

Occurrence of zearalenone contamination in some cereals in Egypt

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Abstract

Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* species, which may invade crops in the field. Zearalenone can, after crop harvesting, be detected both in grain and products thereof. The toxicity of zearalenone has recently been reviewed by the European Food Safety Authority (EFSA) and a tolerable daily intake (TDI) of 0.25µg/kg body weight was established. Cereals represent a staple food for the Egypt population, therefore the aim of this study was to investigate sixty (60) samples of cereals (wheat, barley white corn and yellow corn) purchased from markets of Cairo city were analyzed for the presence of zearalenone using High Performance Liquid Chromatography (HPLC) to compare the levels of contamination by zearalenone with the European norms. Zearalenone was found in wheat samples (40%) with a mean level of 1.55µg/kg. ZEN was found in 4 out of the 15 analyzed samples of barley with mean 1.25µg/kg. On the other hand twenty percent (20%) of white corn samples are contaminated with ZEA with mean 1.7µg/kg. The highest ZEA contamination levels were found in yellow corn samples with ranged from 2.5 to 3.7µg/kg.

All the samples under study were within the permissible limits (100µg/kg) as recommended by the European Union. And also when compared with maximum limits of (50–1000) µg/kg in foods and animal feeds in countries of the world regulating ZEA as in 2003 according to Food and Agriculture Organization.

Keywords: Zearalenone, occurrence, cereals and *Fusarium*

1. Introduction

Zearalenone (ZEA) is a mycotoxin that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zae*), *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti* and *Fusarium semitectum* [5,24]. It exists widely in many cereal crops such as: wheat, corn barley, wheat, oats, sorghum and sesame seeds, as well as in hay and corn silage. These are all ingredients in many food products for human or animal nutrition [12,23,25].

The International Agency for Research on Cancer (IARC) has categorized ZEA as a class 2A carcinogen [19]. The toxicity of zearalenone has recently been reviewed by the European Food Safety Authority (EFSA) and a tolerable daily intake (TDI) of 0.25µg/kg body weight was established [15]. High contaminations of the raw materials are an ongoing problem. Regulatory issues are not available in the field of food exhibition and retailing, and mycotoxin problems have already been associated with some food contamination in some areas in Africa [27].

In Egypt, several commodities were reported to contain ZEA especially corn, wheat and rice [1] and walnut [2]. Corn from Egypt was also found contaminated with high levels of ZEA that ranged from 9.8 to 38.4 mg/kg [11].

In Tunisian Zaied *et al.*, 2012 [26] collected 205 samples of wheat, they found that the incidence of ZEA contamination was 75%. The levels of contamination determined in the positive samples ranged between 3 and 560 µg/kg with a mean value of 60µg/kg. ZEA was regulated in 1996 by 6 countries, but by the year 2003 the toxin ZEA was regulated in foods and animal feeds by 16 countries. Limits for ZEA in maize and other cereals, currently vary from 50 to 1000 µg/kg [16].

Although *Fusarium*-infected cereals standing in the field may accumulate ZEA before harvest time, numerous experiments tend to indicate that the high levels of ZEA reported to occur naturally in some samples of corn-based animal feeds result from improper storage rather than development in the field [21,22]. On the other hand, there is now overwhelming evidence of global contamination of cereals and animals with *Fusarium* mycotoxins particularly ZEA. Trade of these commodities may contribute to the worldwide dispersal of this mycotoxin. The predominant feature of ZEA distribution in cereal grains and animal feed is its occurrence with other *Fusarium* toxins including trichothecenes and fumonisins. This observation is consistent with the confirmed production of ZEA by virtual all toxigenic and plant pathogenic species of *Fusarium* [7].

2. Materials and Methods

2.1. Sampling. Sixty (60) samples of cereals destined for human consumption were randomly purchased during the year 2013. Fifteen samples from each of the (wheat, barley, white corn and yellow corn) were purchased from local markets of Cairo city. Collected samples were milled, conserved in plastic bags and then stored in a dark and dry place until analysis.

