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ISOLATION OF FUNGI FROM *AVICENNIA MARINA*

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Abstract

A total number of 37 species of fungi belonging to 21 genera were isolated from different parts of *Avicennia marina* plant collected from different coastal areas of Pakistan. Of these 16 fungal species are new reports from Pakistan. Of the different plant parts, highest numbers of fungi were isolated from leaves followed by stem and pneumatophore. Greatrr numbers of fungi were observed from Sandspit, West wharf and Boat basin.

Introduction

Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines. Pakistan has a coastline of about 1,000 km. The Indus River delta extends over 250 km from Sir Creek at the Indian Border and Karachi in the west with about 250,000 ha of mangroves (Khan, 1966; Mirza *et al.*, 1983). *Avicennia marina* is the dominant species. The other species that comprises less than 5% are *Aegiceras corniculatum, Burguiera conjugata, Ceriops tagal, C. roxburghiana, Rhizophora mucronata, R. apiculata* and *Sonneratia caseolaris* (Saifullah, 1982). In Pakistan direct utilization of mangroves is restricted to only few types, of this fuel and fodder is the major ones. The consumption of mangroves as fuel and fodder by local people exceeds the sustainable yield (Saifullah, 1997). Approximately there are 125,000 persons in the Indus delta region and most of them rear camels, cattle and goats which either graze or are stall fed on mangrove foliage. The estimated 16,000 camels browse exclusively on *A. marina* foliage (Qureshi, 1995). The mangrove plant part is of various uses like bark used for tanning and dye. Leaves are the source of a black or chestnut dye (Burkill, 1966). It is reported that mangrove is a folk remedy for angina, diabetes, diarrhoea, dysentery, hematuria and haemorrhage (Duke & Wain, 1981). There is a trend to study mangroves as an ecosystem and as such all related living and non-living components are being considered. Fungi make a very important part of the ecosystem along with other microbes of the biomass (Hyde, 1990,1992; Harrison *et al.*, 1994; Jones *et al*., 1988), but unfortunately they have revealed very little information. Fungi growing on mangroves from different parts of the world have been reported (Hyde, 1988, Hyde & Jones, 1988, Jones & Tan, 1987). Mehdi & Saifullah (1992, 2000) reported the species diversity and seasonal occurrence of fungi on seedlings of *A. marina*. Experiments were therefore carried out to study the occurrence of fungi from different parts of *A. marina.*

Materials and Methods

Mangrove plant parts were collected from different coastal areas viz., Clifton, Sandspit, Mai Kalachi, Port Qasim, Korangi naddi, Boatbasin, West wharf, Sommiyani, Rahri, Shahbunder and Ketibunder. Untreated parts of mangrove plants (leaves, stem and pneumatophore) after surface disinfection with 1% $Ca(OCl)_2$ for 10 minutes were placed

on potato dextrose agar (PDA). Five pieces were placed in each Petri dish and the dishes were incubated for 5-7 days at 24±1ºC. The fungi growing on plates were identified after reference to Ellis (1971); Domsch *et al*., (1980); Nelson *et al*., (1983); Raper & Fennel, (1965).

Data were analyzed and subjected to analysis of variance (ANOVA) followed the procedure as given by Gomez & Gomez (1984).

