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SEED-BORNE MYCOFLORA OF WHEAT, SORGHUM AND BARLEY

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Abstract

Standard blotter and Deep Freezing methods were used to study the seed-borne mycoflora of 19 samples of wheat, 27 samples of sorghum and 14 samples of barley. A significant contamination with fungal genera was detected in analyzed samples. Fungi most frequently isolated and identified were *Absidia* sp., *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger, A.sulphureus, Cephalosprium* sp., *Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera dematioidea, D. halodes, D. hawaiiensis, D. tetramera, Fusarium moniliforme, F. oxysporum, F. pallidoroseum, F. subglutinans, Nigrospora oryzae, Penicillium* spp., *Piptocephalis* sp., *Rhizoctonia solani, Rhizopus* sp., *Stemphylium* sp., *Syncephalastrum racemosum, Trichoderma hamatum, Trichothecium roseum and Ulocladium* sp. This is the first report of *Chaetomium globosum* and *D. hawaiiensis* on wheat, *A.sulphureus, Fusarium subglutinans, Nigrospora oryzae, Piptocephalis* sp., *Syncephalastrum racemosum* and *Trichoderma hamatum* on sorghum and *A. niger, Cephalosprium* sp., *Cladosporium herbarum, Drechslera dematioidea, D. tetramera, Trichothecium roseum, Stemphylium* sp., and *Ulocladium* sp., are new records on barley. There does not appear to be any previous report of *Absidia* sp., *Aspergillus sulphureus, Fusarium subglutinans* and *Rhizoctonia solani* on wheat in Pakistan. Deep freezing method showed better results for isolation of *Alternaria alternata, Cladosporium herbarum, Drechslera* spp., and *Fusarium* spp.

Introduction

Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens (Agarwal & Sinclair, 1996).

Seed-borne diseases have been found to affect the growth and productivity of crop plants (Kubiak & Korbas, 1999; Weber *et al*., 2001; Dawson & Bateman, 2001). A seed-borne pathogen present externally or internally or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Khanzada *et al*., 2002; Bateman & Kwasna, 1999).

Wheat is one of the main staple food of man and is grown in almost all the temperate and subtropical regions of the world. Seed-borne mycoflora of wheat reported recently included *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum, Stemphylium botryosum* (Nirenberg *et al.*, 1994; Glazek, 1997; Mirza & Qureshi,1978).

Seed-borne mycoflora of sorghum reported from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* sp., *Fusarium moniliforme, F.oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora* sp., *Phoma* sp., and *Rhizopus* sp., (Abdullah and Kadhum, 1987; Ahmed *et al.*, 1992).

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Some of the seed-borne mycoflora of barley include *Alternaria alternata, Aspergillus candidus, A. flavus, Curvularia lunata, Drechslera halodes, Fusarium moniliforme, F. pallidoroseum, F.solani* and *Ulocladium* sp., (Richardson, 1983; Ahmed *et al*., 1997).

Besides, the mold fungi which grow on the seed substratum produce mycotoxins which are hazardous to man and animals (Halt, 1994). Studies were carried out to study the composition of seed-borne mycoflora occurring in wheat, sorghum and barley grains which are the main crops grown in Pakistan.

Materials and Methods

Seed samples of *Triticum aestivum* (19 samples), *Sorghum vulgare* (27 samples) and *Hordeum vulgare* (14 samples) collected from different localities of Pakistan viz., Karachi, Lahore, Islamabad and Faisalabad were used for the isolation and detection of seed-borne fungi. Blotter Method recommended by International Seed Testing Association (Anon., 1966) and Deep Freezing Method (Limonard, 1968) were used for the isolation of fungi.

Blotter Method: Four hundred seeds of each sample were plated on three layers of moistened blotters placed in 90 mm diam., Petri plates @ 25 seeds/plate. The plates were incubated for 7 days at 20°C ± 1°C. Then were examined under the microscope for seed-borne mycoflora.

Deep Freezing Method: Twenty five seeds per plate were plated on three layers of moistened blotters. The seeds were incubated for one day at 22 °C followed by 24 hours of freezing at -20 °C. The plates were then placed for 4-5 days at 22 °C ± 1 °C.

After incubation the growth characters as well as percentage of infection were recorded. The isolated *Fusarium* spp., were maintained on Spezieller Nahrastoffarmer agar (SNA) medium (Nirenberg, 1976; Hashmi & Thrane, 1990) whereas other fungi were maintained on PDA. The fungi were identified after reference to Barnett & Hunter (1972), Booth (1971), Nelson *et al.*, (1983).

