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Investigation of fungus associated within co-occurrence of aflatoxins and ochratoxin a in cereals from Egypt

Abstract

The present study was prepared to investigate fungi associated within Co-occurrence of aflatoxins (AFs) andochratoxin A (OTA) in some cereals using High Performance Liquid Chromatography (HPLC) to compare the levels of contamination with the Egyptian standard (ES) and European unions (EU) regulation. Sixty cereals samples (white corn, yellow corn, wheat and polished rice) were collected from different localities in Qalubyia governorate. The results indicated that Total Fungal count (TFC) isolated were 535 isolates in non-sterilized cereals and 276 isolated with sterilized cereals. Also data showed that the most isolated fungal species were identified as. *Aspergillus*, *Penicillium*, *Fusarium*, Rhizopus, Alternaria and Cephalosporium Allergy. It was observed that *Aspergillus* spp. (*A. flavus, A. niger and A. parasiticus*) were the most frequent in all samples. When testing the ability of isolated fungi especially *Aspergillus* flavus, *Aspergillus* parasitic us to produce for AFs as well as *Aspergillus niger* and *Aspergillus* ochraceous for OTA. Results clarified 24 isolates of *A. flavus* and10 isolates of *Aspergillus* parasitic us were producers for AFs. On the other hand the obtained results indicated that 17 and 3isolates of *Aspergillus niger* and *Aspergillus* ochraceous were producers for OTA, respectively. The result indicated that the percentages of the incidence of AFs in samples were 60%, 46.6%, 26.6% and 33.3% with white corn, yellow corn, wheat and rice, respectively. Accordingly to results six samples exceeded the maximum levels of AFs set in the EU and ES. While all the rice samples under study were within the permissible limits as recommended by the ES and EU. On the other hand the incidence of OTA in cereals samples are 40%, 26.6%, 20%and 20% of cereals samples (yellow corn, wheat, rice and white corn), respectively. According to obtained results OTA in samples were less than the regulatory limits as recommended by ES and EU.

Keywords: aflatoxins, ochratoxin A, occurrence, fungi and cereals

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Introduction

Cereals are defined as a grain or edible seed of the grass family, Gramineae.[1] These grains composed of the endosperm, germ, and bran. Cereals are grown in greater quantities and provide more food energy worldwide than any other type of crop. In addition to the cereals are a rich source of protein, carbohydrates, vitamins and minerals for the world population. Generally, Cereals are the most important source of human food. The annual world crop of cereals exceeds 2,000 million tones, and the production of cereals is still growing.[2] Cereals and other crops are very susceptible to fungal attacks while in the field or during storage. Depending on environmental conditions and other factors, a fungal attack may result in Mycotoxins contamination of the crop.[3] To date, approximately 300 type of Mycotoxins have been identified, however the most important groups in foods and feed are produced by these three genera: aflatoxins (AFs), produced by some *Aspergillus* species, ochratoxin A (OTA) produced by both *Aspergillus* and *Penicillium*, and fumonisin produced by *Fusarium moniliforme* also one mold species may produce many different Mycotoxins, and several species may produce the same Mycotoxins.[4] AFs have been demonstrated to be carcinogenic, thermogenic, teratogenic, and dermatitis to a wide range of organisms, also cause hepatic carcinoma in humans.[5] On the other hand OTA is a Mycotoxins produced by several fungi, such as *A.ochraceus, A. niger A. carbonarius* or and *Penicillium verrucosum* in food and feed products when optimal temperature and humidity conditions are present in the field or in storage units.[6] OTA has been described as nephrotoxic, carcinogenic, teratogenic, immuno toxic and hepatotoxicity in laboratory and domestic animals, as well as the probable causal agent in the development of nephropathies and urothelial tumors in humans.[7] This study aim to investigate fungi associated within Co- occurrence of AFs and OTA in some cereals using HPLC to compare the levels of contamination by with the Egyptian standard (ES) and European unions (EU) regulation.

Materials and methods

Materials

Cereal samples

Sixty (60) samples of cereals destined for human consumption were investigated. Fifteen samples from each of the (White corn, Yellow corn, Wheat and Polished Rice) were collected from different localities in Qalubyia governorate i.e. Benha, Tukh, Qalube, Shibinelqanater and Elqanater el khireia after the planting season and during the harvest period .The Collected samples were conserved in plastic bags and then stored in a dark and dry place until analysis.

