

ASSOCIATED MOLD FUNGI WITH COLLECTED YELLOW CORN GRAINS FROM DIFFERENT EGYPTIAN GOVERNORATES AND THEIR MYCOTOXINS

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ABSTRACT

Isolation trails from grain samples of five yellow corn hybrids collected from different Egyptian governorates verified the occurrence of large number of mold fungi associated the grains of these corn hybrids. The isolated fungi were identified as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. candidus*, *A. ochraceus*, *Aspergillus* sp., *A. versicolor*, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium digitatum* and *Penicillium* sp., as well as some unknown fungi. Among these surveyed governorates, the highest frequency of isolated mold fungi was recorded in Elmenya followed by Sharkiya governorate samples. Meanwhile, the least frequency of isolated mold fungi was recorded on surveyed yellow corn samples of Assuite and Qena governorates. On the other hand, the associated mold fungi were varied in their frequency on harvested grains of the five tested yellow corn hybrids i.e., SX-3062, SX-SHAMS, SX-3080, 3WC-352 and SX-30N11 from governorate to another where the occurrence of these mold fungi of the same hybrid was affected by the source of grain samples collection. It is clear also that occurrence of *Aspergillus* group was prevalent on tested yellow corn grains than the *Fusarium* group and other isolated fungi in all surveyed governorates. No one of *Fusarium* group was detected on collected yellow corn grain samples of Assuite governorate. Meanwhile, *F. moniliforme* did not detect in all tested grain samples of Qena governorate. All collected grain samples of the five tested yellow corn hybrids from Elmenya governorate were found to be contaminated with considerable quantities of aflatoxins. The highest amount of aflatoxins was recorded in grain samples of 3WC-352 collected from Elmenya governorate followed by

SX-SHAMS (Elmenya) and SX-3080 (Giza). On the other hand, *Aspergillus flavus* was able to produce aflatoxins B1, B2, G1 and G2 *in vitro* where the quantities of aflatoxin B1 is higher than B2 meanwhile, G2 was higher than G1. Also, *Aspergillus ochraceus* was able to produce high amount of ochratoxin. Also, *Fusarium moniliforme* produce high quantity of zearalenone mycotoxin.

INTRODUCTION

Maize (*Zea mays* L.) is considered one of the most important cereal crops in the world. It is used mainly in animal and poultry feeding, either as green fodder or a main component of dry feed. Maize constitutes up to 75% of poultry feed. It is used also for human food in rural societies where it is mixed with wheat flour in bread industry. Besides, it is a major component in several important industries such as corn oil, starch, glucose sugar...etc.. (U.S. Feed Grains Council, 1994). Stored ears and corn grains are found to attack by different fungi upon harvest *i.e.* *Fusarium moniliforme*, *F. graminearum*, *F. oxysporum*, *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Nigrospora oryzae*, *Mucor* sp. and *Rhizopus* spp. (Ibrahim & Farag, 1965; Mislivec and Tuite, 1970 and Sabek, 2003). Abbas *et al.* (1988a) isolated 81 isolates of *Fusarium* spp. from corn ears in Minnesota, USA, and were identified as *F. proliferatum* (11 isolates), *F. subglutinans* (*Gibberella fujikuroi* var. *subglutinans* 23 isolates), *F. graminearum* (*Gibberella zea*, 20 isolates), *F. moniliforme* (*Gibberella fujikuroi*, 19 isolates) and *F. oxysporum* 8 isolates. Yasin *et al.* (1992) revealed that the most storage moulds of corn were *Penicillium*, *Aspergillus*, *Fusarium* and *Cladosporium*. Desjardins *et al.* (2000) assessed seed samples of maize (*Zea mays* subsp. *mays*) from Mexico and of teosintes (*Zea* spp.), for infection with *Fusarium* spp. Strains similar in morphology to *Fusarium moniliforme* [*Gibberella fujikuroi*] and *F. subglutinans* [*Gibberella fujikuroi* var. *subglutinans*] were the most frequent isolates from maize and from teosinte species including *Z. diploperennis*, *Z. luxurians*, *Z. mays* subsp. *mexicana* [*Z. mexicana*], and *Z. mays* subsp. *parviglumis* [*Z. parviglumis*]. Medic-Pap *et al.* (2011) reported that, the genus *Fusarium* is the most significant which can cause corn ear and kernel rots. They isolated also the genera of *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria*. Two species from the genus *Fusarium* of those isolated from the tested corn samples were identified as *F. graminearum* and *F.*

moniliforme. Tested hybrids that belonged to different FAO maturity groups showed differences in susceptibility to ear and kernel rot.

