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SEED BORNE FUNGI ASSOCIATED WITH BOTTLE GOURD (*LAGENARIA SICERARIA* (MOL.) STANDL.

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Abstract

Using ISTA techniques, the seed borne fungi of bottle gourd (*Lagenaria siceraria* was studied. A total of 22 genera and 45species of fungi were isolated, of which 35 have not hitherto been recorded from seeds of bottle gourd in Pakistan. Both blotter and deep-freezing methods yielded quantitatively as well as qualitatively more fungi than agar plate method. *Lasiodiplodia theobromae, Fusarium semitectum Macrophomina phaseolina* and *Fusarium oxysporum* were most frequently isolated from 33, 91, 50 and 66 % seed samples of bottle gourd respectively.

Introduction

Bottle-gourd (*Lagenaria siceraria* (Mol.) Standl.), a paratropical species of Asian and African origin is cultivated throughout Pakistan all the year round for its young and tender fruits eaten as popular domestic vegetable called Lauki and Kaddu. The pulp has cooling and antibilious affect. The seed oil is applied externally in headache (Nazimuddin & Naqvi, 1984). There are reports of few diseases of bottle gourd eg., powdery mildew, downy mildew, fruit rot, antharacnose, root rot, root knot, insect pest and viral diseases (Kamal & Moghal, 1968; Maholay, 1989; Hafiz, 1986; Zitter *et al*., 1996). Some of the fungi reported from seeds of bottle gourd are *Altenaria alternata, Aspergillus flavus, A. niger, Botryodiplodia theobromae, Chaetomium* sp., *Curvularia lunata, Drechslera tetramera, Fusarium equiseti, F. moniliforme, F. solani, Macrophomina phaseolina, Myrothecium roridum, Rizoctonia solani, Sclerotium rolfsii* and *Trichoderma* sp., (Manthachitra,1971; Maholay & Sohi, 1976; Richardson, 1979; Maholay, 1989; Shakir & Mirza, 1992). The present study describes seed borne fungi of bottle gourd.

Materials and Methods

Using ISTA techniques (Anon., 1976), 10 bottle gourd seed samples collected from different places of Sindh,Baluchistan and Punjab were examined for the 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, 22 genera and 45 species were isolated from 24 samples of bottle gourd seed collected from different parts of Pakistan followed by deep-freezing and agar plate methods where respectively 44 and 31 fungal species were detected (Table 1). Out of 45 fungal species, 35 fungal species viz., *Alternaria raphani* Groves & Skorko, *A. tenuissima* Kunze *ex* Pers., *Aspergillus candidus* Link, *A. quadrilineatus* Thom & Raper, *A. terreus* Thom, *A. wentii* Wehmer., *Chaetomium bostrychoides* Zope., *C. funicola* Cooke, *C. globosum* Kunze *ex* Fr., *C. olivaceum* Cook & Ellis, *C. tortile* Bainier, *Cladosporium cladosporioides* (Fr.) de Vries, *C. sphaerospermum* Penz., *Curvularia clavata* Jain, *C. lunata* (Wakker) Boedijn, *C. tuberculata* Jain, *Doratomyces stemonitis* (Pers. *ex* Fr.) Morton & Smith, *D. halodes (*Drechsler) Subram. & Jain, *D. hawaiiensis* (Bugn.) Subram., & Jain, *D. rostrata* (Drechsl) Richardson & Frasier, *D.* state of *Cochliobolus spicifer* Nelson, *Epicoccum purpurascens* Ehrenb. *ex* Schlechi, *Fusarium oxysporum* Schlecht emend. Snyd. & Hans., *F. semitectum* Berk & Rav., *Gliocladium roseum* Bainier, *Memnoniella echinata* (Riv.) Gallowany, *Myrothecium verrucaria* (Alb. & Schw.) Ditm. *ex* Fr., *Nigrospora oryzae* (Berk & Br.) Petch, *Penicillium purpurogenum* Stoll, *Sordaria fimicola* (Rob.) Ces. & De Not., *S. tetraspora* Winter, *Stachybotrys atra* Corda, *Trichurus spiralis* Hasselring, *Ulocladium atrum* Preuss, and *U. botrytis* Preuss isolated do not appear to have been recorded from seeds of bottle gourd (Anon., 1990-2007; Noble& Richardson, 1968; Richardson, 1979; 1992; Wahid, 1985; Wahid *et al*., 1991; Mathur, 1990; Ahmad, 1993). The average percent incidence and the range of occurrence of fungi in seed samples tested revealed that *Lasiodiplodia theobromae*, *Fusarium semitectum Macrophomina phaseolina* and *Fusarium oxysporum* were most frequent and isolated from 33, 91, 50 and 66 % seed samples of bottle gourd respectively. Most of the seeds infected by *L. theobromae* were dark coloured showed thick mycelial fragments on their surface. Seeds infected by *M. phaseolina* were discoloured with minute black sclerotia and most of them were of reduced size. *L. theobromae* and *M. phaseolina* have been reported to be seed borne on bottle gourd, squash and muskmelon causing black rot of fruit and blackening of seeds (Maholay & Sohi, 1982; Maholay, 1988, 1989).

