Pak. j. life soc. Sci. (2011), 9(1): 1-6

Pakistan Journal of
Life and Social Sciences

Natural Occurrence of Mycotoxins in Corn Grains and Some Corn Products

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Abstract

The present study aimed to make a survey on natural occurrence of such mycotoxins in local and imported corn grains and some corn products stored in El-Minufiya Governorate, Egypt. These results could be summarized: during winter season of 2004, out of 20 local corn grain, there were 2 samples by an average of 10%, and the same (10%) in imported ones contained Aflatoxins. Also, during summer, there were (10%) of local corn grains and (10%) of imported ones contained Aflatoxins. Regarding the occurrence of Ochratoxin A, during winter season, out of 40 local and imported corn grains, 2 positive samples for Ochratoxin A (5%). In summer, 3 positive samples for Ochratoxin A, (7.5%) in local and imported corn. The occurrence of Zearalenone, in winter season, 2 positive samples out of 40 local and imported corns with (5%). However, during summer, 3 positive samples, (7.5%) in local and imported corn grains. Finally It could not found any mycotoxins in local and imported corn groats samples, while, out of 15 of corn products (snacks, cornflakes and popcorn), only one sample of cornflakes contained Aflatoxins B_1 and B_2 at concentrations of 3.4 and 2.7 µg/kg cornflakes, respectively.

Key words: Corn grains, Mycotoxins, Corn products, Aflatoxins, Ochratoxin A, Zearalenone

Introduction

Corn and some other cereals an excellent media for fungi growth and mycotoxin contamination which attack either the plant in field or in cereal grains during the storage (pre- or post-harvested contaminations). Some of these pathogenic fungi produce mycotoxins which are considered secondary metabolites and it can cause diseases for both human and animals (FAO, 1999). Mycotoxins which are toxic secondary metabolites produced by certain genera of fungi.

The disease which is caused by these mycotoxins knows as mycotoxicoses while the infection due to the fungi for human or animals know as mycoses. Aflatoxins, Ochratoxins, Zearalenone and Fumonisins are the most important mycotoxins among the others, because they are more toxic and carcinogenic for animals and more dangerous for human health, (FAO, 2001). So, this study aimed to make a survey on natural occurrence of some mycotoxins (Aflatoxins, Ochratoxin A and Zearalenone) in local and imported corn grains and some corn products (corn flour, snacks, cornflakes and popcorn) stored in El-Minufiya Governorate, Egypt.

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Materials and Methods

Materials

The imported and local corn grains (*Zea mays* L.), total of 40 samples were collected from some local shops in El-Minufiya Governorate namely Mnuf, Quisna, Shibin El-Kom and Tala during winter and summer seasons of 2004, 10 samples from each city, all samples were collected randomly about 1 Kg.

Corn groats 15 samples as raw materials were obtained from the local factories which produced the snacks .The samples of corn products were 15, namely corn flour, snacks, cornflakes and popcorn, 5 samples for each products were purchased from many supermarkets at El-Minufiya Governorate.

Determination of Certain Mycotoxins in Corn Grains

Aflatoxins, Ochratoxin A and Zearalenone were determined in corn grains samples according to the method described by Gallagher and Latch (1977) and we used multi-technique accordance A.O.A.C (1995).

Extraction of Mycotoxins

One kg of each corn grain samples was ground, 100 g of ground samples were blended with 200 ml of methanol: water (8: 2 v/v) for 3 min., at a high speed. Then, filtrate through Whatman No. 2 filter paper, 50 ml of the filtrate were added to 50 ml of a clean up solution which contains of 150 g zinc sulphate, and 50 g phosphotungestic acid, in one litter of distilled water in a 250 ml beaker and mixed using

glass rod for 10 min. The mixture was filtered through Whatman No. 4 filter paper and 75 ml of clear filtrate were shaked with 15 ml of benzene for one minute. Layer of benzene was received into a small vial and evaporated to dryness and the residue was transferred to vial and evaporated off using a stream of nitrogen at a temperature below 60°C. The dry film was used for the determinations of mycotoxins by thin layer chromatography (TLC).