Aflatoxins B₁, B₂, G₁, and G₂ are the main toxins produced by *Aspergillus flavus* and *A. parasiticus*. (Diener and Davis, 1986). Abbas *et al.* (1989b) found that eighty-two cultures of *Fusarium* spp. isolated in 1986 from mouldy maize in Minnesota produced the following mycotoxins: *F. graminearum* [*Gibberella zeae*] isolates, produced deoxynivalenol [vomitoxin], 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and zearalenone while, *F. moniliforme* [*G. fujikuroi*], produced fusarin-C. Meanwhile, *G. fujikuroi*, *F. oxysporum*, *F. proliferatum* and *F. [G. fujikuroi var.] subglutinans* isolates, produced *moniliformin* and *G. fujikuroi*, *F. proliferatum* and *G. fujikuroi* var. *subglutinans* isolates produced fusaric acid. *Fusarium moniliforme* J. Sheld. is a seed-transmitted pathogen of maize that is found throughout much of the world (Leslie 1991). Alp *et al.* (1997) analyzed 47 corn samples collected from Turkey for the presence of fungal and mycotoxin contamination. Aflatoxin B₁ was found in 3 samples (12, 18 and 20 ppb) and aflatoxin G₁ was found in 2 samples (10 and 12 ppb). Of the entire tested samples, 36.2% contained ochratoxin A (120-3840 ppb), 14.9% rubratoxin (150 – 800 ppb) and zearalenone (180- 2200 ppb). Gao *et al.* (1997) studied the natural occurrence of fumonisins and aflatoxins in 30 feed corn. Samples were positive for aflatoxin B₁ and B₂ at maximum levels of 208 and 51 ng/kg, respectively. Aflatoxin G₁ was found in 4 samples (13%) and non-samples contained aflatoxin G₂, but fumonisins was found in 67% of maize samples. Solovey *et al.* (1999) analyzed 38 corn samples collected from different locations in Argentina for presence of mycotoxins (zearalenone, vomitoxin, fumonisins and aflatoxins). Results revealed that aflatoxins in all samples were < 2 ng/kg, zearalenone amount < 50 ng/kg, vomitoxin amount < 50 ng/kg and fumonisins contamination was found in 95% of all samples. Williams *et al.* (2011) said that, aflatoxin, a toxin produced by the fungus *Aspergillus flavus* Link: Fries, occurs naturally in maize (*Zea mays* L.). Aflatoxin is a potent human carcinogen and is also toxic to livestock, pets, and wildlife. When contaminated with aflatoxin, the value of maize grain is markedly reduced.

This work aimed to throw a light on the most common mold fungi associating grains of the commonly cultivated yellow corn hybrids in some Egyptian governorates. Also, throw the light on their mycotoxins production in corn grains.

MATERIALS AND METHODS

1- Source of yellow corn grain hybrid samples:

Samples of five widely cultivated hybrids were collected from harvested yellow corn grains in 6 Egyptian governorates *i.e.*, Dakahlia, Sharkia and Giza which representing north Egypt as well as, Assuit, El - Menia and Qena which representing the upper Egypt. The five corn grain hybrids which collected from each governorate were SX-3062, SX-3080, SX-30N11 and Shams (single cross hybrids) and 3W-352 (three way cross hybrid).

2- Isolation of associated mold fungi with the tested yellow corn grains:

Corn grains (hundred grains) were disinfested by immersing in 5% sodium hypochlorite solution for 3 minutes-washed thoroughly in three changes of sterilized water and dried between sterilized filter paper. Grains were aseptically transferred to ready plates of potato dextrose agar medium-PDA (Christensen, 1957). Plates were then incubated at 25°C and observations were daily recorded up to the 7th day. The emerged fungi were counted, and then purified using the single spore technique (Hansen, 1926) and/or the hyphal tip technique (Riker and Riker, 1936). Representative samples of at least 100 grains of each hybrid were usually used for the isolation of the associated fungi. Pure cultures were maintained on PDA slants at 25°C for 15 days and stored at 8°C. in a refrigerator. Identification of the associated fungi was carried out according to their morphological and microscopical characters as described by Jens *et al.* (1991) and confirmed by Fungal Taxonomy Dept., Plant Pathology Institute, ARC, Egypt.

3- Determination of total Aflatoxins in tested yellow corn grain samples:

Aflatoxins were determined according to AOAC (1990). Hundred grams samples were homogenized in 200 ml methanol: water solution, (8: 2) in blender at high speed for 3 min. The samples were filtered by using filter paper No. 1 then, cleaned using 50 ml of clean up solution (150 g zinc sulphate + 50 g phosphotungestic acid then dissolved in 1000 ml distilled water) and filtered again by using filter paper No. 4. About 75-ml of collected filtrate were put in separating funnel containing 15-ml benzene, then shaken for 5 min. The upper layer was collected in a glass beaker and evaporated till dryness under steam of nitrogen.

Samples and standard aflatoxins (B1, B2, G1 and G2) were spotted on thin layer chromatography (TLC) plates at different concentrations: 2, 5, 7 and 10 µl, the spotted samples on TLC plates were eluted in eluting jar (contained, diethyl ether-methanol-water 96:3:1, respectively) for running. The running of samples were stopped when elution solvent reached the end line then TLC plates dried and examined under ultraviolet detector (UV) wavelength 365 nm.

$$\text{Aflatoxins } \mu\text{g/kg samples} = (S \times Y \times V) / (X \times W)$$

Where: S = μl mycotoxin std. equal to unknown;

Y = concentration of aflatoxins $\mu\text{g/ml}$

V = μl of final dilution of sample

X = μl sample extraction spotted giving fluorescent intensity equal to Aflatoxins

W = weight of sample (100 g).

