

Natural Occurrence of *Fusarium* Mycotoxins (Fumonisin, Zearalenone and T-2 toxin) in Corn for Human Consumption in Yemen

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Abstract

The presence of fumonisins, zearalenone (ZEA) and T-2 toxin has not been regulated in the legislation of Republic of Yemen. Therefore, the data on contamination of cereals, especially corn, which is highly susceptible to contamination by such toxins, are not sufficient. In this regard, fifty corn kernels which showed *Fusarium* contamination and which were obtained from different corn growing areas in Yemen (Ibb, Taiz, Tamar, Sana'a and Al-Hodaida), during the spring- summer (2010 – 2011 and 2011 – 2012) cycles, were further analyzed for the presence of these toxins using thin layer chromatography (TLC) and enzyme linked immunosorbent assay (ELISA) techniques. 72% of samples were contaminated with fumonisins with concentrations ranging from 00.57 to 120.20 µg/g with an average of 9.33 µg/g, 30% were contaminated with ZEA with concentrations ranging from 00.17-03.30 µg/g with an average of 01.04 µg/g, while all samples were T-2 toxin free. The co-occurrence of fumonisins and ZEA was also detected in 15 samples (30%). This study is considered the first preliminary survey dealing with three occurring mycotoxins naturally in fresh corn in Yemen. Thus, may be used as a base for further studies assessment for other cereals as well. And in terms of fumonisins and ZEA these high levels detected do not only cause overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses.

Keywords: Fumonisin, zearalenone, T-2 toxin, ELISA, TLC, toxins and corn.

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INTRODUCTION

Corn is the world's third most important crop after wheat and rice, about half of this is grown in developing countries, where corn flour is a staple food for poor people and corn stalks provide dry season food for farm animals. Cereals including corn are major mycotoxin vector because they are consumed by both humans and animals (Chehri, 2011). Having an ideal nutrients, corn is constantly exposed to toxigenic fungi (Haggag, 2013). Many fungi species have been associated to corn, mainly those related to the genera *Fusarium*, *Penicillium* and *Aspergillus*. Several fungal species could be regarded as post-harvest decay of apple, orange, banana, mango and grape fruits (Abdullah *et al.*, 2016). This is a cause of concern because a considerable number of those genera have species capable of producing a wide array of compounds shown to be toxic to man and animals (Camargos *et al.*, 2000). From which, trichothecenes, zearalenone (ZEA) and fumonisins, are of a great public health and agro-economic significance (Jaksic *et al.*, 2011). These are climate depending, plant

and storage associated problems, also influenced by non-infectious factors (e.g. insect damage, pests attack and bio-availability of micronutrients). Climate represents the key agro-ecosystem driving force of fungal colonization and mycotoxin production (Paterson and Lima, 2010).

Toxins produced by *Fusarium* can begin in infected corn in the field, prior to harvest, as *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissue, depending on the host and *Fusarium* species involved (Chehri, 2011). Fumonisin are a group of environmental, non-fluorescent, water-soluble and polar mycotoxins produced mainly by the moulds *F. verticillioides*, *F. proliferatum*, *F. nygamai*, *F. subglutinans* and other *Fusarium* species (Fandohan *et al.*, 2003).

Fumonisin are classified into 4 main groups the A, B, C and P- series fumonisins. The B- series fumonisins are the most abundant analogs with fumonisins B₁ (FB₁) being the most abundant and account for approximately 70% of total fumonisins found in nature (Wang *et al.*, 2008). Although fumonisins have a relatively simple chemical

structure, their inhibition metabolites, sphingolipids can have complex effects in animal and human systems. Consumption of grains contaminated with fumonisins has been associated with human esophageal cancer and birth defects (Desjardins and Proctor, 2007). The European Commission (EC) has set action limits of 4 µg fumonisins/g for unprocessed corn (Al-Hazmi, 2009).