Results and Discussion

A total number of 37 species of fungi belonging to 21 genera viz., *Absidia corymbifera* (Cohn) Sacc.& Trotter, **Alternaria alternata* (Fr.) Keissler, syn, **A. citri* Ellis & Pierce apud Pierce, **A. raphani*, **A. tenuissima* (Kunze ex Pers.) Wiltshire, **Arthrobotrys* sp., **Aspergillus candidus* Link ex Link, *A.flavus* Link ex Gray, *A. fumigatus* Fres, *A. niger* Van Tieghem, **A. parasiticus* Speare, *A. sulphureus* (Fres.) Thom & Church, **A. sydowii* (Bain. & Sart.) Thom & Church, *A. terreus*, **A. ustus* (Bain.) Thom & Church, **A. wentii* Wehmer, *Botrytis cinerea* Pers. ex Nocca & Balb, *Chaetomium chrispatum* Fuckel, *C. funicola* Cooke, *C. globossum* Kunze ex Steud, *Cladosporium cladosporioides* (Fres.) de Vries, **Cunninghamella* sp., *Drechslera australiensis* (Bugni.) Subram. & Jain ex M.B. Ellis, *D.hawaiiensis* (Bugni.) Subram. & Jain ex M.B. Ellis, *Fusarium oxysporum* Schlechtendahl, *F. solani* (Mart.) Appel & Wollenw, *Humicola fuscoatra* Traaen, **Macrophomina phaseolina* (Tassi) Goid, **Memnoniella echinata* (Riv.) Galloway, **Monilia* sp., *Mucor* sp., **Nigrospora oryzae* Zimmerm, *Penicillium* sp., *Rhizopus stolonifer* (Ecicuh ex Link) Lind, **Scopulariopsis brevicaulis* (Sacc.) Bain, *Syncephalastrum* sp., Schröt and *Trichoderma viride* Pers.ex Gray were isolated from different plant parts of *A. marina* viz., leaves, stem and pneumatophore. Of those fungal species marked with an asterisk are new reports from Pakistan (Mehdi & Saifullah 1992a, 1992b, 2000). Present work showed that 21 genera were observed from *A. marina* as compared to the report of Mehdi & Saifullah (2000) where they isolated 23 species from the seedlings of *A. marina*. Of the different samples of *A.marina* tested from 13 localities, one sample was found to be infected with *F. oxysporum* where 0.4% infection was observed in non-sterilized stem of *A. marina* (Table 1). About 95% samples of *A. marina* plant parts were found to be infected by *A. alternata* with an infection range of 2.2-21, 3-20% in surface sterilized parts and 2-21% in non-sterilized parts of *A. marina*. *M. phaseolina* was found only in surface sterilized stem and root with infection range of 0.2-1%. All plant parts of *A. marina* were infected with *A. flavus* and *A. niger*. Their infection % is higher in leaves and stem followed by pneumatophore in non-sterilized parts (11-36%). Surface sterilization of plant parts with $Ca(OCl)_2$ significantly reduced the incidence of *A. flavus* (4-34%). Infection of *F. solani* was recorded from 11 samples, 21% in surface sterilized stem and 16% infection in non sterilized stem whereas in leaves 10 samples were found to be infected, 17% in surface sterilized and 13% in non sterilized plant part. Of the plating method used for isolation of fungi from different parts, leaves yielded highest number of fungi as compared to stem and pneumatophore. Such similar results were observed by Mehdi & Saifullah (1992) who reported maximum number of species on leaves. In *A. marina* the representatives of Deuteromycotina were the dominant one, the members of Zygomycotina and Ascomycotina were also found but no fungus was observed from oomycota phyllum. Such similar results have been observed by Fatima & Saifullah (2000) that Deuteromycotina was the most common and dominant group of *A. marina*. *A. marina* samples of Sandspit and

Table 1. Isolation of fungi from *Avicennia marina* plant parts.

Name of fungi	Sterilized						Non-sterilized					
	Leaves		Stem		Pneumatophore		Leaves		Stem		Pneumatophore	
	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD
Absidia corymbifera	2	4.23±27.57	2	1.58±19.01	2	1.69±11.3	3	5.56±29.37	2	4.76±38.8	2	2.61±2.8
Alternaria alternata	11	14.97±15.8	14	17.82±19.08	4	17.82±19.0	12	19.81±21.31	14	19.56±16.22	4	2.25±5.92
A.citri	1	0.307± 0.00	1	0.307±0.00	0	0	0	0	1	0.15±0.00	0	0
A.raphanii	0	0	0	0	0	0	1	0.38±0.00	0	0	0	0
A.tenniusima	1	0.07±0.00	0	0	0	0	1	0.307±0.00	0	0	0	0
Arthobotrytus sp.	0	0	1	1.15±0.00	0	0	0	0	0	0	0	0
Aspergillus candidus	5	2.622±3.306	6	3.71±10.59	2	0.28±0.24	4	2.07±2.98	5	4.33±11.39	1	0.30±0.00
A.flavus	15	31.95±34.35	15	31.15±37.56	6	6.46 ±10.94	15	30.89±25.32	15	33.41±26.62	6	17.2±18.11
A.fumigatus	9	10.84±7.53	7	10.25±9.95	3	1.4±2.64	7	13.07±10.08	10	9.91±9.37	2	1.91±11.94
A.niger	14	19.28±16.08	14	13.98±11.84	6	10.19±12.19	15	43.48±30.40	14	46.78±32.5	6	1.46±4.378
A.paraciticus	2	1.53±11.31	2	0.777±3.53	3	2.044±5.36	5	10.44±30.98	3	3.66±14.81	3	0
A.sulphureus	7	8.94±19.08	5	2.71±6.88	1	0.38±0.00	9	11.11±18.74	7	4.06±5.21	3	0
A.sydowii	1	0.46±0.00	2	0.307±0.00	0	0	1	0.69±0.00	2	0.38±0.70	0	0
A.terreus	1	0.38±0.00	1	0.61±0.00	0	0	1	0.53±0.00	1	0.61±0.00	0	1.66±4.37
A.ustus	1	0.38±0.00	1	0.07±0.00	0	0	1	0.61±0.00	0	0	0	0
A.wentii	2	0.69±5.12	3	0.69±2.64	3	1.177±6.55	6	2.354±5.621	7	4.622±5.81	4	0
Botrytis cinera	2	1.46±3.53	2	0.53±2.12	1	1.38±0.00	2	1.07±1.41	0	0	1	2.61±0.00
Chaetomium chrispatum	0	0	0	0	0	0	0	0	1	0.21±0.00	0	0
C.funnicola	0	0	1	0.15±0.00	0	0	0	0	0	0	0	0

