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Survey of Postharvest Fungi Associated with Wheat, Rice and Barley Grains in Riyadh (Saudi Arabia)

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A mycological survey of 60 samples includes three cereal grains (wheat, rice and barley) were collected from different markets located in Riyadh region (Kingdom of Saudi Arabia). The frequencies of isolation for fungi belonging to four genera *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* were calculated. The major genera of fungi isolated according to frequency were *Aspergillus* (27.9%), *Fusarium* (24.7%), *Penicillium* (8.1%) and *Alternaria* (3.88%) in wheat, rice and barley. Analysis of variance showed that the main effect of fungi ($p=0.0001$) was highly significant source of variation in this interaction. Cereal grains samples were plated on agar media and the fungi that grew were identified by cultural and morphological characteristics to genus level. Four species of *Aspergillus* (*A. flavus*, *A. parasiticus*, *A. ochraceus* and *A. niger*); four species of *Fusarium* (*F. verticillioides*, *F. oxysporum*, *F. solani* and *F. semitectum*); three species of *Penicillium* (*P. chrysogenum*, *P. citrinum* and *P. notam*) and two species of *Alternaria* (*A. alternata*, *A. chlamydospora*) were isolated from the grains. Cluster analysis divided the fungi genera into two distinc groups (wheat and barley) and three groups (rice). In all cluster analysis cases *Aspergillus* spp. and *Fusarium* spp. were associated positively because found in one group.

Key words: Wheat, Rice, Barley, Seed-borne fungi.

Cereal grains and associated by-products constitute important sources of energy and protein for human (FAO, 1999). Fungi contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and sub-tropics (CAST, 2003). Cereal grains colonized by moulds there is a significant risk of contamination with the secondary metabolites of these fungi (mycotoxins). Mycotoxins are (unavoidably) consumed or ingested by animals and humans. Production of mycotoxins on crops is highly susceptible to environmental factors (e.g. temperature and available moisture (Paterson and lima, 2010). According to the International Agency for Research on Cancer (IARC), Mycotoxins contamination may be a serious concern for both human and animal health because of their wide range of harmful effects, including carcinogenicity, teratogenicity, and mutagenicity (IARC 1993; 2002). Several researchers have been documented filamentous fungi, mainly *Aspergillus*, *Penicillium*, and *Fusarium* species responsible for common mycotoxin contaminants of many cereal grains (Placinta *et al*., 1999; Broggi *et al*., 2007; Kumar *et al*., 2008; Jakiæ-Dimiæ *et al*., 2009; Al-Hazmi, 21010; Bensassi *et al*., 2011; Lutfullah and Hussain, 2012). Traditionally, fungi have been divided in general into two groups, field or plant pathogenic (*Fusarium* species) and storage or saprophytic

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(genera *Aspergillus* and *Penicillium*) (Campbell and White, 1995, Šimerda 1996).

There is a lack of accurate data on the frequency of fungi isolated from wheat, rice and barley grains in Riyadh region (Saudi Arabia). Because of these reason, it has not been possible to develop effective management strategies to prevent fungal infection and bio-deterioration of grains. Hence, this study was undertaken to identify, determine the distribution and levels of fungi. Also, recognized the frequency of fungi, in order to use this information to consider possible mycotoxin occurrence in this area.

MATERIALS AND METHODS

Isolation and identification of mycotoxigenic fungi in grains

Three types of cereal grains were chosen to study the composition of fungi in wheat, rice and barley grains. 60 samples (250 g each) of each grain type were collected from different markets located in Riyadh, in the Kingdom of Saudi Arabia, were examined for fungi in wheat, rice and barley the samples were enumerated using the direct plating method (Flannigan, 1977).

