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Distribution of VAM Fungi in Tannery Effluent Polluted Soils of Tamil Nadu, India

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In recent years, the disposal of industrial effluents on land has become a common practice in many countries. It is now well recognized that industrial activity over a long period of time has led to enhancement of levels of heavy metals and toxic elements in the soil causing genetical, physiological and ecological problems. It is well established that vesicular-arbuscular mycorrhizal (VAM) fungi form associations with many plants under wide range of soil conditions and tremendously increase the plant growth (Mosse 1981). The mycorrhizal fungi are considered as important tool in afforestation and rehabitation of degraded lands especially in mine spoils, eroded sites, and polluted wastelands.

One of the serious problems of tanneries in India is the disposal of large quantities of waste generated by soaking, washing, pickling and tanning of animal hides. Whether organic or inorganic in composition, the waste materials are mobile and persist in the environment, accumulate through food chains and result in adverse human and ecological effects upon exposure. Toxicity and environmental effects of tannery wastes particularly for their effect on soil properties and ground water table are well documented (Sastry and Prasad 1980). The potential of microbes in detoxicifying these wastes through catabolism or selective accumulation is also a fact (Peyton 1984). There are some reports on VA-mycorrhizae from disturbed and polluted soils (Arnold and Kapustka 1987; Bajwa et al. 1991; Gildon and Tinker 1981; 1983 a, b; Kooman et al. 1990; Leyvel et al. 1991) which suggest the necessary of the selection of tolerant strains.

There is no literature available on the relationship between mycorrhizal fungi and tannery effluent polluted soils. The present study was conducted to assess the mycorrhizal profile of tannery effluent polluted wastelands of North Arcot district, Tamil Nadu, India.

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MATERIALS AND METHODS

North Arcot district, Tamil Nadu, India, not only known for leather exports but also known for the high rate of water pollution due to tanneries, dying industries,electroplating industries, and also agricultural and domestic wastes. Three sites of tannery effluent polluted soils (site-1 : Ranipet; site-2 : Ambur; site-3 : Vaniyambadi) were selected to study the mycorrhizal association of plants in three different seasons (summer, rainy and winter). In all the three study sites, 22 plant species belong to 20 families of angiosperms were selected for the survey for three different seasons. Soil samples of about 1 kg were collected at random from rhizosphere by digging to a depth up to 50 cm with a trowel. The soils were kept in polythene bags, labelled and stored at 4°C until analysis.

Fine root samples were collected from a minimum of 4 plants of each species and fixed in FAA (Formalin 13 ml, glacial acetic acid 5 ml, 50% ethyl alcohol 250 ml). Collections were made every month. Mycorrhizal infection was observed following the method of Phillips and Hayman (1970) as modified by Koske and Gemma (1989). The percentage of root infection was estimated by gridline intersect method (Giovannetti and Mosse 1980).

VAM fungal spores were recovered from the soil samples by wet sieving and decanting method (Gerdemann and Nicolson 1963) followed by modified sucrose centrifugation method (Smith and Skipper 1979). The identification of VAM fungal spores was done according to the manuals of Raman and Mohankumar (1988) and Schenck and Perez (1990). The identification of spores was done based on examination of 10 spores of each species. Total spore number was counted using MPN technique (Porter 1979).

Soil pH (1:2, soil: water) and temperature of the effluent were measured at the field site by a pH meter and a soil thermometer, respectively. Total nitrogen, available potassium (Sankaram 1966), soil organic matter (Walkley and Black 1934), available phosphorus (Olsen et al. 1954) were estimated. Microelements such as Cr, Zn, Cu, and Pb were analysed by using atomic absorption spectrophotometer. For effluent collection from polluted areas, standard procedures were followed for sampling, preservation and analyses of the effluent (APHA 1985).

RESULTS AND DISCUSSION

From the three sites of effluent polluted soils, 15 species of VAM fungal spores were isolated and identified. Names and characteristics of the VAM fungal spores are presented in Table-l. Out of the 22 plant species

screened for VAM fungal colonization, 19 plant species exhibited positive results with percentage of infection differing in each season. The root squashes of 19 test plants contained both arbuscules and vesicles. Young roots of most of the plants were arbuscular in nature and mature roots possessed number of intercellular vesicles and complex hyphal network.