ZEA, which is mainly produced by *F. graminearum*, *F. semitectum* and *F. culmorum*, isn't acutely toxic and haven't been associated with any fatal mycotoxicosis in humans or animals (Desjardins and Proctor, 2007). It is non-steroidal estrogenic mycotoxin, causing alternation in the reproductive tract of laboratory and domestic animals. In humans, it causes premature puberty in children aged between 7 and 8 years (Sekiyama et al., 2005). Due to the health hazards of ZEA, the EC has set a maximum level of 0.35 µg/g of ZEA in unprocessed cereals (Atoui et al., 2011).

T-2 toxin; a trichothecene mycotoxin is a secondary metabolite produced by species of *Fusarium*, *Myrothecium*, *Trichothecium*, *Stachybotrys* and *Verticimonosporium*. Trichothecenes are classified according to their chemical structures, and T-2 is the most toxic among the non-cyclic compounds of the family (Lauren and Greenhalgh, 1987). Exposure to the trichothecene mycotoxins, including T-2, cause emesis, necrotic angina, diarrhea, anorexia, hematological changes, neurological alterations, destruction of the bone marrow and generalized hemorrhages in some cases followed by death (Meloche and Smith, 1995).

The data on contamination of cereals, especially corn, which is highly susceptible to contamination by such toxins

in Yemen, are not sufficient. Heading from this point, the evaluation of such toxins in fresh corn for human consumption was the main goal of the present study.

MATERIALS AND METHODS

Samples collection

A total of 50 samples of corn kernels were obtained from different corn growing areas in Yemen (Ibb, Taiz, Tamar, Sana'a and Al-Hodaida), during the spring-summer (2010 – 2011 and 2011 – 2012) cycles.

Most of these collected samples appeared healthy, while a few samples were mechanically damaged by birds. All kernels were harvested from the two central rows of each cultivar plot, the kernels were shelled and homogenized, then reduced to 250 g for each. The collected samples were brought to the laboratory in clean plastic bags and kept in refrigerator at 4 °C prior to mycotoxins analysis.

Mycotoxins analysis

Corn samples were analyzed for fumonisins, zearalenone and T-2 toxins using an enzyme-linked immunosorbent assay (ELISA), known commercially as MaxSignal, obtained from BIOO Scientific Corporation, USA, according to the manufacturer's instructions. Thin-layer chromatography (TLC) technique was also used to analyze the same samples according to the method described by Gimeno (1979) and as shown briefly in Table 1.

Table 1. Procedure outline for different mycotoxins in maize samples.

Mycotoxins	Extraction solution (ml)	TLC plate	Re-dissolving solutions (ml)	Developing solution (ml)	Spraying reagents
Fumonisin	Acetonitrile:Water (50:50)	silica gel 60 F ₂₅₄	Acetonitrile:Water (70:30)	Chloroform: Methanol: Acetic acid (60:30:10)	P-anisaldehyde (0.5% in methanol/sulfuric acid/acetic acid, 90:5:5)
Zearalenone	Acetonitrile:Water (84:16)	silica gel 60 F ₂₅₄	Toluene: Acetonitrile (97:5)	Toluene: Ethylacetat: Chloroforme (50:25:25)	Iron (III) chloride (3% solution in ethanol)
T-2	Acetonitrile:Water (84:16)	silica gel 60 F ₂₅₄	Toluene: Acetonitrile (97:3)	Toluene: Ethylacetat: Chloroforme (50:25:25)	Concentrated (conc.) H ₂ SO ₄

RESULTS

Among 50 fresh corn samples tested, 72% were contaminated with fumonisins, 30% with ZEA, while T-2 toxin wasn't detected in any tested samples. The highest level of fumonisins reached the value of 120.20 µg/g and the lowest one was 0.57 µg/g (mean= 9.33 µg/g) (Table 2). In comparison, the highest level of ZEA was 3.30 µg/g, while the lowest one was 0.17 µg/g (mean= 1.04 µg/g). All samples tested were T-2 toxin- free. The co-occurrence of

fumonisin and ZEA was recorded in 30% of the tested samples.

