and hepatoprotective properties and has marked depressant action on respiration (Salahdeen *et al*., 2004; Edeoga *et al*., 2005; Ravikumar *et al*., 2005; Saxena & Albert, 2005). Its chemical constitutes are flavonoid, procumbenetin, 1.7% fumaric acid, sitosterol, alkalodies, tannin (Edeoga *et al*., 2005; Mohammad *et al*., 2001). Rajagopal *et al*., (2010) studied least number of endophytic fungi including *Alternaria alternata, Curvularia lunata, Chaetomium* sp., and *Sporormiella minima*.

The basic aim of our present research is to study the rhizosphere mycoflora associated with wild plants.

Materials and Methods

Collection of samples: Twenty two rhizospheric soil samples of wild plants (*Amaranthus viridis*, *Chloris barbata* and *Tridax procumbens*) were collected from different localities of Karachi University Campus. The soil samples were collected brought to the laboratory in sterile polythene bags.

Properties of soil: Soil texture was recorded using Bouyoucos hydrometric method using Gee & Bauder (1986). pH meter (Mettler Toledo mp 220) was used to detected soil pH (Brady, 1990). Soil moisture content was obtained by oven drying the soil and determine the loss in weight (Garrett, 1963) while weight loss method was used to determine organic carbon content of soil (Sparks, 1996).

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 3-5 days at temperature of 30 ± 1°C (Naveenkumar *et al.*, 2011).

b. Serial dilution method: Serial dilution method was carried out according to the method described by Aneja (2001). Soil sample (2.0 g) was suspended in 18 ml sterilized distilled water and mixed well and appropriate in serial dilutions of 1.0 ml aliquots was poured onto sterilized PDA poured Petri dishes containing Benzyl Penicillin (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 3 day 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 & Fennell, 1965; Barnett, 1960; Thom & Raper, 1945). The data were represented as percentage of samples in which a species occured.

Results and Discussion

Rhizospheric soil of wild plants was displayed different properties like *A. viridis* and *C. barbata* have sandy clay loam while *T. procumbens* showed sandy loam soil. Sandy loam soil has low porosity and more permeable as compare to fine textured soil which makes water movement easily drains into and out. These soil are categorized in nutritionally rich but agriculturally problematic due to less aeration (Barbour *et al.*, 1980). Moisture content of both *A. viridis* and *T. procumbens* were ranged from 15-31 while less organic content was recorded from *C. barbata.* Acidic pH was observed by rhizospheric soil of *A. viridis* while *C. barbata* showed basic pH ranged between 7.0 to 7.2. However, all the three wild plants showed more or less same organic carbon ranged between 3.0 to 3.8 (Table 1).

Table 1. Properties of rhizopheric soil of wild plants.

Wild plants	Soil texture	pH	moisture content (%)	Organic carbon (g)
Amaranthus viridis	Sandy clay loam	6.7-6.9	14-29	3.0-3.15
Chloris barbata	Sandy clay loam	7.0-7.2	4-18	3.5-3.9
Tridax procumbens	Sandy loam	6.9-7.4	15-31	3.6-3.8

Table 2. Direct plating technique of rhizosphere soil of wild plants for detection of mycoflora.

Name of fungi	*Amaranthus viridis*	*Chloris barbata*	*Tridax procumbens*
Absidia corymbifera	+	+	+
Alternaria alternata	+	+	+
A. solani	-	+	-
Aspergillus flavus	+	+	+
A. niger	+	+	+
A. ustus	-	-	+
Cladosporium cladosporioides	-	+	-
Curvularia lunata	+	+	+
Drechslera sp.	+	+	-
Fusarium oxysporum	-	+	+
F.solani	-	+	+
Geotrichum sp.	-	+	-
Macrophomina phaseolina	-	+	-
Monodictys sp.	-	+	-
Mucor sp.	+	+	+
Paecilomyces variotii	+	+	+
Penicillium sp.	+	-	-
Phoma eupyrena	+	+	+
Trichoderma harzianum	-	+	-
T. viride	+	+	+
Rhizopus sp.	+	+	+

