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SEED-BORNE MYCOFLORA ASSOCIATED WITH OKRA [*ABELMOSCHUS ESCULENTUS* (L.) MOENCH]

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Abstract

Around 75 species belonging to 31 fungal genera were isolated from the eighteen seed samples collected from thirteen localities of Pakistan. Seed-borne mycoflora associated with the samples were isolated and identified by using ISTA techniques. Agar plate method was found best for the isolation of fungi followed by standard blotter method. Seed samples from the areas of Fatu-chuk, Islamabad, Akora Khattak, Mandibahauddin, and Karachi, respectively were found to be highly infected with fungi. Species of *Aspergillus* and *Chaetomium* were the most dominant fungi. Species of *Fusarium*, *Phoma*, and *Macrophomina phaseolina* were isolated through both agar plate and standard blotter methods. Variation in size of sclerotia of *M. phaseolina* was observed. Surface sterilization of seeds with 1% $Ca(OCl)_2$ has reduced the incidence of storage fungi. 32 species belonging to 21 fungal genera are newly reported from Pakistan.

Key words: Okra, Seed, Mycoflora, ISTA Techniques, Pakistan.

Introduction

Abelmoschus esculentus (L.) Moench, also known as okra, lady's finger or bhindi, is a member of the family malvaceae. The plant is cultivated around the world; In Pakistan it is grown as kharif crop (Anon., 2009). During 2009-2010, the total yield of okra was 0.43 million hectares with production of 4.54 million tons. Pakistan produced 114,657 million tons of okra cultivated on 15,081 hectares (Anon., 2009). Okra plant is among the most heat and drought tolerant plants; however severe frost can damage the pods (Franklin, 1982). Nutritional profile of okra showed that it contains saturated fats, carbohydrates, proteins, vitamin A, B_6, B_{12}, folate, ribofalvin, niacin, pentothenic acid, Vitamin C, and E etc., it also contains magnesium, phosphorous, potassium, zinc, sodium, copper, manganese and selenium. The seeds also contains dietary fiber and sugars (Anon., 2012). Okra seeds can be roasted and ground to form a non-caffeinated substitute for coffee (Austin State Gazette, 1861).A survey of literature showed that numerous pathogenic and saprophytic fungi have been reported on okra seeds. *Alternaria alternata*, *Alternaria* sp., *Aspergillus flavus, A. niger, Chaetomium globosum, Curvularia lunata, C. pallescens, C. robusta, Drechslera hawaiiensis, D. rostrata, Fusarium moniliforme, F. oxysporum, Penicillium* sp., *Rhizopus* sp., *R. nigricans, sclerotium* sp., and *Stachybotrys atra* have been reported from Pakistan (Ahmad *et al*., 1993). Fungi like *Macrophomina phaseolina, Rizoctonia bataticola, R.solani, Fusarium solani, Pythium butteri, Phytophthora palmivora, Cercospora abelmoschii* and *Erysiphe cichoracearum* has been found to attack okra plant (Mithal, 2006; Zahoor *et al*., 2012). Anam *et al*. (2002) reported foot and root rot, Anthracnose and die back, *Cercospora* leaf spot, *Cornyspora* leaf spot and leaf blight on okra plant. These diseases were caused by *F. oxysporum, Colletotrichum dematium, Cercospora abelmoschi, Cornyspora cassiicola and M. phaseolina.* Al-kassim & Monawar (2000) reported *Alternaria alternata, A. niger, F. moniliforme, F. oxysporum, F. solani, Humicola grisea, M. phaseolina, Penicillium digitatum, Penicillium* sp., *Pythium aphanidermatum, Rizoctonia* sp. and *Stemphytium botriosum* on okra seeds from seed from Saudi arabia. *Fusarium moniliforme* besides *Phoma sabdariffae, Alternaria tenius, Colletotrichum* and *Chaetomium* sp. *Curvularia lunata* and *Drechslera tetramera* etc. were isolated from areas of Northern Province, Khartoum province and Suki, using standard blotter method (Sohaib & Baghdadi, 1984). Adebanjo & Shopeju (2002) reported *Botriodiplodia theobromae,Fusarium oxysporum, Mucor mucedo, Rhizopus* sp. and *Trichoderma harziamae* on fresh okra. Heme *et al*. (1990) reported *Botriodiplodia theobromae* as internally deep-seated fungus causing diseases, pre- and post-emergence death in Karnataka, India. Fagbohun & Faleye (2012) isolated 6 fungal species viz., *Rhizopus* sp., *Mucor* sp., *Aspergillus niger*, *A. flavus* and *Neurospora crassa* from sun dried okra pods collected from Egypt. They reported gradual increase in storage fungi and decrease in the nutritional composition of okra. Keeping in view the economic importance of the crop, a study was carried out to check the mycoflora associated with okra seeds in Pakistan.

