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Mycoflora and Mycotoxins of Cereal Grains in Delta, Egypt

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Five cereal grains (wheat, barley, rice, maize and sorghum) were collected from three Egyptian provinces known to be grain producers (Daqahlia, Gharbia and Kafer el-Sheikh). Two species of *Alternaria* (*A. raphani* and *A. tenusinae*); two species of *Aspergillus* (*A. flavus* and *A. niger*); one species of *Cunninghamella* (*C. elegans*); one *Dreschslera* species (*D. myaki*); three *Fusarium* species (*F. graminearum*, *F. moniliform* and *F. solani*); one *Rhizopus* species (*R. stolonifer*) and two species of *Penicillium* (*P. digitatum* and *P. notatum*) were isolated from the grains. The densities of these fungi and their frequencies of occurrence have been investigated. All the fungal isolates were tested for the production of toxic metabolites in culture media and the percentages of toxigenic isolates were calculated. The biological assay of the toxigenic fungal isolates showed significant variations in toxigenic activity. Thin layer chromatography revealed that the most active isolate produces moniliformin in culture media. The effect of culture conditions on the production of moniliformin was studied.

KEYWORDS: ***Aspergillus* species, Cereal grains, Mycoflora, Mycotoxin**

Mycotoxins are secondary metabolites of mold fungi identified in many agricultural products screened for toxigenic molds (Van Egmond, 1981; Clevsrton and Ljunggren, 1985; Aziz, 1987). Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and dermatitic to a wide range of organisms and to cause hepatic carcinoma in man (Wary, 1981; Refai, 1988).

Seedborne fungi are the principal producers of mycotoxins. Contaminated agricultural products; cereals and oil seeds in particular are the main sources of mycotoxins in the animal and human food chains. Clearly, mycotoxins are the result of fungal growth on crops in the field and in storage (Scott *et al.*, 1985). Several researchers have examind the mycoflora of cereals (Adisa, 1994; Mediavilla *et al.*, 1996; Ackermann, 1998; Abramson *et al.*, 1999; Almeida *et al.*, 2002; Czerwieck *et al.*, 2002).

A special attention has been given to the fungal mycoflora of cereal grains and their toxigenic capacity in Egypt (El-Kady *et al.*, 1982; Megalla *et al.*, 1985; Abdel-Hafez *et al.*, 1990; El-Shayeb *et al.*, 1992) and in some arabic countries (Abdel-Hafez, 1984).

One of the best methods to determine the nature and extent of the fungal colonization is the direct plating technique (Christensen and Kaufmann, 1969; Flannigan, 1977; Petters *et al.*, 1988). This method is very useful for the quality evaluation of bulk grain (Trojanowska, 1991). The genera of fungi of greatest importance in causing poisoning in humans and domesticated animals are *Fusarium*, *Aspergillus*, and *Penicillium* (Christensen, 1987; Davis and Diener, 1987; Javis and Williams, 1987).

The present study is a comparative study aiming to survey the grain borne mycoflora of 5 cereal crops (wheat, barely, rice, maize and sorghum) which grown in three provinces (Daqahlia, Gharbia, Kafer el-Sheikh) in the Nile-Delta of Egypt. Moreover, the isolated fungi were screened for the ability to produce mycotoxins. The most active toxigenic fungal isolate has been chosen for further mycotoxin studies.

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Materials and Methods

Collection of samples. Five cereal grains (wheat, barley, rice, maize and sorghum) were collected from three Egyptian provinces known to be grains producers (Daqahlia, Gharbia and Kafer el-Sheikh).

To be representative, samples were collected from 5 different sites inside each province. From a 50 kg sub-lot of cereal, 20 incremental samples were taken to obtain 2.0 kg of aggregate sample. Then 0.5 kg of this sample was taken. Samples of each province were mixed to obtain a 2.5 kg representative sample (Czerwiecki *et al.*, 2002).

Mycological methods. Three different culture media were used to isolate the fungi: (1) Czapek-Dox agar, (2) Malt extract-glucose agar and (3) Potato-dextrose agar (PDA). The antibiotic, chloramphenicol, was added at a concentration of 0.5 mg/*ml* medium before autoclaving to suppress bacterial growth. Fungi in 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 surfacesterilized 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).

