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Incidence of mycobiota and toxigenic fusaria in corn grain samples from Sohag, Egypt

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Abstract: A total of 41 species and one variety related to 11 genera of filamentous fungi were isolated from 80 corn grain samples. The total fungal units emerged from non-surface disinfected (NSD) corn grains were much higher than those emerged from surface disinfected (SD) corn grains. However, the number of isolated species in case of SD corn grains exceeded those in case of NSD corn grains. *Aspergillus* and *Penicillium* were the most predominant genera. *Fusarium* was recovered as the third most common genus of which *F. verticillioide* was the most prevalent species. Thin layer chromatography (TLC) showed that of all corn samples tested only one was contaminated by T-2 toxin while the other 79 samples were completely free of any detectable levels of mycotoxins. Methanol extracts of *Fusarium* isolates grown on corn medium showed that 59 out of the 90 *Fusarium* extracts contained at least one of the following *Fusarium* toxins: fumonisins B_1 and B_1 & B_2, zearalenone and deoxynivalenol. Most of *F. verticillioides* isolates (44 out of 76) were fumonisins producers. The HPLC analysis of crude extracts confirmed the results achieved by TLC plates. The highest yield of fumonisin B_1 (2015 µg/kg corn medium) was obtained from an isolate of *Fusarium verticillioides*.

Key words: Corn, *Fusarium verticillioides*, fumonisins B_1 & B_2, zearalenone, deoxynivalenol.

Introduction

Corn (*Zea mays* L.) is the most widely grown cereal crop and one of the four basic food staples consumed as food or feed in the world as well as in Egypt (Anderson *et al.* 2004 and Naqvi *et al.* 2011). It is also at the forefront of a new green revolution in which plants have a number of high value industrial applications to produce valuable molecules as recombinant pharmaceuticals, industrial enzymes, nutrients, chemical precursors and fuels (Naqvi *et al*. 2011). A variety of toxigenic fungi, including *Aspergillus*, *Penicillium* and *Fusarium* are frequently reported as the most dominant fungal genera isolated from corn (Chulze *et al*. 1996, 1999). *Fusarium* could colonize or infect corn grains during pre- and post-harvest periods (Abdel-Mallek *et al*. 1993, Chulze *et al*. 1996, 1999 and Fandohan *et al*. 2005). *Fusarium verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) Nirenberg were recorded as the highest prolific fumonisin producers (Fandohan *et al*. 2003). Fumonisins B_1, B_2 and B_3 were found in naturally-contaminated foods (Soriano *et al.* 2005). These compounds are structurally similar to sphingoid bases such as sphingosine, which is a component of the sphingolipid molecule, and are able to inhibit ceramide synthase. The free amino group appears to play a specific role in the biological activity of FB_1 (Bolger *et al*. 2001 and Soriano *et al*. 2005). The occurrence of fumomisins in corn had been assocaited with the outbreaks of equine leucoencephalomalacia (ELM), pulmonary edema (PE) and hepatocarcinoma (Harrison *et al*. 1990, Ross *et al*. 1990 and Thiel *et al*. 1992). Due to the little information on the natural occurrence of fumonisins in corn and fumonisin-producing *Fusarium* species in Egypt, this study was conducted.

Materials and Methods

Collection of corn samples

A total of 80 corn grain samples (one Kg each) were collected from different regions of Sohag Governorate. None of the samples was visibly damaged or moldy infected. Samples were transferred into the laboratory, kept in a sterilized polyethylene bag and stored in a refrigerator at 4°C till mycological and mycotoxin analysis.

Isolation and identification of fungi

Two conventional methods were used to isolate fungal species colonizing corn grain samples; direct-plate non-surface disinfected (NSD) method (Pitt *et al.* 1992) and surface disinfected (SD) method (Müllenborn *et al.* 2008). Dichloran-rose-Bengal-chloromphenicol (DRBC) agar medium was used for culturing (Tournas *et al*. 2006). Purified fungal isolates were identified morphologically based on macro- and microscopic characteristics using relevant references (Booth 1977, Nelson *et al.* 1983, Moubasher 1993, Leslie and Summerell 2006 and Moustafa 2006). All *Fusarium* isolates were cultured on potato sucrose agar medium (PSA) after single spore isolation (Booth 1977)

and were re-identified at Assuit University Mycological Center (AUMC).

