Pak. J. Bot., 45(5): 1831-1839, 2013.

MYCOTOXIGENIC FUNGI CONTAMINATING CORN AND SORGHUM GRAINS IN SAUDI ARABIA

MOHAMED A. MAHMOUD[1], MONIRA R. AL-OTHMAN[2], ABEER R. M. ABD EL-AZIZ[2]*

[1]*Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*
[2]*Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 1145, Saudi Arabia*
**Corresponding author's e-mail: aabdelaziz@ksu.edu.sa*

Abstract

The natural occurrence of fungal contamination was evaluated in different sample of corn (*zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) grains. Mycological survey was carried out of four cereals crop yellow and white corn and yellow and red sorghum grains collected from produced and imported grains to the Kingdom of Saudi Arabia. A total of 80 samples of corn and sorghum grains (20 samples per crop) were analyzed by direct plating method on PDA and focusing on the mycotoxigenic fungi *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* genera for ability of these genera to produce mycotoxin. The most frequent isolated fungi from yellow and white corn were *Aspergillus* spp. and *Fusarium* spp. and *Alternaria* spp. were the lowest frequent. The most frequent isolated fungi from yellow and red sorghum were *Aspergillus* spp. and *Fusarium* spp. and *Alternaria* spp. were the lowest frequent. Significant difference was observed between the frequency of fungal isolates from different sample of corn and sorghum. The predominant fungal genera recorded at high frequency were *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*. These results indicate possible health hazards for humans and animals consumption of such contaminated food grain by mytoxigenic fungi.

Introduction

The increasing worldwide concern about food has enhanced interest in fungal contamination and subsequent production of mycotoxins in food products. In this regard, attention is continuously focused on corn (*zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) because there are the most important dietary staple foods and feedstuffs in different regions of the world (Anon., 2004). Corn is the world's third most important crop after rice and wheat. About half of this is grown in developing countries where corn flour is a staple food for poor people and corn stalks provide dry season feed for farm animals (Roige *et al*., 2009). In industrialized countries, corn is largely used as livestock feed and as raw material for industrial products e.g., as feed, silage, breakfast food and processing (breakfast cereals, corn chips, grits and flour), industrial starch and popcorn. Corn is an important feed ingredient for the livestock industry in many countries (Warr *et al*., 2008). Sorghum (*Sorghum bicolor* L. Moench) ranks fifth in worldwide economic importance among cereal crops with an annual production of 60 million tons. Besides being an important food, feed and forage crop, sorghum also provides raw material for the production of starch, fiber, dextrose syrup, biofuels, alcohol, and other products. More than half of the world's sorghum is grown in semi-arid tropics of India and Africa, where it is a staple food for millions of poor people (Anon., 2004). Saudi Arabia product about 183.000 and 272.000 tonnes of corn and sorghum respectively, imported about 2000000 and 152850 tonnes of corn and sorghum in 2009 (Anon., 2010). No region of the world escapes the problem of mycotoxins and their fungi (Lawlor & Lynch 2005). The Food and Agricultural Organization (FAO), estimates that between 25% and 50% of agricultural crops worldwide is contaminated by mycotoxins (Wagacha & Muthomi, 2008). Reports indicate that number of fungal species associated with corn and sorghum belong to the genera *Fusarium*, *Aspergillus* and *Penicillium*, which have been known to produce mycotoxins that cause mycotoxicosis in animals and humans. Several researchers have been documented fungi and their mycotoxins in corn grains For example (Magnoli *et al*., 2007, Miller, 2008, Roige *et al*., 2009, Reddy *et al*., 2010, Wu *et al*., 2011, Niaz *et al*., 2012) and sorghum grains for example (Broggi *et al*., 2007, Palumbo *et al*., 2008, Hussaini *et al*., 2009, Yassin *et al*., 2010, Abdulsalaam & Shenge, 2011). The mycotoxins are produced by fungal action during production, harvest, transportation, storage and food processing (Murphy *et al*., 2006). Mycotoxins have been reported to be carcinogenic, teratogenic, mutagenic, immunosuppressive, tremorgenic, hemorrhagic, hepatotoxic, nephrotoxic, neurotoxic and potentially increased susceptibility to HIV (Anon., 1993; Hashem *et al*., 2012). Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha & Muthomi 2008).

There is a lack of accurate data on the frequency and relative percentage of mycotoxigenic fungi isolated from corn and sorghum grains produced and imported by 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, levels of mycotoxigenic fungi, the frequency and a relative percentage of mycotoxigenic fungi in corn and sorghum grains produced and imported by Saudi Arabia.

Material and Methods

Isolation and identification of mycotoxigenic fungi in grains: Two types of cereal grains were chosen to study the composition of mycotoxigenic fungi in corn and sorghum grains, and these were corn (yellow and white), sorghum (yellow and red). 40 samples (250 g each) of each grain type were collected from different markets located in Riyadh, Saudi Arabia, were examined for mycotoxigenic fungi in corn and sorghum 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 using keys and manuals (Barnett & Hunter 1972; Booth 1977; Keith 1996; Mathur & Kongsdal 2003; Singh *et al*., 1999; Summerell *et al*., 2003; Leslie & Summerell 2006). The frequency of fungi and relative percentage 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.