The percentage of mycorrhizal colonization from three study sites varied between 11 and 64%. The percentage increased in summer season and declined in rainy season (Table-2). The soils of study sites were found to be clayey loam and alkaline (pH 7.6 - 8.1) (Table-3). The per cent organic matter varied between 22 to 29%. Total nitrogen (132.5-177.5 kg/ha), available phosphorus (72.4-97.5 kg/ha), available potassium (K 320-345 kg/ha) were considered low, the amounts of microelements present were in higher concentrations in the polluted soils. The chemical analysis of effluent samples is presented in Table- 4. The pH of the effluents was alkaline, ranged from 8.2 to 8.8. BOD, COD, total sulphides, total chlorides and chromium were high in samples of all the 3 sites.

Analysis of rhizosphere soil of the sites, revealed the presence of 15 VAM fungal species and they belong to 4 VAM genera namely, *Glomus* (9),*Gigaspora* (3), *Scutellospora* (3), and *Sclerocystis* (1). Out of 22 plant species examined, 19 plants were mycorrhizal. Among the plant species examined, *Prosopis juliflora, Azadiracta indica, Heliotropium zeylanicum* were associated with maximum number of VAM fungi. Many VAM fungal species were found to be associated with more than one plant species, among them *Gl. fasciculatum, Gl. geosporum, Gigaspora gigantea* were found to be associated with maximum number of plant species, which were incidently called common endophytes in polluted soils examined.

The polluted soils and their effluents were found to be alkaline. It is known that polluted soils of particular depth (0-30 cm) was considered to be not only region of maximum mycorrhizal incidence but also reported to be rich in heavy metals (Lepp and Dickinson, 1977).

In the present study, 15 species of VAM fungi were reported in polluted habitat, whereas Gildon and Tinker (1981) have isolated only *Glomus mosseae* and Dueck *et al.* (1986) have isolated *Gl. fasciculatum* alone. VAM sporulation was maximum in winter but declined in other seasons. This is in agreement with Steffeldt and Vogt (1975) and Mattew *et al.* (1990). During rainy season not only the number of spores, the per cent infection also decreased. Wet condition in the soil usually retard the VAM fungal infection (Hayman, 1983).

Table 1. Description of VAM fungal species isolated from tannery effluent polluted soils of North Arcot district, Tamil Nadu, India.

Fungal Species	Specie code	Spore shape	Spore size (µm)	Nature of the spore	No.of wall group	Spore Wall width (µm)	Wall Morpho-logy	Nature & hyphal dia
GLOMUS (9)								
G.aggregatum	LAGR	Globose-Sub globose	26-97	Yellow -brown	1-2	3-7	Coloured laminated	Straight curved (12 µm)
G.citricola	LCTC	Globose	46-81	Brown-Yellow brown	2	4-8.5	Laminated	Straight (6 µm)
G.constrictum	LCST	Globose-Sub globose	155-279	Dark brown	1	9-12.5	One layered	Straight curved constricted (14 µm)
G.etunicatum	LETC	Globose-Sub globose	76-151	Yellow -brown	1	5-13	Inner Wall laminated	Cylindrial (22 µm)
G.fasciculatum	LFSC	Globose-Sub globose	68-148	Brown	1	7-11	Smooth, thick	Straight (15µm)
G.geosporum	LGSP	Globose-Sub globose	110-285	Dark brown	1	4-18	3-layered	Recurved (22 µm)
G.heterosporum	LHTS	Globose-Sub globose	32-100	Brown	1	2-4	Inner laminated	Frequently branched (6 µm)
G.microcarpum	LMRC	Globose	30-45	Yellow	1	4-6	Laminated	Straight
G.multicaule	LMTC	Globose-Sub globose	152-160	Dark-brown	1	9-20	Finely laminated	Straight (36 µm)
GIGASPORA (3)								
Gig. albida	GABD	Spherical	145-320	Yellow	1	4-11	Outer smooth Inseprable	27-47 µm
Gig. gigantea	GGGT	Ellipsoid	330-618	Yellow -green	1	2.5-7	Outer tightly covering inner	40-50 µm
Gig. rosea	GRSA	Globose-sub globose	230-300	Hya-line	1	2.5-7.5	Outer smooth	30-38 µm
SCUTELLOSPORA (2)								
Scu. erythropa	CERT	Globose irregular	225-360	Brown	2	4-16	Membrano us	35-60 µm
Scu. nigra	CNGR	Spherical	300-470	Black	2	nr	Outer pitted	40-100 µm
SCLEROCYSTIS (1)								
Scl. pachycaulis	SPCC Sporo-carp	Globose	172-250	Yellow -brown	2		Peridium unknown	
	Spore	Sub-globose	27-70	Yellow	2	2-5.5	Separable outer layer	