Solvent and chemical

Methanol, acetonitrile, chloroform, sodium sulphate anhydrous, trifluoroacetic acid, sodium hypochlorite (NaClO**)** and sodium chloride, were purchased from Sigma chemical Co.(St. Louis, MO, U.S.A.). All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, M A,

USA).Potato dextrose agar(PDA) and Yeast extract sucrose medium (YES) medium was purchased from Sigma chemical Co. (St Lous, MO, USA). The immune affinity column Aflates® and Ochra test HPLC were obtained from VICAM (Watertown, MA, USA).

Aflatoxins and ochratoxin A standards

AFs and OTA standards were purchased from Sigma, chemical Co. (St. Louis, MO, U.S.A). Stock solutions and standards were prepared and assayed according to AOAC Method 971.22, (2000).[8]

Methods

Isolation and quantification of fungal flora

Potato dextrose agar medium was used for isolation of fungi according to the method described by Harrigan and Margaret.[9] The potato dextrose agar medium (PDA) was dissolved in boiling distilled water previously autoclaved for 15 min at 21ºC also The (PDA) was prepared according to the manufacturer's directions by adding 39g of the dehydrated PDA to 1000 ml of distilled water. The culture media was then mixed and autoclaved at 121ºC for 15 min, 30 ml of the culture media was poured into each Petri dish. Samples were taken for mycological analysis by plating sterilized and un- sterilized cereal grains (corn (white, yellow), wheat, rice) on (PDA) medium.[10] With the help of sterile forceps, the grain of first group were surface sterilized for 5minutes in1% sodium hypochlorite,[11] then rinsed three times with sterilized distilled water. These grains were dried on sterilized filter paper. Second group grains were non- sterilized. Five un –sterilized grains were plated on each plate containing of (PDA) medium amended with antimicrobial agent(50mg streptomycin/L) to suppress growth of bacteria.[12] The plates were incubated at 25±2ºC for 5-7 days after which the numbers of grains showing fungal infection were recorded.

Purification of fungal isolates

Pure cultures of colonies had been obtained by transferring single fungal colony on fresh media plates on potato dextrose agar.[12] Also fungal colonies were sub –cultured on PDA medium.

Identification of Fungal isolates

All fungal isolates were identified at faculty of science Botany Dep. According to Nelson et al.[13] By using light microscope provided with a camera and slides had been continuously observed under various powers of microscope i.e., 10 and40X. based on cultural and morphological characteristics on specific media and available of literature as compared with the description given by Raper and Fennell[14] and Maren and Johan[15] for the genus *Aspergillus*,[13] for *Fusarium*, the isolates of *Penicillium* spp were determined according to Ramirez[16] and Pitt[17] and Barnett and Hunter[18] for the genera of imperfect fungi, All developing fungi were cultured on PDA slants then stored in a refrigerator for further use.

The frequency of fungi and relative percentage of particular species with in a genus of fungi was calculated using the formula of Ghiasian et al.[19]

$$\text{Frequency}(\%) = \frac{\text{Number of samples infected with fungi}}{\text{Total Number of samples analysis}} X100$$

$$\text{Relative percentage}(\%) = \frac{\text{Number of fungal species isolated}}{\text{Total Number of Fungi}} X100$$

Toxicity of some isolated fungi

*Aspergillus*spp (*A. parasitic sand A. flavus*) were tested for their ability to produce A Fsand (*A ochraceous and A niger*) tested for their ability to produce OTA by YES broth for 14 days at 28˚C according to Ling et al.[20] After incubation period, AFs and OTA were extracted from filtrated medium according to the method described by El Banna et al.

Detection and Determination of AFs and OTA in collected samples

AFs and OTA were extracted and cleaned up using the method described in AOAC[8] using the Immunoffinity column **(**Afla Test ®-P affinity column and Ochra Test ®-p affinity column).

Determination of AFs by HPLC

Derivatization: the derivatives of samples and standard were done as follow100µl of trifluoroacetic acid (TFA) was added to samples and mixed well for 30 s and the mixture stand for15 min. 900µl of water: acetonitrile (9:1 v/v) were added and mixed well by vortex for 30 s .and the mixture was used for HPLC analysis . In this step of reconstitution of the dry film, AFB_1 and AFG_1 were converted into other derivatives, AFB2a and AFG2a, respectively (AFG_1 and AFB_1had low fluorescence properties therefore, they were converted to G2a and B2a, which had high fluorescence properties, using (TFA).