Samples of 10 g of each cereal were surface-sterilized in 1% NaOCl for 1 minute and rinsed twice in sterile distilled water. The surface sterilized grains were aseptically transferred onto the solidified agars. A total of 10 plates were plated per sample. Ten grains were plated on each agar plate. Inoculated plates were incubated for seven days at 27°C prior to visual differentiation and counting of colonies. The different fungal colonies on the plates were subcultured on PDA media for identification of species (Raper and Fennel, 1977; Pitt, 1979, 1985; Domsch *et al*., 1981; Nelson *et al*., 1983). The frequency and relative percentage of fungi of particular species with in a genus of fungi was calculated using the formula of Ghiasian *et al.* (2004).

Statistical analysis

The randomized complete block design, with three replicates, was used in this study. Duncan's multiple range test was used to identify differences in frequencies among fungi. Percentage data of isolation frequencies were transformed into $\sqrt{x + 0.5}$ before carrying out analysis of variance (ANOVA) to normalize and stabilize variance. Cluster analysis was performed with the software package SPSS 6.0. Correlation and regression analysis were performed with a computerized program.

$$\text{Relative percentage (\%)} = \frac{\text{Number of fungal species isolated from each isolated}}{\text{Total numberof all fungi isolated}} \times 100$$

RESULTS

The frequencies of four fungi genera were different from sample to sample. The general means of fungi showed that *Aspergillus* spp. were the most frequently isolated genera 30 and *Fusarium* spp. 25; while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (15.01 and 4.16) from wheat grains samples (Table 1). The general means of fungi showed that *Aspergillus* spp. were the most frequently isolated genera (27.03) and *Fusarium* spp. (23.75); while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (12.15 and 2.49) from rice grains samples (Table 2). The general means of fungi showed that *Aspergillus* spp. were the most frequently isolated genera (26.67) and *Fusarium* spp. (25.42); while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (16.60 and 4.99) from barley grains samples (Table 3). In general the *Aspergillus* spp. and *Fusarium* spp. were the most frequently isolated genera for wheat, rice and barley grains samples. Analysis of variance (ANOVA) (Table 4) of isolation four genera from wheat, rice and barley grains showed non significant (p=0.0001) for effect of samples (except wheat) and sample x fungi but very highly significant for fungi genera. Fungi were the only signification source of variation. Therefore, LSD was used to compare between the general means of sample. Due to non significant sample x fungi for wheat, rice and barley grains samples, a least significant difference (LSD) was calculated to compare frequencies of four fungi genera general means within each samples. The general means of fungi showed that the differences in frequencies of four fungi genera isolated from wheat, rice and barley grains samples.

Considering the importance of the *Aspergillus* genus, 87.00% of isolates of *Aspergillus* species were identified up to the species level in wheat grains samples. The study

showed the presence of three *Aspergillus* species includes *A. flavus, A. parasiticus* and *A. niger*. The relative percentage of *A. flavus* (42.25%), *A. parasiticus* (32.90%) and *A. niger* (21.12%) were the dominant *Aspergillus* species with a high relative percentage (Table 5). Number of isolates for *A. flavus, A. parasiticus* and *A. niger* were 30, 17 and 15 isolates. An important observation made in the present investigation is that *A. flavus* and *A. parasiticus* were isolated from almost all the samples. Wheat grains samples contaminated by species of *Fusarium*, (87%) of isolates of *Fusarium* species were identified up to the species level. The study showed the presence of three *Fusarium* species. Relative percentage showed that *F. verticillioides* (41.00%), *F. oxysporum* (26.23%) and *F. solani* (19.67%) were dominant *Fusarium* species. Number of isolates for *F. verticillioides*, *F. oxysporum* and *F. solani* were 25, 16 and 12 isolates. Further, mycological analysis of wheat samples grains for the other field fungi revealed the occurrence of *Penicillium* species, (82.86%) of isolates of *Penicillium* were identified up to the species level. The study showed the presence of three *Penicillium* species. Relative percentage showed that *P. chrysogenum* (34.29%) *P. citrinum* (25.27%) and *P. notatum* (22.85%) were the dominant *Penicillium* species with a high relative percentage. Number of isolates for *P. chrysogenum, P. citrinum* and *P. notatum* were 12, 9 and 8 isolates (Table 5). Mycological analysis of wheat samples grains for the other field fungi revealed the occurrence of *Alternaria* species, (77.78%) of isolates of *Penicillium* were identified up to the species level. The study showed the presence of one *Penicillium* specie. Relative percentage showed that the *A. alternata* (77.78%) was the dominant *Penicillium* species with a high Relative