4- Determination of mycotoxins production ability of some isolated fungi:

In this experiment, two isolates *i.e.*, *Aspergillus flavus* and *Aspergillus ochraceus* were chosen of those isolated from tested grain samples of SX-SHAMS hybrid as well as one isolate of *Fusarium moniliforme* of those isolated from tested grain samples of SX-3062 and collected from Elmenya governorate. These isolates were tested only for their abilities on producing aflatoxins (B1, B2, G1 and G2), ochratoxins and zearalenone (Sigma, USA) in yellow corn grains of the tested hybrids. In this respect, apparently healthy grains were sterilized in an aqueous sodium hypochlorite solution of Clorox 25% for 10 minutes and then washed several times with sterilized distilled water and air dried under aseptic conditions. Sterilized conical flasks (500ml.), each containing 100 grams of the formerly sterilized grains were separately inoculated with 12 discs of actively growing day-old, PDA culture. Flasks were incubated at 25°C for 18 days. Three replicates were used in each treatment. Un-inoculated flasks served as control (Sabet, 1991).

After 18 days from inoculation, samples were homogenized, filtered, cleaned, filtered again, put in separating funnel and the upper layer was collected and evaporated as mentioned before.

Samples and standard aflatoxins (B1, B2, G1 and G2), ochratoxin A and zearalenone (Sigma, USA) were spotted on thin layer chromatography (TLC) plates at different concentrations: 2, 5, 7 and 10 μl , the spotted samples on TLC plates were eluted in eluting jar (contained, diethyl ether-methanol-water 96:3:1, respectively) for running. The running of samples were stopped when elution solvent reached the end line then TLC plates dried and examined under ultraviolet detector (UV) wavelength 365 nm as mentioned above in aflatoxins. The quantities of determined mycotoxins were estimated as mentioned above.

RESULTS

1 - Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Sharkiya governorate:

Data in **Table (1)** reveal that 213 fungal isolates representing five genera were isolated from tested grains of yellow corn hybrids collected from Sharkiya governorate. In this respect, *Aspergillus niger* recorded the highest frequent number among all isolated fungi (48 isolate) followed by

Aspergillus ustus (37 isolate) and *Aspergillus flavus* (26 isolates). Also, *Aspergillus ochraceus* followed by *Aspergillus versicolor* were the least frequent ones among the isolated *Aspergillus* group. Among the isolated *Fusarium* group, *Fusarium moniliforme* scored the highest frequent number (16 isolate) followed by *Fusarium solani* (10 isolates). Also, three different fungal genera i.e., *Humicola* sp. (3 isolate) and *Mucor* sp. (8 isolate) and one isolate of *Penicillium* sp., were recorded on tested grains of yellow corn hybrids collected from Sharkya governorate. On the other hand, the highest frequent number of isolated fungi was recorded on grains of SX-SHAMS hybrid (70 isolate) followed by SX-3080 (45 isolate) whereas the least number of isolated fungi was recorded on tested yellow corn grains of 3WC-352 hybrid (26 isolate).

Table (1): Frequency of isolated fungi from grains of five yellow corn hybrids collected from Sharkya governorate.

Isolated fungi	SX-3062		SX SHAMS		SX-3080		3WC-352		SX-30N11		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	18	48.6	12	17.1	8	17.8	10	40	-	-	48
<i>Aspergillus flavus</i>	-	-	13	18.6	8	17.8	-	-	5	13.9	26
<i>Aspergillus clavatus</i>	12	32.4	-	-	7	15.6	-	-	-	-	19
<i>Aspergillus candidus</i>	-	-	2	2.9	10	22.2	-	-	-	-	12
<i>Aspergillus ustus</i>	-	-	10	14.3	9	20.0	-	-	18	50.0	37
<i>Aspergillus ochraceus</i>	-	-	-	-	-	-	-	-	2	5.6	2
<i>Aspergillus</i> sp.	-	-	3	4.2	3	6.7	8	32	6	16.7	20
<i>Aspergillus versicolor</i>	3	8.1	-	-	-	-	-	-	-	-	3
<i>Fusarium moniliforme</i>	-	-	16	22.9	-	-	-	-	-	-	16
<i>Fusarium solani</i>	-	-	8	11.4	-	-	-	-	2	5.6	10
<i>Fusarium tricinctum</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Fusarium oxysporum</i>	4	10.8	-	-	-	-	3	12	1	2.8	8
<i>Humicola</i> sp.	-	-	3	4.3	-	-	-	-	-	-	3
<i>Mucor</i> sp.	-	-	2	2.9	-	-	4	16	2	5.6	8
<i>Penicillium</i> sp.	-	-	1	1.4	-	-	-	-	-	-	1
Total	37		70		45		25		36		213

Freq. = Frequency

Moreover, the recorded numbers of isolated mold fungi on grains of SX-3062 and SX-30N11 were 37 and 36 isolates respectively. It is clear also that *Fusarium moniliforme* was recorded only on yellow corn grain samples of SX-SHAMS hybrid while *Aspergillus ochraceus* was recorded only on samples of SX-30N11 hybrid whereas, *Aspergillus flavus* was recorded on grain samples of three hybrids i.e., SX-SHAMS, SX-3080 and SX-30N11.

2- Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Dakahlyia governorate:

Data in Table (2) reveal that 142 fungal isolates representing five genera were isolated from tested grain samples of yellow corn hybrids

collected from Dakahlyia governorate. The isolated fungi were identified as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. candidus*, *A. ochraceus*, *Aspergillus* sp., *A. versicolor*, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium digitatum* and *Penicillium* sp. In this respect, *Aspergillus flavus* recorded the highest frequent number among all isolated fungi (30 isolate) followed by *Aspergillus niger* (24 isolate), *Fusarium moniliforme* (19 isolate) and *Fusarium solani* (13 isolate).