Mycotoxin extraction of corn samples

Fifty grams of corn grains were ground and transferred into a 250 ml Erlenmeyer flask. One hundred ml of 96% methanol was added and the contents were shaken on a rotary shaker (200 rpm for 24 h) and filtered through filter paper (Whatman No.1). The residues were washed twice with the solvent (50 ml each) and the methanol extracts were combined, dried over anhydrous sodium sulphate, and evaporated to near dryness under vacuum. The residues were transferred quantitatively to a small vial with low amount of methanol and evaporated to near dryness (Sydenham *et al*. 1993).

Mycotoxins production by *Fusarium* spp.

A total of 70 different isolates of *Fusarium* obtained by NSD and other 20 isolates of the same genus obtained by SD methods were examined for the production of *Fusarium* toxins. Each isolate was cultivated on corn substrate medium. One hundred grams of corn were moistened overnight with 50 ml distilled water. Excess water was decanted and flasks containing corn were autoclaved at 121°C for 1 hr for 2 consecutive days. Three replicates of autoclaved corn cultures were inoculated with 5 ml of aqueous conidial suspensions (10^7 spores) of each isolate tested. Inoculated flasks were incubated at 25°C for two weeks in darkness. Flasks were shaken once every day to prevent corn adhering and to distribute the inoculum. At the end of the incubation period all flasks were transferred into a refrigerator for 2 more weeks. Control sample was not inoculated by conidial suspensions. Fifty grams of corn cultures of each isolate including the control were transferred from flasks and dried at room temperature, ground using an analytical electric mill and transferred into three 125-ml Erlenmeyer flasks containing 50 ml 96% methanol. Flasks were shaken on a rotatory shaker and filtered through filter paper (Whatman No.1). The residues were washed twice with the same solvent (25 ml each). The methanol extracts were combined, dried over anhydrous sodium sulphate and evaporated to near dryness under vacuum. The residues were transferred quantitatively to a small vial with 96% methanol and evaporated to near dryness.

Thin layer chromatographic analysis

For qualitative determination of mycotoxins other than fumonisins, TLC technique adopted by El-Kady and Moubasher (1982) was employed. For fumonisins detection, 10 µl crude extracts were spotted on TLC plate (Aluminium sheet, silica gel, Merck) along with 5 µg of FB_1 standard (purchased from SIGMA-ALDRICH). Spots were dried during application with a flow of warm air and developed using 96% methanol: water (80: 20, v/v). The developed plates were viewed after spraying with 50% concentrated sulphuric acid in methanol under short wave (254 nm) and long wave (365 nm) ultra-violet irradiation. Fumonisins B_1 and B_2 appear as slight bluish green spots under both short and long UV (Mubatanhema *et al*. 1999) at Rf 0.7 and 0.61 respectively.

HPLC analysis

Twenty-four crude extracts of *F. oxysporum* (1), *F. proliferatum* (6), *F. sterilihyphosum* (1), *F. subglutinans* (6) and *F. verticillioides* (10) were tested. Fumonisins were analyzed using Waters Binary Model 1525 HPLC equipped with Waters 2475 multi-wavelength flourescence detector and data workstation software Breeze 2. A phenomenex C18 (250 x 4.6 mm i.d), five um from Waters Corporation (USA), were used along with a mobile phase of Methanol: 0.1M NaH_2PO_4 (75:25, v/v) isocratic system adjusted to pH 3.35 by the addition of phosphoric acid and was filtered through membrane filter. The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at an excision of 335 nm and emission of 440 nm wavelength at the National Research Centre (NRC) in Cairo**.** The purified dry film residue of sample extracts were dissolved in 200 µl methanol of which 50 µl were transferred to a vial and 225 µl O-phithaladehyde reagent (OPA) were added. The mixed solutions of standard as well as sample extracts after derivatization were filtered through a 0.22 nm membrane filter and 10 µl were injected to HPLC apparatus within 1 min of adding OPA reagent. Fumonisin B_1 concentration was calculated from chromatographic peak areas (Horwitz and Latimer 2007).