Materials and Methods

Collection of seed samples: Eighteen samples of okra seeds were collected from various areas of Pakistan viz., Peshawar (1), Fatu-chuk (1), Islamabad (1), Tordher (1), Mardan (2), Karachi (4), Swabi (1), Mandibahuddin (1), Ghotki (1), Sukkur (1), Bunair (2) Akora Khattak (1) and Abbottabad (1).

Isolation of fungi from okra seeds: For the detection of seed-borne fungi ISTA techniques were used (Anon., 1993). By using Standard blotter, agar plate and deep-freezing methods, about four hundred seeds of each sample were tested.

Standard blotter method: Untreated and seeds after treatment with 1% $Ca(OCl)_2$ for 2 minutes were placed on three layers of moistened blotter paper, 20 seeds per Petri dish. The dishes were incubated for 5-7 days at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Agar plate method: Untreated and seeds after treatment with 1% $Ca(OCl)_2$ for 2 minutes were placed on Potato dextrose agar (PDA), 20 seeds per Petri dish. The dishes

were incubated for 5-7 days at 28±2 °C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Deep-freezing method: Untreated and seeds after treatment with 1% $Ca(OCl)_2$ for 2 minutes were placed on three layers of moistened blotter paper, 20 seeds per Petri dish were incubated for 24h, each at 28±2°C and -2°C followed by 5 days incubation at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Identification of fungi: Mycoflora growing on seeds were identified after referencing to Barnett and Hunter (1998), Domsch *et al*. (1980), Ellis (1971), Gilman (1950), Hanlin (1989), MycoBank (2013), Nelson *et al*. (1983), Raper *et al*. (1965).

Analysis of data: Data was subjected to analysis of variance (ANOVA) following the procedures as suggested by Gomez & Gomez (1984).

