# Mycoflora Of Barley ( Hordeum Vulgare L.) At Different Locations In Hail Area- Saudi Arabia

Elham S. Dawood, Modhi K. Elshamry

**Abstract:** 400 grain samples collected from barley 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 265 of external mycoflora and 517 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.60.9 - 40.5%, RD 48.3 - 40.9%and A. niger Fr. 52.7- 48.6- % and RD 38.7- 41.9% as external – internal mycoflora mycoflora respectively). All the species of Ulocladium and Alternaria were predominant as internal mycoflora .The most predominant species of Ulocladium and Alternaria were U. atrium (Fr 89% -75.5%and RD %-79- 62.5% as internal – external mycoflora respectively) and Alternaria alternate (Fr. 60% - 46.6 % and RD. 55-32.3as external–internal mycoflora respectively).

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

## Introduction:

Barley (Hordeum vulgare L.) like most of the economically important crops, is prone to diseases. Considering its world-wide production and average annual production of 31145 tones, Area 6225 h. in Hail area [1].Barley is an annual cereal grain, which for serves as a major animal feed crop, with smaller Amounts used for malting and in health food. It is a member of the grass family poaceae. It is considered the second main crop in Saudi Arabia, its uses as human food and animal fodder. The infection of barley seeds before and after maturity is greatest at high relative humidity[2]. Several seed-borne fungi, including species of the genera Fusarium, Alternaria, Aspergillus and Penicillium have been considered as important pathogens of cereal grains [3]. Seed borne mycoflora is one of the major components reducing the Barely yield. Mycoflora associated with seeds both internally and externally are responsible for 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 [4]. Yield losses due to seed borne fungi have been reported between 15 to 90% of untreated seeds grown in field [5] Seed borne pathogens of barley include Alternaria alternata, Cladosporium oxysporum, Curvularia lunata, Drechslera sorokiniana, D.tetramera, Fusarium graminearum, Helminthosporium sativum, and post-harvest fungi include species of Aspergillus and Penicillium [6]. Genera of Fusarium, Alternaria, Drechslera, Stemphylium, Curvularia, Cladosporium, Rhizopus, Aspergillus and Penicillium has been the most common isolated fungi from Barley seeds [6]. For the mangement of crop disease the major step is to use disease free and certified seed. Germination test of seeds is significant in identifying seed borne pathogen associated with barley grains and provides valuable information regarding mycoflora and their efficient control. Most of the previous studies of the mycoflora of barley 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. The aim of this study is to identify the isolated fungi associated with barley 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 distribution percentage.


- *Elham S. dawood Associate Professor in biotechnology (MSc. And PhD) Department of life Science Faculty of Education, Nile Valley university, Correspondence, E-mail elhamdawood@hotmail.com or eldawood@nilevalley.edu.sd*
- *Modhi Khalif Motni Faiz Alshamary Lecturer of microbiology, Department of Biology ,Hail university Saudi Arabia*


## Materials and Methods:-

Collection of Seed Samples: A total of 400 samples of barley grains were collected from four framers in Hail which were located at 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 barley 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 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 medium) and then identified on the basis of morphology under microscope [8].

**Isolation of Internal fungi:**
For isolation of the internal mycoflora, subsamples of barley 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:

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

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

**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].

## Results:
Average relative density and frequency of external and internal mycoflora associated with barley grains, which were collected from four locations at Hail area revealed five 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 barley grains as internal mycoflora at all locations ( Fr. range 37.5 -91.3% and RD. 30-88.9). Ulocladium spp. was the second predominant of internal mycoflora (Fr.17.8 – 50% R.D.11.3 0 - 40%). Also Pencillium sp. showed higher frequency and relative densities as internal mycoflora (Fr.0.00 - 41.7% and RD 0.00 -35.8%).The most prevalent genus as external mycoflora was Aspergillus spp. (Fr. 23-84.4% and RD.14.6-30%). Alternaria spp.were slightly similar as external and internal mycoflora. 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 three different species( A. niger, A. flavus and A. nidulus) with high frequency and relative density table 2. All these species were isolated as external and internal mycoflora. except A.nidulus which was isolated as external mycoflora only. The study showed that, Aspergillus flavus was the most predominant species of Aspergillus Fr. 60.9-40.5 %, RD.40.9-48.3% as external and internal mycoflora respectively). while Aspergillus niger the second predominant species (Fr. 52.7 % -48.6 % and R.D 38.7 - 41.9 as external and internal mycoflora respectively). The results of A. nidules showed the incidence of its contamination of barley grains as external mycofloraonly (Fr. 0.00 -20.7 and R.D. 0.00-12.9%). 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 four species of Alternaria (A.alternata, A.lonipes ,A. raphani and A. ramulosa) which were isolated as external and internal isolates except A. longipes and A. raphani which were 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.60-40.6 % and R.D 55- 32.3 as external and internal respectively). A raphani Fr.55-58.5 and R.D.45-44.1% , while A.longipes and A. ramulosa were isolated as internal mycoflora only ( table 3). Statistical analysis showed no significant difference between frequencies of external and internal species of Alternaria at P = 0.05 but significant difference between relative densities of the internal and external mycoflora was showed at P=0.05 . The incidence of Ulocladium species was shown in table 4 which revealed three species (Ulocladium atrium, U. botrytis, U. alternariae and U.audemansii). All these species were isolated as both internal and external mycoflora except U. alternariae and U. audemansii which were isolated as internal mycoflora only. Statistical analysis showed no significant difference between external and internal mycoflora between the species of Ulocladium at P = 0.05. The most predominant specie was U. atrium ((Fr. 75.5 % - 89% and R.D 62.5%-79% as external and internal respectively),all Ulocladium. species showed high incidence as internal mycoflora.

