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CONTAMINANT MYCOFLORA AND AFLATOXINS IN SOME CEREAL GRAINS

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Abstract: The mycoflora of 45 samples cereal grains (wheat, corn and sorghum) were collected from three governorates namely; El-Minia, Assiut and Sohag. These samples were studied using dilution plate method on glucose Czapeks agar medium at 28°C. Twenty-eight species belonging to thirteen genera were identified during this investigation. The broadest spectrum of genera and species was recorded in wheat (16 species and 8 genera); followed by sorghum (15 species and 8 genera) and corn grains (15 species and 8 genera). *Aspergillus, Penicillium* and *Fusarium* were the most common genera in the wheat and corn. Aflatoxins assay using thin layer chromatographic analysis revealed that, cereal grains were contaminated by aflatoxins B_1 and B_2 (1, 3 and 2 samples of wheat, corn and sorghum, respectively) and one sample of corn was contaminated by B_1, B_2, G_1 and G_2.

Key words: mycoflora, aflatoxins, cereal grains.

Introduction

Cereals grains play exceptionally important role in human nourishment. The deterioration of cereal grains during storage caused by a large number of fungi produced mycotoxins which become accumulated in different concentrations (Kaushal and Deepak, 1998). Among these mycotoxins were the aflatoxins which are secondary metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*. Their recognition as potent carcinogens in human and some animals had made them the subject of government legislation as well as valuable tools, in studying cancer (Moss and Smith, 1985). High moisture and relative humidity are essential for growth of fungi and caused the deterioration of cereal grains during storage. The production of aflatoxins and other mycotoxins in cereals and their hazards on human health called for the study of mycoflora of cereal grains (Christensen, 1963; Christensen and Kaufmann, 1965, Flannigan, 1970, 1974; Yap and Kulshreshtha, 1975; Fischer *et al.*, 1995; Kaushal and Deepak, 1998).

The present investigation was designed to study the mycoflora of wheat, corn and sorghum in three Governorate of Egypt namely: El-Minia, Assiut and Sohag. The production of aflatoxins B_1, B_2, G_1 and G_2 in grains were studied as well.

Materials and Methods

I- Materials

Fifteen samples of each wheat, corn and sorghum grains of the crops of 2002 and 2003 were investigated. One-half kg of each were collected from different grain stores located in three studied governorates namely: El-Minia, Assiut and Sohag. Five samples of each grain were collected from each Governorate. Each sample was placed in polyethylene bag, transferred to the laboratory and kept at 4°C until tested.

II- Methods

a. Determination of cereal grain borne fungi:

This was made by using dilution-plate method as described by Christensen (1963). Twenty gms of each sample were placed in sterile conical flask containing 180 ml of sterile distilled water and shaken by hand with a rotating motion for 10 minutes. One ml of the water suspension was transferred to sterile petri-dish. Five plates were poured with glucose-Czapek's agar medium + rose bengal (1/15000) as bacteriostatic agent for each cereal grain sample. The plates were incubated at 28°C for 7-10 days and the developing fungi were identified and counted. The colonies of slow-growing fungi were transferred to slants to ensure precise counting. Fungi were identified according to Raper and Thom (1949), Raper and Funnell (1965) and Ellis (1971).

The following criteria are used chiefly for differentiation and identification of fungi:

1- Hyphae septate or nonseptate.

2- Mycelium clear or dark (smoky).

3- Mycelium colored or colorless.

4- Type of asexual spores.

5- Characteristic of the spore head.

6- Appearance of sporangiophores or conidiophores

7- Microscopic appearance of the asexual spores.

8- Presence of special structure (stolon, rhizoids, foot cell etc ...).

b. Determination of moisture content:

Moisture content of the studied samples was determined according to the method described by A.O.A.C. (1980).

c. Extraction, purification and detection of aflatoxins:

The aflatoxins were assayed by the A.O.A.C. (1980) method, the extraction was performed using chloroform-water mixture and the obtained extracts were purified by column chromatography containing anhydrous $NaSO_4$ and silica gel. Detection of aflatoxins were carried out by comparing the fluorescent of sample spots with those of standard

aflatoxins (B_1, B_2, G_1 & G_2) on thin layer chromatographic plates under U.V. light (366 nm) as outlined in A.O.A.C. (1980).

Results and Discussion

1- Mycoflora and moisture content of cereal grains:

The moisture content of the tested grains was considerably moderate and ranged between 7.90-9.60%, 8.17-10.88% and 8.19-11.87% in wheat, corn and sorghum, respectively (Table 1). The higher moisture content was observed in sample No. 28 of sorghum recording relatively high counts of fungi (166.0 colonies per gm). Meanwhile, samples No. 6 and 22 had above 10% of moisture content in corn samples having relatively the richest total count of fungi (416 and 192 colonies per gm, respectively). Similar observation was reported by Davis *et al.* (1986) and Kaushal and Deepak (1998). They found that the corn exposed to high moisture was more susceptible to infection by fungi particulary *Aspergillus flavus*.