+ = Fungi observed, - = Fungi not observed

By direct plate method, 21 species belonging to 16 genera were isolated from rhizospheric soil of wild plants. A total number of 11 genera belonging to 12 species including *Absidia corymbifera* (Cohn) Sacc .& Trotter, *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link ex Gray, *A. niger* Van Tieghem, *Curvularia lunata* (Wakker) Boedijn , *Drechslera* sp., (Bugni.) Subram. & Jain ex M.B. Ellis, *Mucor* sp., (Ecicuh ex link) Lind, *Paecilomyces variotii* Bain, *Penicillium* sp., Link ex Fr., *Phoma eupyrena* (Boerema and weler), *Trichoderma viride* Pers.ex Gray and *Rhizopus* sp., (Ecicuh ex link) Lind were isolated from twenty two samples of *A. viridis* collected from Karachi University Campus (Table 2).

Total number of 15 genera belonging to 19 species namely *Absidia corymbifera, Alternaria alternata, A. solani, Aspergillus flavus, A.niger, Cladosporium cladosporioides., Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, F. solani* (Mart.) Appel & Wollenw,, *Geotrichum* sp., *Macrophomina phaseolina, Monodictys* sp., *Mucor* sp., (Ecicuh ex link) Lind, *Paecilomyces variotii, Phoma eupyrena* (Boerema and weler), *Trichoderma harzianum, T. viride* and *Rhizopus* sp., were isolated from twenty two samples of *C. barbata*. However, 10 genera belonging to 13 species were observed from *T. procumbens* plant includes *Absidia corymbifera* (Cohn) Sacc. and Trotter, *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus, A.niger, A.ustus, Curvularia lunata* (Wakker) Boedijn, *Fusarium oxysporum, F. solani* (Mart.) Appel and Wollenw, *Mucor* sp., (Ecicuh ex link) Lind, *Paecilomyces variotii, Phoma eupyrena* (Boerema and weler), *Trichoderma viride* Pers.ex Gray and *Rhizopus* sp., (Ecicuh ex link) Lind (Table 2). Less species were recorded from *A. viridis* while *A. corymbifera, A. alternata, A. flavus, A. niger, C. lunata, Mucor* sp., *P. variotii, P. eupyrena, T. viridi* and *Rhizopus* sp., were isolated from all wild plants. According to Jarosz & Davelos (1995) fungi present in soil may be harmful and causing different plant diseases while some other fungi which antagonize plant pathogens, decompose plant residues and provide nutrients to plant which was helpful in plant growth.

By serial dilution method, 28 species belonging to 20 genera were isolated from rhizospheric soil of wild plants where 22 fungal species belonging to 13 genera were isolated from *A. viridis*, 26 fungal species belonging to 19 genera were isolated from *C. barbata* and *T. procumbens*, yielded 27 fungal species belonging to 18 genera (Table 3). Shivanna & Vasanthakumari (2011) reported that *C. barbata* could provide shelter for a wide range of fungal species in the rhizosphere and presence vary depending on the season, root region and soil nutrient situation. *Aspergillus* spp., particularly *A. flavipes, A. flavus, A. niger, A. ustus* and *A. wentii* were isolated from rhizospheric soil of all three wild plants out of which 100% samples showed presence of *A. niger* in *C. barbata*. Pathogenic fungi like *F. oxysporum, F. solani, M. phaseolina, P. eupyrena, C. lunata, Chaetomium* sp., were also isolated of which *C. lunata* showed highest infection % (18.18%) from all three wild plants.

Table 3. Serial dilution technique of rhizosphere soil of wild plants for detection of mycoflora. Each value in the table is obtained by calculating the percentage of samples in which a species occured.