Of the *Fusarium* spp., *F. semitectum* and *F. oxysporum* were frequently isolated followed by *F. solani* and *F. moniliforme*. *Fusarium* is a highly pathogenic fungus and its different species have been reported to cause seed rot, seedling blight and wilt in a number of cucurbitaceous crops (Booth, 1971). Similarly among *Drechslera* spp., *Drechslera* state of *Cochlibolus spicifer* and *D. halodes* showed seed rot and brown rot symptoms in seedlings. *Alternaria alternata* and *Curvularia lunata* caused delay or reduction in seed germination and is due to decay of seeds. Similar results have been reported by Mishra & Prakash (1975).

Use of 2% $NaOCl_2$ as seed disinfectant helped appreciably in minimizing the incidence of superficial and fast growing as well as common seed borne fungi like *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Rhizopus* spp., *Doratomyces stemonitis, Cephaliophora irregularis, Epicoccum purpurascense, Trichurus spiralis, Memnoniella echinata* and *Stachybotrys atra*. Whereas other seed borne fungi like *Curvularia spp*., *Drechslera* spp., *Fusarium* spp., *Myrothecium* spp., *Sodaria* spp., *Ulocladium* spp., and *Lasiodiplodia theobromae* were detected in greater frequency in seeds treated with 2% $NaOCl_2$. These observations are in conformity with the findings of Limonard (1968) and Khan *et al*., (1988).

Table 1. Occurrence of fungi on bottle-gourd seeds by using blotter, deep freezing and agar plate methods.

Fungi	SI	Blotter		SI	Deep freezing		SI	Agar plate	
		Control	Treated		Control	Treated		Control	Treated
Alternaria alternata	20	3.7±0.2 (0.5-9.5)	3.9±0.2 (0.3-7.5)	9	3.8±0.2 (0.5-7.8)	3.7±0.2 (1.5-9.0)	9	3.7±0.2 (1.0-7.5)	3.4±0.1 (0.5-6.8)
A. raphani	4	1.6±0.02 (1.0)	0.9±0.3 (0.5-1.3)	2	0.7±0.0 (0.5)	0.8±0.3 (0.3- 1.0)	-	- -	- -
A. tenuissima	10	1.3±0.2 (0.5-2.0)	1.0±0.2 (0.5-1.5)	4	1.6±0.3 (1.0-2.0)	0.9±0.1 (0.3±1.0)	-	- -	- -
Aspergillus candidus	4	1.45±0.2 (1.0-1.5)	- -	1	1.3±0.0 (1.3)	0.5±0.0 (0.5)	1	2.8±0.0 (2.8)	0.8±0.0 (0.8)
A. flavus	21	9.5±0.8 (0.5-49.8)	8.5±0.5 (0.5-39.3)	11	10.0±0.9 (1.0-38.0)	7.7±0.6 (0.3-43.3)	11	15.4±0.9 (3.8-59.3)	14.5±0.9 (3.0-61.8)
A. fumigatus	8	1.9±0.8 (2.5-7.3)	1.0±0.0 (1.0)	8	1.0±0.8 (0.5-3.8)	1.1±0.5 (0.5-1.3)	3	5.4±0.9 (0.5-7.3)	4.0±0.5 (1.0-3.5)
A. niger	24	13.9±0.8 (2.5-57.3)	3.8±0.4 (0.5-32.0)	23	9.7±0.8 (0.5-63.8)	6.9±0.5 (0.5-36.3)	24	19.8±0.9 (5.5-77.3)	9.0±0.5 (1.0-30.5)
A. quardilineatus	6	2.8±0.4 (2.0-4.2)	1.1±0.1 (1.0-1.3)	3	1.1±0.1 (0.8-1.5)	0.3±0.0 (0.3)	3	3.1±0.4 (2.0-4.0)	2.0±0.4 (1.5-2.5)
A. terreus	7	4.8±0.8 (1.0-7.5)	2.7±0.5 (0.5-5.5)	7	2.7±0.5 (1.0-3.5)	0.7±0.5 (0.5-1.5)	7	5.9±0.7 (1.0-7.9)	3.7±0.5 (0.5-5.5)
A. wentii	10	5.9±0.5 (0.5-7.3)	1.8±0.2 (0.3-2.8)	8	3.6±0.3 (0.5-5.0)	1.8±0.1 (1.0-2.3)	12	6.9±0.9 (1.5-11.0)	3.3±0.6 (0.5-5.5)
Chaetomium bostrychoides	6	0.8±0.1 (0.5-1.0)	0.3±0.0 (0.3)	-	- -	- -	2	- -	0.4±0.1 (0.3-5.0)
C. funicola	4	3.8±3.0 (0.5-8.0)	2.5±1.1 (0.5-3.0)	4	2.3±0.5 (0.5-4.0)	1.5±0.0 (1.5)	-	- -	- -
C. globosum	14	2.7±0.2 (0.3-5.0)	2.8±0.3 (0.5-5.5)	14	3.5±0.6 (0.3-6.7)	3.7±0.6 (1.0-5.5)	9	2.6±0.1 (1.5- 2.9)	2.0±0.2 (0.3-3.0)
C. olivaceum	12	1.4±0.2 (0.5-3.0)	0.8±0.1 (0.3-1.5)	10	1.7±0.3 (1.0-4.0)	1.3±0.4 (0.5-2.5)	2	- -	- (0.0-0.5)
C. tortile	4	0.7±0.1 (0.5-1.0)	1.3±0.1 (1.0-1.5)	3	0.7±0.1 (0.5-1.0)	1.2±0.3 (0.5-2.0)	-	- -	- -