Table (2): Frequency of isolated fungi from grains of five yellow corn hybrids collected from Dakahlyia governorate.

Isolated fungi	SX 3062		SX SHAMS		SX 3080		3WC 352		SX 30N11		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	1	20	3	3.6	-	-	12	54.5	8	33.3	24
<i>Aspergillus flavus</i>	-	-	28	33.7	2	25	-	-	-	-	30
<i>Aspergillus clavatus</i>	-	-	2	2.4	-	-	2	9.1	-	-	4
<i>Aspergillus candidus</i>	-	-	5	6.0	-	-	-	-	3	12.5	8
<i>Aspergillus ochraceus</i>	-	-	3	3.6	-	-	2	9.1	6	25.0	11
<i>Aspergillus</i> sp.	-	-	-	-	3	37.5	5	22.7	-	-	8
<i>Aspergillus versicolor</i>	-	-	-	-	1	12.5	1	4.5	-	-	2
<i>Fusarium moniliforme</i>	1	20	18	21.7	-	-	-	-	-	-	19
<i>Fusarium solani</i>	-	-	8	9.6	-	-	-	-	5	20.8	13
<i>Fusarium oxysporum</i>	3	60	-	-	2	25	-	-	-	-	5
<i>Humicola</i> sp.	-	-	3	3.6	-	-	-	-	-	-	3
<i>Mucor</i> sp.	-	-	10	12.0	-	-	-	-	-	-	10
<i>Penicillium digitatum</i>	-	-	-	-	-	-	-	-	2	8.3	2
<i>Penicillium</i> sp.	-	-	3	3.6	-	-	-	-	-	-	3
Total	5		83		8		22		24		142

Freq. = Frequency

Also, *Penicillium digitatum* and *Aspergillus versicolor* recorded the least frequent numbers (2 isolates) among all isolated mold fungi. On the other hand, the greatest frequent number of isolated mold fungi was recorded on grain samples of SX-SHAMS hybrid (83 isolate) followed by SX -30N11 (24 isolate) and 3WC- 352 hybrid (22 isolate) whereas the least number of isolated fungi was recorded on tested yellow corn grains of hybrid SX -3062 (5 isolates) and SX -3080 (8 isolates). It is clear also that *Aspergillus flavus* was recorded on grain samples of SX -SHAMS and SX -3080 hybrids whereas, *Aspergillus ochraceus* was recorded on grain samples of three hybrids i.e., SX -SHAMS, 3WC 352 and SX -30N11. Meanwhile, *Fusarium moniliforme* was recorded on yellow corn grain samples of SX -SHAMS and SX- 3062 hybrids.

3- Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Giza governorate:

Data in Table (3) reveal that 166 fungal isolates belonging to five genera as well as other unknown group were isolated from tested grain samples of yellow corn hybrids collected from Giza governorate. These

isolates were identified as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. candidus*, *A. ustus*, *A. ochraceus*, *Aspergillus* sp., *A. versicolor*, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium funiculosum*, *Penicillium digitatum* and *Penicillium* sp. as well as, 3 isolates were unknown. Moreover, *Aspergillus niger* recorded the highest frequent number among all isolated fungi (39 isolate) followed by *Aspergillus flavus* (30 isolate), *Mucor* sp. (14 isolate). Also, *Penicillium funiculosum* and *Penicillium digitatum* scored the lowest frequent number among all isolated fungi. On the other hand, the highest frequent number of isolated fungi was recorded on grains of yellow corn SX-3080 hybrid (81 isolate) followed by SX- SHAMS (37 isolate), meanwhile the least frequent number of isolated fungi was recorded on grain samples of SX-3062 hybrid (12 isolate). It is clear also that *Aspergillus flavus* was recorded on grain samples of all tested hybrids except on grains of SX-3062 hybrid. Meanwhile, *Aspergillus ochraceus* was recorded on grain samples of three hybrids i.e., SX-3080, 3WC-352 and SX -30N11. Meanwhile, *Fusarium moniliforme* was recorded on yellow corn grain samples of SX-3062 and 3WC-352.

Table (3): Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Giza governorate

Isolated fungi	SX -3062		SX SHAMS		SX- 3080		3WC- 352		SX- 30N11		Total
	*Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	5	41.6	3	8.1	20	24.6	5	31.3	6	30.0	39
<i>Aspergillus flavus</i>	-	-	16	43.2	9	11.1	3	18.8	2	10.0	30
<i>Aspergillus clavatus</i>	-	-	1	2.7	9	11.1	-	-	-	-	10
<i>Aspergillus candidus</i>	-	-	2	5.4	-	-	-	-	2	10.0	4
<i>Aspergillus ustus</i>	-	-	-	-	6	7.4	-	-	-	-	6
<i>Aspergillus ochraceus</i>	-	-	-	-	5	6.1	1	6.3	1	5.0	7
<i>Aspergillus</i> sp.	3	25	4	10.8	3	3.7	-	-	1	5.0	11
<i>Aspergillus versicolor</i>	-	-	-	-	2	2.4	-	-	1	5.0	3
<i>Fusarium moniliforme</i>	1	8.3	-	-	-	-	3	18.8	-	-	4
<i>Fusarium solani</i>	-	-	-	-	3	3.7	-	-	-	-	3
<i>Fusarium oxysporum</i>	1	8.3	3	8.1	2	2.4	2	21.5	2	10.0	10
<i>Humicola</i> sp.	-	-	3	8.1	8	9.8	-	-	1	5.0	12
<i>Mucor</i> sp.	-	-	-	-	10	12.3	-	-	4	20.0	14
<i>Penicillium funiculosum</i>	-	-	2	5.4	-	-	-	-	-	-	2
<i>Penicillium digitatum</i>	-	-	2	5.4	-	-	-	-	-	-	2
<i>Penicillium</i> sp.	2	16.6	1	2.7	1	1.2	2	12.5	-	-	6
Unknown	-	-	-	-	3	3.7	-	-	-	-	3
Total	12		37		81		16		20		166