Fungi	*Amaranthus viridis*	*Chloris barbata*	*Tridax procumbens*
Absidia corymbifera	4.54	9.09	4.54
Alternaria alternata	9.09	-	13.63
A.solani	4.54	13.63	-
Aspergillus clavatus	-	4.54	9.09
A. flavipes	4.54	4.54	4.54
A. flavus	36.36	63.63	50.00
A.fumigatus	18.18	18.18	-
A. niger	59.09	100	72.72
A.ustus	4.54	4.54	13.6
A.wentii	9.09	9.09	22.72
Chaetomium sp.	-	9.09	4.54
Cladosporium cladosporioides	4.54	9.09	-
Cunninghamella elegans	-	4.54	4.54
Curvularia lunata	18.18	18.18	18.18
Drechslera sp.	13.6	13.6	4.54
Fusarium oxysporum	4.54	4.54	9.09
F.solani	13.6	9.09	13.63
Geotrichum sp.	-	9.09	9.09
Humicola sp.	-	-	13.63
Macrophomina phaseolina	-	9.09	4.54
Monoascus sp.	-	13.63	4.54
Monodictys sp.	4.54	4.54	-
Mucor sp.	-	-	9.09
Paecilomyces variotii	18.18	31.81	27.27
Penecillium sp.	-	4.54	-
Phoma eupyrena	13.6	9.09	18.18
Trichoderma viride	31.81	22.72	31.31
Rhizopus sp.	9.09	-	-

In comparison to direct plating method (19 species), highest number of fungi were recorded from serial dilution method (28 species). Our findings were supported by Naveenkumar *et al*., (2011); Tariq *et al*., (2008) in which serial dilution method showed maximum number of fungi (20 species) as compared to direct plating method (18 species) and Warcup method. Serial dilution method is step wise method and due to its simpler, cheaper and helpful method, the results obtained are more manageable (Aneja, 2001). The rhizospheric soil of wild plants supports an abundance of diverse saprophytic microorganisms. This could be due to high input of organic carbon compounds into the soil through the process of rhizodeposition (Rovira, 1956; Merckx *et al.,* 1987). Among the most frequently occurring fungal species in the rhizosphere and rhizoplane of grasses, *Aspergillus flavus*, *Clonostachys rosea*, *Penicillium citrinum* and *Trichoderma harzianum* have also been frequently isolated from other species of grasses (Abdel-Hafez, 1982; Al-NurEl-Amin & Saadabi, 2007). Present experimental result also showed infection of *A. flavus*, *Penicillium* sp., and *Trichoderma harzianum.*

Our data suggested that methods used in detection of rhizosphere soil mycoflora were effective in large number of fungi isolation and these play vital roles in biomass turnover and form an important part of ecosystem (Jones & Hyde, 1988). Extensive study on these fungi associated with wild plants of different areas of city is required which showed a better picture of mycoflora associated with *A. viridis, C. barbata* and *T. procumbens.*

References

Abdel-Hafez, S.I.I. 1982. Rhizosphere and rhizoplane fungi of *Triticum vulgare* cultivated in Saudi Arabia. *Mycopathologia,* 78: 79-86.

Algesboopathi, C. 2009. Ethanomedicinal plants and their utilization by villagers in Kumaragiri hills of Salem District of Tamil Nadu. *Afr. J. Traditional Complementary and Alternative Medicines,* 6(3): 222-227.

Al-NurEl-Amin and A.M.A. Saadabi. 2007. Contribution to the knowledge of soil fungi in Sudan rhizosphere mycoflora of sugarcane at Kenana sugar estate. *International Journal of Botany,* 3: 97-102.

Aneja, K.R. 2001. *Experiments in Microbiology, Plant pathology and biotechnology*. New age international publishers. Vol 4. pp. 157-162.