HPLC conditions: The mobile phase consists of Acetonitrile/ Water/ methanol (40:240:120 V/V/V) was used. 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 excitation wavelength of 365 nm and an emission wavelength of 450 nm. AFs concentration in samples was determined from the standard curve, using peak area for quantitation. High Performance Liquid Chromatography (HPLC)system (Waters) equipped with model 600 delivery system (Water), and the data were integrated and recorded by millennium chromatography manger software 210 (Waters, Milford MA 0175). Reverse phase hyper clone 5µ ODS C_{18} column (2.5mm X 30cm). The High Performance Liquid Chromatography (HPLC) system consisted of Waters Binary pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Watres 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze 2.A phenomena C_{18} (250x 4.6 mmi.d), 5 um from Waters corporation (USA). On the other hand OTA determined using HPLC analysis according.[21]

Results and discussion

The results recorded in Tables 1&2 revealed that the mycoflora contaminated samples were grown on PDA disinfected and non-disinfected grains. The Total Fungal count (TFC) isolated were 535 isolates in non-sterilized grains and 276 isolated with sterilized cereals. Also data showed that the most isolated fungal species were identified as. *Aspergillus, Pencillium, Fusarium, Rhizopus, Alternaria* and *Cephalosporium roseogrisum*. It was observed that *Aspergillus* spp. (*A. flavus, A. niger and A. parasiticus*) were the most frequent in all samples. We observed *Aspergillus*spp was the most frequent and counted *A. flavus* (79) and *A. niger* (43) in surface sterilized cereals while non–sterilized cereals *A. flavus* (135) and *A. niger* (125).The surface sterilization by sodium hypochlorite (NaOCl) leads to decrease in total number of fungi as *A. flavus, Fusarium* spp. However it didn't kill *Alternaria alternate* Ramakrishna et al.,[22] also Nwangburuka et