Freq. = Frequency

4- Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Elmenya governorate:

Data in Table (4) reveal that 251 fungal isolates belonging to five genera as well as other unknown group were isolated from tested grain

samples of yellow corn hybrids collected from Elmenya governorate. This number of isolated fungi represents the highest frequent number of which isolated fungi from grain samples of the different governorates. These isolates were identified as *Aspergillus niger*; *A. flavus*, *A. clavatus*, *A. candidus*, *A. ustus*, *A. ochraceus*, *Aspergillus* sp., *A. versicolor*, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium funiculosum*, and *Penicillium* sp. as well as, 9 unknown isolates. Also, *Aspergillus niger* scored the highest frequent one (49 isolate) followed by *A. flavus* (33 isolate) and *A. clavatus* (27 isolate). However, *A. versicolor* and *Penicillium funiculosum* recorded the lowest frequencies (3 isolate). On the other hand, the highest frequent numbers of isolated fungi were scored on garin samples of from SX-3062 hybrid (108 isolate) followed by SX-3080 hybrid (54 isolate) and SX-SHAMS hybrid (46 isolate) respectively. Meanwhile, the least frequent number was recorded on grains of 3WC-352.

Table (4): Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Elmenya governorate

Isolated fungi	SX-3062		SX-SHAMS		SX-3080		3WC-352		SX-30N11		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	22	20.4	-	-	15	27.8	1	4.8	11	50	49
<i>Aspergillus flavus</i>	9	8.3	9	19.6	9	16.7	3	14.3	3	13.6	33
<i>Aspergillus clavatus</i>	15	13.9	9	19.6	3	5.6	-	-	-	-	27
<i>Aspergillus candidus</i>	10	9.3	2	4.3	-	-	3	14.3	-	-	15
<i>Aspergillus ustus</i>	3	2.8	-	-	8	14.8	-	-	-	-	11
<i>Aspergillus ochraceus</i>	6	5.6	9	19.6	2	3.7	2	9.5	4	18.1	23
<i>Aspergillus</i> sp.	8	7.4	-	-	-	-	2	9.5	1	4.5	11
<i>Aspergillus versicolor</i>	-	-	3	6.5	-	-	-	-	-	-	3
<i>Fusarium moniliforme</i>	8	7.4	-	-	-	-	3	14.3	1	4.5	12
<i>Fusarium solani</i>	8	7.4	2	4.3	2	3.7	-	-	-	-	12
<i>Fusarium oxysporum</i>	-	-	4	8.7	6	11.1	2	9.5	-	-	12
<i>Humicola</i> sp.	5	4.6	3	6.5	5	9.2	-	-	-	-	13
<i>Mucor</i> sp.	10	9.3	-	-	2	3.7	-	-	-	-	12
<i>Penicillium funiculosum</i>	-	-	-	-	-	-	3	14.3	-	-	3
<i>Penicillium</i> sp.	-	-	2	4.3	-	-	2	9.5	2	9.0	6
Unknown	4	3.7	3	6.5	2	3.7	-	-	-	-	9
Total	108		46		54		21		22		251

Freq. = Frequency

5- Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Assuite governorate:

Data in Table (5) reveal that 79 fungal isolates belonging to three genera as well as other unknown group were isolated from tested grain samples of yellow corn hybrids collected from Assuite governorate. These isolated fungi were identified as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. candidus*, *A. ustus*, *A. ochraceus*, *Aspergillus* sp., *Humicola* sp. and *Mucor* sp. as well as 2 unknown isolates. Moreover, *Aspergillus clavatus* recorded the highest frequent number (17 isolate) followed by *A. niger* and *A. ustus* (16 isolates). On the other hand, the highest frequent number of isolated fungi was recorded on grain samples of SX-30N11 (25 isolate) followed by SX-3080 (22 isolate) while, the least frequent number was recorded on grain samples of 3WC-352 (5 isolates). No one of Fusarium group was detected in collected yellow corn grain samples of this governorate.