The results given in Table 2 show that twenty eight species belonging to thirteen genera were identified from all tested samples. Wheat grains were relatively the highest in the total count fungi followed by sorghum and corn. The broadest spectrum of genera and species was recorded in wheat (8 genera and 16 species), followed by sorghum (8 genera and 15 species) and corn (8 genera and 15 species) as shown in Tables 2 & 3. The broadest spectrum of genera and species was recorded in corn sample No. 9 (3 genera and 5 species), followed by samples No. 19, 20, 24 and 44 (3 genera and 4 species). The least number (1 genera and 1 species) was recorded in sample No. 11, 27 and 29 of sorghum and 34 of wheat. From these results it could be noticed that irregular correlation between the fungal count and the spectrum of genera and species (Table 2).

Aspergillus was the most common genus in all tested grains collected from the three studied governorates (Table 3). This genus was represented by 11 species (7 species in wheat followed by 6 species in corn and 5 species in sorghum). *Aspergillus niger* was consistely the most common species in the three studied grains and was represented in 9, 6 and 9 samples in wheat, corn and sorghum, respectively. The highest count of *A. niger* was recorded in sample No. 18 (126 colonies per gm) of wheat obtained from Assiut governorate. *Aspergillus flavus* occupied the second place in the three grains. It occurred in 6, 9 and 7 samples in wheat, corn and sorghum, respectively. The highest count of *A. flavus* was detected in No. 6 of corn (366 colonies per gm) collected from El-Minia governorate. *Aspergillus fumigatus* was recovered from 4, 4 and 5 of wheat, corn and sorghum samples, respectively. El-Kady *et al.* (1982) isolated *A. niger* as the most

Table (1): Mean values of moisture content and aflatoxins identified in the three studied grains in the three studied governorates.

Sample No.	Governorate	Grain sample	Moisture content %	Aflatoxins identified
1	El-Minia	Wheat	8.44	
2	,,	,,	7.90	
3	,,	,,	8.45	
4	,,	,,	8.29	
5	,,	,,	8.10	
6	,,	Corn	10.21	aflatoxin B_1 & B_2
7	,,	,,	8.334	
8	,,	,,	9.99	aflatoxin B_1 & B_2
9	,,	,,	8.41	
10	,,	,,	8.87	
11	,,	Sorghum	8.20	
12	,,	,,	8.38	
13	,,	,,	9.26	
14	,,	,,	8.15	
15	,,	,,	8.90	
16	Assiut	Wheat	9.35	
17	,,	,,	8.83	
18	,,	,,	9.31	
19	,,	,,	9.50	
20	,,	,,	9.50	
21	,,	Corn	8.94	
22	,,	,,	10.88	aflatoxin B_1 & B_2, G_1 & G_2
23	,,	,,	8.19	
24	,,	,,	8.32	
25	,,	,,	8.17	
26	,,	Sorghum	9.29	
27	,,	,,	9.10	
28	,,	,,	11.87	aflatoxin B_1 & B_2
29	,,	,,	8.44	
30	,,	,,	9.20	
31	Sohag	Wheat	8.91	
32	,,	,,	8.84	
33	,,	,,	9.60	aflatoxin B_1 & B_2
34	,,	,,	8.91	
35	,,	,,	9.40	
36	,,	Corn	9.88	
37	,,	,,	9.72	
38	,,	,,	8.38	
39	,,	,,	9.57	
40	,,	,,	9.66	
41	,,	Sorghum	8.11	
42	,,	,,	8.30	
43	,,	,,	9.33	aflatoxin B_1 & B_2
44	,,	,,	8.85	
45	,,	,,	8.19	

common species in barley, wheat, corn and sorghum and was represented in 88-100% of the samples and they found, *A. flavus* and *A. fumigatus* occupied the second and third place in four grains. *A. terreus* was isolated from wheat and corn in the three studied samples and was not recorded in sorghum. These four species were consistently the most common species in Egyptian seeds and grains (Abdel-Kader *et al.*, 1979; El-Kady *et al.*, 1982 and El-Maghraby and El-Maraghy, 1987).The remaining species viz. *A. versicolor, A. candidus, A. chevalieri, A. tamarii, A. nidulans, A. ochraceus* and *A. wentii* were rare as shown in Table 2.