Screening of isolated fungi for toxigenicity. The isolated fungi were screened for their ability to produce toxic metabolites in liquid cultures as described by Aziz *et al.* (1998). Erlenmeyer flasks (250 *ml*) containing 50 *ml* of yeast extract sucrose medium (2% yeast extract and 20% sucrose) were inoculated with about 10^6 spores per *ml* and incubated with shaking at 28°C for 7 days. After incubation, the fungal cultures were mixed with 120 *ml* of chloroform : water (100 : 10, v/v) and were shaken vigorously by rotary shaker (200 rpm) overnight. The extracts were sequentially filtered through anhydrous sodium sulfate. The chloroform extracts were collected and then dried in a water bath at 50°C until dryness then dissolved in 1 *ml* chloroform and stored frozen until use.

The resulting extracts were tested for toxicity on two genera of bacteria (*Escherchia coli* and *Bacillus subtilis*) which were kindly provided by Bacteriology Lab., Botany Dept., Faculty of Science, Mansoura University. The bacterial isolates were cultivated on nutrient agar plates. Two hundred *ml* of each fungal extract were added to the plates in 0.5 *ml* diameter pore. Three plates were used as replicates for each fungal extract. The plates were incubated at 37±2°C for 24 hr and then the diameters of the clear zones were measured.

Toxin identification and quantification. Only culture extracts of the most active *Fusarium* isolate (Fm5-bd) was subjected to separation and identification procedures. TLC (thin layer chromatography) was performed following the method described by Tseng *et al.*, 1995. The concentrated sample extract and standards were spotted on TLC plates (aluminium silica gel 60, Art. 5553, Merk, Germany) and developed in toluene-ethyl acetate-88% formic acid (6 : 2 : 1) until the solvent front was 1 cm from the top of the plate. The plates were sprayed with 20% $AlCl_3$ in MeOH and air-dried. The dried plates were heated at 110°C for 10 min. The toxins were identified as fluorescent spots at the same height (Rf value) as the standard spots under an ultraviolet (365 nm) lamp. The concentration of the mycotoxin was measured with a flurodensitometer CD-60 at an excitation wavelength of 365 nm and emission wavelength of 443 nm (Aziz *et al.*, 1998). The amount of mycotoxin extraction given is the mean of three replicates on one TLC plate.

The effect of culture conditions on the mycotoxin production. Two media, Czapek Dox and PDA were prepared, inoculated and incubated. Czapek Dox medium was used for different periods, at different temperatures or adjusted at different pHs. The carbon or the nitrogen source in Czapek Dox medium was substituted using equimolars and equipercents of different sources. Each factor was studied separately. In all cases, the dry weights and toxin concentrations were determined.

Results and Discussion

Fungal mycoflora. The percentages of total counts (% TC) and the frequencies of isolation (F%) of the isolated fungi are summarized in Table 1. In general, a total of 12 species of fungi belonging to 7 genera were isolated and identified from five cereal grains; wheat, barley, rice, sorghum and maize. Two species of *Alternaria* (*A. raphani* and *A. tenusinae*); two species of *Aspergillus* (*A. flavus* and *A. niger*); one species of *Cunninghamella* (*C. elegans*); one *Dreschslera* species (*D. myaki*); three *Fusarium* species (*F. graminearum*, *F. moniliforme* and *F. solani*); one *Rhizopus* species (*R. stolonifer*) and two species of *Penicillium* (*P. digitatum* and *P. notatum*) were isolated.

In case of wheat grains, *Aspergilllus* was the predominant and most frequent fungus in the three provinces. The percentages of total count of *Aspergillus* were 47.2, 46.6 and 57.9% in Daqahlia, Gharbia and Kafer el-Sheikh respectively. Its frequencies of isolation were 80%, 90% and 90% in Daqahlia, Gharbia and Kafer el-Sheikh respectively. *Rhizopus stolonifer* followed *Aspergillus* in domination of wheat grains in both Daqahlia (21.3%) and Gharbia (28%) but *Alternaria* followed it in domination (32.2%) in Kafer el-Sheikh. *Drescheslera*, *Fusarium* and *Penicillium* were less in dominations and frequencies. These results comes in accordance with that of Kacaniova and Tancinova (2001) who found that out of 24 genera isolated from feeding wheat, *Aspergillus*, *Acremonium*, *Alternaria*, *Aureobasidium*, *Cladosporium*, *Penicillium*, *Rhizopus* and *Ulocladium* were the most frequently isolated. The results in this study are also in agreement with that of El-Kady *et al.* (1982).