Results and Discussion

The occurrence of fungi most frequently encountered is recorded in terms of mean value with standard error (Table 1).

a. **Wheat:** From 19 samples of wheat, 12 genera and 21 species of fungi viz., *Absidia* sp., *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger, A.sulphureus, Cephalosprium* sp., *Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera halodes, D. hawaiiensis, D. tetramera, Fusarium moniliforme, F. oxysporum, F. pallidoroseum, F. subglutinans, Penicillium* spp., *Rhizoctonia solani and Rhizopus* sp., were isolated and identified. Of the fungi isolated *Alternaria alternata, Aspergillus flavus, A. niger, Cephalosporium* sp., *Penicillium* spp., *and Rhizopus* sp., were found to be predominant. There does not appear to be any previous report of *Chaetomium globosum* and *Drechslera hawaiiensis* on wheat, whereas, *Absidia* sp., *Aspergillus sulphureus, Fusarium subglutinans* and *Rhizoctonia solani* appeared to be new report on wheat from Pakistan (Khanzada *et al*., 2002; Ahmed *et al*., 1992; Richardson, 1983; Ahmed *et al*., 1997; Ghaffar & Abbas, 1972; Glazek, 1997; Mirza & Qureshi, 1978; Weber *et al*., 2001).

Table 1. Mean infection percentage of seed-borne mycoflora of wheat, sorghum and barley studied by two different testing methods.

Place of collection/ Fungi isolated Karachi	Blotter method			Deep freezing method		
	Wheat	Sorghum	Barley	Wheat	Sorghum	Barley
Absidia sp.	1.13 ± 1.13	----	----	0.53 ± 0.53	----	----
Alternaria alternata	7.6 ± 2.80	2.62 ± 1.09	7.63 ± 7.63	12.2 ± 6.99	0.68 ± 0.45	16.5 ± 16.2
Aspergillus candidus	4.97 ± 2.82	6.95 ± 5.46	1.13 ± 0.72	0.43 ± 0.23	5.4 ± 3.30	----
A .flavus	5.6 ± 2.09	16.98 ± 3.99	1.25 ± 0.75	1.33 ± 0.55	7.62 ± 2.20	0.13 ± 0.13
A. niger	4.17 ± 1.23	12.28 ± 3.26	2.63 ± 2.01	1.63 ± 0.99	4.1 ± 1.06	0.63 ± 0.63
A. sulphureus	0.13 ± 0.09	4.97 ± 3.18	----	0.00 ± 0.00	2.80 ± 1.69	----
Aspergillus spp.	1.27 ± 0.64	7.2 ± 2.41	1.5 ± 1.5	0.27 ± 0.15	7.47 ± 2.66	0.5 ± 0.5
Cephalosporium sp.	1.63 ± 1.64	----	3.5 ± 2.06	4.33 ± 4.34	----	0.5 ± 0.3
Chaetomium globosum	0.00 ± 0.00	----	----	0.13 ± 0.13	----	----
Cladosporium herbarum	0.13 ± 0.78	----	----	0.97 ± 0.45	0.27 ± 0.27	0.25 ± 0.25
Curvularia lunata	----	0.97 ± 0.55	----	0.03 ± 0.03	0.45 ± 0.45	----
Drechslera dematioidea	----	----	0.13 ± 0.13	----	----	0.25 ± 0.25
D. halodes	0.03 ± 0.03	0.97 ± 0.55	----	0.03 ± 0.03	0.45 ± 0.45	0.13 ± 0.13
D. hawaiiensis	0.20 ± 0.20	0.02 ± 0.02	----	0.03 ± 0.03	0.03 ± 0.03	----
D. tetramera	----	2.38 ± 0.83	0.75 ± 0.75	0.20 ± 0.14	3.25 ± 1.18	0.88 ± 0.88
Fusarium moniliforme	0.13 ± 1.10	2.97 ± 1.12	0.25 ± 0.25	0.87 ± 0.52	4.05 ± 1.59	0.13 ± 0.13
F. oxysporum	----	----	----	0.07 ± 0.07	----	----
F. pallidoroseum	0.03 ± 0.03	----	0.13 ± 0.13	0.03 ± 0.03	----	---
F. solani	0.07 ± 0.07	----	----	0.10 ± 0.07	----	----
F. subglutinans	----	2.43 ± 1.00	----	----	3.42 ± 1.28	----
Nigrospora oryzae	----	0.02 ± 0.02	----	----	---	----
Penicillium spp.	11.77 ± 3.42	16.57 ± 4.97	8.75 ± 5.57	7.95 ± 2.83	20.58 ± 6.05	12.0 ± 10.68
Piptocephalis sp.	----	17.67 ± 6.75	----	-----	6.4 ± 6.05	----

Table 1 (Cont'd.)