al.[23] reported that sodium hypochlorite pretreatment at concentration 4%and 6%inhibited the population of fungal growth**.** The results of total fungal frequency isolated from non – sterilized cereals given in Figure 1 revealed that white corn samples were found to be infected with fungi the highly frequent species were *A. flavus* (93.33)%, *A. niger* (73.33)% *pencillium* spp. (73.33)%, *Fusarium* spp.(60)% and *A. parasiticus* (20)% of the total fungi on PDA medium However yellow corn samples the highly frequent species were *A. niger* (93.33)%, *A. flavus* (86.66)%, *pencillium spp*. (66.66)%, *A. parasiticus* (60)%, *fusarium spp.*(46.66)%, *R. nigricans* (40)%, and *A. ochraceous* (26.66)% of the total isolated fungi, while in Wheat samples the highly frequent species were *A. flavus* (86.66)%, *Penicillium* spp.(73.33)%, *A. niger* (53.33)%, *A. parasitic us* (33.33)% *fusarium spp.*(33.33)% and *Alternaria (*46.66)%,although the highly frequent species were *A. niger* (100)%, *A. flavus* (73.33)%, *pencillium spp.* (66.66) %, *Fusarium* spp.(33.33)%, *A. parasiticus* (26.66)%, *Alternaria* (40)% and *A. ochraceous* (6.66)% of the total fungi on PDA medium in un sterilized cereals. The frequency isolated from sterilized cereals given Figure 2 white corn samples were found to be infected with fungi the highly frequent species were *A. flavus* (80)%, *A. niger* (40)% *Penicillium spp*. (40)%, *Fusarium* spp.(26.66)% and *A. parasitic us* (13.33)% of the total fungi on PDA medium in sterilized cereals. However yellow corn samples the highly frequent species were *A. flavus* (73.33)%, *A. parasitic us* (66.66), *A. niger* (53.33)%, *Penicillium spp.* (53.33)%, *R. nigricans* (40)%, *Fusarium* spp.(20)%, and *A. ochraceous* (20)% of the total fungi in sterilized cereals, while in Wheat samples the highly frequent species weres *A. flavus* (53.33)%, *Penicillium spp*. (53.33)%, *A. parasiticus* (33.33)%, *Alternaria* (33.33)%, *A. niger* (26.66)% and *Fusarium* spp.(26.66)%, and of the total fungi on PDA medium in sterilized cereals but in rice samples the highly frequent species were *A. flavus* (46.66)%, *Penicillium spp*. (46.66)%, *A. niger* (40)%, *A. parasitic us* (40)%, *Fusarium* spp.(13.33)%, *Alternaria* (20)% and *A. ochraceous* (6.66)% of the total fungi on PDA medium in sterilized cereals. All cereal samples under study contaminated with fungi due to cereals contain high starch, fiber, nutritious protein and lipids rich in essential fatty acids.[24] Among the presented fungi *A. flavus, A. niger, Penicillium*spp. and *Fusarium*spp. Were the most common fungi in the investigated samples these results are in agreement with Bandara et al.[25] and Dabassa.[26] Protein, fiber and fat in rice enhance fungal growth and toxin production. The highest counts of fungi were present in corn and rice grains while the lower numbers were found in wheat. Different fungi were isolated with different frequencies and percentages, this according to the food stuff and the fungus. Generally corn showed more contamination with fungi because of corn harvested and stored under humid and warm climatic conditions and because of the growth of many fungi and pests results in rapid deterioration of the cereals, also fungal growth of corn is mainly affected by moisture content, temperature, relative humidity, storage conditions and insect pests. In addition level of contamination depends on the type of cereal grain, The differences in nutritional composition of the grain, that vary according to the type of cereals in which yellow corn samples were more susceptible to fungal attack than white corn due to the white corn endosperm being harder than that of yellow corn, which results in less fungal infection of the endosperm Suleiman et al.,[27] Pearson et al.[28] The total fungal populations of grains were increased significantly by increasing the storage period Shahin et al.[29] Fungal growth elevates respiration that releases heat and moisture into the surrounding environment in the stored grain mass. The increased moisture content and temperature of the surrounding of corn results in a hot spot of increasing moldy grain. In Egypt the weather give chance for the growth of *Aspergillus* species and other fungi on grains due to it characterize by high temperature and high relative humidity. Also Contamination occurs through small amounts of spores contaminating the grain as it is going into storage from the harvest in handling and storage equipment or from spores already present in storage structures. Mycotoxigenic fungi can infect rice samples due to rice is an aquatic plant usually harvested at very high moisture levels, between 35 and 50%.[30] As a result, Mycotoxins-producing fungi or spores contaminate rice grains in the field and during harvest. Also Mycotoxins will be produced if environmental conditions are favorable.[31] In addition that rice cultivation is usually conducted in subtropical environments, which are characteristically warm and humid. Rice is generally dried after harvesting, due to inappropriate storage conditions, rice can be an ideal substrate for Mycotoxins-producing fungi. Mycotoxins contamination is a major problem in the tropics and sub-tropics areas, where climatic conditions and storage practices are favorable to fungal growth and toxin production.[5] However wheat grains cultivated in winter season (November/December) and harvested in summer which enhanced the chances of pre-harvest contamination. Also the farmers have used old traditional farming practices which can enhance fungal infections. *Aspergillus* species is favored by high temperatures and dry conditions. And also *Aspergillus* ear rot is typically associated with drought stress, and Fungi were noticed due to mechanically damaged seed. Low frequency of *Fusarium* spp. in cereals due to *fusarium* favor low temperature and appear in cooling region and the most important toxigenic fungi occurring in the moderate climatic regions of North America and Europe were *Fusarium* fungi.[32]

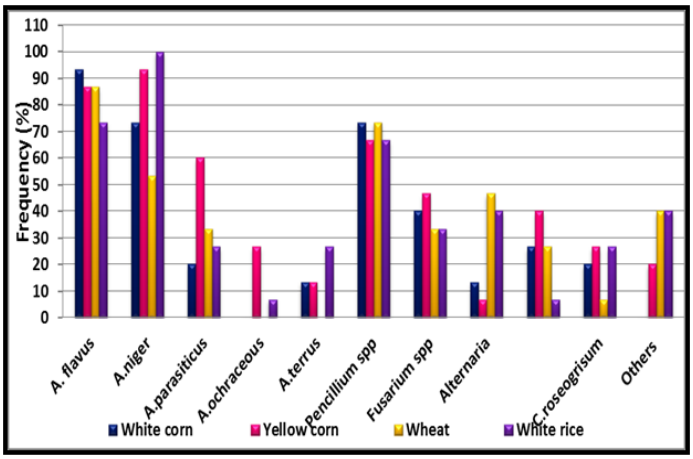

Figure 1 The frequency of fungi from unsterilized cereals samples.

