

## Evaluation of Aflatoxin Contamination in Maize from Mazandaran Province in Iran

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### Abstract

**Background:** In Iran, maize is cultivated in wide areas including northern provinces. It is one of the most important crops for poultry and livestock feeding, which is susceptible to aflatoxins (AFs) contamination. So, pre-harvest maize samples from Mazandaran province, north of Iran were analyzed for AFs contamination.

### Objective:

**Methods:** Thirty-five pre-harvest maize samples were analyzed using immunoaffinity column and reversed-phase liquid chromatography with post-column derivatization.

**Results:** Data revealed that incidence of AFB<sub>1</sub>, AFB<sub>2</sub> and total aflatoxins (AFT) in maize samples were 66%, 54% and 63% with mean of  $9.5 \pm 16.3$ ,  $1.7 \pm 2.6$  and  $10.4 \pm 18.4$  ng/g, respectively.

**Conclusion:** The mean level of AFB<sub>1</sub> in samples was higher than the Iranian maximum tolerated level (MTL). But, AFT mean level (10.4 ng/g) was lower than the Iranian and US (20 ng/g) MTLs.

**Keywords:** Maize, Aflatoxins, HPLC, Mazandaran, Iran

## Introduction

Aflatoxins (AFs) which are produced by the common fungi *Aspergillus flavus* and *A. parasiticus* both in pre- and post-harvest stages are one group of extremely toxic mycotoxins [1]. The most surveys indicate that AFs contamination occurs primarily in maize, peanut and pistachio [2]. Maize crop in Iran is mostly used for livestock and poultry feeding. The worldwide maximum tolerated levels (MTL) of AFs in feed for dairy cattle varies from 5 to 50 ng/g for AFB<sub>1</sub> and 0 - 50 ng/g for total AFs (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) [3]. In Iran, the MTL of AFs in maize intended for livestock and poultry feeding are 5 ng/g and 20 ng/g for AFB<sub>1</sub> and total aflatoxins (AFT), respectively [3,4].

Contamination of food and feed in Iran with most common mycotoxins have been extensively studied [5-14]. Maize is cultivated in wide areas including northern provinces in Iran where humid atmosphere is suitable for growth of *Aspergillus* fungi. In this regard, it has been reported that 89% and 66% of corn samples collected from Mazandaran and Golestan provinces in 1998, contained AFB<sub>1</sub> and AFB<sub>2</sub>, respectively [7]. Mycotoxin contamination of maize due to bioaccumulation, may transfer high amounts of toxins to next links of the food chain and finally human. The aim of this study was to determine the variation of AF levels in maize crop from Mazandaran province.

## Materials and methods

### Sampling and sample preparation

Thirty five visually healthy maize samples belonging to hybrid cultivar SC-704, were randomly collected just before harvesting from farms of the Dasht-e-naz Agricultural Corporation in Mazandaran province, north of

Iran. Each sample consisted of 5 kg of granulated maize ears collected randomly from 10 different places of each farm (0.5 kg, approximately three ears per site). Maize ears were shelled, granulated and after drying, milled and kept at 4°C in order to analysis.

### Aflatoxin Standards

A stock solution of each individual AF, was prepared in toluene-acetonitrile (9+1) in concentration of 10 µg/ml. Concentrations of standard solutions were determined using an ultraviolet (UV) spectrophotometer. Intermediate standards for calibration curve, were prepared in toluene-acetonitrile (9+1) with concentration of 1 µg/ml for each AFB<sub>1</sub> and AFG<sub>1</sub> and 0.2 µg/ml for each AFB<sub>2</sub> and AFG<sub>2</sub> [15].

### Experimental

AFs in the corn samples were extracted by immunoaffinity columns and analyzed using an AOAC method 999.07 [15] and their AFs contents determined by reversed-phase liquid chromatography with post-column derivatization using electrochemically generated bromine (Kobra cell) and fluorescence detector. AFG<sub>2</sub> was eluted first followed by AFG<sub>1</sub>, AFB<sub>2</sub> and AFB<sub>1</sub>. The LOD for AFB<sub>1</sub> and AFT were 0.1 ng/g and 0.4 ng/g, respectively. Internal quality control including spiking blank corn samples with AFs at two levels, was performed with each series of samples and recoveries were recorded [16].

## Results

Obtained results were summarized in the tables 1 and 2. Table 1 presents occurrence of AFs in 35 pre-harvest maize samples from Mazandaran province, which include aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> and total.



**Table 1: Occurrence of AFs in the preharvest maize samples from Mazandaran province**

	AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	AFT
Mean (ng/g)*	9.5	1.7	0.15	0.08	10.4
Standard deviation (ng/g)*	16.3	2.67	-	-	18.4
Median (ng/g)*	3.76	0.8	-	-	4.18
Incidence (%)	66	54	3	3	63
Range (ng/g)*	0.2-78	0.1-11	-	-	0.12-89

\* Mean, STD, range and median were calculated in positive AF samples.