Place of collection/ Fungi isolated Karachi	Blotter method			Deep freezing method		
	Wheat	Sorghum	Barley	Wheat	Sorghum	Barley
Rhizoctonia solani	----	----	----	0.33 ± 0.33	----	----
Rhizopus sp.	37.97 ± 5.87	20.12 ± 4.19	40.25 ± 7.32	13.1 ± 4.96	10.25 ± 3.33	0.5 ± 0.5
Stemphylium sp.	----	----	----	----	----	0.13 ± 0.13
Syncephalastrum racemosum	----	2.67 ± 1.88	----	----	4.47 ± 4.33	----
Trichoderma sp.	----	0.23 ± 0.16	----	----	----	----
Trichothecium roseum	----	----	0.75 ± 0.75	----	----	1.0 ± 1.0
Trichothecium sp.	----	0.75 ± 0.50	---	----	----	0.13 ± 0.13
Ulocladium sp.	----	----	---	----	----	----
Faisalabad						
Alternaria alternata	10.17 ± 2.67	----	----	12.16 ± 2.40	---	----
Aspergillus candidus	1.67 ± 0.83	----	----	0.33 ± 0.37	---	----
A .flavus	4.67 ± 1.04	----	----	1.83 ± 1.21	---	---
A. niger	11.17 ± 2.50	14.00 ± 1.00	----	8.00 ± 7.05	1.50 ± 0.50	---
Curvularia sp.	-----	4.50 ± 1.49	----	----	---	---
Cephalosporium sp.	8.17 ± 8.93	---	0.25 ± 0.25	21.67 ± 23.69	---	0.50 ± 0.29
Cladosporium sp.	0.17 ± 0.18	---	----	0.67 ± 0.73	---	----
Drechslera. hawaiiensis	1.00 ± 1.09	----	----	1.14 ± 0.87	----	----
Drechslera sp.	----	----	----	----	5.50 ± 0.50	----
Fusarium moniliforme	0.5 ± 3.38	16.00 ± 3.01	0.50 ± 0.29	2.83 ± 0.82	17.00 ± 0.00	1.00 ± 0.00
F. subglutinans	----	----	----	-----	0.50 ± 0.50	----
Penicillium spp.	34.5 ± 15.58	----	10.00 ± 2.35	33.17 ± 11.63	----	----
Rhizopus sp.	73.00 ± 16.55	----	1.25 ± 0.42	33.83 ± 9.40	----	0.5 ± 0.29

Table 1 (Cont'd.)

Place of collection/ Fungi isolated Karachi	Blotter method			Deep freezing method		
	Wheat	Sorghum	Barley	Wheat	Sorghum	Barley
Lahore						
Alternaria alternata	----	5.50 ± 0.87	0.75 ± 0.48	----	5.00 ± 0.41	0.50 ± 0.29
Aspergillus candidus	6.25 ± 0.63	---	----	11.75 ± 3.12	---	---
A. niger	0.5 ± 0.29	----	----	0.75 ± 0.25	---	----
Curvularia sp.	----	7.50 ± 2.26	----	----	5.75 ± 0.48	---
Drechslera sp.	----	16.50 ± 2.33	----	----	7.50 ± 1.56	---
Fusarium equiseti	----	----	0.25 ± 0.25	----	-----	1.25 ± 0.25
F. moniliforme	----	3.50 ± 0.65	----	----	5.00 ± 1.08	---
F. subglutinans	----	17.25 ± 2.40	----	----	10.50 ± 2.73	---
Penicillium spp.	1.5 ± 0.65	----	----	0.5 ±0.29	---	---
Rhizopus sp	16.25 ± 1.11	8.75 ± 2.56	----	1.5 ± 0.05	5.50 ± 2.02	---
Islamabad						
Absidia sp.	1.13 ± 1.13	----	---	0.53 ± 0.53	---	----
Alternaria alternata	7.6 ± 2.80	2.50 ± 0.50	0.25 ± 0.25	12.2 ± 6.99	3.00 ± 2.01	0.25 ± 0.25
Cephalosporium sp.	----	2.50 ± 0.50	----	---	3.00 ± 1.00	----
Curvularia lunata	----	0.50 ± 0.50	0.5 ± 0.29	---	---	1.25 ± 0.25
Drechslera tetramera	----	1.50 ± 0.50	1.00 ± 0.41	----	----	----
Aspergillus candidus	4.97 ± 2.82	---	----	0.43 ± 0.23	---	----
A .flavus	5.6 ± 2.09	---	----	1.33 ± 0.55	---	----
A. niger	4.17 ± 1.23	----	----	1.63 ± 0.99	---	----
A. sulphureus	0.13 ± 0.13	----	----	0.00 ± 0.00	----	1.50 ± 0.29
Fusarium moniliforme	1.27 ± 0.64	1.50 ± 0.50	1.00 ± 0.00	0.27 ± 0.15	.150 ± 0.50	1.25 ± 0.25
F. subglutinans	----	---	1.00 ± 1.00	----	2.50 ± 0.50	---
Penicillium spp.	1.63 ± 1.64	---	---	4.33 ± 4.34	----	---
Rhizopus sp.	0.00 ± 0.00	---	---	0.13 ± 0.13	----	----