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

$$\text{Frequency (\%)} = \frac{\text{Number of samples infected with fungi}}{\text{Total Number of samples analysis}} \times 100$$

Results

The frequencies of 4 fungi genera differed from sample to sample. The general means of fungi showed that *Fusarium* spp., were the most frequently isolated genera (31.74) and *Aspergillus* spp. (30.83); while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (13.75 and 1.66) from yellow corn grains samples (Table 1). The general means of fungi showed that *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. was the most frequently isolated genus (27.09, 26.67 and 15.0) while *Alternaria* spp. was the least frequently isolated genera (1.24) from white corn grains samples (Table 2). The general means of fungi showed that *Aspergillus* spp. and *Fusarium* spp. were the most frequently isolated genera (32.49 and 31.24), while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (6.66 and 2.49) from yellow sorghum grains samples (Table 3). The general means of fungi showed that *Aspergillus* spp. and *Fusarium* spp. were the most frequently isolated genera (31.66 and 31.24), while *Penicillium* spp. and *Alternaria* spp. were the least frequently isolated genera (5.41 and 1.62) from red sorghum grains samples (Table 4). In general the *Aspergillus* spp. and *Fusarium* spp. were the most frequently isolated genera for corn and sorghum grains samples. Analysis of variance (ANOVA) (Table 5) of isolation four genera from corn and sorghum grains showed non significant (p=0.01) effect of samples 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 corn and sorghum 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 corn and sorghum grains samples.

Considering the importance of the *Fusarium* genus, 86.25% of isolates of *Fusarium* species were identified up to the species level in yellow and white corn grains samples. The study showed the presence of three *Fusarium* species includes *F. oxysporum, F. monilinforme* and *F. solani*. The relative percentage showed *F. oxysporum* (38.88%) and *F. monilinforme* (31.95%) were the dominant *Fusarium* species with a high relative percentage followed by *F. solani* (15.97%) (Table 6). Number of isolates for *F. oxysporum, F. monilinforme* and *F. solani* were 56, 46 and 23 respectively. An important observation made in the present investigation was that *F. oxysporum* and *F. monilinforme* were isolated from almost all the samples with high relative density. Yellow and white corn grains samples contaminated by species of *Aspergillus*, (90.28%) of isolates of *Aspergillus* species were identified up to the species level. The study showed the presence of two *Aspergillus* species. The study of relative percentage showed that the *A. flavus* (50.36%) and *A. niger* (40.28%) were the dominant *Aspergillus* species with a high relative percentage. Number of isolates for *A. flavus* and *A. niger* were 70 and 56. Further, mycological analysis of yellow and white corn samples grains for the other field fungi revealed the occurrence of *P. notatum* (71.01%) and *A. alternata* (71.43%). Number of isolates for *P. notatum* and *A. alternata* were 49 and 5 (Table 6).

The study of relative percentage of yellow and red sorghum grains samples showed four *Fusarium* species were identified (Table 6). *F. verticillioides* (29.33%) and *F. oxysporum* (24.00%) were the dominant *Fusarium* species with a high relative percentage followed by *F. solani* and *F. semitectum* (22.0% and 14.0). Number of isolates for *F. verticillioides, F. oxysporum, F. solani* and *F. semitectum* were 44, 36, 33 and 21 respectively. An important observation made in the present investigation is that *F. verticillioides* was isolated from almost the samples. *Aspergillus* species were contaminated yellow and red sorghum grains samples, (91.22%) of isolates of *Aspergillus* species were identified up to the species level. The study of relative percentage showed that the *A. flavus* (54.73%) and *A. niger* (36.49%) were the dominant *Aspergillus* species with a high relative percentage. Number of isolates for *A. flavus* and *A. niger* were 81 and 54. The study of relative percentage of other fungi revealed the occurrence of *P. citrinum* and *A. alternata* (78.38 and 66.66%). Number of isolates for *P. notatum* and *A. alternata* were 29 and 6 (Table 6).

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

Sample no.	Fungi genera							
	Aspergillus ssp.		*Fusarium* spp.		*Penicillium* spp.		*Alternaria* spp.	
	%[a]	T	%	T	%	T	%	T
1	33.33	5.80	33.33	5.80	8.33	02.97	00.00	00.71
2	33.33	5.80	50.00	7.11	8.33	02.97	08.33	02.97
3	33.33	5.80	33.33	5.80	8.33	02.97	00.00	00.71
4	33.33	5.80	25.00	5.01	00.00	02.71	00.00	00.71
5	33.33	5.80	33.33	5.80	16.70	4.15	00.00	00.71
6	41.66	6.50	50.00	7.11	8.33	02.97	08.33	02.97
7	25.00	5.01	25.00	5.01	8.33	02.97	08.33	02.97
8	25.00	5.01	25.00	5.01	25.00	05.01	00.00	00.71
9	33.33	5.80	41.66	6.50	16.70	04.15	00.00	00.71
10	25.00	5.01	25.00	5.01	16.70	04.15	00.00	00.71
11	33.33	5.80	33.33	5.80	25.00	05.01	00.00	00.71
12	25.00	5.01	33.33	5.80	8.33	02.97	08.33	02.97
13	25.00	5.01	25.00	5.01	16.70	04.15	00.00	00.71
14	33.33	5.80	25.00	5.01	8.33	02.97	00.00	00.71
15	25.00	5.01	33.33	5.80	8.33	02.97	00.00	00.71
16	41.66	6.50	33.33	5.80	16.70	04.15	00.00	00.71
17	25.00	5.01	33.33	5.80	25.00	05.01	00.00	00.71
18	33.33	5.80	50.00	7.11	16.70	04.15	00.00	00.71
19	33.33	5.80	25.00	5.01	8.33	02.97	00.00	00.71
20	25.00	5.01	33.33	5.80	25.00	05.01	00.00	00.71
Mean	30.83	5.55	31.74	5.75	13.75	03.61	01.66	01.62