Results and Discussion

A total of 41 species and only one species variety belonging to 11 fungal genera were isolated from the 80 corn samples collected from Sohag Governorate (Table 1). Although the total fungal units emerged from non-surface disinfected (NSD) corn grains (3224 CFU/ 2000 corn grains) was much higher than those emerged on surface disinfected (SD) corn grains (906 CFU), the number of isolated species in case of SD corn grains (37 species belong to 10 genera) exceeded those isolated in case of NSD

corn grains (33 species belong to 9 genera). Taxa isolated were assigned to three taxonomic classes. Hyphomycetes were represented by 37 species and one species variety representing 8 genera. Zygomycetes were represented by 3 species of 2 genera and Ascomycetes were represented by 1 species of *Neosartorya*. A view on the diversity at the generic level revealed that *Aspergillus* showed the greatest spectrum of species (19 species and one species variety) followed by *Penicillium* (9 species), *Fusarium* (5 species) and *Rhizopus* (2 species). The remaining 6 genera were represented only by one species for each. *Aspergillus* was the most common genus in both NSD and SD corn grains (97.5% and 90% of the samples, respectively). Of the genus 14 and 17 species and one variety (*A. flavus* var. *columnaris*) were isolated and identified from NSD and SD corn samples respectively. *Penicillium* was the second dominant on NSD and SD corn samples recovered from 77.5% and 75% of the samples accounting 15.5% and 23% of total fungi respectively. *Fusarium* occupied the third position (67.5% and 28.8% of the samples, comprising 8.7% and 6% of the total fungi respectively). It was represented by five species (*F. oxysporum, F. proliferatum, F. sterilihyphosum, F. subglutinans* and *F. verticillioides*) of which *F. verticillioides* was the most dominant. Adebajo *et al*. (1994), Kpodo *et al*. (2000), Schneweis *et al*. (2000) and Oudeelferink *et al*. (2001) recorded *Aspergillus* and *Penicillium* followed by *Mucor* and *Fusarium* spp. as the most dominant fungi isolated from corn and other seed and grain samples. Most of these species, except *F. sterilihyphosum* which was isolated for the first time in our mycology laboratory of Sohag, were previously isolated from various types of seeds and grains in Egypt (Abdel-Hafez *et al*. 1992, Abdel-Mallek *et al*. 1993 and Abdel-Sater *et al*. 1995). Kpodo *et al*. (2000) recorded *F. verticillioides* as the predominant *Fusarium* species in 15 corn samples from Ghana. In the present study, *F. proliferatum* and *F. subglutinans* were recorded in 6.25% & 2.5% and 5% and 7.5% of the NSD and SD grains respectively. Only one isolate of *F. oxysporum* was obtained from SD corn grains with a frequency of 1.25% of the samples. Fandohan *et al*. (2005) reported that *F. verticillioides* and *F. proliferatum* were the two *Fusarium* species commonly isolated from the corn samples during the 3-year survey in Benin. As shown in Table (1), *Rhizopus, Cladosporium, Mucor, Acremonium, Alternaria* and *Trichothecium* appeared with moderate and low frequencies ranging between 13.75% and 1.25% of the samples. The previous findings can be correlated with those obtained by Abdel-Mallek *et al*. (1993), Adebajo *et al*. (1994), El-Maghraby *et al*. (1994), Abdel-Sater *et al*. (1995) and El-Shanawany *et al*. (2005).

Natural occurrence of mycotoxins was determined in the 80 corn samples. Only one sample was found to be contaminated with T-2 toxin while the remaining 79 samples were free of any detectable amounts of this mycotoxin. T-2 toxin was widely produced by Egyptian *Fusarium* isolates (El-Maghraby 1984, El-Maghraby *et al*. 1992a & b, El-Maghraby 1996 and Soliman 1999).

Table 1: Colony-forming units (CFU/ 2000 corn grins), percentage fungal density (%D), and frequency (%F) of fungal genera and species recovered from 80 corn grain samples by non-surface disinfected (NSD) and surface-disinfected (SD) grain plate techniques.

