Mycoflora of Wheat (*Triticum aestivum* L.) at Different Locations in Hail Area- Saudi Arabia

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ABSTRACT--- ***600 grain samples collected from wheat fields in Hail area at the northern part of Saudi Arabia was used for this study. Isolation and identification of seed-borne fungi were conducted according to standard tests described by the International Seed Testing Association (ISTA) using YGCA medium. A total of 505 of external mycoflora and 705 of internal mycoflora were grouped into five fungal genera namely, Aspergillus ; Alternaria ; Penillium ; Fusarium and Ulocladium spp. were isolated. Comparsion between frequencies and relative densities of external and internal mycoflora was carried out among the species of the predominant genera. Aspergillus flavus and A. niger reaveled high Fr. and RD of external mycoflora (A. flavus Fr.67.5 - 48.2% , RD. 44.3 - 38.3% and A. niger Fr. 50.8 - 50.8% and RD. 41.4-36.4 respectively). All the species of Ulocladium and Alternaria were predominant as internal mycoflora except Alternaria alternata . The most predominant species of Ulocladium and Alternaria were U. atrium (Fr 84.2% -75.8 and RD 88.4%-76.6 as internal – external mycoflora respectively) and Alternaria alternate (Fr 75.5% - 45.2% and RD. 37.2 - 68.8 as external –internal mycoflora respectively).***

Keywords--- External mycoflora, Internal mycoflora, *Aspergillus* spp., *Alternaria* spp., *Ulocladium* spp.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) a cereal crop belonging to family *Gramineae* and known to have originated from the Fertile Crescent region of the near east, used as a staple food around the world. It ranked first as essential food crop in Saudi Arabia followed by rice and barely but to fulfill requirement, country has to import wheat significantly from other countries [1]. In the year 2004, cultivated area of wheat in Saudi Arabia was 618720 ha with production of about 419220 ton and in Hail area the production was 157220 tons [2]. Seed borne mycoflora is one of the major components reducing the wheat yield. Mycoflora associated with seeds both internally and externally are responsible for most seed disease [3].Yield losses due to seed borne fungi have been reported between 15 to 90% of untreated seeds grown in field [4]. Seed borne pathogens of wheat include *Alternaria alternata*, *Cladosporium oxysporum*, *Curvularia lunata*, *Drechslera*. *sorokiniana*, *D. tetramera*, *Fusarium graminearum*, *Helminthosporium sativu.* Distribution percentage and post-harvest borne pathogen associated with wheat seeds provides valuable information regarding mycoflora and their efficient control. Most of the previous studies of the fungi include species of *Aspergillus* and *Penicillium* [5]. Genera of *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Penicillium* has been the most common isolated fungi from wheat seeds [6]. For the management of crop disease the mycoflora of wheat focused on the internal fungi isolated from disinfected grains. However type of fungi and their distribution should be taken in consideration and therefore the qualitative composition of the mycoflora occurring on the surface and inside the grain may an indicative of its condition. Germination test of seeds is significant in identifying seed abortion, mortality of grains, reduction in germination capacity, seed necrosis and at the end cause destructive to serious diseases during different stages of plant growth The aim of this study is to identify the isolated fungi associated with wheat grains in Hail area , to determine the relationship between internal and external mycoflora and to establish the species of the genera which will record high frequency and relative density.

2. MATERIALS AND METHODS

Collection of Seed Samples: A total of 600 samples of wheat grains were collected from six locations in Hail area (Saudi Arabia). The locations were Nadic and Hatco agricultural companies and the rest were collected from four farmers at different four different villages (Kuta, Shlania, Kafa' and Delahan) during 2006-2008. Samples were collected in sterile plastic bags and kept at 4°C. All the samples were subjected to mycological analysis .