T= transformed value; LSD for white corn grains samples non significant
LSD for fungi = 1.26 (p 0.005) or 1.67 (p 0.01)
[a] percentage data were transformed into $\sqrt{x + 0.5}$ angles before carrying out the analysis of variance

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

Sample no.	Fungi genera							
	Aspergillus ssp.		*Fusarium* spp.		*Penicillium* spp.		*Alternaria* spp.	
	%[a]	T	%	T	%	T	%	T
1	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
2	41.66	06.50	33.33	05.80	08.33	02.97	00.00	00.71
3	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
4	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.71
5	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
6	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
7	25.00	05.01	25.00	05.01	16.70	04.15	08.33	02.97
8	16.70	04.15	25.00	05.01	16.70	04.15	00.00	00.71
9	25.00	05.01	16.70	4.15	25.00	05.01	00.00	00.71
10	16.70	04.15	25.00	05.01	16.70	04.15	00.00	00.71
11	25.00	05.01	16.70	04.15	25.00	05.01	00.00	00.71
12	33.33	05.80	25.00	05.01	16.70	04.15	00.00	00.71
13	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.71
14	16.70	04.15	33.33	05.80	25.00	05.01	08.33	02.97
15	16.70	04.15	16.70	04.15	25.00	05.01	08.33	02.97
16	25.00	05.01	16.70	04.15	16.70	04.15	00.00	00.71
17	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
18	25.00	05.01	16.70	04.15	25.00	05.01	00.00	00.71
19	33.33	5.80	33.33	05.80	16.70	04.15	00.00	00.71
20	16.70	04.15	33.33	05.80	08.33	02.97	00.00	00.71
Mean	27.09	05.18	26.67	05.15	15.00	03.83	01.24	01.04

T= transformed value; LSD for white corn grains samples non significant
LSD for fungi = 1.26 (p 0.005) or 1.67 (p 0.01)
[a] percentage data were transformed into $\sqrt{x + 0.5}$ angles before carrying out the analysis of variance

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

Sample no.	Fungi genera							
	***Aspergillus* ssp.**		***Fusarium* spp.**		***Penicillium* spp.**		***Alternaria* spp.**	
	%[a]	**T**	**%**	**T**	**%**	**T**	**%**	**T**
1	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
2	25.00	05.01	41.66	06.50	08.33	02.97	00.00	00.71
3	58.33	07.67	33.33	05.80	08.33	02.97	08.33	02.97
4	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
5	41.66	06.50	33.33	05.80	08.33	02.97	00.00	00.71
6	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.71
7	50.00	07.11	33.33	05.80	08.33	02.97	08.33	02.97
8	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
9	33.33	05.80	33.33	05.80	00.00	00.71	00.00	00.71
10	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.71
11	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
12	33.33	05.80	25.00	05.01	00.00	00.71	00.00	00.71
13	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.71
14	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
15	33.33	05.80	33.33	05.80	08.33	02.97	08.33	02.97
16	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.71
17	33.33	05.80	33.33	05.80	00.00	00.71	08.33	02.97
18	25.00	05.01	25.00	05.01	08.33	02.97	00.00	00.71
19	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
20	25.00	05.01	33.33	05.80	00.00	00.71	00.00	00.71
Mean	32.49	05.67	31.24	05.59	06.66	02.51	02.49	01.38

T= transformed value; LSD for yellow sorghum grains samples non significant

LSD for fungi = 1.23 (p 0.005) or 1.63 (p 0.01)

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

Table 4. Frequency of (%) four fungi genera from red sorghum grains.

Sample no.	Fungi genera							
	***Aspergillus* ssp.**		***Fusarium* spp.**		***Penicillium* spp.**		***Alternaria* spp.**	
	%[a]	**T**	**%**	**T**	**%**	**T**	**%**	**T**
1	50.00	07.11	33.33	05.80	08.33	02.97	00.00	00.71
2	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
3	33.33	05.80	41.66	06.50	08.33	02.97	00.00	00.71
4	33.33	05.80	25.00	05.01	00.00	00.71	08.33	02.97
5	25.00	05.01	33.33	05.80	08.33	02.97	08.33	02.97
6	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
7	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
8	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
9	33.33	05.80	25.00	05.01	00.00	00.71	08.33	02.97
10	33.33	05.80	33.33	05.80	00.00	00.71	00.00	00.71
11	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
12	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.71
13	33.33	05.80	33.33	05.80	08.33	02.97	00.00	00.71
14	33.33	05.80	25.00	05.01	08.33	02.97	08.33	02.97
15	25.00	05.01	33.33	05.80	00.00	00.71	00.00	00.71
16	33.33	05.80	33.33	05.80	00.00	00.71	00.00	00.71
17	33.33	05.80	25.00	05.01	00.00	00.71	00.00	00.71
18	25.00	05.01	33.33	05.80	08.33	02.97	00.00	00.71
19	33.33	05.80	33.33	05.80	00.00	00.71	00.00	00.71
20	33.33	05.80	25.00	05.01	08.33	02.97	00.00	00.71
Mean	31.66	05.62	31.24	05.59	05.41	02.17	01.66	01.62

T= transformed value; LSD for red sorghum grains samples non significant

LSD for fungi = 1.52 (p 0.005) or 2.02 (p 0.01)

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

Table 5. Analysis of variance of frequency (%) of four fungi genera isolated from corn and sorghum grains.