Fungal genera & species	NSD		SD	
	% D	% F	% D	% F
Acremonium strictum W. Gams	0.155	3.75	0.22	1.25
Alternaria alternate (Fries) Keissler	0.496	8.75	0.22	2.5
Aspergillus	71.03	97.5	66.89	90
A. aculeatus Lizuka	9.71	70	0.44	2.5
A. awamorii Nakazawa	0.093	1.25	2.3	8.75
A. carbonarius (Bainier) Thom	1.58	15	0.99	6.25
A. clavatus Desmazieres	-	-	0.33	3.75
A. ficuum (Reich.) Hennings	-	-	4.64	21.25
A. flavus Link	12.34	72.5	9.8	53.75
A. flavus var. *columnaris* Raper & Fennell	7.54	62.5	3.42	27.5
A. fumigatus Fresenius	2.11	28.75	21.3	53.75
A. japonicus Saito	1.396	13.75	-	-
A. melleus Yukawa	0.96	5	-	-
A. niger van Tieghem	24.26	95	11.59	53.75
A. ochraceus Wilhelm	-	-	1.43	11.25

Table 1: Continued.

Fungal genera & species	NSD		SD	
	% D	% F	% D	% F
A. oryzae (Ahlburg) Cohn	1.78	15	1.99	7.5
A. parasiticus Speare	7.196	62.5	3.64	21.25
A. sulphureus (Fresenius) Thom & Church	-	-	0.55	2.5
A. sydowii (Bainier & Sartory) Thom & Church	0.22	3.75	1.88	3.75
A. tamarii Kita	1.77	23.75	1.2	7.5
A. terreus Thom	0.093	2.5	0.11	1.25
A. versicolor (Vuillemin) Tiraboschi	-	-	0.44	3.75
Cladosporium cladosporioides (Fresenius) de Vries	0.62	13.75	1.55	12.5
Exserohilum rostratum (Drechsler) Leonard & Suggs	-	-	0.11	1.25
Fusarium	8.68	67.5	6.1	28.75
F. oxysporum Schlechtendal	-	-	0.11	1.25
F. proliferatum (Matsush.) Nirenberg	0.47	6.25	0.66	2.5
F. sterilihyphosum Britz, Marasas & Wingfield	0.06	1.25	-	-
F. subglutinans (Wollenweber & Reinking) Nelson *et al.*	0.53	5	1.2	7.5
F. verticillioides (Sacc.) Nirenberg	7.63	60	4.08	21.25
Mucor racemosus Fresenius	0.155	3.75	0.33	3.75
Neosartorya fisherii (Wehmer) Malloch & Cain	-	-	0.66	5
Penicillium	15.48	77.5	22.96	75
P. brevicompactum Dierckx	1.74	18.75	3.64	20
P. chrysogenum Thom	1.33	15	1.2	6.25
P. citrinum Thom	1.09	16.25	0.11	1.25
P. corylophilum Dierckx	3.38	36.25	1.43	10
P. duclauxii Delacroix	3.94	33.75	9.7	45
P. funiculosum Thom	0.465	5	-	-
P. implicatum Biourge	1.12	12.5	1.77	11.25
P. jensenii Zaleski	0.84	10	0.99	6.25
P. pupurogenum Stoll	1.89	17.5	3.2	22.5
Rhizopus	2.7	27.5	1.66	7.5
Rhizopus oryzae Went & Prinsen-Geerligs	1.43	15	1.32	7.5
Rhizopus stolonifer (Ehrenberg) Vuillemin	1.30	13.75	0.33	2.5
Trichothecium roseum (Persoon: Fries) Link	0.34	6.25	-	-
Total CFUs	3224	-	906	-

TLC of the crude extracts of *Fusarium* isolates grown on corn medium showed that 59 out of 76 isolates of *F. verticillioides* had the ability to produce at least one of the following toxins: fumonisins (B_1 and B_2), zearalenone and deoxynivalenol. All tested isolates of *F. proliferatum*, *F. sterilihyphosum* and *F. oxysporum* were non-fumonisin producers and only one out of six isolates of *F. subglutinans* in this study had the ability to produce zearalenone (Table 2). Results obtained in this study were consistent with those reported earlier by other researches who found that many strains of *F. verticillioides* isolated from corn produced fumonisins (Ross *et al*. 1992, Soo *et al*. 1994 and Fadl Allah 1998). Other researchers reported that *F. proliferatum* produced FB_1 (Sanchis *et al*. 1995 and Castella *et al*. 1999). Also, Abbas *et al*. (1999) reported that 15 isolates of *F. proliferatum* isolated from 20 rough rice samples produced fumonisins, moniliformin and beauvericin.