In case of barley grains, the most common fungus isolated was *Rhizopus stolonifer* in Daqahlia and Gharbia; recording percentages of total count 47.0% and 46.6% respectively. Whereas, *Aspergillus* was the most common (53.5%) and most frequent (100%) on barley grains from Kafer el-Sheikh. *Fusarium* was the second fungus in domination and frequency of isolation on barley grains collected from the three provinces. In all cases *Alternaria*, *Cunninghamella* and *Penicillium* were less common on barley grains. *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* were found to be the predominant mycoflora on barley grains collected from several localities in the world (Abramson *et al.*, 1999; El-Kady *et al.*, 1982).

Table 1. Percentages and frequencies of fungi isolated from cereal grains collected from three provinces in Egypt

Cereal	wheat		barley		rice		sorghum		maize	
	[a]%T.C.	[b]F%	%T.C.	F%	%T.C.	F%	%T.C.	F%	%T.C.	F%
Fungi					**Daqahlia**					
Alternaria	13.0	30	6.8	30	12.4	40	73.1	90	–	–
A. raphani	8.3	20	0.8	10	–	–	50	70	–	–
A. tenusinae	4.6	10	6.0	20	12.4	40	23.1	60	–	–
Aspergillus	47.2	80	17.9	90	64.0	80	7.7	30	14.3	70
A. flavus	13.0	70	9.7	80	41.6	80	5.8	30	9.9	60
A. niger	34.3	80	8.2	70	22.5	70	1.9	10	4.4	40
Cunninghamella elegans	–	–	–	–	–	–	5.8	20	–	–
Drechslera myaki	4.6	30	–	–	23.6	–	–	–	–	–
Fusarium	0.93	10	26.9	60	-	80	3.8	20	25.3	90
F. graminearum	–	–	6.0	10	23.6	–	–	–	–	–
F. moniliforme	0.93	10	10.5	20	–	80	3.8	20	25.3	90
F. solani	–	–	10.5	30	–	–	–	–	–	–
Rhizopus stolonifer	21.3	80	47.0	100	–	–	–	–	–	–
Penicillium	13.0	70	1.5	10	–	–	9.6	20	60.4	100
P. digitatum	0.93	10	–	–	–	–	6.6	20	–	–
P. notatum	12.01	70	1.5	10	–	–	1.9	10	60.4	100
					Gharbia					
Alternaria	16.1	40	6.1	30	15.0	90	60.6	80	–	–
A. raphani	–	–	–	–	–	–	22.3	60	–	–
A. tenusinae	16.1	40	60.1	30	15.0	90	38.3	50	–	–
Aspergillus	46.6	90	25	90	69.3	100	27.7	50	18.9	70
A. flavus	11.9	70	8.8	70	25.5	90	16	50	11.5	50
A. niger	34.7	90	16.2	80	43.8	90	11.7	10	7.4	30
Cunninghamella elegans	–	–	5.4	20	–	–	–	–	–	–
Drechslera myaki	–	–	–	–	–	–	5.3	20	–	–
Fusarium	2.5	20	15.5	30	–	–	5.3	20	23.0	10
F. graminearum	–	–	–	–	–	–	–	–	–	–
F. moniliforme	2.5	20	15.5	30	–	–	5.3	20	23.0	10
F. solani	–	–	–	–	–	–	–	–	–	–
Rhizopus stolonifer	28	80	4.6.6	100	6.5	10	1.1	10	–	–
Penicillium	6.8	60	1.4	10	9.2	70	–	–	58.2	90
P. digitatum	–	–	–	–	–	–	–	–	–	–
P. notatom	6.8	60	1.4	10	9.2	70	–	–	58.2	90
					Kafer el-sheikh					
Alternaria	32.2	80	0.7	10	17.5	90	8.33	40	–	–
A. raphani	21.7	60	0.7	10	10.2	60	8.33	40	–	–
A. tenusinae	10.5	40	–	–	7.3	40	–	–	–	–
Aspergillus	57.9	90	53.5	100	53.1	100	8.33	30	13.8	60
A flavus	57.9	90	40.1	90	47.5	100	6.2	30	8.5	50
A. niger	–	–	13.4	30	6.6	10	2.1	10	5.3	40
Cunninghamella elegans	–	–	–	–	–	–	4.2	20	–	–
Drechslera myaki	–	–	–	–	–	–	64.6	80	–	–
Fusarium	7.2	30	45.8	90	28.8	90	8.3	30	24.5	90
F. graminearum	–	–	–	–	–	–	–	–	–	–
F. moniliforme	7.2	30	45.8	90	26.6	90	8.3	30	24.5	90
F. solani	–	–	–	–	2.2	10	–	–	–	–
Rhizopus stolonifer	–	–	–	–	–	–	–	–	–	–
Penicillium	2.6	10	–	–	0.6	10	6.3	20	61.7	100
P. digitatum	–	–	–	–	–	–	–	–	–	–
P. notatum	2.6	10	–	–	0.6	10	6.3	20	61.7	100