Asghar, I., M. Akmal, M. Ishtiaq, M. Maqbool and T. Hussain. 2013. Analysis os soil microbial biomass dynamics in rainfed wheat fields in arid zone of Pakistan. *Pak. J. Bot.,* 45(SI): 389-399.

Barbour, M.G., J.H. Bark and W.D. Pitts. 1980. *Terrestrial Plant Ecology.* Meulo Park, California.

Barea, J., M.J. Pozo and C. Azcon-Aguilar. 2005. Microbial co-operation in the rhizosphere. *Journal of Experimental Botany,* 56(417): 1761-1778.

Barnett, H.L. 1960. *IIlustrated genera of imperfect fungi* (second edition). Burgess Pub. Co. pp. 225.

Booth, C.1971. *The genus Fusarium*. Common Wealth Mycological Institute, Kew Surrey, England. pp. 237.

Brady, N.C. 1990. *The Nature and Properties of Soils.* 10th ed. Macmillan pub. Company. New York.

Bridge, P. and B.M. Spooner. 2001. Soil fungi: diversity and detection. *Plant and Soil,* 232: 147-154.

Domsch, K.W., W. Gams and T. Anderson. 1980. *Compendium of soil fungi*. Academic Press, London. pp. 859.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotech.,* 4(7): 685-688.

Ellis, M.B. 1971. *Dematiaceous hyphomycetes*. CMI, Kew, Surrey, England. pp. 608.

Garrett, S.D. 1963. *Soil fungi and soil fertility.* Oxford, Pergamon Press. pp. 165.

Gee, G.W. and J.M. Bauder. 1986. Particle-size analysis. *In: Methods of Soil Analysis, Part I, Physical and Mineralogical Methods.* Agronomy Monograph No. 9, 2nd Edition. American Society of Agronomy, Madison, WI, USA, pp. 383-411.

Jahangir, M. 2001. Chemical and Biological studies on some members of Asteraceae family and Pseudocalymma elegans, a Native of Brazil. Ph.D. Thesis submitted to the International Centre for Chemical Sciences H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan.

Jansen, P.C.M. 2004. *Amaranthus viridis* L. *In: vegetables/Legumes*. Grubben, G.J.H and O.A. Denton (Eds.). PROTA, Wageningen, Netherlands.

Jarosz, A.M. and A.L. Davelos. 1995. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. *New Phytologist.,* 129: 371-387.

Jones, E.B.G. and K.D. Hyde. 1988. Methods for the study of mangrove marine fungi. *In: mangrove microbiology; Role of microorganisms in nutrient cycling of mangrove soils and waters*. Agate, A.D., C.V. Subramaniam and H. Vannucci (Eds.). UNDP. pp. 9-27.

Khan, I.U., J.K. Muhammad, U.K. Naqib, J.K. Mohammad, R. Habib, B. Zarina and U. Kalim. 2012. Wastewater impact on physiology, biomass and yield of canola (*Brassica napus* L.). *Pak. J. Bot.,* 44: 781-785.

Kirk, J.L., L.A. Beaudette, M. Hart, P. Moutoglis, J.N. Klironomos, H. Lee and J.T. Trevors. 2004. Methods of studying soil microbial diversity. *Journal of Microbiological Methods,* 58: 169-188.

Kozdroj, J. and J.D. Van Elsas. 2000. Application of polymerase chain reaction denaturing gradient gel electrophoresis for comparison of direct and indirect extraction methods of soil DNA used for microbial community finger-printing. *Biology and Fertility of Soils,* 31: 372-378.

Merckx, R., A. Dijkstra, A. Hartog den and J.A. Van Veen. 1987. Production of root derived material and associated microbial growth in soil at different nutrient levels. *Biology and Fertility of Soils,* 5: 126-13.

Mohammad, A., R. Earla and R. Ramidi. 2001. A new flavonoids from the aerial parts of *Tridax procumbens* Linn. *Fitoterapia,* 72(3): 313-315.