Table 1. Frequency (%) of four fungi genera from wheat grains

Sample no.	Fungi genera							
	Aspergillus ssp.		*Fusarium* spp.		*Penicillium* spp.		*Alternaria* spp.	
	%[a]	T	%	T	%	T	%	T
1	41.66	06.50	16.70	04.15	08.33	02.97	08.33	02.97
2	33.33	05.80	25.00	05.01	16.70	04.15	08.33	02.97
3	16.70	04.15	25.00	05.01	25.00	05.01	00.00	00.70
4	33.33	05.80	16.70	04.15	16.70	04.15	00.00	00.70
5	25.00	25.01	25.00	05.01	16.70	04.15	08.33	02.97
6	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
7	33.33	05.80	25.00	05.01	25.00	05.01	00.00	00.70
8	25.00	05.0	25.00	05.01	25.00	05.01	00.00	00.70
9	25.00	05.01	33.33	05.80	16.70	04.15	08.33	02.97
10	33.33	05.80	25.00	05.01	16.70	04.15	08.33	02.97
11	16.70	04.16	16.70	04.15	16.70	04.15	08.33	02.97
12	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.70
13	41.66	06.50	25.00	05.01	16.70	04.15	08.33	02.97
14	33.33	05.80	16.70	04.15	16.70	04.15	00.00	00.70
15	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.70
16	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.70
17	33.33	05.80	33.33	05.80	16.70	04.15	00.00	00.70
18	16.70	04.15	16.70	04.70	08.33	02.97	08.33	02.97
19	25.00	05.01	33.33	06.80	08.33	02.97	08.33	02.97
20	41.66	06.50	25.00	05.01	16.70	04.15	00.00	00.70
Mean	30.00	06.64	25.00	05.07	15.01	03.86	04.16	01.83

T= transformed value; LSD for wheat grains samples non significant
LSD for fungi = 0.53 ($p < 0.005$) or 0.70 ($p < 0.01$)

[a] percentage data were transformed into $\sqrt{x + 0.5}$ angles before carrying out the analysis of variance

percentage. Number of isolates for *A. alternata* was 7 isolates (Table 5).

Mycological analysis of rice samples grains that occurrence of *Aspergillus* species, (86.15%) of isolates of *Aspergillus* were identified up to the species level. The study showed the presence of four *Aspergillus* species includes *A. flavus*, *A. parasiticus A. niger* and *A. ochraceus*. Relative percentage showed *A. flavus* (41.54%), *A. parasiticus* (33.84%), *A. niger* (6.15%) and *A. ochraceus* (4.62) were the dominant *Aspergillus* species (Table 6). Number of isolates for *A. flavus, A. parasiticus*, *A. niger* and *A. ochraceus* were 27, 22, 4 and 3 isolates. An important observation made in the present investigation is that *A. flavus* and *A. parasiticus* were isolated from almost all the samples. Rice grains samples contaminated by species of *Fusarium*, all isolates of *Fusarium* species were identified up to the species level. The study showed the presence of three *Fusarium* species. Relative percentage showed that the *F. solani* (53.70%), *F. oxysporum* (33.33%) and *F. verticillioides* (14.81%) were the dominant *Fusarium* species with a high relative percentage. Number of isolates for *F. solani*, *F. oxysporum* and *F. verticillioides* were 29, 18 and 7 isolates. Further, mycological analysis of rice samples grains for the other field fungi revealed the occurrence of *Penicillium* and *Alternaria* species, all isolates of *Penicillium* and *Alternaria* were identified up to the species level. The study showed the presence one *Penicillium* specie (*P. notatum*) with 8 isolate. The study showed the presence one *Alternaria* specie (*A. alternata*) with 6 isolate (Table 6).