Determination of the toxicity of some isolates to produce of AFs and OTA

All fungal genera i.e. *Aspergillus* group i.e. A. *flavus*, A. *parasiticus*, A. *niger* and A. *ochraceous* which isolated from cereals surface sterilized samples were tested for production AFs and OTA using HPLC. Results in Tables 3&4 clarify 6 isolates of *A. flavus* were producers for AFs in white corn. In case yellow corn 10 isolates of *A. flavus* and one isolates of *A. parasiticus* were produced for AFs. On the other hand results indicated that 4isolates of *A. flavus* in wheat samples produced for AFs. However in rice samples there were 4 isolates of *A. flavus* and 7 isolates of *A. parasitic s*had detectable levels of AFs. In the same context, the ability of fungi A. *niger* and *A. ochraceous* to produce OTA was determined. The results presented in Table 5 explain 4 isolates of *A. niger* in case white corn were producers for OTA. However no isolates of *A. ochraceous* were isolated in white corn. OTA were detected in only 5 isolates of *A. niger* and 2 isolates of *A. ochraceous* of yellow corn. While only 2 isolates of *A. niger* in wheat samples were producers for toxin. Although in rice samples 6 isolates of *A. niger* and one (1) isolates of *A. ochraceous* had detectable levels of

OTA. The variations in Toxin Type among aflatoxigenic fungi this due to metabolic behavior of strains according to the molecular genetics and phylogenetic relationships *A. flavus* differ from *A. parasitic us* by loss of a portion of the gene, aflU (cypA), involved in G type AFs production as well as *A. flavus* and *A. parasiticus* group forms a polyphyletic assemblage containing isolates of different morph types and having the ability to produce AFs.[33] The production of AFs by *A. flavus, A. parasitic us* dependon possession of AFs genes, the isolates varied widely Due to prescience of seven toxigenic gene (aflR, aflS, aflQ, aflP, aflD, aflM, and aflO) The isolates of toxigenic *A. flavus* possessed at least 5 (out of 7) aflatoxigenic genes ofall aflatoxigenic genes were not detected in most of the isolates there were variation in AFs type.[34] In *A. flavus* and *A. parasitic us*, AFs pathway genes are clustered within a 75-kb region of the fungal genome on chromosome III.[35,36] AF biosynthesis is coded by a 80 kb long DNA sequence. As a cluster containing 30 putative genes characterized in both *A. flavus* and *A. parasiticus*. On the other hand *A. niger* plays an important role in OTA production due to it contain the polypeptide synthase gene which it plays an important role in the biosynthesis of OTA, in the pathway prior to the phenylalanine ligation step**.** The application of PCR assays for detection of ochre toxigenic fungi by targeting the metabolic pathway genes Polyketide Synthase (pks) specific to toxin chemo type. A single fragment of about 549 bp was produced with all positive ochratoxigenic *A. Ochraceus* and *A. niger* while No product was observed with genomic DNA from all negative ochratoxigenic isolates of *A. ochraceus* and *A. niger*.[37,38]

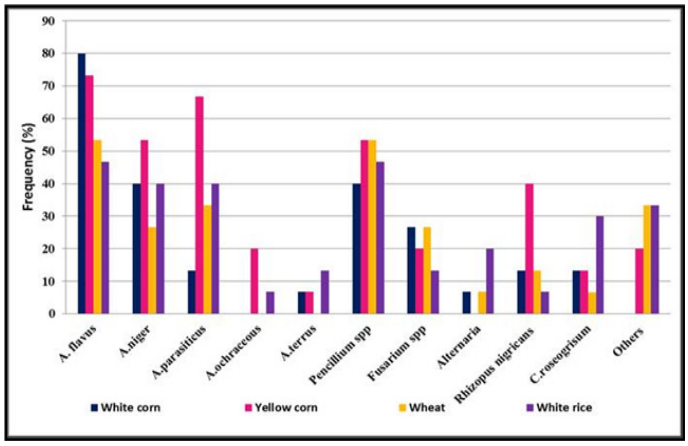

Figure 2 The frequency of fungi from sterilized cereals samples by 1% NaOCl.