Naveenkumar, K.J., B. Thippeswamy, B.V. Thirumalesh, K. Pradeepa and Venkatesh. 2011. Comparative study of fungal diversity in the agricultural and non-agricultural soil in Bhadravathi taluk, Shimoga district, Karnataka, India. *Journal of Research in Biology,* 2: 129-134.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species, An illustrated manual of identification*. The University Press, University Park, Pennsylvania, pp. 203.

Prescott, L.M., J.P. Harley and D.A. Klein. 2005. The epidemiology of infectious disease. *In: Microbiology.* Prescott, L.M.., J.P. Harley and D.A. Klein (Eds.). McGraw-Hill, New York, USA, 6th edition. pp. 821-843.

Rajagopal, K., S. Kalavathy, S. Kokila, S. Karthikeyan, G. Kathiravan, R. Prasad and P. Balasubraminan. 2010. Diversity of fungal endophytes in few medicinal herbs of South India. *Asian J. Exp. Biol. Sci.,* 1(2): 415-418.

Raper, K.B. and D.I. Fennell. 1965. *The Genus Aspergillus*. The Williams and Wilkins Company Baltimore, pp. 686.

Ravikumar, V., K.S. Shivashangari and T. Devaki. 2005. Hepatoprotective activity of *Tridax procumbens* against d-galactosamine/lipopolysaccharideinduced hepatitis in rats. *J. Ethnopharmacol.,* 101: 55-60.

Rovira, A. D. 1956. Interactions between plant roots and soil micro-organisms. *Annual Review of Microbiology,* 19: 241-266.

Salahdeen, H.M., O.K. Yemitan and A.R.A. Alada. 2004. Effect of aqueous leaf extract of *Tridax procumbens* on blood pressure and heart rate in rats. *Afr. J. Biomed. Res.,* 7: 27-29.

Saxena, V.K. and S. Albert. 2005. $-Sitosterol-3-O-$-Dxylopyranoside from flowers of *Tridax procumbens* Linn. *J. Chem. Sci.,* 117: 263-266.

Shabbir, A., B. Rukhsana, S. Shazia and S. Sobiya. 2006. Fungal flora associated with seeds of some common weeds and their impact on seed germination. *Mycopath.,* 4(1): 55-56.

Shivanna, M.B. and M.M. Vasanthakumari. 2011. Temporal and spatial variability of rhizosphere and rhizoplane fungal communities in grasses of the subfamily Chloridoideae in the Lakkavalli region of the Western Ghats in India. *Mycosphere,* 2(3): 255-271.

Sparks, D.L. 1996. *Methods of soil analysis, part 3, Chemical methods*. Soil Science Society of America (SSSA), Book series 5.

Suresh, C.K. and D.J. Bagyaraj. 2002. Mycorrhiza-microbe interaction*:* Effect on rhizosphere. *In: Arbuscular Mycorrhizae: Interactions in plants, Rhizosphere and soils.* Sharma, A.K. and B.N. Johri (Eds.). Science Publishers Inc., Hampshire, pp. 7-28.

Sylvia, D., J. Fuhrmann, P. Hartel and D. Zuberer. 2005. *Principles and Applications of soil Microbiology.* Pearson Education Inc, New Jersey.

Tariq, M., S. Dawar and F. S. Mehdi. 2008. Studies on the rhizosphere mycoflora of mangroves. *Turk. J. Bot.,* 32: 97-101.

Thom, C. and K.B. Raper. 1945. *A manual of the Aspergilli.* The Williams and Wilkins Company, Baltimore, Md, 373.

Thorn, G. 1997. The fungi in soil. *In: Modern Soil Microbiology.* Van Elsas, J.D., J.T. Trevors and E.M.H. Wellington (Eds.). New York, Marcel Dekker, 63-127.

Whistler, W.A. 1988. *Checklist of the weed flora of Western Polynesia*. Technical Paper No. 194, South Pacific Commission, Noumea, New Caledonia, 69.

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