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SEED BORNE FUNGI ASSOCIATED WITH BITTER GOURD (*MOMORDICA CHARANTIA* LINN.)

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Abstract

Using ISTA techniques, the seed borne fungi of bitter gourd (*Momordica charantia* Linn.) was studied. A total of 15 genera and 29 species of fungi were isolated, of which 25 have not hitherto been recorded from seeds of bitter gourd in Pakistan. The blotter method was found to be most suitable technique for detection of fungi in bitter gourd. Deep-freezing method was preferable for the detection of *Fusarium* spp., *Myrothecium* spp. and *Penicillium purpurogenum*.

Introduction

Bitter-gourd (*Momordica charantia* Linn.) is extensively cultivated during warm season for its fruits which although bitter are used as a vegetable. They are stomachic, carminative and used in rheumatism, gout and diseases of liver and spleen (Nazimuddin & Naqvi, 1984). Few disease have been reported on bitter gourd eg., leaf spot (*Cercospora* spp., and *Myrothecium roridum*), Powdery mildew (*Oidium* sp.), white rot of fruit (*Sclerotium rolfsii*) and *Rizoctonia solani* fruit rot (Khan & Kamal, 1962; 1963; Maholay, 1986; Ali *et al.*,1988). Fungi reported from seeds of bitter gourd are *Alternaria* sp., *Aspergillus* sp., *Colletotrichum lagenarium*, *Coleophoma empetri*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Rhizoctonia solani*, *Rhizopus* sp., and *Sclerotium rolfsii* (Manthachitra, 1971; Maholay, 1986; Nair, 1982; Mathur, 1990). The present study describes seed borne fungi of bitter gourd.

Materials and Methods

Using ISTA techniques (Anon., 1976), 10 bitter gourd seed samples collected from different places of Sindh, Baluchistan and Punjab were examined for he seed borne mycoflora. For standard blotter and deep freezing methods, seeds before and after treatment with 2% $NaOCl_2$ for 2 minutes, were placed on three layers of moistened blotters, 10 seeds per Petri dish. The dishes were incubated at 24°C in 12 h alternating cycle of light and darkness for 7 days. In deep freezing method the treated and untreated seeds were incubated for 1 day each at 20°C and -20°C followed by 5 days incubation at 24°C. Fungi growing on seeds were identified after reference to Barnett & Hunter (1977), Booth (1971), Ellis (1971), Nelson *et al.*, (1983) and Raper & Fennel (1965).

Result and Discussion

Using blotter method, 15 genera and 29 fungal species were isolated from 10 samples of bitter gourd collected from different parts of Pakistan (Table 1). Of the fungi isolated 25 species viz., *Alternaria alternata* (Fr.) Keisler, *A. tenuissima* Kunze ex Pers.,

Table 1. Occurrence of fungi on bitter-gourd seeds using blotter and deep-freezing methods.