Fumonisin had been detected in all Provinces under study with variable levels ranging between 0.57-120.20 µg/g, while ZEA was detected in all Provinces except Taiz where it was completely absent (Table 3). The highest mean of fumonisins was recorded in Al-Hodaida Province (24.94 µg/g), followed by Taiz (4.93 µg/g), Sana'a (3.21 µg/g), Ibb (3.01 µg/g) and Tamar (2.27 µg/g). The highest

incidence of fumonisins was recorded in Al-Hodaida (100% of the tested samples), followed by Ibb (80%), while its incidence was 60% in each of Sana'a, Thamar and Taiz. ZEA was detected in 60% of Al-Hodaida samples, followed by Ibb, Sana'a and Thamar with 50%, 30% and 10%, respectively. The highest mean of ZEA recorded in Thamar (3.30 $\mu\text{g/g}$), Al-Hodaida ranked second (1.08 $\mu\text{g/g}$), Sana'a

(1.06 $\mu\text{g/g}$) and finally Ibb (0.52 $\mu\text{g/g}$), while Taiz showed negative results for ZEA (Figure 1). It is worth mentioning that T-2 toxin couldn't be detected at all in any of the 5 Provinces, from which samples were collected (Table 3).

Table 2. Natural occurrence and levels of *Fusarium* toxins in fresh corn samples collected from different Yemeni Provinces

Toxin	Samples analyzed	Positive samples	Incidence (%)	Range of toxins ($\mu\text{g/g}$)	Mean level ($\mu\text{g/g}$)
FUM	50	36	72	0.57-120.20	9.33
ZEA	50	15	30	0.17-3.30	1.04
T-2	50	00	00	00	00

FUM: fumonisins; ZEA: zearalenone.

Table 3. Comparison of natural occurrence and levels of *Fusarium* toxins in fresh corn samples collected from different Yemeni Provinces

Corn- growing areas	FUM				ZEA			
	R. ($\mu\text{g/g}$)	M. ($\mu\text{g/g}$)	P.S.	%	R. ($\mu\text{g/g}$)	M. ($\mu\text{g/g}$)	P.S.	%
Sana'a	1.40-6.39	3.21	6	60	0.17-02.70	1.06	3	30
Ibb	1.40-5.80	3.01	8	80	0.40-00.70	0.52	5	50
Thamar	0.57-4.59	2.27	6	60	3.30	3.30	1	10
Taiz	1.58-13.31	4.93	6	60	0.00	0.00	00.00	00.00
Al-Hodaida	1.78-120.20	24.94	10	100	0.32-01.81	1.08	6	60

R: Range; M: Mean; P.S: Positive samples; %: Incidence.

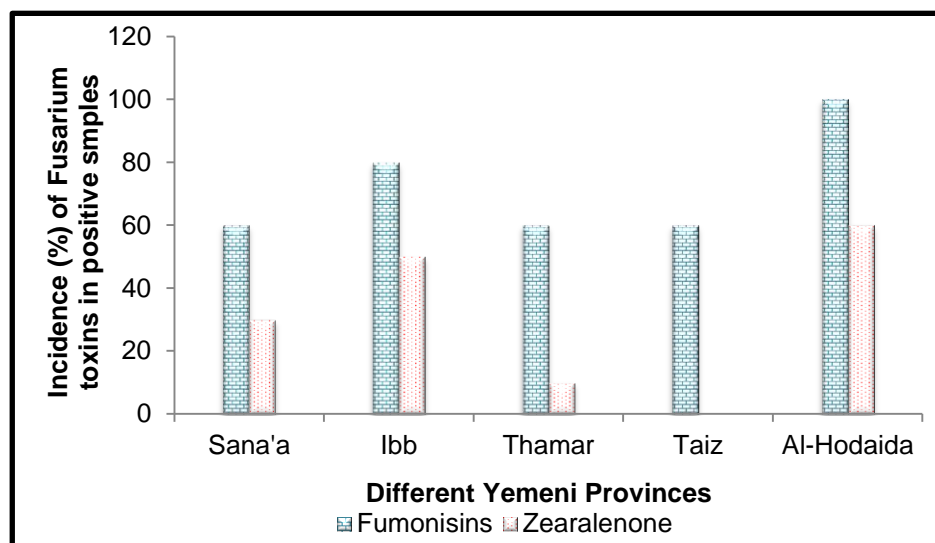


Fig. 1. Incidence (% of positive samples) of natural occurrence of *Fusarium* toxins (fumonisins and ZEA) in fresh corn samples collected from different Yemeni Provinces

DISCUSSION

Mycotoxins have become a key problem of food chain, starting in the field and ending on the table (Richard, 2007).

