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MYCOFLORA ASSOCIATED WITH LENTIL (*LENS ESCULENTA* MOENCH) SEEDS FROM FIVE LOCALITIES OF PUNJAB, PAKISTAN

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Abstract

Twenty five samples of lentil (*Lens esculenta* Moench) seed collected from the markets of Faisalabad, Dera Ghazi Khan, Bahawalpur, Jampur and Rojhan were analyzed for externally and internally seed-borne fungi. The infestation of untreated seed samples varied from 43.3 to 51.4% with an average of 46.96%, while the seeds treated with 2% Mercuric chloride solution showed the infestation from 18.0 to 22.4% with an average of 20.4% on blotter paper. On PDA the infestation of untreated seed samples were from 48.2 to 58.8% with an average of 52.52%, while the seeds treated with 2% Mercuric chloride solution, showed an infestation of 18.8 to 23.6% with an average of 20.87%.The fungi isolated from treated seeds were *Fusarium moniliforme, Alternaria alternata, Mucor hiemalis, Chaetomium* spp., *Penicillium citrinum, Aspergillus niger, A. flavus, A .terreus* and *Nigrospora* spp., *F. moniliforme, A. alternata, M. hiemalis, Chaetomium* spp., and *A. niger* were common in all samples while *P. citrinum, A. flavus, A. terreus* and *Nigrospora* spp., were only isolated from untreated seed.

Introduction

Lentil (*Lens esculenta* Moench), of the family *Leguminosae* is an important pulse crop of Pakistan grown in rabi season, and is generally cultivated on marginal type of land with low fertility. Lentil in the world is known by many names viz., adas (Arabic), masur (Hindi), mercimek (Turkish) and heramame (Japanese). The protein in lentils contains significant concentrations of lysine, a limiting amino acid in cereals. Cereals are relatively rich in tryptophan and the sulfur-containing amino acids. Therefore, when cereals and lentils are consumed together in a balanced diet, they provide adequate amounts of essential amino acids in the human diet (Summerfield, 1981; Summerfield & Muehlbauer, 1982). In Pakistan it is cultivated on an area of 48.8 thousand hectares and the production is 25.9 thousand tones with an average yield of 603 kg/hectare (Anon., 2006). The average yield at national level is very low because of various agronomic and environmental factors as well as attack of various diseases particularly seed-borne diseases. Nobel de Tempe & Neergaard (1958) registered approximately 900 seed-borne diseases of different crop plants including various bean crops from all over the world. Richardson (1979) gave a list of seed-borne diseases of lentil, according to which *Botrytis* spp., and *F. oxysporum* were isolated from lentil seed from Czechoslovakia and *Uromyces fabae* from debris mixed with seed from India. Vishunath & Shukla (1979) observed the frequency of fungi isolated from lentil seeds and found *A. alternata, A. tenuissima, A. flauvs, A. niger, F. equiseti, F. oxysporum and Rhozoctonia bataticola* both externally and internally. Sumar & Howard (1983) studied varying number of seed samples of lentil and beans for seed-borne micro-organisms by blotter and agar plate

methods and found *Alternaria, Penicillium, Rhizopus, Fusarium* and *Botrytis* species most common. Abdel-Hafez (1984) isolated *Aspergillus* (16 species + two varieties), *Penicillium* (14 species), *Rhizopus* (1 species) and Yeasts, followed by *Fusarium* (3 species), *Mucor* (4 species) and *Drechslera* (3 species). Of these *A.niger, A. flavus, P. citrinum, R. stolonifer, F. moniliforme, F. oxysporum, Mucor hiemalis* or *M. racemosus* and *Drechslera spicifera* were the most prevalent species in the seeds of bean, broad bean, lentil, lupine and pea. Abdel-Hafez (1988) found that species of *Aspergillus, Penicillium, Rhizopus, Mucor* and *Fusarium* were predominant among 22 genera isolated from lentil seed in Egypt. Arun & Mathew (1991) studied mycoflora of pigeon pea seeds and isolated *R. nigricans, A. niger, A. fumigatus, A. alternata, Macrophomina phaseolina, F. pallidoroseum, Chaetomium* spp., *Drechslera* spp., and *Phoma* spp. Singh & Tripathy, (1999) studied the mycoflora associated with stored seeds of lentil using the blotter paper technique and the agar plate method on three different media (Czapeck's Dox agar, malt agar and potato dextrose agar) and isolated *A. alternata, A. flavus, A. niger, C. globosum, Cladosporium herbarum, F. oxysporum, Paecilomyces variotii, P. chrysogenum, P. italicum, R. arrhizu* and *P. chrysogenum.* Arshad *et al*., (2005) studied the seeds of four pulses viz. chickpea (*Cicer arietinum* L.) black and white, mungbean (*Vigna radiata* (L.) Wilczek), mashbean (*Vigna mungo* (L.) Hepper) and lentil (*Lens esculenta* Moench), collected from different shops of Lahore, for associated mycoflora and found seven fungi comprising of *A. niger* van Tieghem, *A. flavus* Link ex Gray, *A. fumigatus* Fresenius, *A. terreus* Thom., *F. equiseti* (Corda) Saccardo, *Syncephalastrum racemosum* Cohn ex Schroeter and *Rhizopus* spp. It is obvious from the above that seeds harbour different types of micro-organisms which may results in their decay resulting into pre- and post-emergence death of seedlings. Seedlings during storage are also affected due to their high moisture contents and adverse storage conditions. Lentil seed, like other cultivated crops, carries many fungi and in turn suffers many seed borne diseases. Information on seed-borne diseases is, however, very meager, therefore, the present studies were undertaken to explore mycoflora associated with lentil seeds.