Result

Around 75 species belonging to 31 fungal genera viz., *Absidia corymbifera* (Cohn) Sacc. & Trotter, *A. glauca* Hagem, *Acremonium cerealis* (Karst).W.Gams. , *A. furcatum* F. & V. Moreau ex W. Gams, *A. kiliense* Grutz, *Acremonium* species Link ex Fr., *Alternaria brassicicola* (Schw) Wiltshire, *Alternaria* species Nees ex Fr. Nees., *Aphanoascus fulvescens* (cooke) Apinis, *Aspergillus alutaceous* Berk. & Curt., *A. candidus* Link ex Link, *A. clavatus* Desm., *A. flavus* Link ex Gray., *A.fumigatus* Fres., *A. glaucus*. Mich ex Fr., *A. japonicus Saito*., *A. mellus* Yukawa, *A.niger* Van Tieghem., *A. oryzae* (Ahlburg) Cohn., *A. parasiticus* Speare, *A. sclerotium* Huber, *A. sulphureus* Thom & Church, *A. sydowii* (Bain. & sart.) Thom & Church, *A. terreus* Thom, *A. ustus* (Bain.) Thom & church, *A.versicolor* (Vuill.) Tiraboschi, *A. wentii* Wehmer, *Botryotrichum piluliferum* Sacc. & March. *Cephaliophora irregularis* Thaxter., *Chaetomium bostrychodes* Zopf., *C. cochliodes* Pall., *C. crispatum* (Fuckel)Fuckel, *C. elatum* Kunze ex Steud., *C. funicola* Cooke, *C. globosum* Kunze ex steud., *C. indicum* Corda, *C. spirale* , *Chaetomium* species Kunze ex Fr., *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M.B. Ellis, *D. papendorfii* (Van der Aa) M.B. Ellis. Comb. Nov., *Emericella nidulans* (Eidam) Vuill., *E. nivea* Wiley & Simmons, *E.rugulosa* (Thom & Raper) C.R. Benjamin, *Eurotium chevalieri* Mangin, *E. herbariorum* (Wiggers) Link ex Gray, *Eurotium* spp., Link ex Gray, *F. oxysporum* Schlecht. emend. Sny. & Hans., *Fusarium verticilliodes* (Sacc.) Nirenberg (Formerly *Fusarium moniliforme* Sheld.), *Lophotrichus ampullus* R.K. Benjamin, *Macrophomina phaseolina* (Tassi) Goid, *Microascus cirosus* Zukal, *M. trignosporous* C.W. Emmons & B.O. Dodge, *Melanospora* sp. Corda, *Monoascus* sp. Van Tiegh, *Mucor hiemalis* Wehmer, *M. mucedo* Mich. Ex St. Am., *Mucor* sp. Mich.ex St.Am., *Myrothecium cinctum* (Corda) Sacc., *M. verrucaria* (Alb. & Schw.) Ditm.ex Steudel, *Neocosmospora* sp E. F. Sm., *Nigrospora oryzae* Hudson, *N. sphaerica* (Sacc.) Mason, *Papulaspora irregularis* Hotson, *Penicillium nigricans* Bain ex Thom, *Penicillium* Link ex Fr., *Phoma glomerata* (corda) Wollen W & Hochapfel. , *Phoma* sp. Sacc., *Pseudoeurotium zonatum* Van Beyma, *Rhizopus oryzae* Went & Prinsen Geerligs, *R. stolonifer* (Ehrenb. Ex Link) Lind, *Scopulariopsis brevicaulis* (Sacc.) Bain, *Sordaria* sp. Ces. & De Not., *Syncephalastrum* sp. Schört and *Trichoderma hamatum* (Bonord.) Bain., *Verticillium* sp., Nees ex link., were isolated from the seed samples collected from various areas of Pakistan by using ISTA techniques. 59 species of 28 fungal genera were isolated through agar plate method; 35 species of 18 fungal genera were isolated by using blotter method, while Deep-freezing method yielded 5 species belonging to 3 genera. *Aspergillus niger* followed by *A. flavus* and *Chaetomium globosum* were the most dominant fungi in all three methods used. Pathogenic fungi like *F. oxysporum, F. verticilliodes, Macrophomina phaseolina,* and *Phoma* species favoured growth on agar plate mainly. Four different sizes of sclerotia of *M. phaseolina* were observed on seeds. Species of *Chaetomium, Eurotium, Emericella, Lophotrichus, Microascus, Monoascus,* and *Sordaria* were isolated through blotter method. Keeping in view the work reported by Ahmad *et al.* (1993), 32 fungal species belonging to 21 genera are newly reported from Pakistan (Table 1).

Discussion

Seed samples collected from the areas of Fatu-chuk, Islamabad, Akora Khattak, Mandibahauddin, and Karachi were found to be highly infected with both pathogenic and saprophytic fungi. Agar plate method was found best for the isolation of fungi. Surface sterilization of seeds with 1% $Ca(OCl)_2$ has greatly reduced the incidence of storage fungi, these results were also reported by Wilson (1915), and he suggested calcium hypochlorite as seed sterilizer. *Fusarium* spp., *M. phaseolina* and *Phoma* spp., had caused rot and decay of seeds and seedlings. *M. phaseolina* has produced charcoal rot symptoms on seeds. Seeds which were highly infected with fungi failed to germinate. Such similar results were also reported by Rahim *et al*. (2013).

From consumption point of view presence of so many fungi indicates greater threat to human health, because fungi are known to produce mycotoxins, which in turns reduces the quality of seeds in terms of germination, viability, consumption, and trade value (Agarwal & Sinclair, 1996). Mycotoxins are carcinogenic and produce health damaging affects on both humans and livestock. Around 25% of the world food crop is affected by mycotoxins each year (Mannon & Johnson, 1985). Reduction in okra seeds production has been observed in the previous years of the country and being agricultural state, Pakistan can not afford such losses. Similar results were also made by Lee *et al*. (2000) in their survey of Vietnam market. Youssef (2008) studied the mycological status of sundried okra fruit and found that it was highly contaminated with fungal spores and contained higher levels of toxins when tested.

Okra is one of the important economic crop of Pakistan.The seeds of okra are sensitive and are highly susceptible to rot, decay and deterioration by mycoflora, insects and other organisms, which reduce the quality of crop seeds. Steps must be taken to reduce the risk of damage to future crop of the country, due to mycoflora associated with the seeds.