Table 2: Mycotoxins detected by TLC analysis of different *Fusarium* isolates.

Fusarium species	No. of isolates tested		No. of mycotoxigenic isolates			
	NSD	SD	F(B_1)	F (B_1& B_2)	DON	ZEA
F. oxysporum	-	1	-	-	-	-
F. proliferatum	4	2	-	-	-	-
F. sterilihyphosum	1	-	-	-	-	-
F. subglutinans	1	5	-	-	-	1*
F. verticillioides	64*	12**	13*&3**	22*&7**	13*&5**	12*&1**

**Fusarium* isolates collected from non-surface-disinfected corn samples.
*** Fusarium* isolates collected from surface-disinfected corn samples.

Figures (1 & 2) showed the HPLC Chromatograms of fumonosin B1 standard and the crude extracts of fumonosin-producing and non-producing isolates. The HPLC analysis of 24 crude extracts of *F. oxysporum* (one isolate), *F. proliferatum* (6), *F. sterilihyphosum* (1), *F. subglutinans* (6) and *F. verticillioides* (10) proved that none of the selected *Fusarium* isolate extracts but *F. verticillioides* produced FB_1 ranged between 128 to 2015 µg//kg corn medium (Figs. 1 & 2). Many researches in Asian and European countries reported that strains of *F. verticillioides* produced FB_1, FB_2, and FB_3 (Lee *et al*. 1994, Visconti and Doko 1994 and Ghiasian *et al*. 2005). Also, a Taiwanese study reported that 66% of isolated *F. verticillioides* strains produced FB_1 and/or FB_2 (Tseng *et al*. 1995). Rheeder *et al.* (2002) reported that *F. verticillioides* and *F. proliferatum* had been recognized as the highest fumonisin producers although a few *F. verticillioides* isolates from Nepal (Nelson *et al*. 1991) do not produce any fumonisins. Several isolates of this species from Southeast Asia (Miller *et al*. 1993) produced fumonisins at low levels, whereas some isolates from Australia (Nelson *et al*. 1991) produced only trace quantities of FB_1.

In conclusion: although no detectable amounts of fumonisins were found in the corn grain samples tested, *F. verticillioides* was isolated as one of the most prevalent fungal species that contaminated these collected samples and proved to produce fumonisins for the first time in our laboratory and thus further investigation is needed.