Parameters and Source of variation [a]	D.F	M.S	F. value	P F
Yellow corn grains				
Replication	2	0.509	0.123	
sample (S)	19	0.813	0.197	
fungi (F)	3	57.374	13.916	0.000
S x F	57	2.222	0.539	
Error	266	4.123		
White corn grains				
Replication	2	7.428	2.070	0.133
sample (S)	19	0.355	0.099	
fungi (F)	3	72.889	20.322	0.000
S x F	57	0.563	0.1570	
Error	266	0.157		
Yellow sorghum grains				
Replication	2	4.120	0.8975	
sample (S)	19	0.817	0.1718	
fungi (F)	3	73.360	15.980	0.000
S x F	57	1.154	0.2515	
Error	266	4.591		
Red sorghum grains				
Replication	2	2.364	0.562	
sample (S)	19	1.371	0.316	
fungi (F)	3	57.666	13.323	0.000
S x F	57	0.883	0.203	
Error	267	4.328		

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

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

Name of the fungi genera and crop	Total No. of isolates	Relative percentage
Fusarium species isolated from yellow and white corn grains samples		
F. oxysporum	56	38.88
F. monilinforme	46	31.95
F. solani	23	15.97
Fusarium species	19	13.19
Aspergillus species isolated from yellow and white corn grains samples		
A. flavus	70	50.36
A. niger	56	40.28
Aspergillus species	13	09.35
Penicillium species isolated from yellow and white corn grains samples		
P. notatum	49	71.01
Penicillium species	20	28.99
Alternaria species isolated from yellow and white corn grains samples		
Alternaria alternata	5	71.43
Alternaria species	2	28.57
Fusarium species isolated from yellow and red sorghum grains samples		
F. verticillioides	44	29.33
F. oxysporum	36	24.00
F. solani	33	22.00
F. semitectum	21	14.00
Fusarium species	16	10.67
Aspergillus species isolated from yellow and red sorghum grains samples		
A. flavus	81	54.73
A. niger	54	36.49
Aspergillus species	13	08.78
Penicillium species isolated from yellow and red sorghum grains samples		
P. citrinum	29	78.38
Penicillium species	8	21.62
Alternaria species isolated from yellow and red sorghum grains samples		
Alternaria alternata	6	66.66
Alternaria species	3	33.34

Table 7. The presence of four fungi genera in grain samples.

Number of samples	yellow corn 20	white corn 20	yellow sorghum 20	red sorghum 20	Total 80
Microorganism	Num. positive [a]				num. positive [f]
	PCT positive samples [b]				PCT num. positive
	Max. frequency [c]				
	Min. frequency [d]				
	Mean frequency [e]				
Aspergillus spp.	20	20	20	20	80
	100	100	100	100	100
	41.66	41.66	58.33	50	
	25	16.70	25	25	
	30.83	27.09	32.49	31.00	
Fusarium spp.	20	20	20	20	80
	100	100	100	100	100
	50	33.33	41.66	41.66	
	25.00	16.70	25.00	25	
	31.74	26.67	31.24	31.24	
Penicillium spp.	19	20	17	14	70
	95	100	85	70	87.5
	25	25	8.33	8.33	
	0	8.33	0	0	
	13.75	15	6.66	5.41	
Alternaria spp.	4	3	8	4	19
	20	15	40	20	32.72
	8.33	8.33	8.33	8.33	
	0	0	0	0.0	
	1.66	1.24	2.49	1.66	

[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

The microorganisms, isolated from all 60 samples are shown in Table 7. The leading contaminants among fungi were *Aspergillus* spp. and *Fusarium* spp. Which were detected in all samples (100%) followed by *Penicillium* spp. and *Alternaria* spp in some samples (70% and 32.72%). *Aspergillus* spp. *Fusarium* spp. were highest contaminated for yellow and white corn samples (30.83 and 27.09) and (31.74 and 26.67) respectively. *Aspergillus* spp. *Fusarium* spp. were highest contaminated for yellow and red sorghum samples (32.49 and 31) and (31.24) respectively. *Alternaria* spp. were lowest contaminated for yellow and white corn samples (1.66 and 1.24). *Alternaria* spp. were lowest contaminated for yellow and red samples (2.49 and 1.66).

In the present study, the Phenogram of four fungi genera isolated from 20 yellow corn grains samples based on isolation frequencies (Fig. 1) showed the presence of two unrelated groups of fungi. The first group includes *Aspergillus* species, *Fusarium* species and *Penicillium* species within this group, fungi were classified to two subgroups. The fungi under this subgroup were associated positively and have high similarity level (98%). The second subgroup includes *Penicillium* spp were having similarity level (65%) with first subgroup. The second group includes *Alternaria* species were have low similarity level (40%) with first group.