Isolation of external fungi (Seed washing method):

This test was used to study fungal inoculums located on the surface of wheat seed. 50 g of seed samples were taken in a 200 ml beaker containing 50 ml sterilized distilled water and 1 to 2 drops of Tween 80, shaken for 10 min over a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 15 min. [7]. From 1/10 and 1/100 dilution 0.1ml of the suspension was cultured on YGCA (Yeast Extract Glucose Chloroamphenicol Agar) . The plates were incubated under altering periods of 12h darkness of day light at 28± 2°C for 4 -7 days. The fungal colonies that developed were counted and those of different species were subcultured on PDA (Potato Dextrose Agar medium) and then identified on the basis of morphology under microscope [8].

Isolation of Internal fungi :

For isolation of the internal mycoflora, subsamples of wheat grains from each sample were surface sterilized using commercial 5% aqueous solution of sodium hypochlorite for 2 minutes and rinsed in two changes of sterile distilled water. The seeds were plated on sterile Yeast Extract Glucose Chloroamphenicol Agar (YGCA). Forty five grains were plated and incubated under altering periods of 12h darkness of daylight at 28± 2°C for 4 -7 days. The fungal colonies that developed were counted and those of different species were subcultured on PDA (Potato Dextrose Agar).

Identification of fungi :

Isolates of fungi were identified according to the following authorities: *Fusarium* spp., according to Nelson *et al.* [9]; *Penicillium* spp., *Aspergillus* spp., and other fungi according to Pitt and Hocking [10]. The isolation frequency (Fr.) and relative density (RD.) of species were calculated according to González *et al*. [11] as follows**:**

$$\text{Fr. (\%)} = \frac{\text{No. of samples of occurrence of a species}}{\text{Total No. of samples}} \times 100$$

$$\text{RD. (\%)} = \frac{\text{No. of isolated genus or species}}{\text{Total No. of isolated fungi}} \times 100$$

Statistical analysis :

Asymptotic tests for equality of proportions were used to compare internal and external frequencies and relative densities. [12], and the Fischer exact test was used to analyze possible differences in the isolation frequencies of fungal species. The analysis was performed by using software SPSS [13].

3. RESULTS

Average relative density and frequency of external and internal mycoflora associated with wheat grains, which were collected from six locations at Hail area revealed 5 genera such as *Aspergillus*, *Ulocladium*, *Alternaria*, *Penicillium* and *Fusarium* and are shown in table1. Based on the percentage frequency and relative density the members of genus *Fusarium* spp. were predominantly isolated from corn grains as internal mycoflora at all locations except Delehan(Fr. range 7.2-89% and RD. 9.1- 65.6) . *Pencillium* spp. also showed higher frequency and relative densities as external mycoflora (Fr 10-45and RD.6.2-35%).The second most prevalent genus as external mycoflora was *Aspergillus* spp.(Fr. 8.3-33.3 and RD.6.2- 19). Comparison between external and internal fungal mould (*Penicillium* spp. and *Aspergillus* spp.) showed significant different at P=0.05. Other genera isolated as significant components of the internal and external mycoflora included *Fusarium* spp., *Alternaria* spp. and *Ulocladium* spp.

The incidence of *Aspergillus* species on agar (YGCA) revealed the occurrence of four different *Aspergillus* species with high frequency and relative density table 2. All these species were isolated as external and internal mycoflora except *Aspergillus terrus* which was isolated only as external mycoflora. The study showed that, *Aspergillus flavus* was the most predominant species *of Aspergillus* (Fr. 67.5% - 48.2 % and R.D. 44.3- 38.3 as external and internal respectively) while *Aspergillus niger* the second predominant species (Fr. 50.8% - 52.6 % and R.D 36.4 - 41.4 as external and internal respectively). The results of *A. nidules* showed the incidence of its contamination of wheat grains as external and internal mycoflora are close. Statistical analysis between external and internal species of *Aspergillus* showed significant difference at P = 0.05 and this emphasized the predominant of mold as external isolates. Table 3. Showed five species of *Alternaria* which were isolated as external and internal isolates except *A. citri* which was isolated only as internal isolates. *Alternaria atlernata* the most predominant species among the species of *Alternaria* ,it recorded high Fr. and RD. as external mycoflora when compared to internal one (Fr.75.5% - 45.2% and R.D 68.8 - 37.2 as external and internal respectively). The rest of *Alternaria specie s* (*A raphani* , A.