Fungi	Blotter Method					
	Control			Treated		
	Mean, SX±	St.D	Variance	Mean, SX±	St.D	Variance
Alternaria alternata	13.37 ± 8.37	16.74	280.229	17.75 ± 7.52	15.05	226.417
A. tenuissima	9.75 ± 0.0	-	-	8.25 ± 0.0	-	-
Aspergillus candidus	15.0 ± 10.0	14.14	200.00	-	-	-
A. flavus	25.28 ± 7.9	22.46	504.651	22.60 ± 7.83	22.15	490.751
A. niger	14.08 ± 6.64	16.27	264.767	8.75 ± 3.75	8.49	72.125
A. tamarii	4.75 ± 0.0	-	-	0.75 ± 0.0	-	-
A. terreus	8.25 ± 0.0	-	-	2.0 ± 0.0	-	-
A. wenteii	12.35 ± 2.84	7.54	56.830	7.57 ± 2.36	6.25	39.09
Chaetomium funicola	4.75 ± 0.0	-	-	1.25 ± 0.0	-	-
C. globosum	4.25 ± 0.0	-	-	1.8 ± 0.0	-	-
C. murorum	16.75 ± 0.0	-	-	2.75 ± 0.0	-	-
C. olivaceum	15.12 ± 8.68	28.26	452.342	10.58 ± 7.28	17.84	318.392
Cladosporium cladosporioides	8.42 ± 1.67	2.89	8.396	4.50 ± 1.68	3.37	11.417
C. oxysporum	6.25 ± 0.0	-	-	2.75 ± 0.0	-	-
C. sphaerospermum	2.0 ± 1.3	2.38	5.688	1.25 ± 0.0	-	-
Drechslera state of Cochliobolus spicifer	3.5 ± 0.25	0.43	0.188	5.0 ± 0.72	1.25	1.563
Fusarium moniliforme	13.25 ± 0.0	-	-	8.58 ± 4.77	8.27	68.396
F. oxysporum	5.25 ± 1.5	2.12	4.500	4.85 ± 1.06	2.37	5.644
F. semitectum	15.8 ± 8.76	17.52	306.932	13.68 ± 8.05	16.09	259.141
F. solani	-	-	-	4.33 ± 1.01	1.75	36.125
Memnoniella echinata	7.5 ± 4.25	6.01	36.125	3.86 ± 1.87	2.65	7.031
Myrothecium roridum	7.0 ± 6.0	8.48	72.00	2.25 ± 0.0	-	-
M. verrucaria	-	-	-	-	-	-
Nigrospora oryzae	3.25 ± 0.0	-	-	2.0 ± 0.0	-	-
Penicillium purpurogenum	-	-	-	2.5 ± 0.50	0.71	0.50
Penicillium sp.	3.87 ± 1.87	2.65	1.875	-	-	-
Rhizoctonia solani	7.0 ± 6.0	8.48	72.000	2.25 ± 0.0	-	-
Rhizopus spp.	37.4 ± 16.38	36.6	1341.894	13.47 ± 4.62	14.61	213.492
Stachybotrys atra	10.75 ± 7.5	10.61	112.50	4.75 ± 3.08	5.34	28.563
Stemphylium sp.	11.25 ± 0.0	-	-	2.08 ± 0.98	1.70	2.896
Trichurus spiralis	1.75 ± 0.0	-	-	-	-	-

Table 1. (Cont.'d.)

Fungi	Deep Freezing Method					
	Control			Treated		
	Mean, SX±	St.D	Variance	Mean, SX±	St.D	Variance
Alternaria alternata	16.16 ± 6.33	10.95	120.021	15.08 ± 5.33	9.23	85.146
A. tenuissima	4.0 ± 3.25	4.59	21.125	3.13 ± 1.87	2.65	7.031
Aspergillus candidus	1.5 ± 0.0	-	-	2.0 ± 0.0	-	-
A. flavus	19.18 ± 6.91	19.54	382.192	19.0 ± 7.68	21.73	472.00
A. niger	9.05 ± 3.71	8.29	68.825	9.1 ± 3.59	7.19	51.766
A. tamarii	3.5 ± 0.5	-	-	3.25 ± 0.0	-	-
A. terreus	4.75 ± 0.0	-	-	3.25 ± 0.0	-	-
A. wenteii	9.85 ± 1.89	5.03	25.268	7.42 ± 2.05	5.43	29.452
Chaetomium funicola	0.5 ± 0.0	-	-	0.5 ± 0.0	-	-
C. globosum	3.37 ± 2.87	4.06	16.531	5.0 ± 0.0	-	-
C. murorum	16.75 ± 0.0	-	-	2.75 ± 0.0	-	-
C. olivaceum	15.40 ± 10.55	23.61	557.394	12.7 ± 9.10	20.35	414.419
Cladosporium cladosporioides	5.56 ± 1.88	3.76	14.182	3.83 ± 0.87	1.51	2.271
C. oxysporum	-	-	-	-	-	-
C. sphaerospermum	1.25 ± 0.0	-	-	2.75 ± 0.0	-	-
Drechslera state of Cochliobolus spicifera	2.50 ± 0.38	0.66	0,438	3.0 ± 0.25	0.35	0.125
Fusarium moniliforme	5.58 ± 4.23	7.34	53.896	8.66 ± 4.22	7.31	53.521
F. oxysporum	4.20 ± 1.31	2.92	8.544	7.40 ± 1.45	3.25	10.581
F. semitectum	14.31 ± 7.15	14.30	204.599	18.31 ± 9.68	19.37	375.474
F. solani	2.08 ± 0.58	1.01	1.021	5.15 ± 1.77	3.95	15.675
Memnoniella echinata	4.50 ± 2.50	3.53	12.500	3.37 ± 2.87	4.06	16.531
Myrothecium roridum	7.21 ± 2.43	6.43	41.426	8.21 ± 2.79	7.40	54.780
M. verrucaria	6.18 ± 2.47	4.95	24.557	7.75 ± 3.22	6.45	41.583
Nigrospora oryzae	3.62 ± 1.37	1.94	3.781	3.50 ± 0.75	1.07	1.125
Penicillium purpurogenum	4.00 ± 2.26	3.93	15.438	3.81 ± 1.72	3.45	11.891
Penicillium sp.	-	-	-	-	-	-
Rhizoctonia solani	4.58 ± 3.71	6.43	41.396	2.83 ± 1.72	2.98	8.896
Rhizopus spp.	15.48 ± 6.24	12.48	155.901	8.81 ± 3.98	7.96	63.432
Stachybotrys atra	7.68 ± 4.81	9.63	92.891	1.91 ± 0.44	0.763	0.583
Stemphylium sp.	3.41 ± 1.96	3.40	11.583	5.75 ± 2.29	3.968	15.750
Trichurus spiralis	-	-	-	-	-	-