Preparation of Mycotoxins Standards (Aflatoxins B_1, B_2, G_1 and G2, Ochratoxin A and Zearalenone)

Aflatoxins Standard

The preparation of Aflatoxins standard was carried out according to the methods described in **A.O.A.C.** (1995). The crystals of Aflatoxins B_1, B_2, G_1 and G_2 were diluted by using benzene-acetonitrile (98: 2 v/v) to obtain a concentration of10 µg/ml (stock solution). The concentration of the prepared solution was adjusted by using the spectrophotometer at wavelength of the maximum absorption closed to 350 nm and using the following equation:

$$\mu g \text{ Aflatoxins/ml} = \frac{A \times MW \times 1000 \times CF}{E}$$

Where:

A = Absorbency at the wavelength of the maximum absorption.

MW = Molecular weight of Aflatoxins B_1, B_2, G_1 and G_2.

CF = Correction factor for particular instrument and cells.

E = Absorbability of Aflatoxins in benzene-acetonitrile (98: 2 v/v).

The suitable concentration which used for TLC was 0.5 µg Aflatoxins /ml

Ochratoxin A and Zearalenone Standards

Ochratoxin A and Zearalenone were dissolved in benzene-acetic acid (99: 1 v/v) to obtain the requested concentration by using spectrophotometer at a wavelength of maximum absorption closed to 317 nm and the previous equation was also used. The suitable concentration was 5 µg/ml of solvent.

The Quantitative Assay

The quantitative assay was carried out according to the methods mentioned in A.O.A.C. (1995). The previously obtained extract was dissolved in 200 ml benzene-acetic acid (99: 1 v/v). Twenty µl from the extract of each standard of Aflatoxins (B_1, B_2, G_1 and G_2), Ochratoxin A and Zearalenone at different concentrations were spotted on TLC plate with the spots of samples . The intensities of fluorescence of all mycotoxins spots were estimated by using a Fluorodensitometer (Densitometer-TLC-100 Vitatron). The amounts of all mycotoxins were determined by comparing the densitometer reading of each sample with those of standard solutions using the following equation:

$$\mu g/kg = \frac{S.Y.V.}{X.W}$$

Where: S = µl of mycotoxin standard equal to unknown.

Y = Concentration of standard mycotoxin in µg/ml.

V = Dilution factor (µl).

X = µl of sample extract giving fluorescent intensity equal to standard.

W = Weight in g. of the original sample found in the final extract.

Results and Discussions

Natural Occurrence of Some Mycotoxins in Local and Imported Corn Grains: 1.Natural Occurrence of Aflatoxins

Results showed the natural occurrence of Aflatoxins in local and imported corn grains in winter season of 2004 (Table 1). The results indicated that two samples out of 20 samples were positive for Aflatoxins B_1 and B2 in local corn grains achieved from Quisna and Tala shops. Two samples out of 20 were positive for Aflatoxins, one sample from Shibin El-Kom contained Aflatoxins B_1 and B2, and one sample from Tala with Aflatoxins G_1 and G2 in the imported corn grains. This represents 10% Aflatoxin positive samples for both local and imported corn grains. The concentrations of Aflatoxin B_1 and B2 were ranged from 5.8 to 7.5 and 2.7 to 4.1µg/kg, respectively in the local and imported corn grains, meanwhile in the imported ones the concentrations of Aflatoxin G_1 and G_2 were 8.1 and 3.2 µg/kg, respectively.

The occurrence of Aflatoxins in local and imported corn grain samples in summer season of 2004 (Table 2). The results demonstrated that two out of 20 local corn samples were positive for Aflatoxins, where one sample from Mnuf contained aflatoxins B_1 and B2, and one sample from Shibin El-Kom with Aflatoxins G_1 and G2 in the local corn grains, with concentrations of 5.3 & 4.2 Aflatoxins B_1 and B2 µg/kg and 4.6 & 2.9 Aflatoxins G_1 and G2 µg/kg corn grain), respectively. While in imported corn grain samples, it was found two positive samples out of 20, with 10 % were positive for Aflatoxin B_1 and $B_2$with concentrations at 6.2 to 7.3 and 2.5 to 3.9 µg/kg of corn grains, respectively in shops from Mnuf and Tala towns.

These results are in a harmony with those recorded by several researchers, Atwa (1997) found that the levels of Aflatoxins B1, B2, G1 and G2 in the tested corn samples which were incident 33.3, 0.0, 40 and 10% for the samples collected from different countries namely USA, Canada, Australia and Egypt,

Table 1 Survey of Aflatoxins in local and imported corn grains obtained from El-Minufiya Governorate during winter season of, 2004.