Mycological analysis of barley samples grains that occurrence of *Aspergillus* species, (81.25%) of isolates of *Aspergillus* were identified up to the species level. The study showed the

Table 2. Frequency (%) of four fungi genera from rice grains

Sample no.	Fungi genera							
	Aspergillus ssp.		*Fusarium* spp.		*Penicillium* spp.		*Alternaria* spp.	
	%[a]	T	%	T	%	T	%	T
1	33.33	05.80	25.00	05.01	08.33	04.16	08.33	02.97
2	16.70	04.16	33.33	05.80	08.33	04.16	08.33	02.97
3	25.00	05.01	25.00	05.01	08.33	04.16	00.00	00.70
4	33.33	05.80	25.00	05.01	00.00	00.70	00.00	00.70
5	33.33	05.80	25.00	05.01	00.00	00.70	00.00	00.70
6	25.00	05.01	33.33	05.80	00.00	00.70	00.00	00.70
7	25.00	05.01	16.70	04.16	08.33	04.16	08.33	02.97
8	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.70
9	16.70	04.16	16.70	04.16	00.00	00.70	00.00	00.70
10	25.00	05.01	25.00	05.01	00.00	00.70	00.00	00.70
11	33.33	05.80	25.00	05.01	00.00	00.70	08.33	02.97
12	33.33	05.80	25.00	05.01	00.00	00.70	08.33	02.97
13	25.00	05.01	16.70	04.16	00.00	00.70	00.00	00.70
14	50.00	07.10	16.70	04.16	08.33	02.97	00.00	00.70
15	16.70	04.16	25.00	05.01	00.00	00.70	00.00	00.70
16	16.70	04.16	25.00	05.01	00.00	00.70	00.00	00.70
17	25.00	05.01	25.00	05.01	00.00	00.70	00.00	00.70
18	25.00	05.01	25.00	05.01	08.33	02.97	08.33	02.97
19	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.70
20	33.33	05.80	16.70	04.16	00.00	00.70	00.00	00.70
Mean	27.08	05.18	23.75	04.87	03.33	01.85	02.49	01.38

T= transformed value; LSD for rice grains samples non significant
LSD for fungi = 1.68 ($p < 0.005$) or 2.25 ($p < 0.01$)

a percentage data were transformed into $\sqrt{x+0.5}$ angles before carrying out the analysis of variance

presence of two *Aspergillus* species includes *A. niger* and *A. flavus*. Relative percentage showed *A. niger* (54.69%) and *A. flavus* (26.56%) were the dominant *Aspergillus* species with a high relative percentage (Table 7). Number of isolates for *A. niger* and *A. flavus* were 35 and 17 isolates. An important observation made in the present investigation is that *A. niger* and *A. flavus* were isolated from almost all the samples. Barley grains samples contaminated by species of *Fusarium*, all isolates of *Fusarium* species were identified up to the species level. The study showed the presence of three *Fusarium* species. Relative percentage showed that the *F. solani* (53.70%), *F. oxysporum* (33.33%) and *F. verticillioides* (14.81%) were the dominant *Fusarium* species with a high relative percentage. Number of isolates for *F. solani*, *F. oxysporum* and *F. verticillioides* were 29, 18 and 7 isolates. Further, mycological analysis of barley samples grains for the other field fungi revealed the occurrence of *Penicillium* and *Alternaria* species, all isolates of *Penicillium* and *Alternaria* were identified up to the species level. The study showed the presence one *Penicillium* specie (*P. notatum*) with 8 isolate. The study showed the presence one *Alternaria* specie (*A. alternata*) with 6 isolate (Table 7).