[a] $\%T.C. = \frac{\text{no. of isolates}}{\text{total no. of fungal isolates}} \times 100$

[b] $\%F = \frac{\text{no. of times of isolation}}{\text{no. of samples}} \times 100$

[c] (–): no fungal species detected.

In case of rice grains, *Aspergillus* was the predominant and most frequent in all cases. Its percentages of total count were 64.0, 69.3 and 53.1 and its frequencies of isolation were 80%, 100% and 100% for Daqahlia, Gharbia and Kafer el-Sheikh respectively. *Fusarium* was the second in domination and frequency on rice grains collected from both Daqahlia and Kafer el-Skeikh while it was not recorded at all on rice grains collected from Gharbia. *Alternaria* was less common while *Penicillium* was rare in all cases. Mycoflora is a major cause of deterioration in rough rice and results in a loss in quality, economic value and perhaps in quantity. Some of the most common fungal genera found in rice are *Bipolaris*, *Pyricularia*, *Nigrospora*, *Fusarium*, *Alternaria*, *Cladosporium*, *Trichothecium* and *Epicoccum* (Hernàndez *et al.*, 1968). Furthermore, they have found that the deterioration caused by micro-organisms in rice after storage is largely caused by species of the genera *Aspergillus* and *Penicillium*.

Alternaria was predominant on sorghum grains from Daqahlia and Gharbia (73.1% and 60.6%) respectively while *Dreschslera myaki* was predominant on sorghum grains from Kafer el-Sheikh (64.6%). *Aspergillus* followed *Alternaria* in domination of sorghum grains from Gharbia (27.7%). *Cunninghamella*, *Fusarium* and *Penicillium* were rare on sorghum grains in all cases. Williams and Rao (1981) listed the species most frequently isolated in studies of mycoflora associated with sorghum grain. Subsequent studies list much the same spectra of fungal species. Prominent among these are species of *Alternaria*, *Drechslera*, *Cladosporium*, and *Olpitrichum*. A pictorial guide of fungi commonly associated with sorghum grain has been recently published (Navi *et al.*, 1999).

Penicillium predominated maize grains in all cases. Its percentages of total count were 60.4, 58.2 and 61.7 for Daqahlia, Gharbia and Kafer el-Sheikh, respectively. It is followed by *Fusarium* and *Aspergillus* in domination and frequency on maize grains in all cases. Almeida *et al.* (2000) found that the fungal population of freshly harvested corn grains collected from three regions in Brazil composed mainly of *Fusarium*, *Penicillium*, and *Aspergillus* species and among the genera *Fusarium* and *Aspergillus*, the most frequently isolated species were *F. moniliforme* and *A. flavus*, respectively. Moreover, it has been reported that the most serious maize contamination problems are due to *Aspergillus flavus* and *Fusarium moniliforme* (Marasas *et al.*, 1981 and Marin *et al.*, 1998).