Materials and Methods

Four hundred seeds from each tested sample were taken at random out of those 25 seeds were placed without treatment in sterilized Petri plates containing three sterilized blotter paper. Another four hundred seeds from same sample were disinfested with 0.2% Mercuric chloride solution and placed 25 seeds in same way as described above. Similarly two other sets of 400 seeds from each sample following same procedure were plated on PDA. Numbers of fungi were recorded after 10 days in case of blotter paper while after 5-7 days on PDA. The isolated fungi were transferred to culture slants for further growth and identification upto species level.

Results

The infestation of untreated seed samples varied from 43.4 to 51.4% with an average of 46.96%, whereas infestation of treated samples varied from 18.0 to 22.4% with an average of 20.4% on blotter paper. On the other hand, on PDA, infestation of untreated seed samples varied from 48.2 to 58.8% with an average of 52.52%, whereas infestation of treated samples varied from 18.8 to 23.6% with an average of 20.87%.The fungi isolated from lentil seeds were *F.moniliforme* 10.6 %, *A. alternata* 16.9%, *M. hiemalis* 4.5%, *Chaetomium* spp., 4.9%, *P. citrinum* 8.29%, *A. niger* 15.2%, *A. flavus* 19.3%, *A. terreus* 10.9% and *Nigrospora* spp., 0.4%. *F. moniliforme, A. alternata, M. hiemalis,*

Chaetomium spp., and *A. niger* were common in all samples. *P. citrinum, A. flavus, A. terreus* and *Nigrospora* spp., were most prevalent on untreated seeds. Percent infestation of infested seeds collected from different localities is given in Table 1.

External and internal infestation percentage of different seed-borne fungi in treated seed samples from different localities is given in Table 2, whereas internal infestation percentage of different seed-borne fungi in treated seed samples from different localities is given in Table 3.

Table 1. Percent infestation in seed samples collected from different localities.

Locality	No. of samples	Percent infestation	
		External	Internal
Faisalabad	5	26.2	21.4
Dera Ghazi Khan	5	25.8	22.4
Bahawalpur	5	32.3	19.8
Jampur	5	27.7	19.4
Rojhan	5	29.4	20.3
Average		**28.28**	**20.66**

Table 2. External and internal infestation percentage of different seed-borne fungi in untreated seed samples of different localities.

Fungi	Percentage infestation on			
	Blotter paper		PDA	
	Range	Average	Range	Average
Fusarium moniliforme	8.27-12.71	11.49	9.8-11.45	10.55
Alternaria alternata	12.2-21.2	17.39	9.7-22.7	16.8
Mucor hiemalis	1.2-5.2	3.9	3.0-7.3	5.5
Chaetomium spp.	2.7-6.5	4.3	5.1-9.2	7.0
Penicillium citrinum	5.6-11.7	8.2	5.1-12.3	8.3
Aspergillus niger	13.5-21.7	17.7	12.1-18.9	16.0
Aspergillus flavus	17.0-21.7	19.7	18.0-21.6	18.9
Aspergillus terreus	10.3-12.4	11.1	9.7-12.5	10.7
Nigrospora spp.	0.1-0.5	0.4	0.1-0.3	0.2

Table 3. Internal infestation percentage of different seed-borne fungi in treated seed samples of different localities.

Fungi	Percentage infestation on			
	Blotter paper		PDA	
	Range	Average	Range	Average
Fusarium moniliforme	6.9-17.1	10.3	8.6-11.5	10.1
Alternaria alternata	10.7-20.6	16.0	15.4-20.0	17.7
Mucor hiemalis	3.07-5.4	3.4	3.2-6.9	5.13
Chaetomium spp.	2.1-5.1	3.7	3.7-6.3	4.6
Penicillium citrinum	-	-	-	-
Aspergillus niger	11.2-17.3	14.4	10.2-15.0	12.8
Aspergillus flavus	-	-	-	-
Aspergillus terreus	-	-	-	-
Nigrospora spp.	-	-	-	-

Discussion

The external infestation of lentil seed in tested samples varied from 26.2 to 32.2% with an average of 28.2%. Even surface disinfestations of these seeds with 0.2% solution of Mercuric chloride for two minutes did not check infestation completely except that of *P. citrinum*, *Aspergillus* spp., and *Nigrospora* spp. The surface disinfested seeds gave infestation varying from 19.6 to 22.4% with an average of 20.4% which clearly indicated that the fungi were present deep within the seed. The fungi isolated from the infested seed were *Aspergillus* spp., *F. moniliformae, A. alternata*, *P. citrinum, M. hiemalis*, *Chaetomium* spp., and *Nigrospora* present singly or in combination with each other. Similar results have already been reported by Vishunavat & Shukla (1979), Abdel-Hafez (1988), Arun & Mathew (1991) and Sumar & Howard (1983). *A. niger, A. flavus and A. terreus* were recorded from all samples in higher percentage ranging from 13.5 to 21.7%, 17.0 to 21.5% and 10.3 to 12.4% with an average of 17.8, 19.6 and 11.1%. The same fungi were also reported by Vishunavat & Shukla (1983). The present studies showed that different pathogenic fungi are associated with lentil seeds, therefore it is indispensable to treat the seeds with systemic fungicides.

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