Table 1. (Cont'd.).

Fungi	SI	Blotter		SI	Deep freezing		SI	Agar plate	
		Control	Treated		Control	Treated		Control	Treated
Cladosporium cladosporioides	14	1.9±0.2 (1.0-4.0)	2.8±0.1 (0.3-1.5)	14	1.4±0.1 (0.3-2.5)	0.8±0.2 (0.5-1.5)	14	3.6±0.2 (2.0-5.5)	1.8±0.1 (0.5-3.0)
C. sphaerospermum	10	2.9±0.7 (0.5-5.9)	1.1±0.4 (0.3-1.9)	8	1.8±0.4 (0.3-4.7)	1.3±0.5 (0.3- 1.8)	10	2.9±0.4 (0.5-5.8)	1.7±0.33 (0.5-3.6)
Curvularia clavata	8	1.4±0.7 (0.5-2.0)	2.5±0.4 (0.5-3.5)	8	1.9±0.4 (0.8-4.3)	3.9±0.5 (1.0±7.0)	8	3.6±0.4 (2.2-6.3)	6.9±0.8 (3.0-8.5)
C. lunata	18	3.4±0.5 (0.4-8.5)	3.9±0.4 (0.4-8.9)	10	2.6±0.5 (0.5-4.5)	3.2±0.4 (0.5-4.5)	18	4.6±0.7 (0.5-9.0)	6.0±0.3 (2.0-10.8)
C. tuberculata	2	2.0±0.0 (2.0)	2.5±0.0 (2.5)	-	- -	- -	2	2.8±0.0 (2.8)	3.5±0.0 (3.5)
Doratomyces stemonitis	4	5.7±1.1 (2.8-12.0)	3.9±1.9 1.3-6.5)	3	2.3±0.7 (0.5-4.5)	1.8±0.9 (0.5-3.0)	3	1.6±0.6 (0.3-3.5)	- -
D. halodes	7	3.0±0.2 (1.3-4.8)	4.3±0.2 (2.0-5.0)	7	2.9±0.3 (0.5-5.0)	5.0±0.3 (2.8-6.9)	5	- -	0.5±0.2 (0.5-1.5)
D. hawaiiensis	8	4.6±0.4 (1.5-17.3)	3.6±0.4 (0.5-4.5)	8	4.8±0.7 (2.0-6.8)	2.7±0.6 (0.5-4.8)	6	0.6±0.1 (0.5-0.8)	1.0±0.2 (0.5-1.5)
D. rostrata	10	0.8±0.4 (0.5-1.0)	0.9±0.1 (0.3-2.0)	10	1.9±0.1 (0.8-2.3)	2.2±0.2 (1.3±3.8)	8	0.7±0.1 (0.3-0.5)	0.6-0.1 (0.3-1.0)
D. state of C. spicifer	22	3.2±0.2 (0.5-5.8)	4.2±0.2 (1.0-8.5)	22	3.1±0.1 (0.5-3.8)	3.5±0.2 (0.3-7.3)	22	4.8±0.2 (0.5-8.3)	6.0±0.3 (0.3-9.3)
Epicococum purpurascens	4	1.0±0.7 (0.5-1.5)	- -	8	0.8±0.1 (0.3-1.0)	0.6±0.3 (0.3-0.8)	-	- -	- -
Fusarium equiseti	10	1.8±0.1 (0.3-1.5)	2.3±0.4 (0.5-3.8)	12	1.8±0.1 (0.5-1.0)	2.5±0.1 (0.8-2.8)	6	- -	1.4±0.04 (0.3-0.5)
F. moniliforme	5	3.5±0.7 (0.5-8.0)	4.6±0.8 (0.5-10.5)	6	6.0±0.6 (0.5-8.5)	6.6±0.7 (1.0-13.3)	5	2.3±0.2 (0.3-2.5)	3.0±0.3 (1.3-3.8)
F. oxysporum	16	8.9±0.9 (2.0-19.5)	10.3±1.2 (2.5-26.5)	18	10.2±0.9 (3.3-23.0)	10.5±1.2 (0.5-29.3)	16	5.9±0.7 (0.5-13.0)	7.1±0.7 (0.5-16.5)
F. semitectum	22	11.5±1.4 (0.5-48.0)	11.2±1.2 (0.5-49.3)	22	10.9±1.1 (0.8-47.5)	13.8±1.1 (2.0-30.0)	22	6.4±0.7 (0.3-23.8)	8.8±0.9 (0.3-32.3)