The microorganisms, isolated from all 60 samples are shown in Table 8. The leading contaminant among fungi was *Aspergillus* spp. and *Fusarium* spp. detected in all samples (100%) followed by *Penicillium* spp. and *Alternaria* spp in some samples (66.67% and 43.33%). *Aspergillus* spp. *Fusarium* spp. were highest contaminated for wheat, rice and barley (30.00, 27.08 and 26.67) and (25.00, 23.00 and 25.42) respectively. *Alternaria* spp. were lowest contaminated for wheat, rice and barley (4.16, 2.49 and 99).

Table 3. Frequency (%) of four fungi genera from barley grains

Sample no.	Fungi genera							
	Aspergillus ssp.		*Fusarium* spp.		*Penicillium* spp.		*Alternaria* spp.	
	%[a]	T	%	T	%	T	%	T
1	33.33	05.80	25.00	05.01	08.33	02.97	08.33	02.97
2	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
3	16.70	04.16	25.00	05.01	16.70	04.16	00.00	00.70
4	33.33	05.80	25.00	05.01	00.00	00.70	08.33	02.98
5	25.00	05.01	16.70	04.16	00.00	00.70	00.00	00.70
6	33.33	05.80	25.00	05.01	00.00	00.70	00.00	00.70
7	33.33	05.80	33.33	05.80	16.70	04.16	08.33	00.70
8	25.00	05.01	25.00	05.01	00.00	00.70	00.00	00.70
9	25.00	05.01	25.00	05.01	00.00	00.70	08.33	02.97
10	16.70	04.16	16.70	04.16	00.00	00.70	08.33	02.97
11	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
12	25.00	05.01	16.70	04.16	08.33	02.97	00.00	02.97
13	33.33	05.80	25.00	05.01	08.33	02.97	08.33	02.97
14	25.00	05.01	25.00	05.01	08.33	02.97	08.33	02.97
15	25.00	05.01	33.33	05.80	00.00	00.70	08.33	02.97
16	16.70	04.16	25.00	05.01	08.33	02.97	00.00	00.70
17	33.33	05.80	16.70	04.16	08.33	02.97	00.00	00.70
18	25.00	05.01	33.33	05.80	08.33	02.97	08.33	02.97
19	16.70	04.16	25.00	05.01	08.33	02.97	08.33	02.97
20	25.00	05.01	25.00	05.01	00.00	00.70	00.00	00.70
Mean	26.67	05.15	25.42	05.03	05.83	02.18	04.99	02.06

T= transformed value; LSD for rice grains samples non significant
LSD for fungi = 1.68 ($p > 0.005$) or 2.25 ($p > 0.01$)

a percentage data were transformed into $\sqrt{x + 0.5}$ angles before carrying out the analysis of variance

In the present study, the Phenogram of four fungi genera isolated from 20 wheat grains samples based on isolation frequencies (Fig.1) showed that, two separated groups of fungi were identified. The first group includes *Aspergillus* species, *Fusarium* species and *Penicillium* species within this group, fungi were classified to two subgroups. *Aspergillus* species, *Fusarium* species in first subgroup were associated positively and have high similarity level (95%). The second

Table 4. Analysis of variance of frequency (%) of four fungi genera isolated from wheat, rice and barley grains

Parameters and Source of variation [a]	D.F	M.S	F. value	P-F
Wheat grains				
Replication	2	72.677	18.535	0.000
Sample (S)	19	2.496	0.637	
Fungi (F)	3	364.427	92.942	0.000
S x F	57	1.968	0.502	
Error	230	3.921		
Rice grains				
Replication	2	1.134	0.269	
Sample (S)	19	3.769	0.895	
Fungi (F)	3	33.088	7.862	0.000
S x F	57	1.706	0.405	
Error	246	4.209		
Barley grains				
Replication	2	12.436	2.947	0.060
Sample (S)	19	0.890	0.211	
Fungi (F)	3	45.447	10.770	0.000
S x F	57	0.781	0.185	
Error	289	4.220		

[a] replication is random, while each fungi and samples is fixed

Table 5. *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* species isolated from wheat grains samples

