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MYCOFLORA ASSOCIATED WITH LENTIL (*LENS CULINARIS* L.) SEEDS OF PAKISTAN

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Abstract

Twenty one seed samples of lentil (*Lens culinaris* L.) collected from various localities of Pakistan viz., Karachi (12), Sukkur (1), Swabi (1), Mardan (2), Ghazi (1), Wah (1), Faisalabad (1), Lahore(1) and Hub(1) were analyzed for the detection of seed-borne mycoflora using standard blotter, agar-plate and deep-freezing methods as suggested by ISTA. Total number of 42 fungal species belonging to 18 genera were isolated. Of these 8 fungal species viz., *Absidia corymbifera*, *Actinomucor elegans*, *Chrysosporium pannorum, Myrothecium cinctum*, *Oidiodendron truncatum*, *Scopulariopsis acremonium*, *Trichoderma hamatum* and *T. polysporum* are new reports from Pakistan on lentil seeds. Of the three methods used, agar plate method was found to be better for the isolation of fungi from lentil seeds. Surface disinfection by 1% $Ca(OCl)_2$ reduced the incidence of *Aspergillus* spp.

Introduction

Lentil (*Lens culinaris* L.) of the family Fabaceae is native to sub-continent. It is cultivated in sandy loam soil and can be grown in nutrient deficient soil (Summerfield, 1981). Lentils are drought resistant and can be grown in water logged and saline soils (Muehlbaur *et al.*, 2002). In Pakistan it is cultivated as Rabi crop on an area of 30.4 thousand hectares and the production of 14.6 thousands tonnes with an average yield of 480 Kg/hectare during 2007-2008 (Anon, 2007; Hussain *et al.*, 2007). Lentil seeds are rich in protein with concentration averaging 26%; however, there is shortage of certain amino acids including Methionine and Cystine (Muehlbauer *et al.*, 2002). Lentil is a good source of vitamin B and other groups while minerals reported from lentil included calcium, phosphorus, sodium, potassium etc., (Sastri, 1962). Lentil is one of the healthiest foods (Raymond, 2006) and considered as one of the best vegetable source of Iron and important for adolescent and pregnant women (Anon., 2004). A survey of literature showed that many fungal species have been reported from lentil seeds including species of *Alternaria*, *Chaetomium*, *Drechslera*, *Fusarium*, *Phoma*, *Monilia*, *Penicillium*, *Rhizopus*, *Mucor* and *Macrophomina phaseolina* from Pakistan (Ahmed *et al.*, 1993). Richardson (1979) gave a list of seed-borne diseases of lentil according to which *Botrytis* spp., and *Fusarium oxysporum* were isolated from lentil seeds from Czechoslovakia and *Uromyces fabae* from debris mixed with seeds from India. Lentil also suffers from root rot and wilt complex caused by *Pythium, Rhizoctonia, Sclerotium* and *Fusarium* spp., (Muehlbaur *et al.,* 2002). Hussain *et al.*, (2007) isolated *Alternaria alternata, Aspergillus* spp*., Fusarium moniliforme, Mucor hiemalis, Chaetomium* spp*., Penicillium citrinum* and *Nigrospora* spp., from the 25 seed samples collected from various localities of the Punjab, Pakistan. Lentil is one of the important food crops and is consumed as an important part of diet in the sub-continent. Presence of so much storage and pathogenic fungi reduces the quality and quantity of crop, also the application of improper cultural practices and lack of proper storage conditions along with several other problems resulted in yield losses which in turn cause economic losses. In view of the economic importance of the crop, present work was carried out to explore the seed-borne mycoflora associated with lentil (*Lens culinaris* L.).

Materials and Methods

For the detection of seed-borne mycoflora ISTA techniques were used (Anon., 1993). By using standard blotter, agar plate and deep-freezing methods, about 400 seeds of each sample were tested.

Collection of seeds: Lentil seeds (21 samples) were collected from different localities of Pakistan viz., Karachi (12), Sukkur (1), Swabi (1), Mardan (2), Ghazi (1), Wah (1), Faisalabad (1), Lahore(1) and Hub(1).

Standard blotter method: Untreated and seeds after treatment with 1% $Ca(OCl)_2$ for 5 minutes were placed on three layers of moistened blotter paper, 20 seeds per Petri dish. The dishes were incubated for 7 days at 24 ±1°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon. , 1993).

Agar plate method: Untreated seeds and seeds after surface sterilization with 1% $Ca(OCl)_2$ for 5 minutes were placed on potato dextrose agar (PDA), 20 seeds per Petri dish. The dishes were then incubated for 7days at 24±1°C under 12h, alternating cycles of artificial day light (ADL) and darkness (Anon., 1993).

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

Identification of fungi: Fungi growing on seeds were identified after reference to Barnett (1960), Booth (1971), Domsch *et al.,* (1980), Ellis (1971), Nelson *et al*., (1983), Raper & Fennell (1965).