The presence of mycotoxins in food is often as a result of public ignorance about their existence, dumping of food products, lack of regulatory mechanisms and the

introduction of contaminated agricultural commodities into the human food chain during chronic food storage due to drought and economic instability (Bii *et al.*, 2012).

During this investigation, it was possible to notice that 72% of 50 fresh corn samples analyzed were naturally contaminated with fumonisins, 30% with ZEA, while T-2 toxin wasn't detected in any sample. It seems that the incidence of highly contaminated grains with different mycotoxins is an indicator of favourable conditions for grain contamination with mycotoxins producing fungi. Even a short period of time, with exposure to appropriate conditions for growth and production of mycotoxins, may be enough for production of high amounts of mycotoxins (Riazipour *et al.*, 2012).

Accessory evidence, the use of fungicides differentially affects the *Fusarium* species that infect the corn ears, resistance to some fungicides may selectively remove dominant but susceptible non-toxicogenic species, allowing more active colonization by toxicogenic species (Arino *et al.*, 2007). Similar results dealing with corn contamination with *Fusarium* toxins were reported, 75% of fresh corn samples were positive for FB₁ (Perilla and Diaz, 1998). In addition, Hirooka *et al.* (1996) and Orsi *et al.* (2000) found that 97 and 90% of corn samples were contaminated with FB₁, respectively. On the other hand, contamination with FB₁ didn't exceed 50% of the samples tested (Mallmann *et al.*, 2001; Wisniewska-Dmytrow *et al.*, 2004).

In the same trend, 7.5% of pre-harvested corn samples analysed were found to be contaminated with ZEA (Hadiani *et al.*, 2003). Meanwhile, the analysis of 24 corn samples collected from various regions of Tlaxcala, Mexico indicated that approximately 70% of the monitored samples were contaminated with ZEA (Briones-Reyes *et al.*, 2007). In Egypt, Nogaim *et al.* (2011) found that 6.25% of local and imported corn grains tested were contaminated with ZEA. In the contrary, no ZEA was detected in all 65 corn grain samples collected from 7 corn producing areas of Pakistan (Khatoon *et al.*, 2012).

It was worthy to mention that T-2 toxin wasn't detected in any of the samples analyzed during this investigation. Haggler, (1984) supported our findings as he found that not a single sample from the central and Western parts of US was contaminated with T-2 toxin. However, this toxin was detected in only 6.15% of samples with relatively low concentrations in the study of Khatoon *et al.* (2012). The present study revealed that there was a high degree of variability in concentrations of mycotoxins from one province to another. In this respect, Al-Hodida recorded the highest fumonisins values which ranged from 1.78-120.2 µg/g with a mean concentration of 24.94 µg/g, whereas, Tamar recorded the lowest concentrations with a values which ranged between 0.57- 4.59 µg/g with a mean of 2.27 µg/g. The differences in fumonisins levels may due to that the production of mycotoxins in agricultural commodities depends on such factors as geography, season and environmental conditions. In certain geographical areas of

the world, some mycotoxins are produced more readily than others (Khatoon *et al.*, 2012).

The results obtained previously weren't surprising as they were compared with the data recorded by Abbas *et al.* (2006), which indicated that in spite of using Bt-corn hybrids, fertilization, weed control and irrigation to minimize the contributions from nutrient, population and drought stresses, respectively, leaving heat as a major uncontrolled source of stress, fumonisins levels were very high in all hybrids, ranging from 8 to 83.6 ppm which is equal to (µg/g).