Significant difference was observed in seed samples of wheat collected from Islamabad ($p<0.001$) and Faisalabad ($p< 0.001$), whereas samples collected from Karachi showed insignificant difference. Methods for isolation of fungi showed significant difference in samples collected from Karachi ($p<0.05$), Faisalabad ($p<0.001$), Lahore ($p<0.01$) and Islamabad (p0.001). Significant number ($p<0.001$) of fungi were isolated from samples collected from all localities.

b. Sorghum: From 27 samples of sorghum, 14 genera and 23 species of fungi viz., *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger, A.sulphureus, Curvularia* sp., *C. lunata, Cladosporium* sp., *Drechslera* sp., *D. halodes, D. tetramera, D. hawaiiensis, Nigrospora oryzae, Trichoderma hamatum, Trichothecium roseum, Piptocephalis* sp., *Syncephalastrum racemosum, Fusarium moniliforme, F. subglutinans, Penicillium* spp., *and Rhizopus* sp. were isolated and identified. Of these, *Aspergillus candidus, A. flavus, A. niger, Penicillium* spp., *Piptocephalis* sp. *and Rhizopus* sp. were found to be predominant. There does not appear to be any previous report of *Aspergillus sulphureus, Nigrospora oryzae, Trichoderma hamatum, Fusarium subglutinans, Piptocephalis* sp., and *Syncephalastrum racemosum* on sorghum (Ahmed *et al*., 1992; Ahmed *et al*., 1997; Ghaffar & Abbas, 1972; Mirza & Qureshi, 1978; Williams and McDonald, 1983).

Significant difference was observed in seed samples of sorghum collected from Karachi ($p<0.001$). Simple blotter and deep freezing methods for isolation of fungi showed significant difference in samples collected from Karachi ($p<0.01$) and Lahore ($p<0.01$), whereas nonsignificant difference was observed in samples collected from Islamabad and Faisalabad. Significant number ($p<0.001$) fungi were isolated from samples collected from all localities except from Islamabad ($p<0.01$).

c. Barley: From 14 samples of barley, 11 genera and 17 species of fungi viz., *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger, Cephalosprium* sp., *Cladosporium herbarum, Drechslera dematioidea, D. halodes, D. tetramera, Trichothecium roseum, Fusarium moniliforme, F. pallidoroseum, Penicillium* sp., *Stemphylium* sp., and *Ulocladium* sp. were isolated and identified. Of these *Alternaria alternata, Aspergillus niger, Penicillium* spp., *Cephalosprium* sp., and *Rhizopus* sp., were found to be predominant. *Aspergillus niger, Cephalosprium* sp., *Cladosporium herbarum, Drechslera dematioidea, D. tetramera, Trichothecium roseum, Stemphylium* sp., and *Ulocladium* sp., appeared to be new records on barley (Ahmed *et al*., 1992; Ahmed *et al*., 1997; Chong & Sheridan, 1982; Ghaffar & Abbas, 1972; Mirza & Qureshi, 1978).

Significant number ($p<0.001$) of fungi were isolated from seed samples of barley collected from Karachi and Faisalabad. Significant difference ($p<0.001$) was observed in simple blotter and deep freezing methods.

In the present work higher percentage of *Fusarium* spp., and *Drechslera* spp., was found where deep freezing method was used and mean infection range of *Fusarium* also increased (Fakhrunnisa & Hashmi, 1992). This may be partly due to the ease of observation and partly due to the fact that the dead embryo provides nourishment to the developing mycoflora. SNA medium appears satisfactory for the isolation and identification of *Fusarium* spp.

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