Results

Total number of 42 species belonging to 18 genera of fungi viz., *Absidia corymbifera* (Cohn) Sacc. & Trotter, *Actinomucor elegans* (Eidam) C.R. Benjamin & Hesseltine, *Alternaria alternata* (Fr.) Keissler, *A. cheiranthi* (Libert) Bolle, *A. raphani* Groves & Skolko, *A. sonchi* J.J. Davis, *A. tenuissima* (Kunze ex Pers.) Wiltshire, *Aspergillus alutaceus* Berk. & Curt., *A. candidus* Link ex Link, *A. clavatus* Desm., *A. erythrocephalus* Berk. & Curt. , *A. flavus* Link ex Gray, *A. fumigatus* Fres., *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. restrictus* G. Sm., *A. sulphureus* Thom & Church, *A. tamari* Kita, *A. terreus* Thom, *A. ustus* (Bain.) Thom & Church, *A. versicolor* (Vuill.) Tiraboschi, *A. wentii* Wehymer, *Chaetomium indicum* Corda, *Chrysosporium pannorum* (Link) Hughes, *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M.B. Ellis, *D. hawaiiensis* (Bugnic.) Subram. & B.L. Jain, *Fusarium aquaeductuum* (Radlk. & Rabenh) Lagerh., *Fusarium oxysporum* Schlecht., *F. semitectum* Berk. & Ravenel, *Macrophomina phaseolina* (Tassi) Goid, *Monilia* spp., Pers. Ex Fr., *Mucor* spp., Mich. Ex St.-Am., *Myrothecium cinctum* (Corda) Sacc., *Oidiodendron truncatum* Barron, *Penicillium* spp., Link ex Fr., *Rhizoctonia solani* Kühn., *Rhizopus arrhizus* (Fischer), *R. oryzae* Went & Prinsen Geerligs, *R. stolonifer* (Ehrenb. Ex Link) Lind, *Scopulariopsis acremonium* (Delacr.) Vuill., *Trichoderma hamatum* (Bonord.) Bainier and *T. polysporum* Rifai were

isolated from lentil seeds (Table 1). Of the 42 species isolated, *Absidia corymbifera*, *Actinomucor elegans*, *Chrysosporium pannorum, Myrothecium cinctum*, *Oidiodendron truncatum*, *Scopulariopsis acremonium*, *Trichoderma hamatum* and *T. polysporum* were found to be new reports from Pakistan on lentil seeds (Hussain & Ahmed, 1971; Jamal & Ghaffar, 1974; Nayeemullah, 1977; Khan *et al.*, 1984; Illyas, 1990; Rafique, 1991; Hussain *et al.*, 2007).

Aspergillus flavus was isolated on all the samples followed by *A. fumigatus* and *A. niger*. Of the 21 samples tested, Bhutta village (Karachi) and Ghazi (Khyber Pakhtunkhwa) samples were found to be infected with the pathogenic fungi including *F. oxysporum, F. solani, D. australiensis, Monilia* spp., *Absidia corymbifera*, *Chaetomium indicum*, *Myrothecium cinctum* and *S. acremonium* (Table 1). Sterilized (0.14 %) and non sterilized seeds (0.095 %) of only one sample from New Hali Road (Karachi) was found to be infected with *R. solani.* Of the three methods used, agar plate method yielded highest number of fungi. Surface disinfection of seeds by 1% $Ca(OCl)_2$ reduced the microbial infestation. In blotter method, *A. flavus* was dominant on non sterilized seeds with an infection range of 8.62%, in sterilized seeds and *A. fumigatus* was dominant with an infection range of 11.07%. On agar plate method, 28.89% infestation was observed by *A. flavus* in non sterilized seeds while 23.69% infestation was observed by *A. fumigatus* on surface sterilized seeds. 8.26% infestation of *A. flavus* was observed on non sterilized seeds by deep-freezing method. Other fungi observed on both non sterilized and sterilized seeds included species of *Absidia, Alternaria, Chaetomium, Drechslera, Fusarium, Monilia, Mucor, Myrothecium, Oidiodendron, Penicillium, R. solani*, *Rhizopus*, *Scopulariopsis* and *Trichoderma*. Out of 21 samples tested, 7 samples were found to be infected with *Aspergillus* spp., by deep freezing method. *Penicillium* spp., was isolated from deep freezing method (0.02%) and agar plate method (0.047–0.52%). Highest number of pathogenic fungi like *M. phaseolina* (0.2%), *F. aquaeductuum* (0.02%), *F. oxysporum* (0.02 %) were observed by deep freezing method (Table 1).