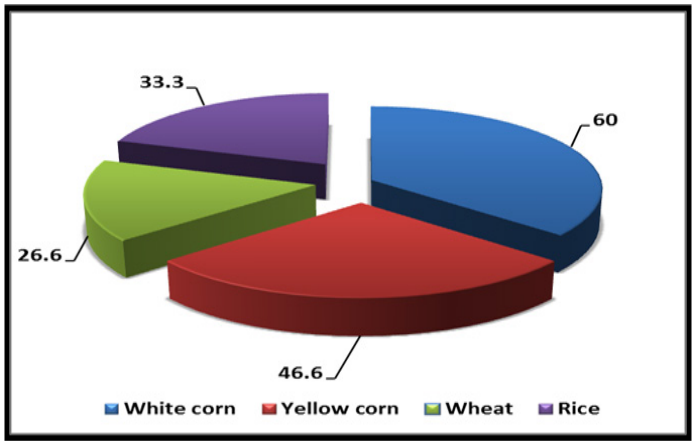

Figure 3 Percentages of the incidence of AFs in cereals samples.

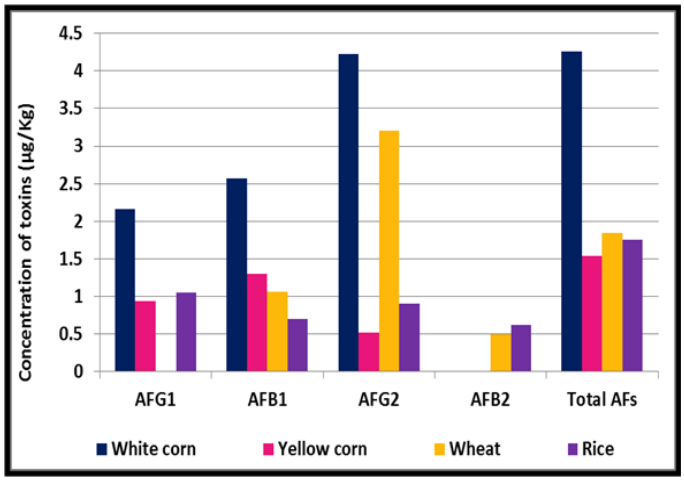

Figure 4 Mean concentrations of AFs in cereals samples.

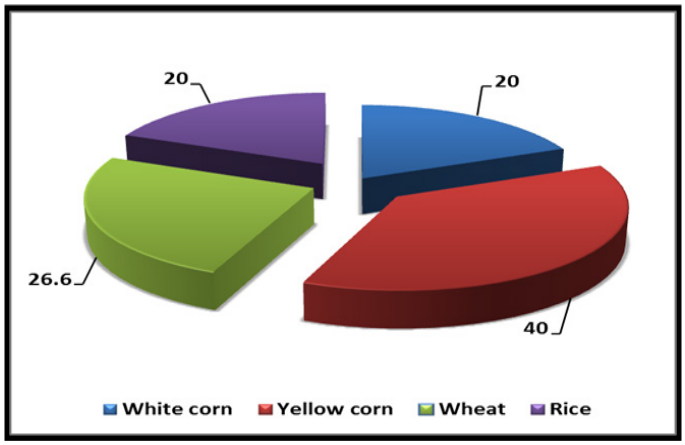

Figure 5 Percentages of the incidence of OTA in cereals samples.

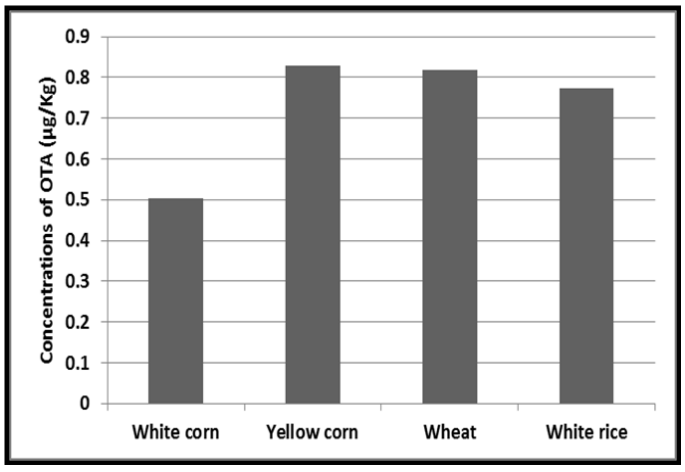

Figure 6 Mean concentrations of OTA in cereals samples.