Name of the fungi genera	Total no. of isolates	Relative percentage
Aspergillus species		
A. flavus	30	42.25
A. parasiticus	17	32.90
A. niger	15	21.12
Aspergillus species	9	12.68
Fusarium species		
F. verticillioides	25	41.00
F. oxysporum	16	26.23
F. solani	12	19.67
Fusarium species	8	13.10
Penicillium species		
P. chrysogenum	12	34.29
P. citrinum	9	25.72
P. notatum	8	22.85
Penicillium species	6	17.14
Alternaria species		
A. alternata	7	77.78
Alternaria species	2	22.22

subgroup includes *Penicillium* spp were having similarity level (35%) with first subgroup. The second group include *Alternaria* species were have low similarity level (10%) with first group.

The Phenogram of four fungi genera isolated from 20 rice grains samples based on isolation frequencies (Fig.2) showed that, three separated groups of four fungi were identified. The first group includes *Aspergillus* species and *Fusarium* species. The fungi in this group were associated positively and have high similarity level (98%). The second group include only *Penicillium* species were have low similarity level (15%) with first group. The third group include only *Alternaria* species were have low similarity level (15%) with first group and second group.

The Phenogram of four fungi genera isolated from 20 barley grains samples based on isolation frequencies (Fig.3) showed that, two separated groups of fungi were identified. The first group includes *Aspergillus* species and *Fusarium* species. The fungi in this group were associated positively and have high similarity level (95%). The second group includes *Penicillium* species and *Alternaria* species were have similarity level (80%) with first group. Similarity level between first group and second group were have low similarity level (10%).

Table 6. *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* species isolated from rice grains samples

Name of the fungi genera	Total no. of isolates	Relative percentage
Aspergillus species		
A. flavus	27	41.54
A. parasiticus	22	33.84
A. niger	4	06.15
A. ochraceus	3	04.62
Aspergillus species	9	13.85
Fusarium species		
F. solani	29	53.70
F. oxysporum	18	33.33
F. verticillioides (monilinforme)	7	14.81
Penicillium species		
P. notatum	8	100
Alternaria species		
A. alternata	6	100

Table 7. *Aspergillus, Fusarium, Penicillium* and *Alternaria* species isolated from barley grains samples

Name of the fungi genera	Total no. of isolates	Relative percentage
Aspergillus species		
A. niger	35	54.69
A. flavus	17	26.56
Aspergillus species	12	18.75
Fusarium species		
F. oxysporum	25	40.98
F. verticillioides	21	32.81
F. semitectum	15	59.59
Penicillium species		
P. notatum	10	66.67
P. chrysogenum	5	33.33
Alternaria species		
A. alternata	7	70.00
A. chlamydospora	3	30.00

Table 8. The presence of four fungi genera in grain samples

Number of samples	Wheat 20	Rice 20	Barley 20	Total 60
Microorganism	Num. positive [a]			num. positive [f]
	PCT positive samples [b]			PCT num. positive
	Max. frequency [c]			
	Min. frequency [d]			
	Mean frequency [e]			
Aspergillus	20	20	20	60
	100	100	100	100
	41.66	50	33.33	
	16.70	16.70	16.70	
	30.00	27.08	26.67	
Fusarium	20	20	20	60
	100	100	100	100
	33.33	25.00	33.33	
	16.70	16.70	16.70	
	25.00	23.00	25.42	
Penicillium	20	8	12	40
	100	40	60	66.67
	25.00	08.33	16.70	
	08.33	00.00	00.00	
	15.01	03.33	05.83	
Alternaria	8	6	12	26
	40	30	60	43.33
	08.33	08.33	08.33	
	00.00	00.00	00.00	
	04.16	02.49	04.99	

[a] Number of positive samples.
[b] Percentage of positive samples
[c] Maximum level of frequency
[d] Minimum level of frequency
[e] Mean level frequency
[f] Total number of positive samples regarding one microorganism.