Table (5): Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Assuite governorate

Isolated fungi	SX 3062		SX SHAMS		SX 3080		3WC 352		SX 30N11		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	3	42.8	8	40.0	-	-	3	60.0	2	8.0	16
<i>Aspergillus flavus</i>	2	28.6	-	-	-	-	-	-	-	-	2
<i>Aspergillus clavatus</i>	-	-	-	-	2	9.0	-	-	15	60.0	17
<i>Aspergillus candidus</i>	-	-	6	30.0	2	9.0	-	-	-	-	8
<i>Aspergillus ustus</i>	-	-	6	30.0	2	9.0	-	-	8	32.0	16
<i>Aspergillus ochraceus</i>	-	-	-	-	1	4.5	-	-	-	-	1
<i>Aspergillus</i> sp.	2	28.6	-	-	2	9.0	2	40.0	-	-	6
<i>Humicola</i> sp.	-	-	-	-	9	40.9	-	-	-	-	9
<i>Mucor</i> sp.	-	-	-	-	2	9.0	-	-	-	-	2
Unknown	-	-	-	-	2	9.0	-	-	-	-	2
Total	7		20		22		5		25		79

Freq. = Frequency

6- Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Qena governorate:

Data in Table (6) reveal that only 74 fungal isolates belonging to five genera were isolated from tested grain samples of yellow corn hybrids collected from Qena governorate. The isolated fungi were identified as *Aspergillus niger*, *A. flavus*, *A. candidus*, *A. ustus*, *A. ochraceus*,

Aspergillus sp., *A. versicolor*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium funiculosum* and *Penicillium digitatum*. Among isolated fungi, *Aspergillus niger* was the most prevalent pathogen (26 isolate) followed by *A. flavus* (16 isolate) and *Fusarium oxysporum* (10 isolate). Meanwhile, *A. candidus*, *Penicillium funiculosum* and *Penicillium digitatum* scored the least frequent number among all isolated fungi. On the other hand, the highest frequent number of isolated fungi was recorded on grain samples of SX-3062 hybrid (24 isolate) followed by 3WC- 352 hybrid (23 isolate). Meanwhile, the least frequent number of isolated fungi was recorded on grain samples of SX-3080 (5 isolates). Also, it is clear that *A. flavus* was recorded only on grains of SX-3062 and 3WC-352 hybrids while, *A. ochraceus* was isolated from grain samples of SX-3062 and SX- 30N11 hybrids. Meanwhile, *F. moniliforme* did not detected in all tested grain samples of Qena governorate.

Table (6): Frequency of isolated mold fungi from grains of five yellow corn hybrids collected from Qena governorate

Isolated fungi	SX- 3062		SX- SHAMS		SX -3080		3WC- 352		SX- 30N11		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
<i>Aspergillus niger</i>	18	75.0	3	30.0	2	40	-	-	3	25	26
<i>Aspergillus flavus</i>	3	12.5	-	-	-	-	13	56.5	-	-	16
<i>Aspergillus candidus</i>	-	-	-	-	-	-	1	4.3	-	-	1
<i>Aspergillus ustus</i>	-	-	-	-	-	-	2	8.7	-	-	2
<i>Aspergillus ochraceus</i>	2	8.4	-	-	-	-	-	-	1	8.3	3
<i>Aspergillus</i> sp.	1	4.2	2	20.0	-	-	-	-	3	25	6
<i>Aspergillus versicolor</i>	-	-	-	-	-	-	2	8.7	-	-	2
<i>Fusarium solani</i>	-	-	-	-	-	-	2	8.7	-	-	2
<i>Fusarium oxysporum</i>	-	-	1	10.0	3	60	3	13.0	3	25	10
<i>Humicola</i> sp.	-	-	2	20.0	-	-	-	-	-	-	2
<i>Mucor</i> sp.	-	-	2	20.0	-	-	-	-	-	-	2
<i>Penicillium funiculosum</i>	-	-	-	-	-	-	-	-	1	8.3	1
<i>Penicillium digitatum</i>	-	-	-	-	-	-	-	-	1	8.3	1
Total	24		10		5		23		12		74

Freq. = Frequency

7- Aflatoxins productivity in grain samples of collected yellow corn hybrids from different governorates:

Results in Table (7) clear that many grain samples of different yellow corn hybrids were found to be contaminated with different quantities of aflatoxins. In this respect, all collected grain samples of the

five tested yellow corn hybrids from Elmenya governorate were found to be contaminated with quantities of aflatoxins ranged between 10.0-22.0 ppb. The highest amount of aflatoxins was recorded in grain samples of 3WC-352 hybrid collected from Elmenya governorate (22.0 ppb) followed by SX-SHAMS (Elmenya) and SX-3080 (Giza) to be 20 ppb. On the other hand, the least amount of aflatoxins was 10 ppb which recorded in grains of SX-3062 (Qena), SX-SHAMS (Dakahliya and Assuite), 3WC-352 (Dakahliya) and SX-30N11 (Giza and Elmenya). Among the five hybrid samples, grain samples of SX-SHAMS in all Governorates except Qena were found to be contaminated with aflatoxins. On the other hand, the highest amount of aflatoxins was recorded in Elmenya samples (average 19.6 ppb). Meanwhile, the least amount of aflatoxins was recorded in Assuite samples (average 2.0 ppb). As for hybrids, the highest amount of Aflatoxins was recorded in grain samples of SX-SHAMS (average 12.17 ppb). Whereas, the least amount of aflatoxins was recorded in grain samples of SX-30N11 (average 3.33ppb).

Table (7): Aflatoxins productivity (ppb) in grain samples of yellow corn hybrids collected from some Egyptian governorates.