Co- occurrence of AFs and OTA in samples cereals

This study aimed to investigate sixty samples of cereals for Co-occurrence of AFs and OTA using HPLC to compare the levels of contamination by with the Egyptian standard (ES) and European unions (EU) regulation. Occurrence of AFs in yellow corn, white corn, wheat and rice shown in Figure 3 Showed the result indicated

that the percentages of the incidence of AFs in samples were 60%, 46.6%, 26.6% and 33.3% in white corn, yellow corn, wheat and rice, respectively. The maximum level of AFs and AFB_1 in cereals according to (EU) and (ES) were 4 and 2μg/kg, respectively. Accordingly, four samples with white corn exceeded the maximum levels set in the EU; having AFB_1 concentrations of 3.75, 3.21, 2.89 and 2.35μg/kg. In addition the total of AFs in these four samples was 7.85, 6.96, 4.39 and 4.3μg/kg. Also when compared with maximum limits they found that one samples with yellow corn and wheat samples was exceeded the maximum levels in case of yellow corn the AFB_1 was 4.40μg/kg, total AFs 4.94μg/kg. On the other hand the wheat sample AFB_1 was 2.025 μg/kg and AFs 5.48μg/kg. While all the rice samples under study were within the permissible limits as recommended by the ES and EU as shown Figure 4. On the other hand the incidence of OTA in cereals samples are 40%, 26.6%, 20%and 20% of cereals samples (yellow corn, wheat, rice and white corn), respectively (Figure 5). The results have shown that yellow corn samples found to be highly contaminated with OTA with average mean 0.828 (μg/Kg) followed by wheat samples with average mean 0.817(μg/Kg) and rice samples with average mean 0.773(μg/Kg) while white corn have shown least contamination with OTA (Figure 6). According to obtained results OTA in samples were less than the regulatory limits as recommended by ES and EU of 5 (μg/Kg). Level contamination often varied considerably depending on the substrate or media where the species or strains grow. Moreover, the subtropical climate of Egypt is conducive to fungus growth, resulting in contamination of grains during pre- and post-harvest stages. Fungal spores may infect grains in the field and at harvest. Infected grain may later contaminate those products already in storage leading to contamination. Occurrences of AFs and OTA in cereals samples may be due to less awareness towards good agricultural practices as well as the climatic conditions of Egypt are more favorable for fungal growth either in field or during storage. Therefore, it is necessary to create awareness in farmers towards OTA or AFs producing fungi along with proper handling and storage of grains so that exposure risk to OTA can be minimized at large. Cereals and other crops are exposed to fungal attack in the field (pre-harvest) or during storage and this would result in the production of AFs. Also the climatic and storage conditions practices also play important role in fungal attack and Mycotoxins production.[39] Due to improper storage conditions in urban areas of Egypt. It also has been reported that increased AFs formation was registered by heavy rains during the storage, by delayed storage and high moisture contents.[5] Tirado et al.[40] reported that the AFsare expected to become more prevalent with climate change in countries with temperate climate which have not faced with this problem before. Finally the differences in the concentrations of AFs in samples cereal may be due to origin and year of harvest.[41–44]

Table 1 Fungal contamination levels in unsterilized cereals

Name of the fungi	Cereals (15 samples from each of grain type)*											
	White corn			Yellow corn			Wheat			White rice		
	No. of samples infected	No. of isolates	R.P (%)	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P
A. flavus	14	43	31.6	13	39	25.3	13	32	28.6	11	21	15.8
A.niger	11	33	24.3	14	35	22.7	8	17	15.2	15	40	30.1
A.parasiticus	3	7	5.1	9	12	7.8	5	7	6.2	4	8	6.0
A.ochraceous	0	0	0	4	6	3.8	0	0	0	1	1	0.75
A.terrus	2	3	2.2	2	2	1.3	0	0	0	4	4	3.0
Pencilliumspp.	11	30	22.1	10	22	14.3	11	14	12.5	10	16	12.0
Fusariumspp.	9	8	5.9	7	13	8.5	5	7	6.2	5	6	4.5
Alternaria	2	2	1.5	1	1	0.6	7	11	9.8	6	8	6.0
R.nigricans	4	6	4.4	6	8	5.2	4	6	5.4	1	3	2.2
C.roseogrisum	3	4	2.9	4	7	4.5	1	1	0.9	4	5	3.8
Others	0	0	0	3	9	5.8	6	17	15.2	6	21	15.8
Total fungi count /5seeds	**136**			**154**			**112**			**133**		