tenuissima A.longipes and A. citri) recorded high Fr. and RD. as internal mycoflora than the external mycoflora (table 3). Statistical analysis showed no significant difference between external and internal species of *Alternaria* at P = 0.05.
The incidence of *Ulocladium* species was shown in table 4. Which revealed three species (*Ulocladium atrium, U. botrytis* and *U. alternariae*)**.** All these species were isolated as both internal and external mycoflora except *U. alternariae* which was isolated as internal mycoflora only. The most predominant specie was *U. atrium* ((Fr. 75.8% - 84.2% and R.D76.6%-88.4% as external and internal respectively) , all *Ulocladium*. species showed high incidence as internal mycoflora. Statistical analysis showed significant difference between external and internal of two species of *Ulocladium* (*U. atrium and U. botrytis*) at P = 0.05 but no significant difference between external and internal isolates of *Ulocladium alternariae* at P = 0.05

Table (1): average frequency and relative density between external and internal mycoflora of wheat grains at different locations in Hail area

Genera of external mycoflora	Delahan		Shalania		Khota		Kafa		Hatco		Nadic	
	%RD.	%Fr.	% RD.	% Fr.	% RD.	% Fr.	% RD.	% Fr.	% RD.	% Fr.	% RD.	% Fr.
Aspergillus spp.	12.5**	* 22.5	6.2**	8.3*	15.4**	25.5*	13.6**	16*	17.5**	27.5*	19**	33.3*
Penicillium sp.	6**	10*	1.7**	5.5*	12.7**	25*	12.3**	15*	15**	25*	35**	45*
Alternaria spp.	6.1**	* 11.5	1.8**	6.5*	8.3**	13.3*	5.7**	9.3*	3**	7*	2..5**	5*
Ulocladium spp.	10.7**	* 16.4	4.7**	9.5*	12.5**	20*	4.5**	7*	25**	35*	7**	10 *
Fusarium spp.	1.3**	2*	15**	20*	10**	15*	7.5**	15*	2.5**	5*	5**	10*
Total No. of isolates	85		60		105		65		95		95	
Total No. of grains	100		100		100		100		100		100	
Genera of internal mycoflora	Delahan		Shalania		Khota		Kafa		Hatco		Nadic	
	%RD.	%Fr.	% RD.	% Fr.	% RD.	% Fr.	% RD.	% Fr	% RD	% Fr	% RD	% Fr
Aspergillus spp.	5.9**	6.5*	3.5**	7.5*	4.5**	9.5*	3.2**	8*	6**	12*	5.5**	4*
Penicillium sp.	1.5**	4*	2**	5*	3.6**	4.2 *	1**	3*	3.3**	4.3*	4**	7*
Alternaria spp.	7.8**	12.3*	7.5**	12.4*	8.6**	20.6	8.6**	16.2*	16.5**	33*	10.5**	15*
Ulocladium spp.	25**	35*	10.5**	16.2*	20.5**	27.5*	11.3**	17.5 *	35**	45*	7.3**	12.5*
Fusarium spp.	7.2**	9.1*	48.6**	4 .65*	20.9*	57.9*	65.6**	81.7*	45.2**	59.6*	46**	89*
Total No. of isolates	50		115		95		120		165		160	
Total No. of grains	100		100		100		100		100		100	

Fr. = Frequency RD. = Relative density

* Significant difference between Fr. of external and internal mycoflora at P= 0.05

** Significant difference between RD. of external and internal mycoflora at P= 0.05

Table 2: Comparison between relative density and frequency of different species of genus *Aspergillus*