Mycotoxin production in culture media. All the fungal isolates were tested for the production of toxic metabolites in culture media and the percentages of toxigenic isolates were represented in Table 2. The 71 fungal isolates which showed positive toxicity were given code numbers and names then represented in Table 3. The isolates names composed of two parts, the first part denotes the genus and species and the second part denotes the grain and the province. For example, "-wd" means wheat and Daqahlia. Isolate1 is *Alternaria tenuisinae* and isolates 2-51 are *Aspergillus flavus*. Isolate52 is *Fusarium graminearum*, isolates 53-70 are *Fusarium moniliforme*

Table 2. Percentages (%) of toxic isolates of fungi isolated from cereal grains collected from three provinces in Egypt

Province	Daqahlia					Ghrbia					Kafer el-sheikh				
Cereal	wheat	barley	rice	sorghum	maize	wheat	barley	rice	sorghum	maize	wheat	barley	rice	sorghum	Maize
fungi															
Alternaria	7.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A. raphani	–[a]	–	–	–	–	–	–	0.9	–	–	–	–	–	–	–
A. tenusinae	50	–	5.3	–	7.7	3.6	–	2.6	–	–	34.1	–	–	–	–
Aspergillus	9.8	4.2	8.1	–	12.5	14.4	–	–	–	–	34.1	2.6	4.3	–	7.7
A. flavus	35.7	7.7	–	–	–	–	–	–	–	–	–	3.5	4.8	–	12.5
A. niger	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cunninghamella elegans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dreschslera myaki	–	–	–	–	8.7		–	–	–	–	–	–	–	–	–
Fusarium	–	11.1	–	–	–	–	43.5	–	–	7.1	–	3.1	2.0	–	34.8
F. graminearum	–	12.5	–	–	8.7	–	–	–	–	–	–	–	–	–	–
F. moniliforme	–	21.4	–	–	–	–	43.5	–	–	7.1	–	3.1	–	–	34.8
F. solani	–	–	–	–	–	–	–	–	–	–	–	–	25	–	–
Rhizopus stolonifer	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Penicilium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. digitatum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. notatum	–	–	–	–		–	–	–	–	–	–	–	–	–	–

[a] –: no toxic isolates found.

Table 3. Biological assay of the whole culture extracts of the toxigenic fungal isolates (data are the means of three replicates)

Fungal isolate		Clear zone diam. (Cm.)		Fungal isolate		Clear zone diam. (Cm.)	
No.	name	*B. subtilis*	*E. coli*	No.	name	*B. subtilis*	*E. coli*
1	Altten-wd	2.5[a]	1.6[b]	37	Af36-wk	2.6	1.6
2	Af1-bk	2.3	1.6	38	Af37-wk	2.6	1.6
3	Af2-bk	2.7	2.1	39	Af38-wk	2.4	1.2
4	Af3-bd	2.4	2.0	40	Af39-wk	1.7	1.1
5	Af4-mk	2.2	1.8	41	Af40-wk	1.7	0.7
6	Af5-md	2.1	1.7	42	Af41-wk	1.6	1.6
7	Af6-rk	2.5	1.7	43	Af42-wk	1.6	1.2
8	Af7-rk	2.3	1.6	44	Af43-wk	1.5	1.1
9	Af8-rk	2.5	1.7	45	Af44-wd	2.6	1.8
10	Af9-rk	2.6	1.6	46	Af45-wd	2.6	1.8
11	Af10-rd	2.6	1.7	47	Af46-wd	1.7	1.2
12	Af11-rd	2.5	2.1	48	Af47-wd	1.9	1.4
13	Af12-rd	2.5	1.9	49	Af48-wd	2.8	1.5
14	Af13-rg	2.4	1.6	50	Af49-wg	2.7	1.6
15	Af14-wk	2.1	1.3	51	Af50-wg	1.9	1.2
16	Af15-wk	2.0	1.2	52	Fg-bd	2.6	1.7
17	Af16-wk	1.9	1.3	53	Fm1-bd	2.5	1.8
18	Af17-wk	1.9	1.1	54	**Fm2-bk**	**3.6*****	**2.4*****
19	Af18-wk	1.8	1.1	55	Fm3-bk	2.9	1.9
20	Af19-wk	2.6	1.9	56	Fm4-bd	1.7	1.4
21	Af20-wk	2.7	1.6	57	**Fm5-bd**	**4.2*****	**2.8*****
22	Af21-wk	2.4	1.4	58	Fm6-bd	2.8	1.7
23	Af22-wk	2.6	1.4	59	Fm7-bg	1.6	1.1
24	Af23-wk	2.7	1.6	60	**Fm8mk**	**3.2****	1.2
25	Af24-wk	1.8	1.2	61	Fm9-mk	2.6	1.1
26	Af25-wk	1.9	1.2	62	Fm10-mk	2.6	1.3
27	Af26-wk	1.8	1.1	63	Fm11-mk	2.5	1.1
28	Af27-wk	1.8	1.3	64	Fm12-mk	2.7	1.2
29	Af28-wk	1.8	1.4	65	**Fm13-mk**	**3.2****	1.7
30	Af29-wk	2.2	1.4	66	**Fm14-mk**	**3.2****	1.2
31	Af30-wk	2.6	1.6	67	**Fm15-mk**	**3.11****	1.3
32	Af31-wk	2.6	1.5	68	Fm16-md	2.6	1.5
33	Af32-wk	1.7	1.2	69	Fm17-md	2.5	1.4
34	Af33-wk	1.6	1.6	70	Fm18-mg	2.5	1.2
35	Af34-wk	1.2	0.7	71	Fs-rk	1.9	1.3
36	Af35-wk	2.3	1.2				