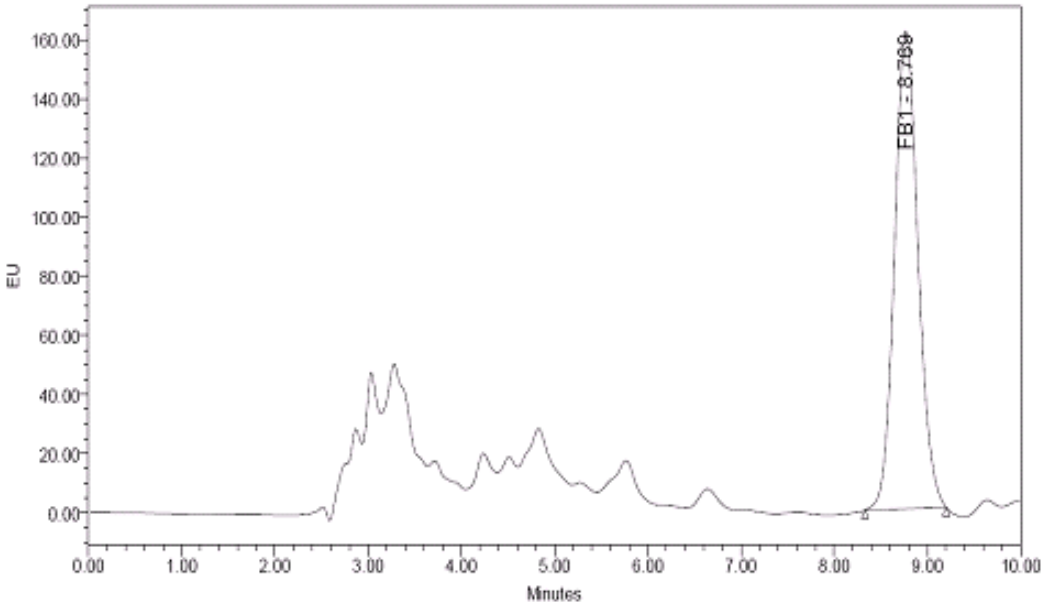
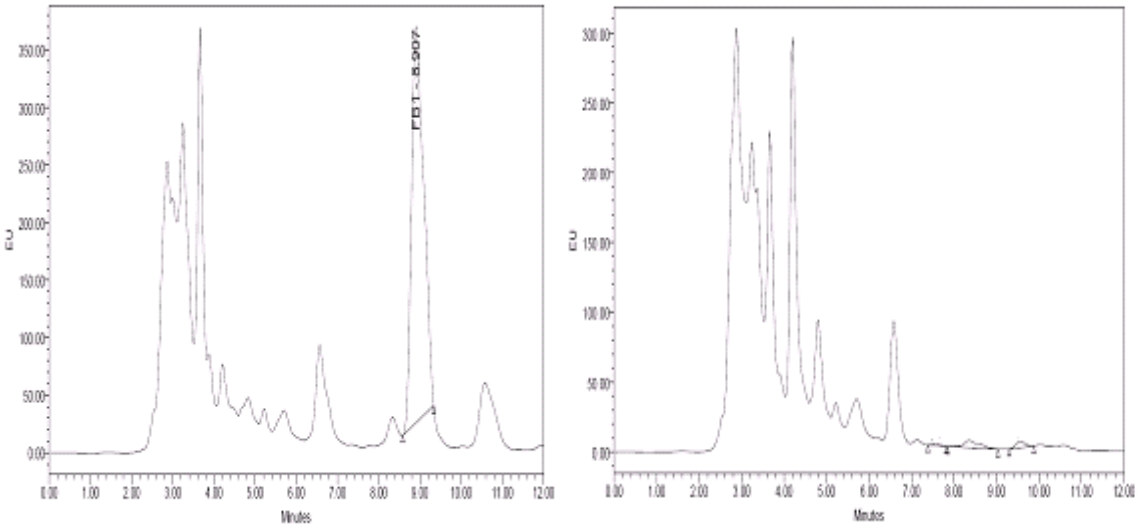


Figure 1: Chromatograms of FB_1 standard solution derivatized with OPA reagent



(a) (b)

Figure 2: Chromatograms of crude extracts of fumonisin-producing (a) and non-producing (b) isolates derivatized with OPA reagent.

References

Abbas HK, Cartwright RD, Xie W, Mirocha CJ, Richard JL, Dvorak TJ, Sciumbato GL and Shier WT (1999): Mycotoxin production by *Fusarium proliferatum* isolates from rice with *Fusarium* sheath rot disease. Mycopathologia 147: 97-104.

Abdel-Hafez SII, El-Kady I, Mazen M and El-Maghraby O (1992): Effect of temperature and moisture content on germination capacity and paddy grain-borne fungi from Egypt. Abhath Al-Yarmouk; Pure Science and Engineering 1: 91-105.

Abdel-Mallek AY, El-Maraghy SSM and Hasan HAH (1993): Mycotoxin-produing potential of some *Aspergillus*, *Penicillium* and *Fusarium* isolates found on corn grains and sun-flower seeds in Egypt. Islamic Academy of Science 6(3): 189-192.

Abdel-Sater MA, Hemida SK, Eraky SA and Nasser MM (1995): Distribution of fungi on two mite species and their habitats in Egypt. Folia Microbiologica 40(3): 304-313.

Adebajo LO, Idown AA and Adensy OO (1994): Mycoflora and mycotoxins production in Nigeria corn and corn based snacks. Mycopathologia 126(3): 183-192.

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR and Daszak P (2004): Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution 19:535-544.

Bolger M, Coker RD, DiNovi M, Gaylor D, Gelderblom W and Olsen M (2001): Safety evaluation of certain mycotoxins in food, Edited by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), FAO, Rome, pp. 103-280.

Booth C (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Key, Surrey, England.

Booth C (1977): *Fusarium*: Laboratory Guide to the Identification of Major Species. Commonwealth Mycological Institute, Kew, Surrey, England.