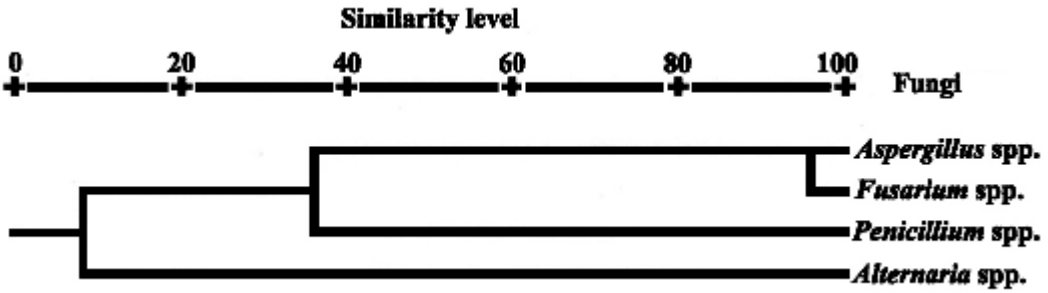

Fig. 1. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from wheat grains samples

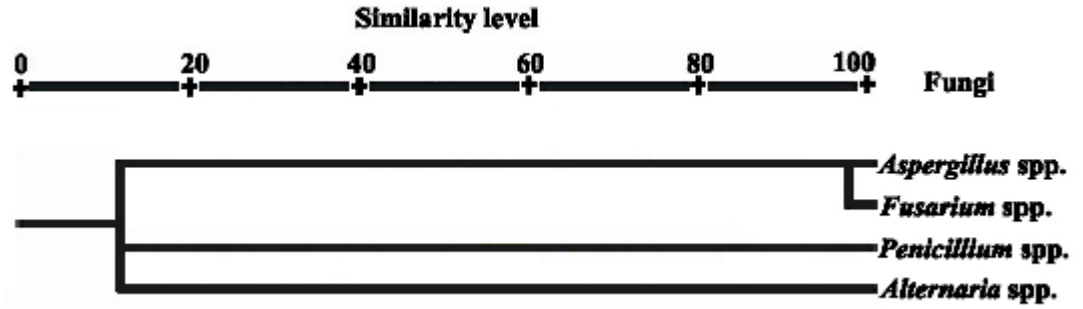

Fig. 2. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from rice grains samples

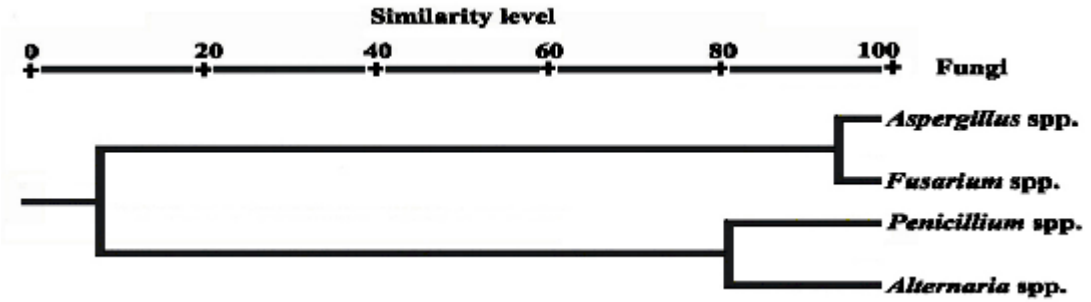

Fig. 3. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from barley grains samples