The data represented in Table 3 indicated that *Penicillium* was the second most dominant genus. It was isolated from all tested samples except wheat obtained from El-Minia and Sohag governorates. From this genus 5 species were identified and the most common species was *P. chrysogenum* which was recorded in one sample of each three grains (No. 6, 19, 45 of corn, wheat and sorghum, respectively) with relatively low counts. According to Abdel-Kader *et al.* (1979) 21 species of *Penicillium* were isolated from Egyptian barley grain of which *P. citrinum* and *P. chrysogenum* were the most common. Moreover, El-Kady *et al.* (1982) isolated 17 species of *Penicillium* from barley, sorghum, corn and wheat and the most common species were *P. capsulatum, P. chrysogenum* and *P. corylophilum*. The remaining species were rare as listed in Table 2.

Fusarium occupied the third place represented by two species namely *F. oxysporum* and *F. moniliformi*. It was completely absent in tested samples of sorghum. These two species were also recovered in Egyptian seeds and grains by Moubasher *et al.* (1972) and El-Kady (1982).

Rhizopus was not found in wheat samples collected from El-Minia and Assiut governorates and corn samples obtained from El-Minia. *Rhizopus* was the only genus isolated from samples No. 11 and 27 of sorghum collected from El-Minia and Assiut governorates, respectively.

Nine species namely *Cladosporium herbarum, Alternaria alternata, Curvularia lunata, Trichothecium roseum, Mucor racemosus, Phoma herbarum, Trichoderma viride, Myrothecium verrucaria* and *Scopulariopsis brevicaulis* were isolated sporadically.

2- Aflatoxins of cereal grains:

Thin layer chromatographic analysis revealed that aflatoxins (B_1, B_2, G_1 and G_2) were detected in the extracts of the different studied samples (Table 1).

Aflatoxins B_1 and B_2 were detected in 6 samples namely; one sample of wheat, three of corn and two of sorghum. While aflatoxins B_1, B_2, G_1 and G_2 were detected in the extract of

Table (2): Isolated genera and species and their counts and total fungal count in samples of the three studied grains.

Sample No.	Genera & species	Count (colonies)	Total fungal count	Sample No.	Genera & species	Count (colonies)	Total fungal count
1	*Aspergillus niger*	94	106	19	*A. flavus*	64	76
	A. fumigatus	12			*A. tamarii*	6	
2	*A. niger*	56	68		*P. chrysogenum*	4	
	A. terreus	12			*Phoma herbarum*	2	
3	*A. flavus*	96	112	20	*A. niger*	102	132
	A. fumigatus	14			*A. terreus*	16	
	Fusarium moniliformi	2			*P. citrinum*	12	
4	*A. flavus*	56	90		*Alternari alternata*	2	
	A. niger	22		21	*A. fumigatus*	70	126
	Fusarium oxysporum	12			*A. niger*	32	
5	*A. niger*	74	104		*R. stolonifer*	24	
	A. versicolor	22		22	*A. flavus*	162	192
	Curvularia lunata	8			*A. niger*	16	
6	*A. flavus*	365	416		*Cladosporium herbarum*	14	
	Trichothecium roseum	22		23	*A. flavus*	266	278
	Penicillium chrysogenum	28			*P. funiculosum*	12	
7	*A. niger*	70	112	24	*A. flavus*	46	76
	A. terreus	24			*A. nidulans*	16	
	P. fumiculosum	18			*F. oxysporum*	8	
8	*A. flavus*	78	100		*Alternaria alternata*	6	
	F. oxysporum	22		25	*A. flavus*	86	120
9	*A. flavus*	72	130		*A. niger*	24	
	A. terreus	44			*Trichothecium roseum*	10	
	A. candidus	6		26	*A. niger*	72	106
	P. funiculosum	4			*A. flavus*	34	
	F. oxysporum	4		27	*Rhizopus stolonifer*	36	36
10	*A. flavus*	82	112	28	*A. flavus*	142	166
	A. fumigatus	34			*P. cyclopium*	24	
	Trichothecium roseum	6		29	*A. niger*	90	90
11	*Rhizopus stolonifer*	44	44	30	*A. flavus*	56	76
12	*A. niger*	106	132		*A. ochraceus*	12	
	A. chevalieri	18			*Myrothecium verrucaria*	8	
	R. stolonifer	8		31	*A. fumigatus*	104	126
13	*A. flavus*	104	180		*R. stolonifer*	22	
	A. niger	72		32	*A. flavus*	64	106
	P. chrysogenum	4			*A. wentii*	42	
14	*A. fumigatus*	78	96	33	*A. flavus*	182	264
	Cladosporium herbarum	18			*A. terreus*	60	
15	*A. niger*	110	170		*F. oxysporum*	22	
	A. fumigatus	42		34	*A. niger*	106	106
	Curvularia lunata	18		35	*A. niger*	118	152
16	*A. flavus*	50	78		*A. terreus*	34	
	A. niger	28		36	*A. fumigatus*	98	146
17	*A. fumigatus*	74	90		*F. oxysporum*	32	
	F. oxysporum	16			*C. herbarum*	16	
18	*A. niger*	126	148	37	*A. niger*	118	134
	Mucor racemosus	22			*R. stolonifer*	16	

Table (2): Cont.

