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DETECTION OF SEED-BORNE MYCOFLORA IN *PINUS GERARDIANA*

ZAKIA BILGRAMI AND ABDUL GHAFFAR

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Using agar plate, blotter and deep freezing method, 14 genera and 26 species of fungi were isolated from fourteen seed samples of *Pinus gerardiana*. About 70% of the samples were infected with *Aspergillus flavus* and *A. niger* with an infection range of 4 to 56% and 4 to 100% respectively. Agar plate method yielded greater number of fungi (19) as compared to deep freezing method (13) and blotter method (12). Deep freezing method was found superior to standard blotter and agar plate method for the detection of *Alternaria, Cladosporium* and *Fusarium* spp.

Introduction

Pinus gerardiana seed have carminative, stimulant and exporant properties. The seeds are greatly valued as a dessert and are eaten raw and roasted. The kernels contain 7.5% moisture; 15.9% protein, 49.9% fat; 21.6% carbohydrate; 2.2% fibre and 2.9% mineral matter including calcium, phosphorus and iron (Krishnamurthi, 1969). Although mold fungi are known to deteriorate the seed and produce mycotoxins (Rodricks, 1976) there does not appear to be any report on the seed-borne mycoflora of *P. gerardiana* (Neergaard, 1977; Richardson, 1979). Studies on the detection of mycoflora of *P. gerardiana* seed are presented in this paper.

Materials and Methods

Fourteen samples of *P. gerardiana* seeds collected from different localities of Pakistan viz., Islamabad (2), Quetta (1), Muslimbagh (1), Risalpur (1), Swat (1), Peshawar (1), Lahore (1) Hyderabad (1) and Karachi (5) were used. Using ISTA techniques (Anon., 1976), 150 seeds from each sample were tested. For the standard blotter technique, untreated seed and seed after treatment with 1% $Ca(OCl)_2$ for 2 min., were placed on three layers of moistend blotter with 5 seeds per Petri dish. For the agar plate method, the treated and untreated seeds were plated on potato dextrose agar (PDA), 5 seeds per Petri dish and the dishes were incubated at 24°C for 5 days. In the deep freezing method, the treated and untreated seeds were incubated for one day each at 24°C and at -4°C followed by 5 days incubation at 24°C. Fungi growing from seeds were identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Nelson *et al.*, (1983). Raper & Thom (1949) and Thom & Raper (1945).

Results and Discussion

A total number of 14 genera and 26 species of fungi isolated from *P. gerardiana* seed were *Alternaria alternata, A. tenuissima, Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. sulphureus, A. terreus, A. wentii, Aspergillus* spp., *Botryodiplodia theobromae, Chaetomium* sp., *Cladosporium* sp., *Drechslera australiensis, D. hawaiensis,*

Table 1. Detection of the seed-borne mycoflora in *Pinus gerardiana*.

Fungi	Surface sterilized									Surface non-sterilized								
	Agar plate method			Blotter method			D.f. method			Agar plate method			Blotter method			D.f. method		
	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD
Alternaria alternata	-	-	-	-	-	-	2	.64 (4-5)	1.64	1	.28 (4)	1.0	1	.28 (4)	1.0	-	-	-
A. tenuissima	-	-	-	-	-	-	2	1.14 (4-12)	3.3	-	-	-	-	-	-	2	3.14 (20-24)	8.0
Aspergillus candidus	1	.7 (10)	2.6	2	2.28 (10-16)	5.8	1	.85 (12)	3.2	-	-	-	5	2.8 (4-16)	4.8	2	2 (8-20)	5.6
A. clavatus	2	7.4 (16-88)	23.5	2	3.7 (4-48)	12.7	2	4 (4-52)	13.8	2	3.14 (20-24)	8.0	1	2.8 (40)	10.6	2	2.8 (4-36)	9.5
A. flavus	10	7.5 (4-24)	7.7	9	7.2 (4-32)	8.8	2	.64 (4-5)	1.64	6	4.8 (4-24)	8.0	9	12 (4-56)	15.7	4	4.3 (5-36)	9.8
A. fumigatus	3	1.5 (4-12)	3.5	-	-	-	-	-	-	1	.85 (12)	3.2	-	-	-	2	.64 (4-5)	1.6
A. niger	10	20.8 (4-100)	32.2	7	9.4 (4-60)	16.9	1	.57 (8)	2.1	8	22.8 (8-100)	30.8	8	10.8 (8-48)	14.0	6	2.8 (4-20)	5.3
A. sulphureces	1	.57 (8)	2.1	1	.57 (8)	2.1	-	-	-	1	.28 (4)	1.0	2	2 (8-20)	5.6	-	-	-
A. terreus	2	1.14 (4-12)	3.3	2	3.2 (5-40)	10.6	-	-	-	1	.28 (4)	1.0	2	1.14 (4-12)	3.3	4	2.28 (4-16)	4.6
A. wentii	4	20.2 (8-100)	39.3	3	17.8 (50-100)	37.2	2	.6	1.6 (4-5)	4	24.8 (56-100)	42.0	4	22.8 (20-100)	42.1	2	2.57 (12-24)	6.9
Aspergillus spp.	1	.28 (4)	1.0	-	-	-	2	.85 (14-8)	2.3	-	-	-	-	-	-	1	.28 (4)	1.0