Discussion

Agar plate method was found better in terms of percentage recovery of fungal species where it yielded 38 species belonging to 18 genera of fungi. Kumar *et al.,* (2002), Hussain *et al*, (2007) suggested agar plate method with PDA to be better than blotter method in terms of percentage recovery of fungi in lentil seeds. Limonard (1968) reported that intrafungal antagonism becomes a problem in agar plate method, Tempe (1970) reported that quick growing of saprophytic fungi like *Aspergillus* and *Cladosporium* spp., adhering to seed surface becomes troublesome especially in the detection of slow growing fungi present internally. Surface disinfestations by 1% $Ca(OCl)_2$ reduced the incidence of quick growing saprophytic and mold fungi along with other microbial organisms. Similar results were also reported by Tariq *et al.,* (2005) on soy bean, Kumar *et al*., (2002) on lentil, Dawar & Ghaffar (1991) on sunflower seeds, Niaz & Dawar (2009) on maize. Present results showed that deep freezing method was found to be best for the detection of *F. aquaeductuum*, *F. oxysporum*, *M. phaseolina*, *Monilia* spp., *Penicillium* spp., *Scopulariopsis acremonium*, *Trichoderma hamatum* and *T. polysporum.* Niaz & Dawar (2009) reported that deep freezing method was considered best for the isolation of *Drechslera* spp., *Fusarium* spp., and *Penicillium* spp. Deep freezing method was considered to be most suitable for the detectation of *Fusarium* spp., (Mathur *et al.*, 1975).

Table 1. Isolation of fungi isolated from lentil.

Name of fungi	Sterilized seeds						Non-sterilized seeds					
	Blotter method		Agar plate		Deep freezing		Blotter method		Agar plate		Deep Freezing	
	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD
**Absidia corymbifera*	-	-	-	-	-	-	1	0.143±0	-	-	-	-
**Actinomucor elegans*	-	-	1	0.05±0	-	-	-	-	1	0.071±0	-	-
Alternaria alternata	1	0.26±2.22	1	0.2±0	1	0.02±0	-	-	-	-	1	0.02±0
A. cheiranthi	-	-	1	0.02 ± 0	-	-	-	-	-	-	-	-
A. raphani	1	0.05 ±0	-	-	-	-	-	-	-	-	-	-
A. sonchi	-	-	-	-	-	-	-	-	-	-	1	0.0458± 0
A.tenuissima	-	-	2	0.05± 0	-	-	-	-	1	0.07 ± 0	1	0.023 ± 0
Aspergillus alutaceus	-	-	1	0.02 ± 0	-	-	-	-	-	-	-	-
A. candidus	3	0.095± 0	7	1.43 ± 2.7	2	0.071 ± 0.71	1	0.19 ± 0	8	1.05 ± 2.2	2	0.071±0.71
A. clavatus	1	0.24 ± 0	-	-	-	-	2	0.26 ± 2.9	-	-	1	0.14 ± 0
A. erythrocephalus	-	-	1	0.02 ± 0	-	-	1	0.29 ± 0.6	-	-	-	-
A. flavus	16	9.05±7.6	21	22.07 ± 6.29	7	9.5 ± 4.7	20	8.62 ± 7.4	20	28.89± 6.3	7	8.26 ±6.3
A. fumigatus	17	11.07± 6.31	17	23.69 ± 7.63	5	9.5 ± 5.8	14	7.95 ± 5.1	20	19.17±11.8	5	3.43 ± 4.9
A. niger	14	1.95 ± 1.29	18	10.33 ± 5.9	2	0.97 ± 5.5	13	1.76 ± 2.1	20	11.29 ± 3.9	1	0.90 ± 4.10
A. restrictus	-	-	1	0.02 ± 1	-	-	-	-	-	-	-	-
A. sulphureus	-	-	1	0.05 ± 0	-	-	-	-	1	0.07 ± 0	-	-
A. tamarii	-	-	1	0.02 ± 0	-	-	1	0.095 ± 0	-	-	-	-
A. terreus	-	-	5	5.57 ± 7.5	-	0.309 ± 0	1	0.07 ± 0	9	2.10 ± 5.9	1	0.095 ± 0
A. ustus	-	-	-	-	-	-	-	-	1	0.05 ± 0	-	-
A. versicolor	1	0.024 ± 0	1	0.02 ± 0	-	-	-	-	-	-	1	0.047±0
A. wentii	1	0.024 ± 0	6	0.5 ± 1.5	3	0.714 ± 1.5	4	0. ± 0.38	7	0.95 ± 3.1	3	0.214 ± 0.8
A. ochraceus	-	-	4	0.38 ± 2.4	-	-	1	0.24 ± 4.2	4	0.36 ± 2.4	1	0.071 ± 0

Table 1. (Cont'd.).