nr - not reported

Table 2. Seasonal variations in mycorrhizal profile of plant species occurring in tannery effluent polluted soils of North Arcot district, Tamil Nadu, India

Plant species	Hat.	Frq.	Summer		Rainy		Winter		VAM fungal species
			% inf	Sp.pop	% inf	Sp.pop.	% inf	Sp.pop	
SITE-1									
Asclepiadaceae *Calotropis gigantea* (L.) R.Br.	Sh	+	32	68	24	67	24	97	LCST,LFSC, GGGT
Boraginaceae *Heliotropium zeylanicum* (Burm.f.) Lam.	Hb	+++	40	87	30	82	36	104	LCST,LFSC LGSP,GGGT
Compositae *Parthenium hysterophorus* L.	Hb	++	49	90	32	72	41	116	LFSC,LGSP GGGT, SPKS
Cyperaceae *Cyperus* sp.	Hb	++	-	26	-	18	-	20	LFSC, CERT
Euphorbiaceae *Croton sparsiflorus Morong.*	Hb	++	24	101	20	93	22	105	LCTC, LFSC, GABD
Malvaceae *Sida cardifolia* L.	Hb	++	16	120	16	108	12	135	LFSC, LMRC, LMTC
Meliaceae *Azadirachta indica* Adc.Juss.	Hb	++	59	198	51	178	57	203	LFSC, LGSP, GGGT, CERT, SPKS
Mimosoideae *Prosopis juliflora* (Sw.) DC.	Sh	+++	56	186	52	157	48	205	LAGR,LFSC, LGSP, GGGT, CERT, CNGR
Pedaliaceae *Pedalium murex* L.	Hb	++	52	204	50	194	50	218	LETC,LFSC, LGSP, LHTS
Zygophyllaceae *Tribulus terrestris* L.	Hb	+	27	118	18	100	19	102	CAGR, LCTC, LGSP, GABD
SITE-2	Hb	+	23	146	20	148	20	191	LCTC, LGSP, GRSA, SPKS
Achyranthaceae *Achyranthus Lanata L.*	Hb	+	23	146	20	148	20	191	LCTC, LGSP, GRSA, SPKS
Amaranthaceae *Amaranthus viridis* L.	Hb	+	20	98	11	60	16	74	LFSC, LGSP
Aristolochiaceae *Aristolochia bracteata Retz.*	Hb	+	-	20	-	12	-	30	LHTS, LGSP
Boraginaceae *Heliotropium zeylanicum* (Brum.f) Lam.	Hb	+++	54	201	42	192	52	226	LAGR,LCST, LFSC,LGSP
Cactaceae *Opuntia stricta* (Haw).Haw	Sh	+	28	79	23	70	28	100	LAGR,LFSC
Caesalpinoideae *Cassia auriculata* L.	Hb	+	48	104	45	92	50	128	LAGR,LCST,GABD
Compositae *Eclipta alba* (L.)Hassk.	Hb	+	44	103	32	98	39	110	LGSP,LMRC, LMTC,CERT,

Table 2. Continued.