Sources of corn grains	No. of positive samples *	% positive samples	Aflatoxins concentration µg/kg			
			AFB_1	AFB_2	AFG_1	AFG_2
Local						
Mnuf	0.0	0.0	0.0	0.0	0.0	0.0
Quisna	1.0	20	5.8	3.6	0.0	0.0
Shibin El-Kom	0.0	0.0	0.0	0.0	0.0	0.0
Tala	1.0	20	7.5	4.1	0.0	0.0
Imported						
Mnuf	0.0	0.0	0.0	0.0	0.0	0.0
Quisna	0.0	0.0	0.0	0.0	0.0	0.0
Shibin El-Kom	1.0	20	6.9	2.7	0.0	0.0
Tala	1.0	20	0.0	0.0	8.1	3.2

*Aflatoxins determination was carried out in 5 corn grains samples from each location.

Table 2 Survey of Aflatoxins in local and imported corn grains obtained from El-Minufiya Governorate during summer season of, 2004.

Sources of corn grains	No. of positive samples *	% positive samples	Aflatoxins concentration µg/kg			
			AFB_1	AFB_2	AFG_1	AFG_2
Local						
Mnuf	1.0	20	5.3	4.2	0.0	0.0
Quisna	0.0	0.0	0.0	0.0	0.0	0.0
Shibin El-Kom	1.0	20	0.0	0.0	4.6	2.9
Tala	0.0	0.0	0.0	0.0	0.0	0.0
Imported						
Mnuf	1.0	20	7.3	2.5	0.0	0.0
Quisna	0.0	0.0	0.0	0.0	0.0	0.0
Shibin El-Kom	0.0	0.0	0.0	0.0	0.0	0.0
Tala	1.0	20	6.2	3.9	0.0	0.0

*Aflatoxins determination was carried out in 5 corn grains samples from each location.

Table 3 Survey of Ochratoxin A in local and imported corn grains obtained from El-Minufiya Governorate during winter and summer seasons of, 2004.

Sources of corn grains	Winter season			Summer season		
	No. of positive samples *	% of positive samples	Conc. of Ochratoxin A µg/kg	No. of positive samples	% of positive samples	Conc. of Ochratoxin A µg/kg
Local						
Mnuf	0.0	0.0	0.0	0.0	0.0	0.0
Quisna	1.0	20	11.9	1.0	20	15
Shibin El-Kom	1.0	20	14.3	0.0	0.0	0.0
Tala	0.0	0.0	0.0	0.0	0.0	0.0
Imported						
Mnuf	0.0	0.0	0.0	0.0	0.0	0.0
Quisna	0.0	0.0	0.0	0.0	0.0	0.0
Shibin El-Kom	0.0	0.0	0.0	1.0	20	10.5
Tala	0.0	0.0	0.0	1.0	20	9.3

*The total number of samples taken was 5 either in winter or in summer season from each location.

respectively. Also, Rasti et al. (2000) determined the level of contamination in imported and native corn [maize] with Aflatoxin B1 in central feed silos of Isfahan in Iran. The results showed that most of the samples were contaminated with Aflatoxin B1with a range of 0.0 to 9.9 µg/kg, and an average of 5.1 µg/kg, which was much lower than the tolerance level. In this regard, Escobar and Sanchez-Regueiro (2002) found that 17.04 % of a total 4594 analyzed samples presented Aflatoxin B1, and the biggest

percentages were in sorghum and groundnut with 83.3 and 40.4%, respectively. Maize, oat, wheat, and soya are fundamental raw ingredients in the elaboration of concentrates. Percentages of contamination with Aflatoxin B1 of 23.3, 10.7, 25, and 4.6 were found in maize, oat, wheat, and soya, respectively.

Natural Occurrence of Ochratoxin A

Data showed the occurrence of Ochratoxin A in corn grains stored during winter and summer seasons of, 2004 (Table 3). These results indicated that, in local corn grains, it was found only 3 positive samples for Ochratoxin A out of 40 local corn grain samples. These 3 positive samples were from the shops at Quisna and Shibin El-Kom, where two samples in winter and one sample in summer season, and induced a concentration of Ochratoxin A at a range of 11.9 to 15 µg/kg corn grains. However, two samples from 40 samples were positive to Ochratoxin A in imported corn grains during summer season. The imported corn gave two positive samples with a concentration of 9.3 and 10.5 µg of Ochratoxin A per/ kg of corn grains in the shops of Shibin El-Kom and Tala.