The Phenogram of 4 fungi genera isolated from 20 white corn grains samples based on isolation frequencies (Fig. 2) showed that, two unrelated 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. The first subgroup include *Aspergillus* species and *Fusarium* spp were have high similarity level (98%). The fungi under every subgroup were associated positively, like first subgroup includes *Aspergillus* species and *Fusarium* spp. The second subgroup includes *Penicillium* spp were have low similarity level (20%) with first subgroup. The second group includes *Alternaria* species were have very low similarity level (10%) with first group.

The Phenogram of four fungi genera isolated from 20 yellow sorghum grains samples based on isolation frequencies (Fig. 3) showed that, three unrelated groups of fungi were identified. The first group includes *Aspergillus* species and *Fusarium* species. The fungi under this group were associated positively and have high similarity level (98%). The second group includes only *Penicillium* species were have low similarity level (22%) with first group. The third group includes only *Alternaria* species were have low similarity level (22%) with first group and second group.

The cluster analysis of 4 fungi genera isolated from 20 red sorghum grains samples based on isolation frequencies (Fig. 4) showed that, two unrelated groups of fungi were identified. The first group includes *Aspergillus* species; *Fusarium* specie were having high similarity level (98%). The second group includes *Penicillium* species and *Alternaria* species were having low similarity level (20%). The fungi under every group were associated positively.

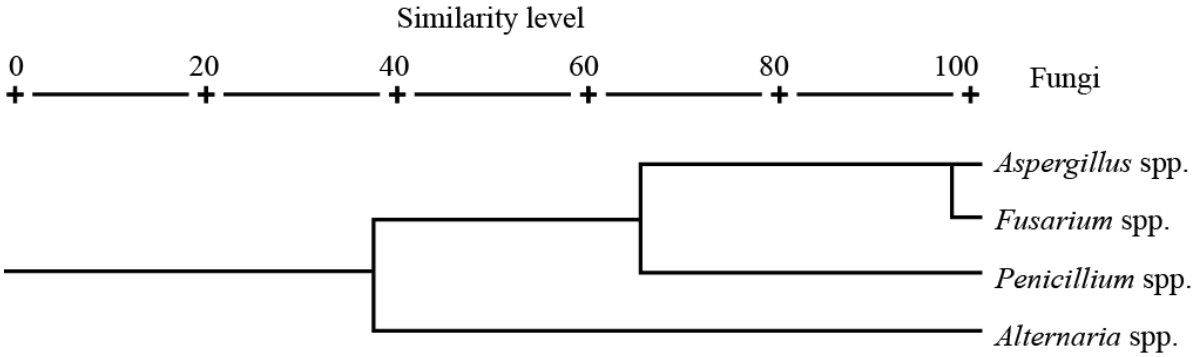

Fig. 1. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from yellow corn grains samples.

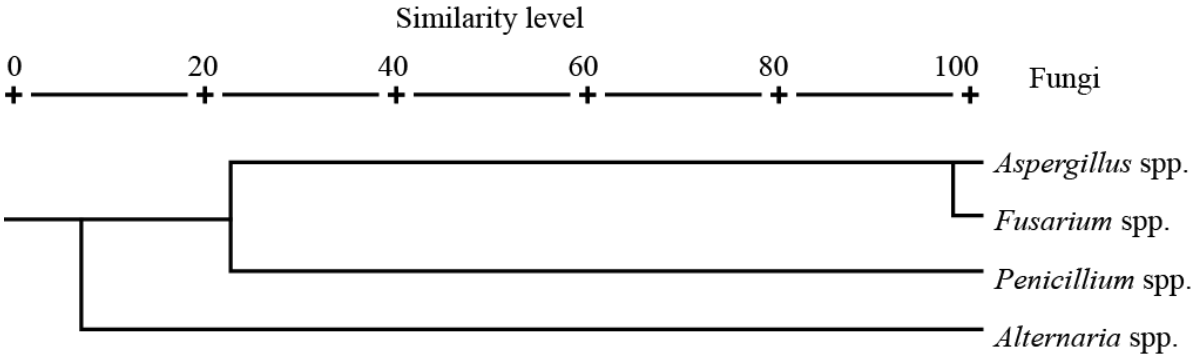

Fig. 2. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of six fungi genera from white corn grains samples

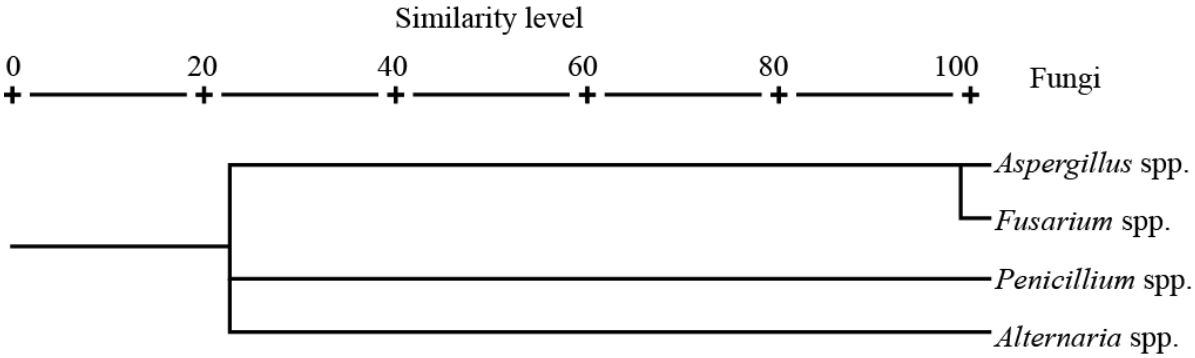

Fig. 3. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from yellow sorghum grains samples.