Castella G, Bragulat MR, Cabanes FJ (1999): Fumonisin production by *Fusarium* species isolated from cereals and feeds in Spain. Food Protection 62: 811-813.

Chulze S, Ramírez ML, Farnochi MC, Pascale M, Visconti A and March G (1996): *Fusarium* and fumonisin occurrence in Argentinian corn at different ear maturity stages. Journal of Agricultural and Food Chemistry 44: 2797-2801.

Chulze S, Etcheverry MG, Lecumberry SE, Magnoli C, Dalcero A, Ramírez ML, Pascale M and Rodríguez MI (1999): Fumonisin production on irradiated corn kernels: effect of inoculum size. Food Protection 62: 814-817.

El-Kady IA and Moubasher MH (1982): Toxigenicity and toxins of *Stachybotrys chartarum* isolates from wheat straw samples in Egypt. Experimental Mycology 6: 25-31.

El-Maghraby OMO (1984): Mycoflora and trichothecene toxins of paddy (*Oryza sativa*

L.) grains in Egypt. Ph. D. Thesis, Botany Deptartment, Faculty of Science, Assuit University, Egypt.

El-Maghraby OMO (1994): Transformation of trichoverrols A and B to macrocyclic trichothecenes by *Stachybotrys* spp. Bulletin of the Faculty of Sceince, Assiut University 23: 71-84.

El-Maghraby OMO (1996): Mycotoxins and mycoflora of rice in Egypt with special reference to trichothecenes production and control. Natural Toxins 5(1): 49-59.

El-Maghraby OMO, El-Sawi NM and Mohamed MM (1992a): T-2 toxin control and toxigenicity on adrenal gland. 4th National Conference on Biochemistry, Cairo, Egypt, pp. 149-163

El-Maghraby OMO, Saber SM and Aboul-Nasr MB (1992b): Contamination of pea (*Pisum sativum* L.) seeds by fungi, mycotoxins and resistance of cultivars to mycotoxins accumulation. Sohag Pure and applied Science Bulletin, Faculty of Science, Egypt 8: 137-155.

El-Shanawany AA, Mostafa ME and Barakat A (2005): Fungal populations and mycotoxins in silage in Assuit and Sohag Governorates in Egypt, with a special reference to characteristic aspergilli toxins. Mycopathologia 159: 281-289.

Fadl Allah EM (1998): Occurrence and toxigenicity of *Fusarium moniliforme* from freshly-harvested maize ears with special refrences to fumonisin production in Egypt. Mycopathologia 140: 99-103.

Fandohan P, Gnonlonfin B, Hell K, Marasas WFO and Wingfield MJ (2005): Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. Food Microbiology 99:173-183.

Ghiasian SA, Rezayat SM, Kord-Bacheh P, Maghsood AH, Yazdanpanah H, Shephard GS, van der Westhuizen I, Vismer HF and Marasas WFO (2005): Fumonisin production by *Fusarium* species isolated from freshly harvested corn in Iran. Mycopathologia 159: 31-40.

Harrison LR, Colvin BM, Green JT, Newman LE and Cole JRJr (1990): Pulmonary edema and hyfrotorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. Journal of Veterinary Diagnostic Investigation 2: 217-221.

Horwitz W and George LGJr (2007): Official methods of analysis of the Association of Analytical Chemists AOAC. 18th ed., AOAC Int., Gaithersburg, MD, USA.

Kpodo K, Thrane U and Hald B (2000): Fusaria and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. Food Microbiology 61: 147-157.

Lee U-S, Lee M-Y, Shin K-S, Min Y-S, Cho C-M, Ueno Y (1994): Production of fumonisin B_1 and B_2 by *Fusarium moniliforme* isolated from Korean corn kernels for feed. Mycotoxin Research 10: 67-72.

Leslie JF and Summerell BA (2006): The *Fusarium* Laboratory Manual. Blackwell Publishing Ames, Iowa, USA, 388 pp.

Miller JD, Savard ME, Sibilia A, Rapior S, Hocking AD, and Pitt JJ (1993): Production of fumonisins and fusarins by *Fusarium moniliforme* from Southeast Asia. Mycologia 85: 385-391.