DISCUSSION

These results indicated that the type of fungal contamination of the wheat, rice and barley grains, at Saudi Arabia belongs to four genera (*Aspergillus*, fusarium, *Penicillium* and *Alternaria*) were qualitatively and quantitatively. Cereal grains contamination by fungi is a worldwide problem where several reports documented the presence of these fungi in samples of United States, Argentina, Spain, Italy, Serbia, Tunisia, Saudi Arabia, India and Australia (Morciaa *et al.*, 2012; Broggi *et al*., 2007; Jurado, 2003; Covarelli *et al*., 2011; Jakiæ-Dimiæ *et al.,* 2009; Bensassi *et al.,* 2011; Al-Hazmi, 2010; Kumar *et al*., 2008; Berghofer *et al* ., 2003). Fungi surface and internal infected wheat seeds were isolated by Flotation method and freezing blotter. Collected 279 samples and isolated 20 fungi species, which belonging to 8 different genus. Both surface and internal wheat seeds mycoflora were included *Alternaria* spp. (79.1%), *Penicillium* spp. (78.7%), *Aspergillus* spp. and (75.4%) *Fusarium* spp. (33.7%) respectively (Abedi-Tizaki *et al*., 2011). Collected thirty samples only eighteen samples (60%) showed fungal contamination that belongs to six genera (*Aspergillus*, *Penicillium*, *Alternaria*, *Emericella*, *Eurotium* and *Acremonium*). *Aspergillus* species were the most dominant species in the infected samples where they represented 70.33% of the total infected species. Samples were collected from food stores and mills located at Jeddah region in Saudi Arabia (Al- Hazmi, 2010). The mycological profile of wheat flour occurrence fungal contamination of the retail wheat flour selling in different markets at Jeddah (Kingdom of Saudi Arabia). The most common genera were *Aspergillus* (isolated from 70% of the tested samples), *Penicillium* (30%), *Fusarium* (20%), *Alternaria* (18%) and *Eurotium* (14%), (Gashgari *et al.,* 2010). Standard blotter and Deep Freezing methods were used to study the seed-borne mycoflora of 19 samples of wheat. A significant contamination with fungal genera was analyzed and the fungi most frequently isolated and identified were *Aspergillus flavus*, *A. niger*, *Fusarium moniliforme*, *F. oxysporum*, *Alternaria*

alternata, *Rhizoctonia solani* and *Penicillium* spp. (Fakhrunnisa *et al*., 2006). Thirty rice bran samples were collected from different factories in Mazandran province, northern Iran. Samples containing 15 specimens were conserved for one year in the storage and the other one was not subjected to storage. The frequency of toxigenic fungi isolated from old and new samples were *Aspergillus* spp. (41%), *Fusarium* spp. (35%), *Penicillium* spp. (20%) and *Alternaria* spp. (15%). Fungi most frequently isolated and identified were *Aspergillus fumigates*, *A. tereus*, *A. flavus*, *A. niger Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *F. equesti* and *Alternaria alternaria* (Zaboli and Khosravi, 2010). The one hundred and ninety six rice samples collected from field (28), market (84) and store (84). The commonest field fungi were *Alternaria* spp. (14/28), *Aspergillus niger* (8/28), *A. parasiticus* (7/28) *Fusarium* spp. (8/28), and *Penicillium* spp. (7/28). Mostly contaminated from the store were *Pencillium* (69/84), *A. flavus* (63/84), *A. niger* (46/84), *A. parasiticus* (43/84), *Fusarium verticillioides* (14/84), *F. oxysporum* (7/84), and *Alternaria* spp. (37/84). The major fungal contaminants of marketed rice in the state were *A. parasiticus* (45/84), *A. flavus* (41/84), *A. niger* (34/84), *Pencillium* spp. (40/84), *Fusarium* spp. (33/84) and *Alternaria* spp. (29/84). (Makun *et al*., 2007). Standard blotter and Deep Freezing methods were used to study the seed-borne mycoflora of 14 samples of barley, 11 genera and 17 species of fungi viz., *Alternaria alternata*, *Aspergillus* sp., *A. candidus*, *A. flavus*, *A. niger*, *Fusarium moniliforme*, *F. pallidoroseum*, *Penicillium* sp. were isolated and identified. Of these *Alternaria alternata*, *Aspergillus niger*, *Penicillium* spp. were found to be predominant (Fakhrunnisa *et al*., 2006).

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