2.2. Solvent & Chemical. Methanol, acetonitrile, 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).

2.3. Zearalenone standard: One milligram of ZEA was purchased from Sigma, chemical Co. (St. Louis, MO, U.S.A).

2.4. Apparatus: High Performance Liquid Chromatography (HPLC) system consisted of Waters Binary pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze 2.A phenomenonex C₁₈ (250x 4.6 mm i.d), 5 µm from Waters corporation (USA). Blender; filter paper-reeve angel 802, 32cm diameter, pre-pleated (Whatman Inc, Clifton, NT). Auto sampler vial (5, 10 ml) with Teflon lined crimp top (hew lett- pakard, avondale, PA); the immunoaffinity column ZearalaTest™ HPLC were obtained from VICAM (Watertown, MA, USA).

2.5. Methods:

2.5.1. Extraction procedure. Twenty five grams of finally ground samples with 2g salt sodium chloride were placed in a blender jar. 125 ml of acetonitrile: water (75:25 v/v) was added. After covering the jar, blending was carried out at high speed for 2 min. The extract was poured into fluted filter paper, and the filtrate was collected in a clean vessel.

2.5.2. Extract Dilution. Pipet or pour 10 ml filtered extract into a clean vessel. Dilute extract with 10mL of purified water and mix well. Filter dilute extract through glass microfiber filter into a glass syringe barrel using markings on barrel to measure 10 ml.

2.5.3. Immunoaffinity Chromatography. Pass 10 mL filtered diluted extract (10 ml =1g sample equivalent) completely through ZearalaTest™ affinity column at a rate of about 1-2 drops/second until air comes through column. Pass 20 ml of purified water through the column at a rate of about 2 drops/second. Elute affinity column by passing 1.5 ml HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1ml) in a glass vial. Evaporated to dryness under stream of nitrogen and was determination of HPLC.

3.5.4. HPLC/fluorescence Analysis. The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual

injector, a Watres 2475 Multi- Wavelength Fluorescence Detector.

The fluorescence detector was operated at wavelength of 274 nm for excision and 440 nm for emission, and a data workstation with software Breeze 2. A phenomenex C₁₈ (250 X 4.6 mm i.d.), 5 µm from waters corporation (USA).

The mobile phase consisted of a mixture of acetonitrile: water: methanol (48:50:3, v/v/v). A 20 µl of the reconstituted extract was injected onto the column at a flow rate of 1.0 ml/min. ZEA content in samples was calculated from chromatographic peak areas using the standard curve.

3.Results and Discussion

3.1. Method validation. The average recoveries of ZEA were in the range of 84.2 ± 1.5, 91.3±2. 5 and 93.8 % for wheat spiking at levels 10, 25 and 100µg/kg, respectively, on the other hand the best recovery values was barley (95.2 ± 2.5%) at the spiking level of 100 g/kg. The precision was estimated by the relative standard deviation (RSDr) of the recovery, being at 1.8, 3.1 and 2.8 %, where the wheat was related with the spiking level of 10, 25 and 100µg/kg, respectively, while with the same concentrations in barley (RSDr) of the recovery, being in the range 3.1-3.9%.

Table 1. Recovery of zearalenone added (10µg/kg) to wheat, barley, white corn and yellow corn.

Samples	Level added (µg/kg)	Mean Recovery ^a (%)	SD ^b (%)	RSDr ^c (%)
Wheat	10	84.2	1.5	1.8
Barley		88.8	2.5	3.1
White corn		83.7	2.0	1.5
Yellow corn		81.5	2.7	3.0

^a Average recovery of the 3 replicates at the spiking level; ^b Standard deviation, n =3; ^c Relative standard deviation.

Table 2. Recovery of zearalenone added (25µg/kg) to wheat, barley, white corn and yellow corn

Samples	Level added (µg/kg)	Mean Recovery ^a (%)	SD ^b (%)	RSDr ^d (%)
Wheat	25	91.3	2.5	3.0
Barley		93.5	3.5	3.9
White corn		90.3	2.2	2.8
Yellow corn		88.7	4.1	4.6

^a Average recovery of the 3 replicates at the spiking level; ^b Standard deviation, n =3; ^c Relative standard deviation.

On the other hand mean recoveries for ZEA added to white and yellow corn at levels of 10, 25 and 100µg/kg were 83.7±2, 90.3±2.2 and 92.5±2% with white corn, respectively, the mean recoveries for ZEA with yellow corn were 81.5±2.7, 88.7±4.4 and 90.5±3.7% in the same concentrations.