[a]$LSD_{0.05}$ for *B. subtilis*=0.18.
[b]$LSD_{0.05}$ for *E. coli*=0.12.
***=Significantly very heigh at *P* value 0.01.
**=Significantly high at *P* vaue 0.05.

and isolate 71 is *Fusarium solani*.

The results in Table 2 showed that the toxigenic isolates related only to *Alternaria*, *Aspergillus*, and *Fusarium*. 7.1% of *Alternaria* isolates from wheat grains in Dakahlia were found to be toxigenic. No other *Alternaria* isolates were found to be toxigenic. The production of mycotoxins by *Alternaria* isolated from Chinese wheat kernels was investigated by Li *et al.* (2001).

None of the fungal isolates recorded on sorghum grains collected from any province showed toxigenicity.

Toxigenic *Aspergillus* isolates were found on all tested grains (except barley and maize from Gharbia). All the toxigenic isolates of *Aspergillus* were related to *A. flavus*. The highest persentages of toxigenic *A. flavus* isolates were found on wheat grains collected from Daqahlia (35.7) and Kafer el-Sheikh (34.1). Adisa (1994) isolated two toxigenic *Aspergillus* species associated with wheat and maize grains in Nigeria.

No toxigenic *Fusarium* isolates were found on wheat grains. While toxigenic isolates were found on barley and maize grains collected from the three provinces and only rice grains of Kafer el-Shiekh. In earlier studies on isolates of *Fusarium* species from Canadian cereal grains, Frarber *et al.* (1988) found that cultures of both *F. moniliforme* and *F. subglutinans* produced mycotoxins, whereas *F. graminearum* from western Canadian wheat did not.

The biological assay of the 71 toxigenic fungal isolates recorded in this study (Table 3), showed a significant vari-

ations in toxigenic activity. Some *Fusarium moniliforme* isolates (Fm2-bk and Fm-bd) which isolated from barley grains showed significantly very high activity. Similarly, the isolates Fm8-mk, Fm13-mk, Fm14-mk and Fm15-mk which isolated from maize grains showed a high significant activity.

Mycotoxin identification. Only culture extract from the most active *Fusarium moniliforme* isolate (Fm5-bd) was subjected to thin layer chromatography. The toxic spots were found to be moniliformin. The production of moniliformin mycotoxin by *Fusarrium moniliforme* has been reported before (Farber *et al.*, 1988; Fotso *et al.*, 2002).

The effect of culture conditions on mycotoxin production. Czpeck Dox medium was found to be the best medium in the production of moniliformin. The concentration of moniliformin mycotoxin increases with the increase of incubation period up to 15 days then declines (Fig. 1). Similarly the fungus dry weight increases with time until 18 days then remain constant. The optimum temperature for moniliformin production was 28°C and the optimum pH was 7 (Fig. 3). The best carbon source for moniliformin production was glucose followed by maltose (Fig. 4) and the best nitrogen source was peptone followed by yeast extract and beef extract (Fig. 5). Lillehoj and Elling (1983) found that laboratory studies in cultures and controlled environments can provide preliminary

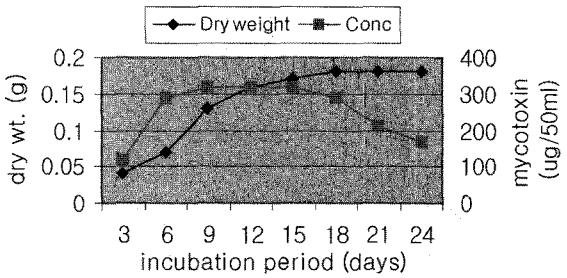

Fig. 1. The effect of incubation period on dry weight and mycotoxin concentration.