Moubasher AH (1993): Soil Fungi in Qatar and Other Arab Countries. Scientific and Applied Research Center, University of Qatar, 566 pp.

Moustafa AF (2006): Zygomycetes of Egypt. In: Fungi of Egypt, AUMC Descriptions No. 1.

Mubatanhema W, Moss MO, Frank MJ and Wilson DM (1999): Prevalence of *Fusarium* species of the *Liseola* section on Zimbabwean corn and their ability to produce the mycotoxins zearalenone, moniliformin and fumonisin B_1. Mycopathologia 148: 157-163.

Müllenborn C, Steiner U, Ludwig M, and Oerke EC (2008): Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. European Journal of Plant Pathology 120: 157–166.

Naqvi S, Ramessar K, Farré G, Sabalza M, Miralpeix B, Twyman RM, Capell T, Zhu C and Christou P (2011): High-value products from transgenic maize. Biotechnology Advances 29: 40-53.

Nelson PE, Plattner RD, Shackelford DD and Desjardins AE (1991): Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Applied and Environmental Microbiology 57: 2410-2412.

Nelson PE, Toussoum TA and Marasas WFO (1983): *Fusarium* species an illustrated manual for identification. The Pennsylvania State University Press, London.

Oude Elferink SJWH, Driehuis F, Gottsschal JC and Spoelstra SF (2001): Silage fermentation processes and their manipulation. FAO Corporate Document

Repository, produced by Agriculture and Consumer Protection Department.

Pitt JI, Hocking AD, Samson RA and King AD (1992): Recommended methods for mycological examination of foods. In: Samson RA, Hocking AD, Pitt JI and King AD (eds.), Modern Methods in Food Mycology. Elsevier, Amsterdam, pp. 365-368.

Rheeder JP, Marasas WFO, Vismer HF (2002): Production of fumonisin analogs by *Fusarium* species. Applied and Environmental Microbiology 68: 2101-2105.

Ross PF, Nelson PE, Richard JL, Osweiler GD, Rice LG, Plattner RD and Wilson TM (1990). Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and pulmonary edema syndrome in swine. Applied and Environmental Microbiology 56:3225-3226.

Ross PF, Rice LG, Osweiler GD, Nelson PE, Richard JL and Wilson TM (1992): A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 117: 109-111.

Sanchis V, Abadias M, Oncins L, Sala N, Vinas I and Canela R (1995): Fumonisin B_1 and B_2 and toxigenic *Fusarium* strains in feeds from the Spanish market. Food Microbiology 27: 37-44.

Schneweis I, Meyer KM, Rmansdorfer S and Bauer J (2000): Mycophenolic acid in silage. Applied and Environmental Microbiology 66(8): 3639-3641.

Soliman S (1999): Mycoflora and mycotoxin of sorghum grains in Egypt. Ph. D. Thesis, faculty of Science at Sohag, South Valley University.

Soo LU, Yur LM, Sop SK, Sik MY, Min CC and Ueno Y (1994): Production of fumonisin B_1 and B_2 by *Fusarium moniliforme* isolated from Korean corn kernels for feed. Mycotoxin Research 10: 67-72.

Soriano JM, Gonzálea L and Catalá AI (2005): Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. Progress in Lipid Research 44: 345-356.

Sydenham EW, Shephard GS, Thiel PG, Marasas WFO, Rheeder JP, Peralta Sanhueza CE, Gonzalez HHL and Resnik SL (1993): Fumonisins in Argentinian field trial corn. Journal of Agricultural and Food Chemistry 41: 891–895.

Thiel PG, Marasas WFO, Sydenham EW, Shepard GS and Gelderblom WCA (1992): The implications of naturally-occurring levels of fumonisins in corn for human and animal health. Mycopathologia 117: 3-9.

Tournas VH, Heeres J and Burgess L (2006): Moulds and yeasts in fruit salads and fruit juices. Food Microbiology 23(7): 684-688.

Tseng TC, Lee KL, Deng TS, Liu CY and Huang JW (1995): Production of fumonisins by *Fusarium* species in Taiwan. Mycopathologia 130: 117-121.

Visconti A and Doko B (1994): Survey of fumonisin production by *Fusarium* isolated from cereals in Europe. Journal of the Association of Official Agricultural Chemists 77: 546-550.