Table 1. Isolation of fungi from *Abelmoschus esculentus* (L.) Moench. (Okra) using ISTA techniques.

Name of Fungi	Standard blotter method				Agar plate method				Deep-freezing method			
	NSt		SSt		NSt		SSt		NSt		SSt	
	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD
Absidia corymbifera	1	0.028 ± 0.0	1	0.083 ± 0.0	3	0.194 ± 0.288		-		-		-
*A glauca**	1	0.222 ± 0.0		-	1	0.056 ± 0.0	1	0.056 ± 0.0		-		-
*Acremonium cerealis**		-		-	1	0.028 ± 0.0		-		-		-
*A furcatum**		-		-		-	1	0.139 ± 0.0		-		-
*A kiliense**	1	0.111 ± 0.0		-		-		-		-		-
Acremonuim sp.*		-		-		-	1	0.22 ± 0.0		-		-
Alternaria brassicicola		-		-		-	1	0.028 ± 0.0		-		-
Alternaria spp.		-		-		-	1	0.028 ± 0.0		-		-
*Aphanoascus fulvescens**		-		-		-	1	0.028 ± 0.0		-		-
Aspergillus alutaceous		-		-	1	0.083 ± 0.0	2	0.11 ± 0.707		-		-
A.candidus	1	0.028 ± 0.0	1	0.056 ± 0.0		-		-		-		-
A.clavatus		-		-		-	1	0.028 ± 0.0		-		-
A.flavus	18	5.67 ± 4.33	13	4.417 ± 5.207	17	16.167 ± 16.133	18	13.67 ± 19.45	3	0.11 ± 1.0	1	0.38 ± 0.0
A.fumigatus		-		-	1	0.083 ± 0.0	6	0.61 ± 1.88		-		-
A.glauca		-		-		-	1	0.056 ± 0.0		-		-
A.japonicus		-		-		-	3	0.139 ± 0.0		-		-
A.mellus		-		-		-	1	0.028 ± 0.0		-		-
A.niger	10	0.805 ± 1.9	9	1.028 ± 3.079	18	32.11 ± 20.65	15	12.78 ± 21.67	2	0.11 ± 0.0		0.056 ± 0.0
A.oryzae	1	0.166 ± 0.0	1	0.028 ± 0.0	1	2.78 ± 0.0		-		-		-
A.parasiticus		-		-	1	0.056 ± 0.0	1	0.028 ± 0.0		-		-
A.sclerotium		-		-		-	1	0.056 ± 0.0		-		-
A. sulphureus	1	0.056 ± 0.0	1	0.028 ± 0.0		-	1	0.028 ± 0.0		-		-
A. sydowii		-		-		-	1	0.028 ± 0.0		-		-
A.terreus		-		-	1	0.56 ± 6.7	4	0.5 ± 4.04		-		-
A.versicolor		-	1	0.028 ± 0.0	1	0.028 ± 0.0	1	0.083 ± 0.707		-		-
A.ustus		-	1	0.083 ± 0.0		-		-		-		-
A.wentii	1	0.028 ± 0.0		-	2	0.056 ± 0.0	4	0.67 ± 0.479		-		-
*Botryotrichum piluliferum**	2	0.166 ± 1.441	2	0.139 ± 1.06		-		-		-		-
*Cephaliospora irregularis**	1	0.28 ± 0.0		-		-		-		-		-
Chaetmoium bostrychodes	2	0.22 ± 0.0	1	0.083 ± 0.0		-		-		-		-
C.cochliodes	1	1.611 ± 0.0	2	0.083 ± 4.90		-		-		-		-
C.crispatum	2	1.528 ± 8.8	2	0.75 ± 7.42		-	3	0.36 ± 2.46		-		-
C.elatum	1	3.0 ± 24.1	5	3.38 ± 8.507		-	3	0.11 ± 0.28		-		-
C.funicola		-		-		-	1	0.028 ± 0.0		-		-
C.globosum	8	1.33 ± 7.19	6	1.56 ± 3.507	1	0.083 ± 0.0	4	1.083 ± 7.79	1	0.11 ± 0.0		0.16 ± 0.0
C.indicum	1	0.083 ± 0.0	1	0.083 ± 0.0		-	1	0.056 ± 0.0		-		-
C.spirale	1	0.22 ± 0.0		-		-		-		-		-
Chaetmoium spp.	8	1.94 ± 6.105	5	1.22 ± 4.05		-	6	0.72 ± 2.33		-		-

Table 1. (Cont'd.).