***Table (1):*** *Average Frequency and Relative Density between External and Internal Mycoflora of Barley Grains at Different Locations in Hail area*

| Genera of Eternal mycoflora | Delehan | | Shalania | | Kuta | | Kafa | |
|---|---|---|---|---|---|---|---|---|
| | R.D.% | Fr.% | R.D% | Fr% | R.D% | Fr% | R.D% | Fr% |
| Aspergillus spp. | 30* | **84.4 | 18.75** | 30* | 14.63** | 23* | 20.17** | 28.3* |
| Penicillium sp. | 7** | 10* | 2.5** | 5* | 3.5** | 7* | - | - |
| Alternaria spp. | 11** | 17.5* | 7.5** | 15* | 16** | 22.5* | 2.5** | 5* |
| Ulocladium spp. | 11.7** | 20* | 3.4** | 6* | 4.2** | 8.5* | - | - |
| Fusarium spp. | 7.5** | 10* | 2.5** | 5* | 20** | 30* | 2.5** | 5* |
| Total No. of Isolates | 65 | | 65 | | 60 | | 75 | |
| Total No. of Grains | 100 | | 100 | | 100 | | 100 | |
| Genera of Internal mycoflora | Delehan | | Shalania | | Kuta | | Kafa | |
| | % R.D | Fr% | % R.D | Fr% | % R.D | Fr% | R.D.% | Fr% |
| Aspergillus spp. | 27.3** | 8.6* | 3** | 6* | 2** | 4.8* | 10.5** | 12.3* |
| Penicillium sp. | 5** | 8* | 4** | 7* | - | - | 35.8** | 41.7* |
| Alternaria spp. | 15** | 24* | 8.4** | 15.7* | 10.2** | 17.4* | 6.1** | 9.1* |
| Ulocladium spp. | 40** | 50* | 13.3** | 20* | 11.4** | 17.8* | 21.5** | 25.2* |
| Fusarium spp. | 30** | 37.5* | 88.9** | 93.1* | 42.4** | 57.9* | 50** | 58.4* |
| Total No. of Isolates | 115 | | 132 | | 122 | | 148 | |
| Total No. of Grains | 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 spp.*

| Different Species of Aspergillus | External Fungi | | | Internal Fungi | | |
|---|---|---|---|---|---|---|
| | R.D% | Fr.% | Total No. of Isolates | R.D% | Fr.% | Total No. of Isolates |
| A.flavus | 48.3 | 60.9 | 150 | 40.9 | 40.5 | 30.4 |
| A.niger | 38.7 | 52.7 | 120 | 41.9 | 48.6 | 31 |
| A.nidules | 12.9 | 20.7 | 40 | 0.00 | 0.00 | 0.00 |

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

| Different species of Alternaria | External Fungi | | | Internal Fungi | | |
|---|---|---|---|---|---|---|
| | R.D% | Fr.% | Total No. of Isolate | R.D% | Fr.% | Total No. of Isolates |
| A. aternata. | 55** | 60 | 55 | 32.3** | 46.6 | 53.7 |
| A. longipes | 0.00** | 0.00 | 0.00 | 18.5** | 34.2 | 30.8 |
| A.raphani | 45 | 55 | 45 | 44.1 | 58.5 | 73.2 |
| A.ramulosa | 0.00** | 0.00 | 0.00 | 5.1** | 8.9 | 84 |