*using PDA media RP, relative percentage; Relative percentage (%) = (Number of fungal species isolated / Total Number of fungi isolated) x 100

Table 2 Fungal contamination levels in sterilized cereals by 1% NaOCl

Name of the fungi	Cereals (15 samples from each of grain type)*											
	White corn			Yellow corn			Wheat			White rice		
	No. of samples infected	No. of isolates	R.P (%)	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P
A. flavus	12	27	45.0	11	29	32.9	8	11	20.4	7	12	16.2
A.niger	6	11	18.3	8	14	15.9	4	4	7.5	6	14	18.9
A.parasiticus	2	5	8.3	10	12	13.6	5	7	12.9	6	12	16.2

Table Continued

Name of the fungi	Cereals (15 samples from each of grain type)*											
	White corn			Yellow corn			Wheat			White rice		
	No. of samples infected	No. of isolates	R.P (%)	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P	No. of samples infected	No. of isolates	R.P
A.ochraceous	0	0	0	3	3	3.4	0	0	0	1	1	1.3
A.terrus	1	1	1.77	1	1	1.1	0	0	0	2	2	2.7
Pencilliumspp.	6	6	10.0	8	10	11.4	8	9	16.6	7	11	14.9
Fusariumspp.	4	4	6.7	3	4	4.5	4	5	9.2	2	2	2.7
Alternaria	1	1	1.7	0	0	0	5	6	11.1	3	3	4.1
R. nigricans	2	3	5.0	6	7	7.9	2	4	7.4	1	3	4.1
C.roseogrisum	2	2	3.3	2	2	2.3	1	1	1.8	3	3	4.1
Others	0	0	0.0	3	6	6.8	5	7	12.9	5	11	14.
Total fungi count /5seeds	**60**			**88**			**54**			**74**		

*using PDA media R.P, relative percentage; Relative percentage (%) = (Number of fungal species isolated / Total Number of fungi isolated) x 100

Table 3 Toxicity of *A. flavus* isolated from cereal samples

Cereal Types	Numbers	Concentration of AFs (ng/ml)							
		AFG1		AFB1		AFG2		AFB2	
		Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
White corn	27(6)*	1.77	0.715	28.9	0.26	1.35	0.28	2.4	0.36
Yellow corn	29(10)*	2	0.172	38.88	0.33	0.11	0.026	5.64	0.037
Wheat	11(4)*	5.15	1.31	8.13	1.59	2.79	0.65	3.75	0.59
Rice	12(4)*	0.381	0.105	16.67	1.56	0	0	0.197	

*: Number of +ve samples.

Table 4 Toxicity of *A. parasiticus*isolated from cereal samples

Cereal Types	Numbers	Concentration of AFs (ng/ml)							
		AFG_1		AFB_1		AFG_2		AFB_2	
		Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
White corn	5(2)*	5.1	1.7	9.52	1.5	2.4	0.31	2.87	0.73
Yellow corn	12(1)*	0.667		4.34		0.01		0.283	
Wheat	7(0)*	0	0	0	0	0	0	0	0
Rice	12(7)*	1.67	0.127	71.11	3.33	3.34	0.13	6.25	0.404

*: Number of +ve samples.

Table 5 Toxicity of ochratoxigenic fungi isolated from cereals samples

Cereal Types	Numbers		Concentration of OTA(ng/ml)			
			OTA produced by. *A.niger*		OTA produced by *A. Ochraceous*	
	A. niger	*A. Ochraceous*	Max.	Min.	Max.	Min.
White corn	11(4)*	0	4.36	2.61		
Yellow corn	14(5)*	3(2)*	3.77	1.59	10.1	6.78
Wheat	4(2)*	0	7.31	3.26		
Rice	14(6)*	1(1)*	4.38	0.07	5.71	

*: Number of +ve samples.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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