So how the expectations will be about fumonisins levels for a crop that exposed to all previous stresses and additional unrecognized sources of stress may have been present, besides it might be mechanically damaged. In this trend, it was reported that weather conditions influence fumonisins contamination of corn, although *Fusarium* spp. are found in a wider range of climate conditions. Heat stress during the period when the kernel is developing (i.e., between silking and blacklayer), particularly night time temperatures above 20°C, is a major factor in mycotoxin contamination (Abbas *et al.*, 2006).

Perilla and Diaz (1998) found that the mean levels of FB₁ in corn and corn based products for human consumption reached 218 µg/kg and the range was 24 – 2170 µg/kg. In another study, the levels of fumonisins in the positive samples ranged between 0.46 and 9.95 µg/g for FB₁ and from 0.14 to 3.06 µg/g for FB₂. The total fumonisins concentration ranged from 0.46 to 14.08 µg/g. These data confirmed that FB₁ is the major fumonisin found under natural conditions (Gonzalez *et al.*, 1999). Camargos *et al.* (2000) found that all samples tested were contaminated with fumonisins with concentrations ranging from 1.63 to 25.69 µg/g with an average of 5.61 µg/g for FB₁ and from 0.38 - 8.60 µg/g with an average of 1.86 µg/g for FB₂. In Venezuela, fumonisins content ranged from 0.80 to 10.00 ppm in Portuguesa State, and from 1.50 to 13.00 ppm in Guárico state (Mazzani *et al.*, 2001).

ZEA levels as shown in this investigation were higher in Tamar Province with the only contaminated sample (03.30 µg/g), followed by Al-Hodaida (00.32-01.81 µg/g and a mean of 01.08 µg/g), Sana'a (00.17-02.70 µg/g with a mean of 01.06 µg/g) and finally Ibb (00.40 – 00.70 µg/g and mean of 00.52 µg/g). All samples analyzed from Taiz province were ZEA-free. It seemed that continuous hot and dry weather, followed by periods of high humidity, resulted in the occurrence of ZEA (Wisniewska-Dmytrow *et al.*, 2004). In a similar study, Nogaim *et al.* (2011) found that ZEA concentrations ranged from 5.7 to 9.4 and 7.4 to 8.7 µg/kg in contaminated corn grains, local and imported ones consumed in Egypt, respectively. In addition, Briones-Reyes *et al.* (2007) found that approximately 70% of the monitored corn samples collected from various regions of Tlaxcala, Mexico were contaminated with ZEA, with levels ranging from 3 to 83 ng/kg of corn kernels. On the contrary, no ZEA was detected in all 65 corn grain samples collected

from 7 corn producing areas of Pakistan (Khatoon *et al.*, 2012).

It was obvious from this study that most of the samples analyzed had detectable amounts of both Fumonisin and ZEA that exceeded the legal limits of the EC for unprocessed corn, 4 µg/g and 0.35 µg/g, respectively. The intake of very low levels of mycotoxins do not only cause overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses (Dalcero *et al.*, 1998).

CONCLUSION

As starting in the field and ending on the table is the mode action of mycotoxins in cereals and specially corn, affecting by this mode the safety image of corn grain as a raw material for the food and feed industry. The present study revealed that out of 50 fresh corn samples examined, 72% of them were contaminated with fumonisins, 30% with ZEA, whereas T-2 toxin wasn't detected in any sample. All corn samples of Al-Hodaida Province were contaminated with mycotoxins, coupled with a highest fumonisins concentration value, while ZEA showed the highest concentration value in Tamar corn samples. The lowest values of fumonisins and ZEA were recorded in Tamar and Sana'a corn samples, respectively.

RECOMMENDATIONS

Having carcinogenic potential and poisonous effects, mycotoxins are considered to be the most important regulatory issue. Heading from this point of view, appropriate sanitary measures must be taken to ensure that conditions for growth and toxins production are reduced or eliminated during handling, transportation, packaging and storage of corn. Beside that public should be educated and sensitized on the necessity to observe sound food safety measures. Frequent studies of a broader scope, which show the overall situation in all Yemeni Provinces, taking into consideration climatic and seasonal differences is recommended.

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CONFLICT OF INTEREST

The authors verify having no interest in competition and have no conflicts of interest.

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