Egyptian Governorates	Tested yellow corn hybrids					Mean
	SX-3062	SX-SHAMS	SX-3080	3WC-352	SX-30N11	
Sharkiya	0.0	15.0	0.0	0.0	0.0	3.0
Dakahliya	0.0	10.0	0.0	10.0	0.0	4.0
Giza	0.0	18.0	20.0	0.0	10.0	9.6
Elmenya	18.0	20.0	18.0	22.0	10.0	19.6
Assuite	0.0	10.0	0.0	0.0	0.0	2.0
Qena	10.0	0.0	0.0	12.0	0.0	4.4
Mean	4.67	12.17	6.33	7.33	3.33	

8- Determination the abilities of some isolated mold fungi on producing mycotoxins *in vitro*:

Results in **Table (8)** verify the abilities of tested mold fungi to produce mycotoxins *in vitro*. In this respect, *Aspergillus flavus* was able to produce aflatoxins B1, B2, G1 and G2 *in vitro* with quantities ranged between 244.4 ppb (G2) - 3238.4 ppb (B1). The total amount of determined aflatoxins was 8060.4 ppb meanwhile, *Aspergillus ochraceus* was able to produce high amount of Ochratoxin (800 ppb). Also,

Fusarium moniliforme produced high quantity of zearalenone mycotoxin which reached 1200ppb. Results clear that the determined quantities of aflatoxin B1 is higher than B2 meanwhile, G1 was higher than G2.

Table (8) The abilities of some isolated mold fungi on producing mycotoxins *in vitro*

Fungi	Aflatoxins (ppb)					Ochratoxin (ppb)	Zearalenone (ppb)
	B1	B2	G1	G2	Total		
<i>Aspergillus flavus</i>	3238.4	535.2	4042.4	244.4	8060.4	-	-
<i>Aspergillus ochraceus</i>	-	-	-	-	-	800	-
<i>Fusarium moniliforme</i>	-	-	-	-	-	-	1200

DISCUSSION

Maize (*Zea mays* L.) is considered one of the most important cereal crops in the world. Stored ears and corn grains are found to be attacked by different fungi upon harvest *i.e.* *Fusarium moniliforme*, *F. graminearum*, *F. oxysporum*, *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Nigrospora oryzae*, *Mucor* sp. and *Rhizopus* spp. (Ibrahim & Farag, 1965; Mislivec and Tuite, 1970 and Sabek, 2003).

Isolation trails from grain samples of five yellow corn hybrids collected from different Egyptian governorates verified the occurrence of large number of mold fungi could be associate the harvested grains of these corn hybrids. The isolated fungi were identified as *Aspergillus niger*, *A. flavus*, *A. clavatus*, *A. candidus*, *A. ochraceus*, *Aspergillus* sp., *A. versicolor*, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum*, *Humicola* sp., *Mucor* sp., *Penicillium digitatum* and *Penicillium* sp., as well as some unknown fungi. The occurrence of isolated mold fungi from the different selected Egyptian governorates was varied from governorate to another and from hybrid to another in each one of surveyed governorates. Among these surveyed governorates, the highest frequency of isolated mold fungi was recorded on Elmenya samples followed by Sharkiya governorate samples. Meanwhile, the least frequency of isolated mold fungi was recorded on surveyed yellow corn samples of Assuite and Qena governorates. On the other hand, the associated mold fungi were varied in their frequency on harvested grains of the five tested yellow corn hybrids *i.e.*, SX-3062, SX-SHAMS, SX-3080, 3WC-352 and SX-30N11 from governorate to another where the occurrence of these mold fungi of the same hybrid was affected by the source of grain

samples collection. It is clear also that occurrence of *Aspergillus* group was prevalent in its dominance on tested yellow corn grains than the *Fusarium* group and other isolated fungi in all surveyed governorates. No one of *Fusarium* group was detected on collected yellow corn grain samples of Assuite governorate. Meanwhile, *F. moniliforme* did not detect in all tested grain samples of Qena governorate. The obtained results could be discussed in light the findings of **Hassan and Selim (1982)** who isolated 168 isolates of fungi belonging to 11 genera from ten collected corn samples representing different locations in Egypt. Out of them, 18 isolates were *Aspergillus*, 11 isolates were *Fusarium*, 13 isolates were *Mucor* and *Rhizopus* spp. and 10 isolates were hyphomycetes. Also, **Paul and Mishra (1992)** mentioned that, most common fungi on corn grains were *Alternaria alternata*, *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. terreus*, *Fusarium moniliforme*, *Penicillium* spp. and *Rhizopus nigricans*. The observed field fungi were *Alternaria* spp., *Cladosporium* sp., *Fusarium* spp., *Drechslera maydis* and *Verticillium albo-atrum*. Also, these results are in harmony with those obtained by **Eisa et al. (1996a)** who, mentioned that the most dominant fungi which associated with yellow corn grains according to the occurrence percentage proved to be *Aspergillus flavus*, *Penicillium funiculosum*, *A. terreus*, *Fusarium moniliforme*, *F. oxysporum*, *F. graminearum*, *A. niger*, *Alternaria* sp., *Rhizopus* sp., *Macrophomina phaseolina* and *Fusarium* spp. Similar results were obtained also by **Sabek (2003)** who isolated 217 fungal isolates belonging to 5 genera and 15 species from un-sterilized kernels of two yellow corn hybrids (SC-3062 and DC-Dahab) and one female inbred line (FIL-X). These isolated fungi were identified as *Aspergillus flavus*, *A. niger*, *A. versicolor*, *A. terreus*, *A. ochraceus*, *Fusarium moniliforme*, *F. tabacinum*, *F. solani*, *F. semitectum*, *F. tricinctum*, *F. nivale*, *Penicillium funiculosum*, *Alternaria* sp., and *Rhizopus* spp., as well as, another unknown fungal group.