Name of fungi	Sterilized seeds						Non-sterilized seeds					
	Blotter method		Agar plate		Deep freezing		Blotter method		Agar plate		Deep Freezing	
	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD	NSI	I % ± SD
Chaetomium indicum	-	-	1	0.023±0	-	-	-	-	-	-	-	-
**Chrysosporium pannorum*	-	-	1	0.023±0	-	-	-	-	1	0.023±0	-	-
Drechslera australiensis	-	-	2	0.12±2.12	-	-	-	-	1	0.095 ± 0	-	-
D.hawaiiensis	-	-	1	0.047 ± 0	-	-	-	-	-	-	-	-
Fusarium aquaductum	-	-	1	0.02 ± 0	-	-		-	-	-	1	0.02 ± 0
F.oxysporum	-	-	-	-	1	0.02 ± 0	-	-	1	0.12 ± 0	-	-
F.semitectum	-		-	-	-	-	-	-	4	1 ± 7.8	-	-
Monilia spp	4	0.47 ± 1.56	9	3.33 ± 7.17	1	0.74 ± 0.71	-	-	7	3.43 ± 7.23	2	0.405±2.36
Mucor spp	1	0.167 ± 2.1	2	1017±9.29	-	-	1	0.45±0	4	0.52±6.5	-	-
**Myrothecium* spp	-	-	1	0.047±0	1	0.05±0	-	-	-	-	-	-
**Oidiodendron truncatum*	1	0.024±0	1	0.02±0	1	0.23±0	-	-	-	-	1	0.048±0
Penicillium spp	-	-	1	0.52±11.3	-	-	-	-	1	0.047±0	1	0.02±0
Rhizoctonia solani	-	-	1	0.14±1	-	-	-	-	1	0.095±0	-	-
Rhizopus arrhizus	-	-	1	1±0.0	-	-	-	-	-		-	-
R. oryzae	1	0.047±0	4	1.45±5.04	-	-	3	1.26±10.12	8	2.52±5.5	-	-
R.stolonifer	2	0.19±2.89	6	4.91±6.67	-	-	3	0.14±0.96	3	1.48±4.9	-	-
**Scopulariopsis acremonium*	-	-	1	0.39±0	-	-	-	-	-	-	1	0.167±3.5
**Trichoderma hamatum*	-	-	1	0.41±4.7	1	0.071±0	-	-	1	0.14±0	-	-
**T.polysporum*	-	-	1	0.07± 0	-	-	-	-	-	-	-	-

NSI = No of samples infected
SD = Standard deviation
I % = Infection %
* = New reports on lentil seeds from Pakistan

Presently it was observed that 42 fungal species belonging to 18 genera were isolated from lentil seeds by ISTA techniques whereas El-Nagerabi & El-Shafie (2000) isolated 69 species belonging to 24 genera of fungi from Sudan, Abd-Allah & Hashem (2006) isolated 32 fungal species belonging to 17 genera from the samples of lentil collected from Egypt. Results showed that *A. flavus* was isolated on all the samples followed by *A. fumigatus* and *A. niger*. Purchase (1974), Diener & Davis, (1969) reported that aflatoxins are produced by strains of *A. flavus, A. parasiticus* and *A. niger* and these compounds are designated as aflatoxins B_1 B_2, G_1 and G_2. These aflatoxin are carcinogenic and produces liver cancer. El Maraghy (1988) observed the presence of aflatoxin at 20 mg/kg in lentils. It was observed that lentil seeds allow fungal growth but no aflatoxin was detected from the isolates of *A. flavus* due to the fact that lentil may contain anti-aflatoxigenic factor (Mabrouk & El-Shayeb, 1980). *A. niger* and *A. flavus* were observed to be common allergens and may cause opportunistic invasive infection (Denning, 1998; De Hoog *et al.*, 2000). Of the *Fusarium* spp., isolated important mycotoxin producers like *F. oxysporum* produce zeralenone α and β causing haemorrhage and necrosis in bone marrow. *F. solani* cause corneal ulcer whereas *F. proliferatum* and *F. verticillioides* causes epidemiologically human esophageal cancer (Desjardins *et al.,* 2006). Presently *Penicillium* spp., was observed from lentil seeds. There are many species of *Penicillium* found to be associated with the grain seeds reported to produce mycotoxins that cause mycotoxicoses of domestic animals and man (Scott *et al.,* 1972; Scott, 1978). El-Maghraby & El-Maraghy (1988) observed citrinin production by *P. citrinum* from groundnut seeds. Tanazawaic acid and citrinin produced by 25 isolates of *P. citrinum* (Malmstroem *et al.,* 2000). As lentil is one of the oldest food crops of the world and consumed for its nutritional values, measures should be taken to improve the crop quality and seed-storage conditions. Even the yield of lentil crop is reducing annually due to environmental and various other agronomic factors (Hussain *et al.*, 2007). Steps should be taken on emergency basis to reduce the disease incidence and increase the yield of lentil in Pakistan.

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