Table 1. (Cont'd.).

Fungi	SI	Blotter		SI	Deep freezing		SI	Agar plate	
		Control	Treated		Control	Treated		Control	Treated
F. solani	12	4.9±0.7 (0.5-8.0)	5.5±0.7 (1.5-12.5)	14	3.9±0.5 (0.5-10.3)	6.1±0.7 (1.0- 16.0)	6	3.3±1.3 (0.5-4.3)	3.5±0.7 (1.0-5.0)
Gliocladium roseum	2	0.4±0.1 (0.3-0.5)	- -	2	0.3±0.0 (0.3)	- -	-	- -	- -
Lasiodiplodia thobromae	8	23.7±3.9 (0.3-51.5)	25.3±4.7 (1.5-68.0)	8	26.0±5.2 (0.8-57.3)	30.3±5.6 (1.0-60.3)	8	16.0±10.9 (0.5-41.5)	18.4±12.5 (0.8.44.8)
Macrophomina phaseolina	12	10.±3.9 (0.3-58.5)	11.3±4.1 (0.5-62.0)	10	13.0±5.2 (0.8-59.3)	14.3±5.6 (1.0-64.3)	4	16.0±10.9 (0.5-31.5)	18.4±12.5 (0.8-39.0)
Memnoniella echinata	16	2.6±0.1 (0.5-3.5)	1.5±0.1 (0.3-1.5)	14	0.8±0.1 (0.3-1.5)	0.8±0.1 (0.3-2.3)	-	-	- -
Myrothecium roridum	16	0.8±0.03 (0.3-1.0)	0.9±0.04 (0.5-1.5)	8	1.3±0.1 (0.5-2.5)	1.9±0.1 (1.0-3.5)	-	- -	- -
Nigrospora oryzae	4	2.3±1.1 (0.5-3.5)	1.7±0.0 (1.8)	2	2.8±0.0 (3.0)	2.1±0.0 (2.3)	-	- -	- -
Penicillium purpurogenum	10	1.3±0.1 (0.5-2.0)	2.4±0.3 (1.3-2.8)	10	2.8±0.3 (1.0-4.5)	2.8±0.4 (1.3±4.8)	10	1.1±0.2 (0.5-2.0)	1.7-0.2 (0.5-2.5)
Rhizopus sp.	23	5.8±0.2 (1.8-10.5)	2.6±0.1 (0.5-4.3)	23	3.9±0.1 (0.5-8.0)	1.6±0.1 (0.5-3.0)	23	23.5±0.7 (2.0-30.0)	7.9±0.4 (2.0-20.5)
Sordaria fimicola	3	2.5±0.0 (2.5)	2.0±0.0 (2.0)	3	3.0±1.1 (1.5-5.5)	4.5±0.0 (4.5)	-	- -	- -
S. tetraspora	1	1.1±0.0 (1.1)	2.5±0.0 (2.5)	1	3.5±0.0 (3.5)	2.0±0.0 (2.0)	-	- -	- -
Stachybotrys atra	12	2.7±0.3 (0.5-5.5)	2.3±0.3 1.3-3.5)	10	3.3±0.4 (0.5-6.5)	2.8±0.2 (2.0-3.5)	-	- -	- -
Trichurus spiralis	4	4.5±0.4 (1.0-5.0)	0.9±0.3 (0.5-1.3)	2	2.9±1.1 (1.3-4.5)	1.4±0.6 (0.5-2.3)	-	- -	- -
Ulocladium atrum	4	4.8±1.2 (3.0-6.5)	- -	1	1.0±0.0 (1.0)	0.3±0.0 (0.3)	1	- -	9.3±0.0 (0.3)
U. botrytis	2	2.0±0.4 (1.5-2.5)	- -	2	2.3±0.5 (1.5-3.0)	2.0±0.0 (2.0)	1	0.5±0.0 (0.5)	- -