As for Aflatoxins production in harvested grain samples, it is clear that all collected grain samples of the five tested yellow corn hybrids from Elmenya governorate were found to be contaminated with considerable quantities of aflatoxins. The highest amount of aflatoxins was recorded in grain samples of 3WC-352 collected from Elmenya governorate followed by SX-SHAMS (Elmenya) and SX-3080 (Giza). On the other hand, the least amount of aflatoxins was recorded in grains of SX-3062 (Qena), SX-SHAMS (Dakahliya and Assuite), 3WC-352 (Dakahliya) and SX-30N11 (Giza and Elmenya). All grain samples of SX-SHAMS in all

governorates except Qena were found to be contaminated with aflatoxins. Also, the highest amount of aflatoxins was recorded in Elmenya samples. Meanwhile, the least amount of aflatoxins was recorded in Assuite samples. As for hybrids, the highest amount of Aflatoxins was recorded in grain samples of SX-SHAMS. Whereas, the least amount of aflatoxins was recorded in grain samples of SX-30N11. On the other hand, *Aspergillus flavus* was able to produce aflatoxins B1, B2, G1 and G2 *in vitro* where the quantities of aflatoxin B1 is higher than B2 meanwhile, G1 was higher than G2. Also, *Aspergillus ochraceus* was able to produce high amount of ochratoxin. Also, *Fusarium moniliforme* produce high quantity of zearalenone mycotoxins. The obtained results are in agreement with those obtained by Eisa *et al.*, (1996b) who reported that aflatoxins are widely contaminated the stored and imported corn grains under Egyptian condition and cause a great problems to human and animals. These aflatoxins are mycotoxins produced by mold fungi, belonging essentially to genus *Aspergillus* (*A. parasiticus* and *A. flavus*, etc.). Also, Igawa *et al.* (2007) stated that maize is subject to ear rot caused by toxigenic *Aspergillus* and *Fusarium* species, resulting in contamination with aflatoxins, fumonisins, trichothecenes, and zearalenone.

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فطريات العفن المصاحبة لحبوب الذرة الصفراء المجمعة من محافظات مصر المختلفة وسمومها الفطرية

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*** باحث في شركة مصر بيووير للبيور - مصر.

أكدت تجارب العزل من عينات حبوب ٥ هجن ذرة صفراء مجمعة من محافظات مصر المختلفة تواجد عدد كبير من فطريات العفن مصاحبا لحبوب تلك الهجن. وقد عرفت الفطريات المعزولة بأنها أسبرجلس نيجر، أسبرجلس فلافس، أسبرجلس كلافانتس، أسبرجلس كنديس، أسبرجلس أوكراسيوس، أسبرجلس فرسيكولر، فيوزاريوم مونيليفورم، فيوزاريوم سولاني، هميكولا، ميكور، بنسليوم بالإضافة لمجموعة أخرى من الفطريات التي لم تعرف أو تعرف علي مستوي النوع. وقد سجل أعلى تكرار لفطريات العفن المعزولة علي عينات حبوب محافظتي المنيا والشرقية علي التوالي بينما سجل أقل تكرار للفطريات المعزولة علي عينات الذرة الصفراء المجمعة من محافظتي أسيوط وقنا. كما تباينت فطريات العفن المعزولة في تكرارها علي حبوب الخمس هجن من حبوب الذرة الصفراء المختبرة والمسماه SX-3062, SX-SHAMS, SX-3080, 3WC-352 and SX-30N11 من محافظة إلي أخرى حيث تأثر تواجد تلك الفطريات علي نفس الهجين المختبر بمصدر عينات الحبوب المجمعة. كان واضحا من النتائج سيادة مجموعة الأسبرجلس المعزولة علي حبوب الذرة الصفراء مقارنة بمجموعة الفيوزاريوم والمجموعات الأخرى المعزولة في كل المحافظات الممسوحة. لم يسجل أي تواجد لأي فطر من مجموعة الفيوزاريوم علي عينات حبوب الذرة الصفراء المجمعة من محافظة أسيوط، كما لم يكتشف وجود فطر فيوزاريوم مونيليفورم في كل العينات المختبرة من محافظة قنا. وجدت كل العينات المجمعة لهجن الذرة الصفراء الخمس المختبرة أنها ملوثة بكميات معقولة من الأفلاتوكسينات. وقد سجلت أعلى كمية من الأفلاتوكسينات المقدرة في عينات حبوب الهجين 3WC-352 المجمعة من محافظة المنيا متبوعة بالهجين SX-SHAMS (المنيا) والهجين SX-3080 (الجيزة). وعلي الجانب الآخر كان فطر أسبرجلس فلافس قادرا علي إنتاج الأفلاتوكسينات B1, B2, G1, G2 تحت ظرف المعمل وكانت كميات الأفلاتوكسين B1 أعلى من B2 بينما كان G2 أعلى من G1. كما كان أيضا الفطر أسبرجلس أوكراسيوس قادرا علي إنتاج كمية عالية من سم الأوكراتوكسين بينما استطاع الفطر فيوزاريوم مونيليفورم إنتاج كمية كبيرة من سم الزيرالينون تحت ظروف المعمل.