Name of Fungi	Standard blotter method				Agar plate method				Deep-freezing method			
	NSt		SSt		NSt		SSt		NSt		SSt	
	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD
Drechslera australiensis		-		-		-	1	0.138 ± 0.0		-		-
D.papendorfii		-		-		-	1	0.056 ± 0.0		-		-
*Emericella nidulans**		-		-		-	1	0.028 ± 0.0		-		-
*E.nivea**	1	0.028 ± 0.0		-		-		-		-		-
*E.regulosa**		-		-		-	1	0.028 ± 0.0		-		-
*Eurotium chevalieri**		-		-		-	2	0.25 ± 0.0		-		-
*E.herbariorum**		-		-		-	1	0.028 ± 0.0		-		-
Eurotium spp.*		-		-	1	0.028 ± 0.0	1	0.11 ± 0.0		-		-
Fusarium verticillioides		-		-		-	1	0.138 ± 0.0		-		-
F.oxysporum		-		-		-	1	0.028 ± 0.0		-		-
*Lophotrichus ampullus**	1	0.028 ± 0.0		-		-		-		-		-
Macrophomina phaseolina	1	0.11 ± 0.0	2	0.75 ± 8.48	2	0.861 ± 4.59	4	6.138 ± 29.0		-		-
Melanospora spp.*	1	0.028 ± 0.0		-		-		-		-		-
*Microascus cirosus**	1	0.11 ± 0.0	1	0.194 ± 0.0		-	1	0.028 ± 0.0		-		-
*M.trignosporus**		-		0.028 ± 0.0		-		-		-		-
Monoascus sp.*		-	1	0.139 ± 0.0		-		-		-		-
*Mucor himelis**		-	1	0.56 ± 0.0	1	0.056 ± 0.0	1	0.167 ± 0.0		-		-
*M.mucedo**		-		-		-	1	1.11 ± 0.0		-		-
Mucor sp.*	1	0.306 ± 0.0		-		-		-		-		-
*Myrothecium cinctum**		-		-		-	1	0.11 ± 0.0		-		-
*M.verrucaria**		-	1	0.028 ± 0.0		-	1	0.139 ± 0.0		-		-
Neocosmospora sp.*	2	0.58 ± 6.71	1	0.028 ± 0.0		-		-		-		-
*Nigrospora oryzae**		-		-		-	1	0.028 ± 0.0		-		-
*N.sphaerica**		-		-		-	1	0.028 ± 0.0		-		-
*Papulaspora irregularis**		-		-	1	0.028 ± 0.0		-		-		-
Penicillium nigricans		-		-	1	0.028 ± 0.0		-		-		-
Penicillium sp.		-		-	3	0.33 ± 3.18	2	0.056 ± 0.0		-		-
Phoma glomerata		-		-		-	1	0.28 ± 0.0		-		-
Phoma sp.		-		-	1	0.083 ± 0.0	1	0.11 ± 0.707		-		-
*Pseudoeurotium zonatum**	1	0.028 ± 0.0		-		-	2	0.028 ± 0.0		-		-
Rhizopus oryzae	1	0.88 ± 0.0	1	0.056 ± 0.0	2	0.56 ± 0.0		-	1	0.056 ± 0.0		-
R.stolonifer	10	4.056 ± 12.10	7	1.27 ± 3.72	16	4.167 ± 22.80	14	2.61 ± 18.54	1	0.11 ± 0.0		-
*Scopulariopsis brevicaulis**		-		-		-	1	0.083 ± 0.0		-		-
Sordaria sp.*	3	0.61 ± 3.4	2	2.83 ± 34.64		-		-		-		-
Syncephalastrum sp.*		-		-	1	0.028 ± 0.0		-		-		-
Trichoderma hamatum		-		-	2	0.138 ± 0.354		-		-		-
Verticillium sp.*		-		-		-	1	0.083 ± 0.0		-		-

*=New reports; I % = Infection percentage; NSI = Number of samples infected; NSt = Non-surface sterilized seeds; SSt = Surface sterilized seeds. SD = Standard Deviation.

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