These results agree with those of Hegazy (2000), who found that the highest Ochratoxin A contamination was observed in peanuts collected from Bolak El-Dakrour, which recorded 27.77 and 20.04 µg/kg for unsalted and salted peanuts, respectively. Also, Qahtan (2002) and Ebba (2003) cited approximately the same results.

Natural Occurrence of Zearalenone

Results demonstrated occurrence of Zearalenone in local and imported corn grain samples collected during winter and summer seasons of 2004, from different shops in El-Minufiya Governorate (Table 4).

The results indicated that, local corn grain samples gave only 3 positive samples out of 40 samples; i.e., 7.5 %, whereas imported corn grain samples showed only 2 positive sample; i.e., 5 %. The total positive samples were 5 out of 80, with means of 6.25 % with concentrations of ZEA ranging form 5.7 to 9.4 and 7.4 to 8.7 µg of Zearalenone per kg of corn grains in local and imported corn grains, respectively.

These results sustained those of Silva and Vargas (2001), who carried out a survey of Zearalenone (ZEA) in corn grains obtained from various regions of Brazil, and they analyzed 380 corn samples, where 30 samples 7.8%, were found to be contaminated with ZEA at a range of 46.7 to 719 µg/kg. Study on occurrence of Aflatoxins, i.e., Ochratoxin A and Zearalenone in local and imported corn samples collected from Mnuf, Quisna, Shibin El-Kom, and Tala towns at El-Minufiya Governorate are shown in (Fig. 1 and 2).

Natural Occurrence of Mycotoxins in Corn Products

A survey of mycotoxins Aflatoxins B1, B2, G1and G2, Ochratoxin A and Zearalenone, in some corn products carried out, These results demonstrated that it didn't find any mycotoxin in raw materials (local and imported corn groats) which used for snacks production (because it is free from fats) and collected from local factories in El-Minufiya Governorate. Concerning corn products (snacks, cornflakes and popcorn), it didn't record any mycotoxins in snacks and popcorn, meanwhile only one sample of cornflakes out of 5 samples; i.e., 20 % contained Aflatoxins B1and B2 at low concentrations of 3.2 and 2.7 µg/kg of corn flacks, respectively .The samples of raw material (corn groats) and corn products which collected from El-Minufiya Governorate were 5 samples for each product.

Table 4 Survey of Zearalenone in local and imported corn grains obtained from El-Minufiya Governorate during winter and summer seasons of, 2004.

Sources of corn grains	Winter season			Summer season		
	No. of positive samples *	% of positive samples	Conc. of Zearalenone µg/kg	No. of positive samples	% of positive samples	Conc. of Zearalenone µg/kg
Local						
Mnuf	0.0	0.0	0.0	0.0	0.0	0.0
Quisna	0.0	0.0	0.0	1.0	20	8.2
Shibin El-Kom	0.0	0.0	0.0	1.0	20	9.4
Tala	1.0	20	5.7	0.0	0.0	0.0
Imported						
Mnuf	1.0	20	8.7	0.0	0.0	0.0
Quisna	0.0	0.0	0.0	0.0	0.0	0.0
Shibin El-Kom	0.0	0.0	0.0	0.0	0.0	0.0
Tala	0.0	0.0	0.0	1.0	20	7.4

*The total number of samples taken was 5 either in winter or in summer season from each location.