Different Species of *Aspergillus*	External mycoflora			Internal mycoflora		
	R.D.%	Fr.%	Total No. of Isolates	R.D.%	Fr.%	Total No. of Isolates
A.flavus	44.3**	67.5*	225	38.3**	48.2*	52.2
A.niger	41.4**	52.6*	185	36.4**	50 .8*	56.4
A.nidules	28**	29.7*	58	20.3**	35.6*	27.6
A.terrus	40**	26.7*	40	0**	0*	0

Fr. = Frequency RD. = Relative density
* Significant difference between Fr. of external and internal species of *Aspergillus* at P= 0.05
** Significant difference between RD. of external and internal species of *Aspergillus* at P= 0.05

Table3: Comparison between relative density and frequency of different species of genus *Alternaria*

Different species of *Alternaria*	External mycoflora			Internal mycoflora		
	R.D% .	Fr.%	Total No. of Isolate	R.D.%	Fr.%	Total No. of Isolates
A. aternata.	68.8	75.5	95	37.2	45.2	173.4
A. tenuissima	7.2	15.8	10	13.4	25.6	62.4
A. longipes	22.5	3.7	2	16.5	27.5	76.8
A.raphani	1.4	35.5	31	27.1	40	126.6
A.citri	0	0	0	5.9	7.1	27.6

Fr : Frequency RD: Relative density
* No Significant difference between Fr. of external and internal species of *Alternaria* at P= 0.05
** No Significant difference between RD. of external and internal species of *Alternaria* at P= 0.05

Table 4: Comparison between relative density and frequency of different species of genus *Ulocladium*

Different species of Genus *Ulocladium*	Internal mycoflora			External mycoflora		
	R.D.%	Fr.%	Total No. of Isolates	R.D.%	Fr.%	Total No. of Isolates
U.atrium	88.4**	84.2*	184.3	76.6**	75.8*	127
U.botrytis	21.5**	41.6*	51.6	11.2**	20.5*	16
U.alternariae	1.9	4.8	4.6	0	0	0

Fr.= Frequency RD.= Relative density
* Significant difference between Fr. of external and internal species of *Ulocladium* at P = 0.05
** Significant difference between RD. of external and internal species of *Ulocladium* at P = 0.05

4. DISCUSSION

In the present study, seed-borne mycoflora associated with local wheat grains were isolated and identified. Among the fungal isolates observed during the study period, the molds (*Aspergillus flavus* and *A. niger*) were the most predominant external mycoflora, this results agreed with that reported by [14] and [15] who stated that high degree of mould contamination in stored grains and animal feeds is a measure of their quality assurance. The high level of mold contamination of Saudi wheat grains may be due to the exposure of the grains to dust and atmospheric pollutants. This could be attributed to poor method of storage or contaminated farm equipment or in the soil ([16], as the spores of fungi are easily transmitted via seeds due to cracks ([17]. Species of the genera *Atlernaria and Ulocladium* are predominant as internal mycoflora except *A.alternata* which was predominant as extrnal mycoflora. *Alternaria* and *Ulocladium* are saprophytic or weak parasitic fungi, so they infect wheat grain in the field or during storage so they are consider as pre-harvest and post-harvest infectors [18]. Similar to the findings of our study, [19] whom showed in their research that *Alternaria* spp. was the most prevalent fungi in harvested wheat and, sorghum dusts from Egypt and *Aspergillus* spp. ranked as the second place *A. flavus* has the highest ability to produce toxin in comparison to other *Aspergillus* species and because of this high potential lin toxin production such as Aflatoxin B1 and B2, this species is one of the most important fungus species related to human health and animals [20]. *A. niger* species is also considerable, because this species can produce dangerous toxin such as ochratoxin A [21].

5. CONCLUSION

Comparison between frequency and relative density of external and internal mycoflora which are presented in this study would be of great importance in this region for predicting the extent of post- harvest infection, colonization, deterioration and extent of mycotoxin in wheat grain. These results are highly useful for further studies on toxin producing fungi and their epidemiological significance in wheat crops grown in Hail area and elsewhere of Saudi Arabia

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