According to performance criteria established by Commission Regulation (EC) No 401/2006 [13] this method can be qualified as acceptable.

The limit of detection (LOD) (signal-to-noise ratio=3) was calculated to be 0.15 µg/kg and the limit of quantification (LOQ) (signal-to-noise ratio=10) was 0.4 µg/kg of ZEA in samples.

Table 3. Recovery of zearalenone added (100µg/kg) to wheat, barley, white corn and yellow corn

Samples	Level added ^a (µg/kg)	Mean Recovery ^b (%)	SD ^c (%)	RSDr ^d (%)
Wheat	100	93.8	2.2	2.8
Barley		95.2	2.5	3.5
White corn		92.8	2.0	2.5
Yellow corn		90.5	3.7	4.0

^a Average recovery of the 3 replicates at the spiking level; ^b Standard deviation, n =3; ^c Relative standard deviation

3.2. Occurrence of ZEA. The results of our study about the occurrence of ZEA contamination in wheat, barley, white and yellow corn are presented in (Table 4 and Fig 1). ZEA was detected in only 6 samples of wheat (40%), the levels of contamination ranged between 0.53 and 2.5µg/kg. ZEN was found in 4 out of the 15 analyzed samples of barley. The levels of contamination in these samples ranged from 0.7 to 1.77µg/kg. On the other hand twenty percent (20%) of white corn samples are contaminated with ZEA with ranged from 1.13 to 2.15µg/kg. The highest ZEA contamination levels were found in yellow corn samples with ranged from 2.5 to 3.7µg/kg. All the samples under study were within the permissible limits (100µg/kg) as recommended by the European Union [14] on the other hand these levels are insignificant when compared with maximum limits of 50–1000 µg/kg in foods and animal feeds in countries of the world regulating ZEA as in 2003 [16].

Among the *Fusarium* mycotoxins, deoxynivalenol (DON) and ZEA are of special importance as they are formed at the field prior to harvest and because their occurrence cannot completely be avoided by

plant production minimizing strategies due to the major impact of weather conditions. Especially wheat, triticale and corn grains are vulnerable for *Fusarium* infection and are also frequently higher contaminated with DON and ZEA compared to other cereal grain [9].

Table 4. Occurrence of ZEA in wheat, barley, white and yellow corn from Cairo market

Samples	Number of each sample	Number of positive samples	Zearalenone levels (µg/kg)		
			Min	Max	Average ^a
Wheat	15	6	0.53	2.5	1.55
Barley		4	0.7	1.77	1.25
white corn		3	1.13	2.15	1.7
yellow corn		7	2.5	3.7	3.08

^a Average calculated using of positive samples.

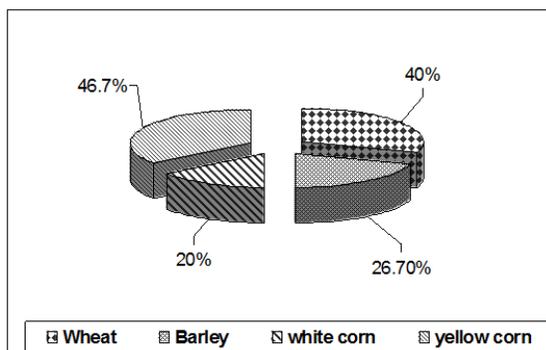


Figure 1. The percentage of occurrence of ZEA in wheat, barley, white and yellow corn.

Previous studies have shown that contamination of cereals with ZEA is a serious problem for consumer health. In Spain, Ibanez-Vea *et al.* (2011) [18] reported that 48% of breakfast cereal samples were contaminated with ZEA, with a mean level of 25.4µg/kg.

The presence of ZEA in the current analyzed samples may be due to the growth of contaminating ZEA producing-fungi and its ability to elaborate ZEA. Generally, in post-harvest situations, fungal growth and mycotoxin formation results from the interactions of several factors in the storage environment [3]. These factors include: moisture content, temperature, time and damage to the seed, O₂ levels fungal infection level, prevalence of toxigenic strains of fungi, and the grain structure [4].