Data shows percentage of infected seed ± standard error. SI = No of sample infected. Numbers in parenthesis indicate infection range.
Control = Non-surface sterilized seed, Treated = Surface sterilized seeds with 2% $NaOCl_2$ for 2 minutes.

Both blotter and deep-freezing methods yielded quantitatively as well as qualitatively more fungi from seeds of bottle gourd. 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) 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 *Drechslera halodes, D. rostrata, Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Myrothecium* spp., *Penicillium purpurogenum* and *Sordaria* spp. These findings corroborate the reports that the deep-freezing method is more suitable for deeply seated seed borne fungi especially *Fusarium* spp., (Mathur *et al*., 1975; Khan *et al*., 1988; Diekmann & Assend, 1987; Dawar, 1994).

Disinfection of the seeds with 2% $NaOCl_2$ lowered the incidence of *Aspergillus* spp., *Cladosporium* spp., and *Rhizopus* sp., whereas these ubiquitous fungi were isolated in high percentage on agar plate method. Agar plate was found most suitable for isolation of *Curvularia* spp., and *Drechslera* state of *Cochliobolus spicifer* where disinfected seeds of bottle gourd were used. Khan *et al*., (1988) preferred the use of agar plate method over the blotter method for isolation of *Curvularia* spp., and *Drechslera* spp., from disinfected seeds of rice. However, in the present studies *Drechslera rostrata* and *D. halodes* were isolated more frequently from bottle gourd seeds by deep-freezing method.

Presence of *Aspergillus* spp., especially *A. niger* and *A. flavus* on seeds of bottle gourd in higher frequencies and its association with ungerminated seeds of bottle gourd confirmed the findings that species of *Aspergillus* though occur as saprophytes may cause low germination in seeds (Christensen, 1967; Shakir & Mirza, 1992; Dawar, 1994). *Macrophomina phaseolina*, a virulent fungus causing charcoal rot and stem rot disease in a number of crops, was present in higher percentage in bottle gourd. *M. phaseolina* has been isolated from seeds of various cucurbitaceous crops and found associated with diseased seeds (Shakir & Mirza, 1992; Wahid, 1985; Shakir *et al*., 1995; Maholay, 1988; 1989). *Lasiodiplodia theobromae* has been reported to cause seed borne disease in watermelon, squash and bottle gourd (Sohi & Maholay, 1974; Maholay & Sohi, 1976; Sultana & Ghaffar 1992). In the present study *Fusarium oxysporum* and *F. solani* were most consistently isolated fungi from bottle gourd seeds and varied in the nature and severity of the symptoms that they induced in emerging seeds and seedlings. *F. solani* has been reported on seeds of bottle gourd, sponge gourd (Shakir *et al*., 1992; 1995; Wahid, 1985; Wahid *et al*., 1991), *Benincasa cerifera* Sav., and *Lagenaria vulgaris* Ser., (Mirza & Qureshi,1978). *F. oxysporum* has been isolated in high frequencies from seeds of water melon (Mclaughlin & Martyn, 1982) and sponge gourd (Shakir *et al*., 1995).

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