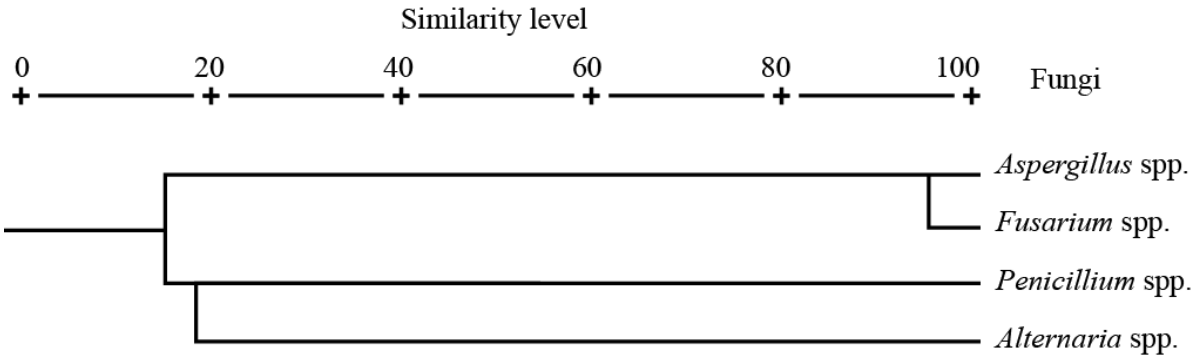

Fig. 4. Phenogram based on average linkage cluster analysis of isolation frequencies (%) of four fungi genera from red sorghum grains samples.

Discussion

These results indicated that the type of fungal contamination of the corn and sorghum grains, at Saudi Arabia, was qualitatively similar to that found in other sorghum producing countries such as the United States (Wu *et al*., 2011), Italy (Covarelli *et al*., 2011), Switzerland (Dorn *et al*., 2011), African countries (Abdulsalaam and Shenge, 2011), Saudi Arabia (Yassin *et al*., 2010), India (Sreenivasa *et al*., 2010), Malaysia (Reddy & Sallah, 2011) and Pakistan (Saleem *et al*., 2012).

The major potential impacts of climate change, food safety and food security have received relatively less attention. Agriculture is profoundly affected by the main climatic factors that may change significantly in the near future: temperature, precipitation, drought, and atmospheric carbon dioxide. A number of agricultural entities could be affected by these climatic factors, including soil quality, crop yields, and the biological environment of crops such as the abundance of pests and plant pathogens. Mycotoxins are among the food-borne risks that are dependent upon climatic conditions. Indeed, the ability of fungi to produce mycotoxins is largely influenced by temperature, relative humidity, insect attack, and stress conditions of the plants (Miraglia *et al.*, 2009). Therefore studies on frequency and their relative percentage of mycotoxigenic fungi are highly useful and required for further studies on toxin producing fungi and their epidemiological significance in corn and sorghum crops. Several genera and species of filamentous fungi produce polyketide-derived mycotoxins that have significant agricultural, epidemiological and economic impact. *Aspergillus*, *Fusarium*, and *Penicillium* genera are mycotoxigenic fungi responsible for the majority of agricultural mycotoxin contamination. These fungi are common components of the microbial flora associated with many agronomic crops, including corn and sorghum (Palumbo *et al*., 2008). Many previous researchers have reported cereal grain during ripening as well as grape development represent food ecosystems that are colonized by mycotoxigenic fungi, which are influenced by abiotic factors such as prevailing temperature, relative humidity, especially at a microclimate level and storage conditions in many regions around the world (Castellari *et al*., 2010, Magan *et al*., 2010, Rocha *et al*., 2009). Previous studies identified genus *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* as mycotoxigenic fungi were isolated from all the samples in this study. It was report as a natural contaminant in corn and sorghum crops and also in many other agricultural commodities (Lino *et al*., 2007; Logrieco *et al*., 2007, Pacin *et al*., 2009; Cunha *et al*., 2009). In general it is some factors effect on imported corn to be more susceptible to fungi contamination. It seems that the traditional methods of handling grains during harvesting in the field, drying process in relevant country and transferring it to other countries lead to mechanical damage of grains. In the condition, broken and ground grains are more vulnerable to fungal attack than whole grains (Odvody *et al*., 1990). On the other hand, the contamination could be due to long term storage of imported corn in poor environmental condition including high moisture and temperature. Corn stored for long time periods are more vulnerable than freshly harvested corn. Insects and rodents may also be contributed to deteriorating the grains rapidly and increasing corn mycoflora during long term storage (Hussein & Brasel, 2001). Therefore, the use of good agricultural practices that would discourage fungal growth and mycotoxin production would be necessary to reduce mycotoxin levels in the corn and corn products. Contact of the corn with the soil should be avoided during harvest and drying to avoid contamination with the fungal inoculum present in the soil. Drying of corn to safe moisture levels of less than 13% and cleaning of stores at the end of each season would reduce chances of infection and mould growth. Other methods of reducing mould and mycotoxin contamination include drying corn on mats and polythene sheets to avoid contact with soil surface (Muthomi *et al*., 2009). If the earth's surface temperatures continue in a warming trend, and other associated climate patterns may be changing, then farmers, food industries, and policymakers should be concerned about changing mycotoxin risks both in the short term and in the long term. In the short term, from year to year, temperature and precipitation may favour or discourage growth of mycotoxigenic fungi and mycotoxin contamination of agricultural products. In the long term, climatic trends may pose longer-term impacts on distribution of fungi, their mycotoxins, and host crop plants (Wu *et al*., 2011).