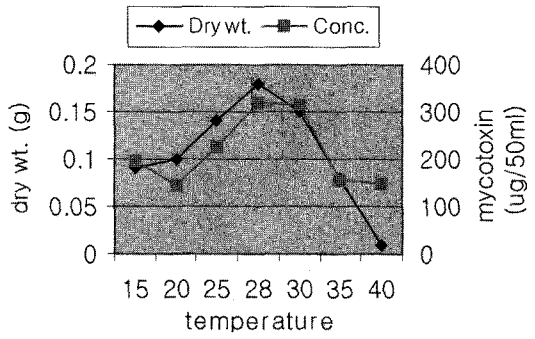

Fig. 2. The effect of temperature on dry weight and mycotoxin concentration.

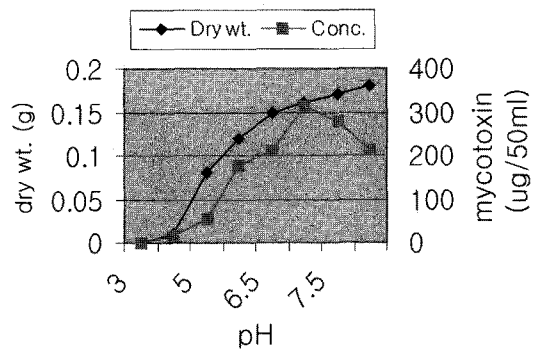

Fig. 3. The effect of pH on dry weight and mycotoxin concentration.

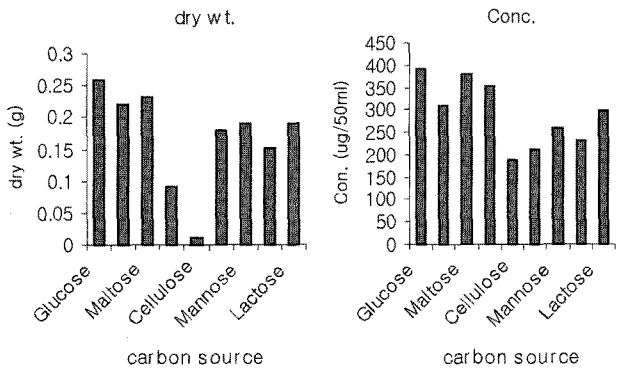

Fig. 4. The effect of carbon source on mycelial dry weight and mycotoxin production.

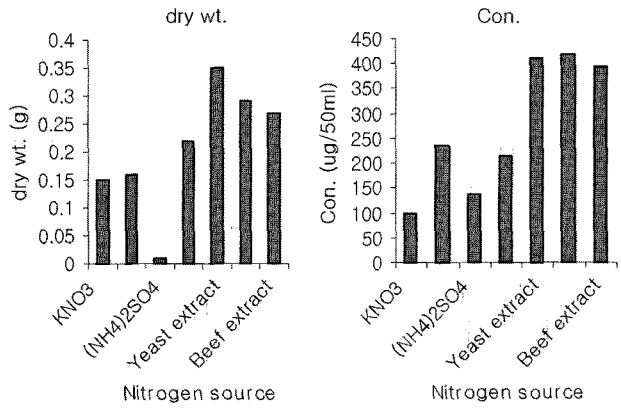

Fig. 5. The effect of nitrogen source on mycelial dry weight and mycotoxin production.

information to account for mycotoxin contamination of cereals.

In conclusion, the mycoflora diversity on freshly harvested Egyptian cereal grains is relatively low, only 12 species of fungi belonging to 7 genera were isolated. The toxigenic isolates related only to *Alternaria*, *Aspergillus* and *Fusarium*. None of the fungal isolates recorded on sorghum grains collected from any province showed toxigenicity. The most toxic isolate Fm2-bk produces moniliformin in the culture medium.

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