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

| Different species of Genus Ulocladium | Internal Fungi | | | External Fungi | | |
|---|---|---|---|---|---|---|
| | R.D% | Fr.% | Total No. of Isolates | R.D% | Fr.% | Total No. of Isolates |
| U.atrium | 79 | 89 | 158.3 | 62.5 | 75.5 | 85 |
| U.botrytis | 0.00 | 0.00 | 0.00 | 37.5 | 45.6 | 43.5 |
| U.alternariae | 12.5 | 25 | 16.7 | 0.00 | 0.00 | 0.00 |
| U.oudemansii | 8.3 | 16.7 | 25.5 | 0.00 | 0.00 | 0.00 |

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

## Discussion:

In the present study, seed-borne mycoflora associated with local barley 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 barley 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. Alternaria and Ulocladium are saprophytic or weak parasitic fungi ,so they infect barley grain in the field or during storage so they are consider as pre-harvest and post-harvest infectors [18]. Both are known as the main causative of black point disease of barley grains [19] . A. flavus has the highest ability to produce toxin in human health and animals [20]. A. niger species is also considerable, because this species can produce dangerous toxin such as ochratoxin A [21].

## Conclusion:

The data on frequency and relative density presented in the 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. This data is of immense value for assessing the possible health hazard in humans and animals upon consumption of such contaminated wheat grains by toxigenic mould.The results of this study 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.

## References

[1] Anonymous,.Agricultural Statistics of Saudi Arabia. Ministry of Agriculture, 2004.

[2] Rabie C.J., Lubben A., Marais G.J., van Vuuren H.J.(1997). Enumeration of fungi in barley. Inter. J. Food Microb., 35: 117–127.

[3] Doohan F.M., Brennan J., Cooke B.M. (2003): Influence of climatic factors on Fusarium species pathogenic to cereals. Eur. J. Plant Path., 109:115 120.

[4] Niaz. and S. Dawar.. Detection of seed borne mycoflora in maize (Zea mays L.). Pak. J. Bot. 41(1): 443-45 , 2009.

[5] M.V.Wiese. Compendium of wheat diseases (3rd Ed). St. Paul, USA: APS Press, 112pp , 1998

[6] Rehman,, K. Sultana, N. Minhas, M. Gulfraz, G.K. Raja, and Z. Anwar.. Study of most prevalent wheat seed-borne mycoflora and its effect on seed nutritional value. Afr. J. Microb. Res. 5(25): 4328-4337, 2011.

[7] SB Mathur, O. Kongsdal .Common laboratory seed health testing. methods for detecting fungi. International Seed Testing Association, Bassersdorf, Switzerland, p. 427,2003

[8] LA Castlebury, DF .Farr .The Genus Tilletia in the United States, Systematic Mycology and Microbiology Laboratory, ARS, USDA., 2011,

[9] R. R. Nelson, and F. A. Haasis, .The perfect stage of Curvularia lunata. Mycologia. 56: 316 317, 1964.

[10] J.I. Pitt and A.D. Hocking,. Fungi and food spoilage. Chapman and Hall, New York, USA. 593 p, 1997 .

[11] HH .Gonzalez, GA. Molto, A .Pacin, SL. Resnik, MJ. Zelayaand M. Masana. Trichothecenes and mycoflora in wheat harvested in nine locations in Buenos Aires province, Arg.. Myco... 165(2):105-14, 2008.

[12] JL. Devore. Probability and Statistics for Engineering and the Sciences. Monterrey, California. Brooks-Cole Publishing Company, 1987: 352-354, 2008.

[13] Statistix Version 1.0. User's Manual. Analytical Software. Borland International Inc. and Fleming Software, 1996.

[14] M. Hoda (2003). Mycoflora and Mycotoxins of Cereal Grains in Delta, Egypt. Mycobio 31(4): 183-190

[15] Nadeem, J. O. Zrary (2014). Isolation, Identification and Pathogenicity of Seed borne fungi of some barley cultivars J. of Zank. Sul.i- Part A, Special Issue, 16: 55-60.

[16] G.N.Agrios, " Plant Pathology ". Academic Press, New York.(2005).

[17] B.W Horn. Colonization of wounded peanut by soil fungi: selectivity for species from Aspergillus section flavi. Myco., 23:425-430, 2005.

[18] Habib, ST. Sahi, Javed, N .Ahmad. Prevalence of seed-borne fungi on wheat during storage and its impact on seed germination. Pak. J. Phyt.. 23(1): 42-47, 2011.

[19] U.L. Diener, R.J. Cole, RA. Hill, Annual.Review Phytopathology, 25: 249, 1987 .

[20] SI .Abdel-Hafez, AH. Moubasher, Shoreit, AA .Ismail. Fungal flora associated with combine harvester wheat and sorghum dusts from Egypt. J Basic Microbiol.;30(7):467-79, 1990

[21] M.L. Abraca,. Bragulat, F.J. Cabanes, App.Env.Micr. , 60:2650, 1994