Acknowledgement

The authors would like to extend their appreciation to the Deanship of the Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-VPP-269.

References

Abdulsalaam, S. and K.C. Shenge. 2011. Seed borne pathogens on farmer-saved sorghum (*Sorghum bicolor* L.) seeds. *J. Stored Prod. Postharv. Res*., 2(2): 24-28.

Anonymous. 1993. *Monograph on the evaluation of carcinogenic risks to humans, some naturally occurring substances*: *food items and constituents, heterocyclic aromatic amines and mycotoxins*. Lyon, France: 56: 489-521.

Anonymous. 2004. *Production year book 2002*. no. 56. FAO Stat. Ser. Pp 176, FAO, Rome.

Anonymous. 2010. *Production year book 2009*. no. 61. FAO Stat. Ser. Pp. 181, FAO, Rome.

Barnett, H.L. and B.B. Hunter. 1972. *Illustrated Genera of Imperfect Fungi*. III ed. Burgess Publishing Company, Minneapolis, Minnesota, USA, pp. 208.

Booth, C. 1977. *Fusarium: Laboratory Guide to the Identification of Major Species*. Commonwealth Mycological Institute. Ferry Lane. Kew, Surrey, England: pp. 4-57.

Broggi, L.E.I., H.H.L. González and S.L.R. Pacin. 2007. *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina, *Rev*. *Iberoam*. *Micol*., 24: 47-51.

Castellari, C., F. Marcos Valle, J. Mutti, L. Cardoso and R. Bartosik. 2010. Toxigenic fungi in corn stored in hermetic plastic bags. *Julius-Kühn-Archiv*, 425: 501-504.

Covarelli, L., G. Beccari and S. Salvi. 2011. Infection by mycotoxigenic fungal species and mycotoxin contamination of corn grain in Umbria, central Italy. *Food Chem*. *Toxicol*., 49(9): 2365-2369.

Dorn, B., H.R. Forrer, E. Jenny, F.E. Wettstein, T.D. Bucheli and S. Vogelgsang. 2011. *Fusarium* species complex and mycotoxins in grain corn from corn hybrid trials and from grower's fields. *J. Appl. Microbiol.*, 111(3): 693-706.

Flannigan, B. 1977. *Enumeration of fungi and assay for ability of degrade structural and storage components of grain*. In: *Biodeterioration investigation techniques*. (Ed.): A.H. Walter. Applied Science, London: pp. 185-199.

Ghiasian, S.A., P.K. Bacheh, S.M. Rezayat, A.H. Maghsood and H. Taherkhani. 2004. Mycoflora of Iranian corn harvested in the main production areas in 2000. *Mycopath.*, 158(1): 113-121.

Hashem Abeer, Abd-Allah E.F. and Alwathnani Hend A. 2012. Effect of propolis on growth, aflatoxins production and lipid metabolism in *Aspergillus parasiticus* spear. *Pak. J. Bot*., 44(3): 1153-1158.

Hussaini, A.M., A.G. Timothy, H.A. Olufunmilayo, A.S. Ezekiel and H.O. Godwin. 2009. Fungi and Some Mycotoxins found in mouldy sorghum in Niger State, Nigeria. *World J. Agri. Sci.*, 5(1): 05-17.

Hussein, S.H. and J.M. Brasel. 2001. Toxicity, metabolism and impact of mycotoxin on humans and animals. *Toxicology*, 167: 101-134.

Keith, S. 1996. *Fuskey-Fusarium Interactive Key*. Agriculture and Agri-Food Canada, Canada. Available from: http://www. ctu.edu.vn/colleges/agri/gtrinh/fuskey.pdf

Lawlor, P.G. and P.B. Lynch. 2005. Mycotoxin management. *African Farming and Food Processing*, 46: 12-13.

Leslie, J.F. and B.A. Summerell. 2006. *The Fusarium, Laboratory Manual*. 1st ed. Blackwell Publishing Professional, USA, Pp 274.

Lino, C.M., L.J. Silva, A. Pena, M. Fernández and J. Mañes. 2007. Occurrence of fumonisins B1 and B2 in *broa*, typical Portuguese corn bread. *Inter. J. Food Microbiol*., 118: 79-82.

Logrieco, A., G. Moretti, G. Perrone and G. Mule. 2007. Biodiversity of complexes of mycotoxigenic fungal species associated with *Fusarium* ear rot of corn and *Aspergillus* rot of grape. *Inter. J. Food Microbiol.*, 119: 11-16.

Magan, N., D. Aldred, R. Hope and D. Mitchell. 2010. Environmental factors and interactions with mycobiota of grain and grapes: effects on growth, deoxynivalenol and ochratoxin production by *Fusarium culmorum* and *Aspergillus carbonarius*. *Toxins*, 2: 353-366.

Magnoli, C.E., A.L. Astoreca, S.M. Chiacchiera and A.M. Dalcero. 2007. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. *Mycopathologia*, 163: 249-260.