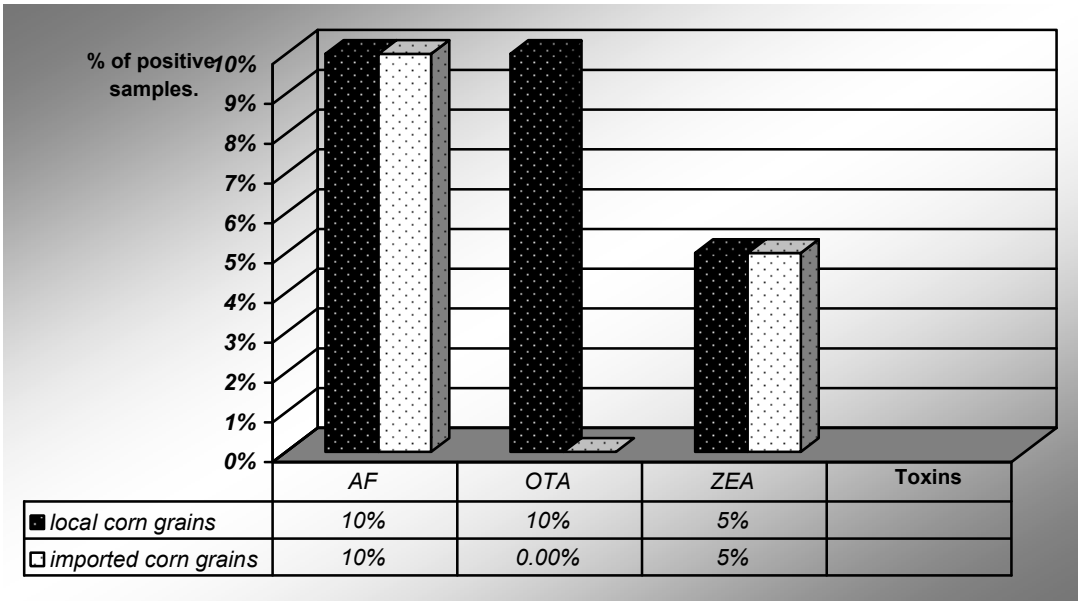

Fig 1 % of positive local and imported corn samples which contain mycotoxins and collected from El-Minufiya Governorate in winter season of 2004.

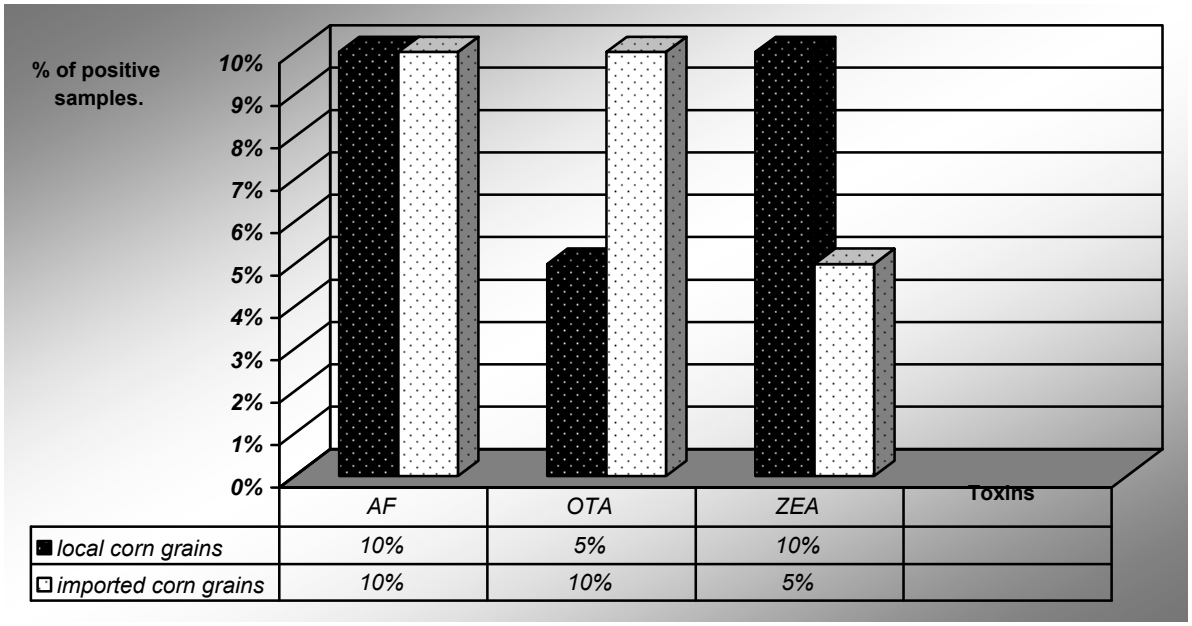

Fig 2 % of positive local and imported corn samples which contain mycotoxins and collected from El-Minufiya Governorate in summer season of 2004.

Conclusion

It could be concluded that the source of corn grains, location of the store and year season influence on the occurrence and the concentration of mycotoxins in corn grains, where local corn grains had nearly the same amounts of mycotoxins which were found in imported ones.

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