Parthenium hysterophorus L.	Hb	++	46	118	38	92	42	156	LCTC,LFSC, LGSP,GGGT,SPKS
Cucurbitaceae *Coccinia indica* Wright & Arn	Vi	+	26	72	18	70	25	100	LETC, LFSC
Malvaceae *Pavonia zeylanica* (L.) Cav.	Sh	+	18	77	18	72	17	89	LCTC, GGGT
Meliaceae *Azadirachta indica Adr. Juss.*	Tr	++	60	194	53	162	54	210	LFSC,LGSP, LHTS, GGGT, CERT
Mimosoideae *Prosopis Juliflora* (Sw.) DC.	Sh	+++	64	168	60	152	61	212	LFSC,LGSP, LHTS, GGGT, CNGR
Pedaliaceae *Pedalium murex L.*	Hb	++	50	172	40	154	48	185	LFSC,LHTS, GGGT
Verbenaceae *Clerodendrum inerme* (L.) Gacrtn.	Hb	+	32	94	21	90	32	99	LFSC, GGGT
SITE-3									
Amaranthaceae *Gomphrena globosa* L.	Hb	+	-	34	-	20	-	99	LFSC, LGSP
Asclepiadaceae *Calotropis gigantia* (L.) R.Br	Sh	++	32	124	21	90	30	151	LFSC, SPKS
Boraginaceae *Heliotropium zeylanicum* (Brum.f.) Lam	Hb	++	54	219	50	201	54	261	CFSC, LGSP, LMTC,GGGT,CERT
Compositae *Parthenium hysterophorus* L.	Hb	++	38	69	33	60	36	99	LFSC,LGSP, GGGT
Cyperaceae *Cyperus* sp	Hb	++	-	45	-	44	-	45	LFSC,LGSP
Mimosoideae *Prosopis juliflora* (Sw.) DC.	Sh	+++	53	205	46	168	55	246	LCST,LFSC, GGGT,CERT,CNGR
Meliaceae *Azadirachta indica* Adr.Juss	Tr	++	52	187	41	159	50	232	LFSC, LGSP,CMRC,GGGT
Solanaceae *Datura metel* L.	Hb	+	30	98	18	79	27	103	LCTC,LFSC, CERT
Vitaceae *Vitis lanata Roxb.*	Sh	+	18	91	16	80	21	118	LAGR,LFSC, LGSP

Hb Herb., Sh - Shrub, Tr-Tree, +++ - Dominant, ++-Sub-dominant. +-Occasionally present.
% int-percentage of infection. Sp.pop - Spore population per 100 g of soil.

Table 3. Physico-chemical characteristics of tannery effluent polluted soils of North Arcot District, Tamil Nadu, India

Study Site	pH	Ec	Organic matter (%)	Soil type	Kg/ha			mg/kg			
					N	P (P_2O_5)	K (K_2O)	Cr	Zn	Cu	Pb
Site-1	7.8	3.2	22	Clayed loam	132.5	22.4	320.0	179	2.1	0.99	1.19
Site-2	7.6	3.0	20	Clayed loam	145.0	97.5	345.0	153	1.9	0.87	0.97
Site-3	8.1	3.9	29	Clayed loam	177.5	92.5	342.5	210	1.8	0.90	1.08

Values are mean of 10 replicates with standard error 2%

Table 4. Chemical analysis of tannery effluents collected from study sites of the North Arcot district, Tamil Nadu, India.

Study Site	pH	Temp (°C)	Total sulphides	Total chlorides	BOD_5 (20°C)	COD	Cr	Zn	Cu	Pb	Phosphates
						ppm					
Site-1	8.9	31	1341	2510	520	702	1761	21.2	1.76	1.51	8.5
Site-2	8.2	30	1021	2318	512	612	1438	20.9	1.81	1.41	8.1
Site-3	8.8	31	1347	2376	575	751	1805	18.1	1.84	1.49	8.7

Values are mean of 10 replicates with standard error 2%

Koske (1987) reported that volatile substances and heavy metals reduced the rate of germination and colonization of VAM fungi. Even in the soils with high concentration of heavy metals, VA mycorrhizal fungi associated with most of the plants and able to survive in the tannery effluent polluted environment. *Prosopis juliflora,* and *Azadirachta indica* are the potential tree species grown well in the tannery polluted environment and associated with maximum number of mycorrhizal fungi. *Glomus* species are prevalant in tannery effluent polluted soils, indicate their adaptability to heavy metal exposure. *Glomus fasciculatum, Gl. geosporum, Gigaspora gigantea* are dominant mycorrhizal symbionts in the polluted environment. Suitable mycorrhizal fungi and host plants will make them sustainable one in the tannery effluent polluted soils.

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