Mathur, S.B. and O. Kongsdal. 2003. *Common laboratory seed health-testing methods for detecting fungi*. International Seed Testing Association, Switzerland. pp. 255.

Miller, D.J. 2008. Mycotoxins in small grains and corn: old problems, new challenges. *Food Additive and Contaminants*, 25(2): 219-230.

Miraglia, M., H.J.P. Marvin, G.A. Kleter, P. Battilani, C. Brera, E. Coni, F. Cubadda, L. Croci, B. De Santis, S. Dekkers, L. Filippi, R.W.A. Hutjes, M.Y. Noordam, M. Pisante, G. Piva, A. Prandini, L. Toti, G.J. Van den Born and A. Vespermann. 2009. Climate change and food safety: an emerging issue with special focus on Europe. *Food Chem. Toxicol.*, 47: 1009-1021.

Cunha, M.E., G.T. García, M.A. Ono, E. Vizoni, O. Kawamura, E.Y. Hirooka and E.Y. Sataque Ono. 2009. Co-ocurrence of mycotoxins in corn samples from the Northern region of Paraná State, Brazil. *Food Chemistry*, 116: 220-226.

Murphy, P.A., S. Hendrich and C.M. Bryant. 2006. Food Mycotoxins - An Update. *J. Food Sci.*, 71(5): 51-65.

Muthomi, J.W., L.N. Njenga, J.K. Gathumbi and G.N. Chemining. 2009. The occurrence of aflatoxins in corn and distribution of mycotoxin-producing fungi in Eastern Kenya. *Plant Pathol. J.*, 8(3): 113-119.

Niaz, I., S. Dawar and Najum-us-Sahar. 2012. Detection of mycotoxins in maize seed samples. *Pak. J. Bot.*, 44(3): 1075-1078.

Odvody, G.N., J.C. Remmers and N.M. Spencer. 1990. Association of kernel splitting with kernel and ear rots of corn a commercial hydride grown in the coastal bend of Texas. *Phytopahology*, 80: 1045-1050.

Pacin, A.M., E.C. Bovier, H.L. González, E.M. Whitechurch, E.J. Martínez and S. Resnik. 2009. Fungal and fumonisins contamination in Argentine mayze (*Zea mays* L.) solo bags. *J. Agri. Food Chemistry*, 57: 2778-2781.

Palumbo, J.D., T.L. O'keeffe and H.K. Abbas. 2008. Microbial interactions with mycotoxigenic fungi and mycotoxins. *Toxin Reviews*, 27: 261-285.

Reddy, K.R.N. and B. Salleh. 2011. Co-occurrence of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. *J. Animal. Veterir.*, 10(5): 668-673.

Reddy, K.R.N., B. Salleh, B. Saad, H.K. Abbas, C.A. Abel and W.T. Shier. 2010. An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews*, 29: 3-26.

Rocha, L.O., V.K. Nakai, R. Braghini, T.A Reis, E. Kobashigawa and B. Corrêa. 2009. Mycoflora and co-occurrence of fumonisins and aflatoxins in freshly harvested corn in different regions of Brazil. *Int. J. Mol. Sci*., 10: 5090-5103.

Roige, M.B., S.M. Aranguren, M.B. Riccio, S. Pereyra, A.L. Soraci and M.O. Tapia. 2009. Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. *Rev. Iberoam Mycol*., 26: 233-237.

Saleem, M.J., R. Bajwa, A. Hannan and T.A. Qaiser. 2012. Maize seed storage mycoflora in Pakistan and its chemical control. *Pak. J. Bot*., 44(2): 807-812.

Singh, K., J.C. Frisrad, U.Thrane and S.B. Mathur. 1999. *An Illustrated Manual on Identification of Some Seed-Borne Aspergilli, Fusaria, Penicillia and their Mycotoxins*. Danish Govt. Institute of Seed Pathology for Developing Countries, Denmark. pp. 322.

Sreenivasa, M.Y., R.S. Dass and G.R. Janardhana. 2010. Survey of postharvest fungi associated with sorghum grains produced in karnataka (India). *J. Pl. Prot. Res.*, 50(3): 335-339.

Summerell, B.A., B. Salleh and J.F. Leslie. 2003. A utilitarian approach to *Fusarium* identification. *Plant Dis*., 87(2): 117-128.

Wagacha, J.M. and J.W. Muthomi. 2008. Mycotoxin problem in Africa: Current Status, implications to food safety and health and possible management strategies. *Inter. J. Food Microbiol.*, 124: 1-12.

Warr, S.G., A.M. Rodriguez and J. Penm. 2008. Changing food consumption and imports in Malaysia. *Res. Rep.*, 8.6, pp. 1-29.

Wu, F., D. Bhatnagar, T. Bui-Klimke, I. Carbone R. Hellmich, G. Munkvold, P. Paul, G. Payne and E. Takle. 2011. Climate change impacts on mycotoxin risks in US corn. *World Mycotoxin J.*, 4(1): 79-93.

Yassin, M.A., A. El-Samawaty, A. Bahkali, M. Moslem, K.A. Abd-Elsalam and K.D. Hyde. 2010. Mycotoxin-producing fungi occurring in sorghum grains from Saudi Arabia. *Fungal Diversity*, 44: 45-52.

(Received for publication 8 May 2012)