**Table 2: Comparison of mycotoxin incidence in maize samples from Mazandaran province in the year of 1998 and in this study**

	AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	AFT	FB <sub>1</sub>	ZEA
Mean (ng/g) in 1998	15.83*	3	Nd	Nd	18.82	2270*	-
Mean (ng/g) in this study	9.48*	1.72	0.15	0.08	10.4	5820*	141*
Frequency (%) in 1998	89	67	0	0	88	100	-
Frequency (%) in this study	66	54	3	3	63	97	7.5

\* Iranian MTL of maize is 5 ng/g for AFB<sub>1</sub>, 1000 ng/g for FB<sub>1</sub> and 200 ng/g for ZEA.

Comparative data of mycotoxins incidence in pre-harvest maize samples from Mazandaran province in the years of 1998 and this study were shown in table 2.

## Discussion

According to presented results in table 1, from 35 analyzed samples, 12 maize samples (34%) were not contaminated with AFB<sub>1</sub> (<LOD). In 10 (28%) samples, level of AFB<sub>1</sub> was higher than Iranian (5 ng/g) MTL [4]. The mean level of AFB<sub>1</sub> in maize samples (9.48 ng/g) was lower than the European Union (EU) MTL for AFB<sub>1</sub> in all feed materials (20 ppb) [3]. Although the mean contamination level of AFB<sub>1</sub> in maize samples (9.48 ng/g) was higher than the Iranian MTL [4] for AFB<sub>1</sub> in maize intended for livestock and poultry feeding (5 ng/g), AFT mean level (10.4 ng/g) was lower than the Iranian and US (20 ng/g) MTLs [3].

Maize is very susceptible for contaminating with two other carcinogenic and estrogenic mycotoxins, namely fumonisins (FM) and zearalenone (ZEA) [14]. The corn

samples analyzed in this study were also contaminated with ZEA and fumonisin B<sub>1</sub> (FB<sub>1</sub>) [9, 17]. In 1998, contamination of corn samples from Mazandaran province with both AFs and fumonisins were reported too [6, 7]. Comparative data of mycotoxins incidence in pre-harvest maize samples from Mazandaran province in the years of 1998 and this study has been shown in table 2. In both years, FB<sub>1</sub> was most abundant mycotoxin (both in terms of frequency and mean), followed by AFB<sub>1</sub>, AFB<sub>2</sub> and ZEA, respectively. Also, 33% and 28% of maize samples from Mazandaran province, in the year of 1998 and this study, respectively, contained AFB<sub>1</sub> level higher than Iranian MTL in maize intended for feed (5 ng/g), and in both years, mean of AFB<sub>1</sub> was higher than this limit. Moreover, it was found that 96% of AFB<sub>1</sub>-contaminated samples contained FB<sub>1</sub> concomitantly, which is consistent with data from Brazilian maize [18, 19]. Also, co-occurrence of AFB<sub>1</sub>+FB<sub>1</sub>+ZEA was found in 8.5% of samples and incidence of AFB<sub>1</sub>+AFB<sub>2</sub>+FB<sub>1</sub> found in 43% of maize samples. This is the first report of



co-occurrence of AFs, FB<sub>1</sub> and ZEA in Iranian maize. Our findings are also comparable with those of Bangladesh maize crop [20], north of Italy [21] and Japanese maize crops [22].

The high level of AFs and FB<sub>1</sub> and low level of ZEA in the collected maize samples in this study, may be due to the drought stress which increased the growth of AFs- and FB<sub>1</sub>-producing fungi and decreased the growth of ZEA-producing fungi. Such correlation has also been found in maize from northern Italy [21]. It should be noted that our findings are related to pre-harvest maize, while mycotoxin production can be increased after storage.

Efforts have been made to manage AFs contamination by promoting Good Agricultural Practice (GAP) principles in the fields and Hazard Analysis and Critical Control Point (HACCP) principles in storage and processing plants. These activities were supported by research projects funded through international and national organizations. In this regard, a FAO project entitled "Management, control and analysis of mycotoxins in foodstuffs and feedstuffs in Iran" was implemented in Iran.

## Conclusion

The mean level of AFB<sub>1</sub> in maize samples (9.48 ng/g) was lower than the European Union (EU) MTL [3] for AFB<sub>1</sub> in all feed materials (20 ppb). Although the mean level of AFB<sub>1</sub> in samples (9.48 ng/g) was higher than the Iranian MTL, AFT mean level (10.4 ng/g) was lower than the Iranian and US (20 ng/g) MTLs [3]. But, co-occurrence of FB<sub>1</sub> with AFB<sub>1</sub> could increase their toxicities. Maize is widely used in Iran as one of the main ingredients of poultry and livestock feedstuffs, and application of proper agricultural and sanitary measures and HACCP principles should lead to production of corn of better quality in Iran.

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