Table 1 (Cont'd)

Fungi	Surface sterilized									Surface non-sterilized								
	Agar plate method			Blotter method			D.f. method			Agar plate method			Blotter method			D.f. method		
	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD
Botryodiplodia theobromae	1	.1 (2)	.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium sp.	2	.5 (4-4)	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium sp.	-	-	-	-	-	-	1	.28 (4)	1.0	-	-	-	-	-	-	2	.92 (5-8)	2.4
Drechslera australiensis	-	-	-	1	1.1 (16)	4.2	-	-	-	-	-	-	1	.28 (4)	1.0	-	-	-
D. hawaiiensis	-	-	-	1	.28 (4)	1.0	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium solani	1	1.7 (24)	6.4	2	1.0 (5-10)	2.9	2	1.57 (10-12)	4.0	2	2.28	7.4 (4-28)	2	1.7	5.36 (4-20)	3	.85	1.7 (0-4)
F. oxysporum	1	.5 (8)	2.1	-	-	-	-	-	-	1	.57 (8)	2.1	-	-	-	-	-	-
Mucor sp.	1	.28 (4)	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monodictys castanae	-	-	-	-	-	-	-	-	-	1	.28 (4)	1.0	-	-	-	-	-	-
Nigrospora sp.	1	.28 (4)	1.0	-	-	-	1	.28 (4)	1.0	-	-	-	-	-	-	-	-	-
Penicillium spp.	6	9.14 (4-52)	15.5	5	11.5 (4-72)	24.9	5	8.0 (4-85)	22.4	5	15.5 (12-66)	24.7	6	8.8 (8-72)	19.0	5	8.7 (4-70)	19.3

Table 1 (Cont'd)

Fungi	Surface sterilized									Surface non-sterilized								
	Agar plate method			Blotter method			D.f. method			Agar plate method			Blotter method			D.f. method		
	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD	NIS	1%	SD
P. decumbens	1	.28 (4)	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus sp.	5	20.2 (4-100)	34.	8	15.2 (4-80)	22.5	2	1.42 (4-16)	4.3	9	40.8 (20-100)	41.3	9	28.5 (16-100)	32.9	3	3.14 (12-20)	6.5
Trichoderma harzianum	-	-	-	-	-	-	-	-	-	1	.85 (12)	3.2	-	-	-	-	-	-
Trichothecum roseum	1	.5 (.8)	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NIS = No. of infected samples out of 14. SD = Standard deviation,

1% = Infeected seed % out of 14 samples tested., ( ) = Infection range between infected samples.

D.f = Deep freezing

Table 2. Infection % of seeds of Pinus gerardiana by *Aspergillus flavus, A. niger, A. wentii* and *Penicillium* spp., collected from different localities of Pakistan.

City	*Aspergillus flavus*						*Aspergillus niger*						*Aspergillus wentii*						*Penicillium* spp..					
	Agar		Blotter		D.f.		Agar		Blotter		D.f.		Agar		Blotter		Deepf		Agar		Blotter		D.f.	
	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
Quetta	12	20	4	32	4	36	4	36	4	20	8	20	-	-	-	-	-	-	4	-	8	4	8	4
Muslimbagh	8	-	12	8	-	-	4	8	20	8	-	4	-	-	-	-	-	-	4	12	-	-	-	4
Lahore	-	-	8	20	-	12	-	-	28	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Islamabad	4	12	-	-	-	-	8	-	12	48	-	4	-	-	-	-	-	-	-	-	72	72	-	24
Islamabad	-	-	4	8	-	8	-	-	-	-	-	4	100	100	100	100	5	-	-	-	-	-	85	70
Swat	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Risalpur	8	-	-	4	-	-	8	12	4	8	-	4	-	-	-	-	-	12	20	32	-	16	4	-
Peshawar	6	-	5	-	-	-	4	-	36	32	-	-	8	56	-	20	-	-	20	66	8	16	-	-
Hyderabad	24	24	12	12	-	-	80	56	60	20	-	4	-	-	-	-	-	-	52	52	68	8	-	-
Karachi	4	-	32	56	-	-	8	8	-	12	-	-	-	-	-	-	-	-	28	56	-	-	12	-
Karachi	20	4	-	-	-	-	32	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
Karachi	16	4	8	12	5	5	-	-	-	-	-	-	100	100	100	100	4	24	-	-	-	-	4	20
Karachi	-	-	-	-	-	-	44	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Karachi	-	4	-	4	-	-	100	100	-	-	-	-	76	92	50	100	-	-	-	-	-	-	-	-

S = Sterilized, N = Non-sterilized, D.f. = Deep freezing

Fusarium solani, F. oxysporum, Mucor sp., *Monodictys castanae, Nigrospora* sp., *Penicillium* spp., *P. decumbens, Rhizopus* sp., *Trichoderma harzianum* and *Trichothecium roseum* (Table.1). This is the first report of fungi found on *P. gerardiana* seed (Richardson, 1979; Neergaard, 1977).