Sample No.	Genera & species	Count (colonies)	Total fungal count	Sample No.	Genera & species	Count (colonies)	Total fungal count
38	*A. flavus*	112	148	43	*A. flavus*	116	180
	P. funiculosum	36			*A. niger*	64	
39	*A. niger*	144	162	44	*A. flavus*	76	130
	A. terreus	18			*A. fumigatus*	48	
40	*A. flavus*	86	132		*Trichoderma viride*	6	
	F. oxysporum	42		45	*A. niger*	84	154
	Trichoderma viride	4			*A. fumigatus*	66	
41	*A. niger*	112	116		*P. chrysogenum*	4	
	Scopulariopsis brevicaulis	4					
42	*A. flavus*	72	92				
	A. niger	16					
	Curvularia lunata	4					

Table (3): Isolated genera of cereal grains collected from El-Minia, Assiut and Sohag governorates.

Cereal \ Governorate	El-Minia	Assiut	Sohag
Wheat	*Aspergillus* *Fusarium* *Curvularia*	*Aspergillus* *Penicillium* *Fusarium* *Mucor* *Phoma* *Alternaria*	*Aspergillus* *Fusarium* *Rhizopus*
Corn	*Aspergillus* *Penicillium* *Fusarium* *Trichothecium*	*Aspergillus* *Penicillium* *Fusarium* *Rhizopus* *Alternaria* *Trichothecium* *Cladosporium*	*Aspergillus* *Penicillium* *Fusarium* *Rhizopus* *Trichoderma* *Cladosporium*
Sorghum	*Aspergillus* *Penicillium* *Rhizopus* *Cladosporium* *Curvularia*	*Aspergillus* *Penicillium* *Rhizopus* *Myrothecium*	*Aspergillus* *Penicillium* *Rhizopus* *Trichoderma* *Curvularia* *Scopulariopsis*

one sample only of corn collected from Assiut Governorate, where these samples were naturally contaminated with *A. flavus*. Among the cereals, corn was one of the richest substrate for aflatoxins elaboration (Payne, 1983; Marsh and Payne, 1984; Kelly, 1987; Sinha, 1990; Widstrom *et al*., 1994). Other cereal grains viz. wheat, barley, oat and sorghum were not very susceptible to extensive preharvest aflatoxin contamination (Anderson *et al*., 1975; Stoloff 1977; McMillian *et al*., 1983; Bekhet *et al.* 1987).

In conclusion, mycological analysis of collected wheat, corn and sorghum samples revealed that these cereals were contaminated with many fungi members especially *Aspergillus* and *Penicillium*. Likewise, *A. flavus* was encounted in 40-60% of tested samples, where the most strains of this fungus are well known aflatoxins-producing, and there are strong correlation between high moisture content and contamination with filamentous fungi, since several of these fungi could produce other harmful mycotoxins to human health. To minimize health hazards from fungi precaution must be adopted during harvest, transportation, storage and processing to avoid contamination and serious deterioration of the grains by filamentous fungi.

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الفلورا الفطرية والافلاتوكسينات الملوثة لثلاثة أنواع من الحبوب

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تـناول هذا البحث دراسة الفطريات الملوثة لعدد وقدره ٤٥ عينة جمعت من ثلاث محافظات هى المنيا ، اسيوط ، سوهاج لثلاثة أنواع من الحبوب (القمح – الذرة الشامية – الذرة الرفيعة) باستخدام طريقة التخفيف على الأطباق على بيئة شابكس المتصلبة عند درجة ٢٨°م. تم عزل ٢٨ نوعاً من الفطريات تنتمى إلى ١٣ جنس . إحتوت حبوب القمح على أكثر الأنواع المعزولة (١٦ نـوع تنتمى إلى ٨ أجناس) يليها الذرة الرفيعة (١٥ نوع تنتمى إلى ٨ أجناس) ثم الذرة الشامية (١٥ نـوع تنـتمى إلى ٨ أجناس) . وقد أظهرت النتائج أن أكثر الأجناس إنتشاراً الاسبرجلس والبنيسيليوم والفيوزاريم على القمح والذرة الشامية أما فى حالة عينات الذرة الرفيعة فكانت أجناس الاسـبرجلس والبنيسـيليوم والرايزوبس هى الأكثر تواجداً . ومن جهة أخرى إستبـان من نتائج التحلـيل الكروماتوجـرافى باستخدام الـ Thin layer chromatography تلوث ست عينات بالافلاتوكسينـات B_1 , B_2 بواقع عينة ، ثلاث عينات ، عينتين فى كل من القمح والذرة الشامية والـذرة الرفـيعة على التوالى بالإضافة إلى تلوث عينة واحدة من الذرة الشامية بالأفلاتوكسينات B_1, B_2, G_1, G_2 .