Data shows percentage of infected seed ± standard error. Control = Non-surface sterilized seed, Treated = Surface sterilized seeds with 2% $NaOCL_2$ for 2 minutes.

Aspergillus candidus Link., *A. flavus* Link & Pers., *A. niger* Van Tiegh., *A. tamarii* Kita, Centr., *A. terreus* Thom, *A. wentii* Wehmer, *Chaetomium funicola* Cooke, *C. globosum* Kunze ex Fr., *C. murorum, C. olivaceum* Cook & Ellis, *Cladosporium cladosporioides* (Fr.) de Vries, *C. oxysporum* Schlecht. Emend. Snyd. & Han., *C. sphaerospermum* Penz., *Drechslera* state of *Cochliobolus spicifer* Nelson, *Memnoniella echinata* (Riv.) Gallowany, *M. verrucaria* (Alb. & Schw.) Ditm. *ex* Fr., *Nigrospora oryzae* (Berk & Br.) Petch, *Penicillium purpurogenum* Stoll, *Rizoctonia solani* Kuhn., *Stachybotrys atra* Corda, *Stemphylium* sp., and *Trichurus spiralis* Hasselring do not appear to have been reported on bitter-gourd seeds (Noble& Richardson, 1968; Richardson, 1979; 1990; Mathur, 1990; Ahmad, 1993).

Aspergillus spp., *Chaetomium* spp., *Cladosporium* spp., *Fusarium semitectum* and *Rhizopus* sp., were most frequent in bitter-gourd seed. *Rhizopus* sp., was consistently isolated from seeds of bitter gourd. Species of *Myrothecium, M. roridum* and *M. verrucaria* were found associated with some discoloured and un-germinated seeds and also with seeds having abnormal seedlings. *Myrothecium roridum*, a common pathogen of cucurbits causes leaf spot and blight (Ali *et al*., 1988). *M. roridum* found associated with seeds of cucurbits has been reported (Sheikh, 1990; Shakir & Mirza, 1992).

The standard blotter method yielded maximum number of fungi. Such similar results have been observed from the detection of seed borne fungi in rice (Khan *et al*., 1988), cotton (Bhutta, 1988), cajanus (Chraya & Ready, 1979) and sunflower (Dawar, 1994). Begum & Momin (2000) reported that blotter method was found useful for detection of most infectious fungi of cucurbits. Deep-freezing method was found most suitable for detection of deep-seated as well as slow growing seed borne fungi like *Fusarium oxysporum, F. semitectum, F. solani, Myrothecium* spp., and *Penicillium purpurogenum*. These findings corroborate the reports that the deep-freezing method is more suitable for deeply seated seed borne fungi (Khan *et al*., 1988; Diekmann & Assad, 1987; Sultana, 2000).Disinfection of the seeds with 2% $NaOCl_2$ lowered the incidence of *Aspergillus* spp., *Cladosporium* spp., and *Rhizopus* sp., and increased the incidence of *Fusarium* spp., and *Myrothecium roridum*. Presence of *Aspergillus* spp., especially *A. niger* and *A. flavus* on seeds of bitter-gourd in higher frequencies and its association with ungerminated seeds of bitter-gourd confirmed the findings that species of *Aspergillus* though occur as saprophytes may cause low germination in seeds (Christensen, 1967; Shakir & Mirza, 1992).

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