On the other hand, other investigators reported that the storage life of grains depends mainly on two physical abiotic factors: temperature and moisture content which are considered to be the most important variables in determining the fungal growth and rate of mycotoxin production. In the same regard [20]. The scarce occurrence of ZEA might be explained by the fact that it is abundantly produced by specific *Fusarium* strains in cool dry climates (temperatures of 10– 15°C) at a certain period of fungal infestation of crops [6], whereas in Egypt, the temperature is high year-round (27–40 °C) and there is relatively high humidity (≥80%) for most of the year. Most often the compound is found in corn. However, it is also found in other important crops such as wheat, barley, sorghum, and rye throughout various countries of the world. In wheat the conditions for the occurrences of ZEA would be essentially the same as for the occurrence of deoxynivalenol as the organism gains entry into the host plant in the same manner. Generally, the *Fusarium* species grow in moist cool conditions and similarly invade crops under these more favorable conditions. As noted above, the same organism produces both of these compounds. This same organism is capable of producing both compounds in corn. The finding of aflatoxin co-occurring with ZEA and deoxynivalenol would imply that two different fungi, *Aspergillus flavus* in the case of aflatoxin and *Fusarium graminearum* in the case of the latter two mycotoxins, established infection. In wheat, sorghum and corn, it is well established that ZEA occurs in pre-harvest grain but in other commodities the surveys are insufficient to determine if the ZEA occurred pre- or post-harvest. Variations in the incidence of ZEA occur with different crop years, cereal crop and perhaps geographical areas. As with other fungi, to avoid growth of *Fusarium graminearum* in grains during storage the moisture levels should be <14%. Perhaps, ZEA can be produced in relatively cool conditions compared to some other mycotoxins but it is likely that most grains mentioned above can become contaminated with ZEA during storage and levels that were present in the grain pre-harvest may increase if the grain is not sufficiently dried and stored. With regard to ZEA, the mean levels observed in this survey were similar to or less than the results found by other researches, although the occurrence was higher.

According to the [17] report, the mean level found in barley samples from six countries was 0.83µg/kg and the contamination frequency was 5%.

The Egyptian population consumes great amounts of cereals and cereal-based food. Indeed, large amounts of cereals commercialized in Egypt are imported and little is known about eventual mycotoxin contamination. Consequently, in Egypt, there are no applicable norms concerning cereal contamination, particularly by ZEA, neither for local nor for imported cereals. Devegowda *et al.*, (1998) [8] reported that approximately 25% of cereals consumed in the world are contaminated by mycotoxins. In general, the extent of contamination is expected to be higher especially when climatic conditions are favorable to mycotoxin contamination.

Several farming practices such as the use of sensitive varieties of wheat, no crop rotation; no-till or reduced tillage enhance fungal attacks and therefore increase mycotoxin accumulation in grains. Unfortunately, farmers do not practice a suitable crop rotation to prevent the accumulation of *Fusarium* in the fields. Finally, the social and economic characteristics of the Egyptian population such as the cooking methods, home-made food storage and the eating habits can probably increase the exposure to ZEA [10].

4. Conclusion:

Mycotoxins and related pathologies have become a worldwide preoccupation and they raise serious economic and sanitary problems. These are also of some concern in Egypt because of climate and geographic situation in addition to the social and economic conditions. In our previous survey on Egypt, we have shown that cereals were contaminated by ZEA and other mycotoxins such as aflatoxins, ochratoxin A and fumonisin. Our present study presents the report evaluating the situation of wheat, barley, yellow corn, white corn, flower corn, flower wheat and molt contamination by ZEA in Egypt. Our findings suggest that ZEA found at such high levels in molt may cause serious health problems for both humans and animals. These levels were less than the permissible limits fixed by the European

Commission Regulation. The limit intended for human consumption is 100 mg/kg [14].

Furthermore, these findings revealed that a sample under the Egyptian agro-climatic conditions is contaminated by ZEA. This situation should spur the Egyptian authorities to set regulatory limits for ZEA and other *Fusarium* toxins in cereals, foods and feeds to ensure food safety.

A similar study is in preparation to evaluate the levels of other mycotoxins that contaminate cereals in addition to ZEA.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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