Table 1. (Cont'd.)

Name of fungi	Sterilized						Non-sterilized					
	Leaves		Stem		Pneumatophore		Leaves		Stem		Pneumatophore	
	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD
C.globossum	3	0.38±0.00	1	0.38±0.00	1	0.15±0.00	2	0.38±1.18	2	0.58±4.00	1	0.07±0.0
Cladosporium cladosporidales	4	2.82±13.19	3	2.23±8.02	1	0.15±0.00	3	15.46±51.11	4	11.0±36.26	1	0.07±0.0
Cunninghamella sp.	2	0.87±3.30	0	0	0	0	2	0.23±0.00	0	0	0	0
Dreshelera austriliansis	10	14.76±23.91	9	13.59±21.47	4	3.37±9.49	10	12.95±15.08	9	15.19 ±23.3	2	1.15±4.9
D.hawaeinsis	1	0.76±0.00	1	1.69±0.00	1	0.76±0.00	1	1.07±0.00	1	0.46±0.00	1	0.38±0.00
Fusarium solani	12	19.88±16.12	13	21.71±21.14	5	5.44 ±10.6	9	11.66±23.69	9	14.86±36.56	3	1.11±4.11
Humicola sp.	1	0.15±0.00	0	0	0	0	0	0	0	0	0	0
Macrophomina phaseolina	0	0	1	0.66±0.0	1	0.44±0.0	0	0.00±0.00	1	0.22±0.00	0	0
Memnoniella echinata	1	0.15±0.00	0	0	1	0.07±0.00	1	0.23±0.00	1	0.53±0.00	0	0
Monilia sp.	0	0	1	0.21±0.00	0	0	1	1.05±0.00	3	0.738±2.61	0	0
Mucor sp.	1	0.25±0.00	2	0.93±1.51	0	0	2	2.05±11.78	3	2.97±7.74	0	0
Nigrospora oryzae	2	1.0± 6.36	5	4.70±11.88	3	2.25±1.34	3	1.0±3.05	5	4.05±10.22	2	015±0.00
Penicillium sp.	4	7.07±32.71	3	4.69±15.27	1	0.23±0.00	4	12.23±40.68	4	16±51.58	1	3.53±0.00
Rhizopus stolonifer	8	7.53± 4.80	10	9.043±12.389	3	2.20±7.34	10	24.66±27.14	15	21.94±18.8.62	5	5.95±12.2
Scopularipsis brevicaulis	0	0	1	0.46±0.00	1	0.307±0.00	0	0	1	0.07±0.00	1	0.30±0.00
Syncephalastrm sp.	0	0	0	0	1	0.15±0.00	0	0	0	0	0	0
Trichoderma sp.	4	2.6±2.06	3	2.46±4.04	3	3.28±8.45	3	3.53±17.785	3	5.0± 25.48	4	6.6±18.02

NSI = Number of samples infected out of 15 samples tested
SD = ± standard deviation
1% = Percentage of infected seeds

Clifton showed the incidence of pathogenic fungi viz., *F.solani*, *F. oxysporum* and *Alternaria* sp. Present results showed that surface sterilization of plant parts of *A.marina* reduced the infection of *A. flavus* and *A. niger* and increased the incidence of pathogenic fungi. The saprophytic fungi like *A.flavus* and *A. niger* were predominant among the isolated fungi. Mehdi & Saifullah (2000) reported that *Aspergillus* were the most diverse genus followed by *Penicillium* sp. Present result showed that highest number of fungi were observed from Sandspit, Boat basin and West Wharf. Mehdi & Saifullah (1992) reported the species diversity in Clifton were more as compared to Korangi Creek. This richness in species diversity may be due to the inflow of city's sewage into the sea which is rich in organic matter which is a base of multiplication of fungi. There is therefore need to study the mycoflora of *A. marina* on a large scale from other areas of Pakistan and also keep the coastal areas clean.

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