About 70% of the samples were infected with *Aspergillus flavus* and *A. niger* showing infection range between 4-56% and 4- 100% respectively. *A. candidus, Alternaria* spp., and *Fusarium solani* were detected in 14% samples showing infection range upto 20%, 24% and 28% respectively. *Penicillium* spp., were detected from 35% samples showing a high infection percentage (4-85%). The samples collected from Sindh showed the highest infection of *A. flavus* and *A. niger* while the samples of Islamabad were heavily infected by *Penicillium* spp., and *A. wentii* (Table 2).

Of the three methods used, the agar plate method yielded the highest number of fungi as compared to blotter and deep freezing method. The deep freezing method was superior to the standard blotter and agar plate method for the detection of *Alternaria, Cladosporium* and *Fusarium* spp. Mathur *et al.*, (1975) working on sorghum seed also found that the deep freezing method was more suitable for the detection of *Fusarium* spp. Similar results were obtained by Dawar & Ghaffar (1991) in sunflower seed. Surface disinfection of seed by 1% $Ca(OCl)_2$ reduced the incidence of *Aspergillus* spp., in the blotter and deep freezing methods which showed an increase in agar plate method. Infection of *Fusarium solani* was less in sterilized seeds.

A number of fungi isolated in the present study are known to produce mycotoxins, harmful for human health. Mycotoxin can cause severe damage to the liver, kidney and nervous system of man even in low dosages (Rodricks, 1976). *Aspergillus flavus* produces aflatoxin B_1, B_2, G_1, G_2 which are carcinogenic and produce liver cancer (Purchase, 1974). *A. candidus* produces citrinin harmful to kidney (Domsch *et al.*, 1980). *Fusarium solani* causes corneal ulcer while *F. oxysporum* produces Zeralenone α and ß causing haemorrhage and necrosis of bone marrow. *A. terreus* attacks human skin and nails and is a parasite in human ear (Domsch, 1980). *A. wentii* produces kojic acid causing cardiovascular and brain disorder. There is thus a need for proper storage of *P. gerardiana* seed and that healthy seeds free from mold fungi are sold in the market for human consumption.

References

Anonymous. 1976. International rules of seed testing. *Proc. Int. Seed. Test. Assoc.*, 4: 3-49.

Barnett, H.L. 1960. *Illusterated genera of Imperfect fungi*. Burgess pub. Co. Minn. PP.241.

Booth, C. 1971. *The genus Fusarium*. CMI, Kew, Surrey, England, pp. 237.

Dawar, S. and A. Ghaffar. 1991. Detection of seed borne mycoflora of sunflower. *Pak. J. Bot.*, 23: 173-178.

Domsch. K.H., W. Gams, and T. Anderson. 1980. Compendium of soil fungi vol.1. Academic Press. pp. 859.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. C.M.I., Kew Surrey, England, pp. 608.

Krishnamurthi, A. 1969. *The wealth of India*. A dictionary of Indian., Raw Materials Vol. VIII. Publication, Information Directorate CSIR New Delhi pp. 394.

Mathur, S.K., S.B. Mathur and P. Neergaard. 1975. Detection of seedborne fungi in sorghum and allocation of *Fusarium moniliforme* in seed. *Seed Sci & Technol.*, 13: 683-690.

Neergaard, P. 1977. *Seed Pathology*, Volume 1. The MacMillan Press Ltd. London. pp. 839.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species. An illustrated manual for identification*. The State Univ. Press,Penn., USA. pp. 203.

Purchase, I.R.H. 1974. *Mycotoxin*. Elsevier Scientific Publ. com. Amsterdam, pp. 443.

Raper, K.B. and C. Thom. 1949. *A manual of the Penicillia*. Williams & Wilkins Co., Baltimore, U.S.A. pp. 875.

Richardson, M.J. 1979. *An Annotated List of Seed-borne Diseases*. Int. Seed Test. Assoc. Zurich, Switzerland, pp. 320.

Rodricks, J.V. 1976. *Mycotoxins and other fungus related food problem*. Advances in chemistry Series 149. American Chemicals Society, Washington.

Thom, C. and K.B. Raper. 1945. *A manual of the Aspergilli*. Williams & Wilkins Co., Baltimore, U.S.A. pp. 373.

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