Evaluation of Aflatoxin Contamination in Maize from Mazandaran Province in Iran

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Receive: 1 Feb. 2009 **Acceptance:** 15 Mar. 2009

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_1, AFB_2 and total aflatoxins (AFT) in maize samples were 66%, 54% and 63% with mean of 9.5 ± 16.3, 1.7 ± 2.6 and 10.4 ± 18.4 ng/g, respectively.

Conclusion: The mean level of AFB_1 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

[DOR: 20.1001.1.2717204.2009.8.29.36.7]

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_1 and 0 - 50 ng/g for total AFs (B_1, B_2, G_1, G_2) [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_1 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_1 and AFB_2, 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_1 and AFG_1 and 0.2 µg/ml for each AFB_2 and AFG_2 [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_2 was eluted first followed by AFG_1, AFB_2 and AFB_1. The LOD for AFB_1 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_1, B_2, G_1 and G_2 and total.

[DOR: 20.1001.1.2717204.2009.8.29.36.7]

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

	AFB_1	AFB_2	AFG_1	AFG_2	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_1	AFB_2	AFG_1	AFG_2	AFT	FB1	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_1, 1000 ng/g for FB_1 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_1 (<LOD). In 10 (28%) samples, level of AFB_1 was higher than Iranian (5 ng/g) MTL [4]. The mean level of AFB_1 in maize samples (9.48 ng/g) was lower than the European Union (EU) MTL for AFB_1 in all feed materials (20 ppb) [3]. Although the mean contamination level of AFB_1 in maize samples (9.48 ng/g) was higher than the Iranian MTL [4] for AFB_1 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_1 (FB_1) [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_1 was most abundant mycotoxin (both in terms of frequency and mean), followed by AFB_1, AFB_2 and ZEA, respectively. Also, 33% and 28% of maize samples from Mazandaran province, in the year of 1998 and this study, respectively, contained AFB_1 level higher than Iranian MTL in maize intended for feed (5 ng/g), and in both years, mean of AFB_1 was higher than this limit. Moreover, it was found that 96% of AFB_1-contaminated samples contained FB_1 concomitantly, which is consistent with data from Brazilian maize [18, 19]. Also, co-occurrence of AFB_1+FB_1+ZEA was found in 8.5% of samples and incidence of AFB_1+AFB_2+FB_1 found in 43% of maize samples. This is the first report of

[DOR: 20.1001.1.2717204.2009.8.29.36.7]

co-occurrence of AFs, FB_1 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_1 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_1-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_1 in maize samples (9.48 ng/g) was lower than the European Union (EU) MTL [3] for AFB_1 in all feed materials (20 ppb). Although the mean level of AFB_1 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_1 with AFB_1 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.

Acknowledgement

This study was made possible by the financial support of the Food and Drug Control Laboratories, Food and Drug deputy, Ministry of Health and Medical Education of Iran. Their support is gratefully acknowledged.

References

1. Gourama H, Bullerman LB. Aspergillus flavus and Aspergillus parasiticus; aflatoxigenic fungi of concern in foods and feeds_ a review. *J. Food Protect* 1995; 58: 1395 - 404.

2. Pittet A. Natural occurrence of mycotoxins in foods and feeds — an updated review. Revue de *Me´dicine Ve´te´rinaire* 1998; 149: 479 – 92.

3. Food and Agricultural Organization of the United Nations. Worldwide mycotoxin regulations. *FAO Food and Nutrition Paper* 2003; No. 64. FAO, Rome.

4. Institute of Standard and Industrial Research of I.R. Iran (ISIRI). Maximum tolerated limits of mycotoxins in foods and feeds. 2002, National Standard No. 5925.

5. Shephard GS, Marasasy WFO, Yazdanpanah H, Rahimian H, Safavi N, Zarghi A, Shafaati A, Rasekh HR. Fumonisin B1 in maize harvested in Iran during 1999. *Food Addit Contam.* 2002; 19 (7): 676 - 9.

[DOR: 20.1001.1.2717204.2009.8.29.36.7]

6. Shephard GS, Marasas WFO, Leggott NL, Yazdanpanah H, Rahimian H, Safavi N. Natural occurrence of fumonisins in corn from Iran. *J. Agric. Food Chem*. 2000; 48: 1860 - 4.

7. Yazdanpanah H, Miraglia M, Calfapietra FR, Brera C. Natural occurrence of aflatoxins and ochratoxin A in corn and barley from Mazandaran and Golestan in north provinces of Iran. *Mycotoxin Res.* 2001; 17: 21 - 30.

8. Yazdanpanah H, Shephard GS, Marasas WFO, van der Westhuizen L, Rahimian H, Safavi SN, Eskandari P, Ghiasian SA. Human dietary exposure to fumonisin B1 from Iranian maize harvested during 1998 – 2000. *Mycopathologia* 2006; 161: 395 – 401.

9. Hadiani MR, Yazdanpanah H, Ghazi-Khansari M, Cheraghali AM., Goodarzi M. Survey of the natural occurrence of zearalenone in maize from northern Iran by thin-layer chromatography densitometry. *Food Addit Contam*. 2003; 20**:** 380 – 5.

10. Cheraghali AM, Mohammadi HR, Amirahmadi M, Yazdanpanah H, Abouhossain G, Zamanian F, Ghazi Khansari M, Afshar M. Incidence of patulin contamination in apple juice produced in Iran. *Food Control* 2005; 16**:** 165 – 7.

11. Cheraghali AM, Yazdanpanah H, Doraki N, Abouhossain G, Hassibi M, Ali-abadi S, Aliakbarpoor M, Amirahmadi M, Askarian A, Fallah N, Hashemi T, Jalali M, Kalantari N, Khodadadi E, Maddah B, Mohit R, Mohseny M, Phaghihy Z, Rahmani A, Setoodeh L, Soleimany E, Zamanian F. Incidence of Aflatoxins in Iran Pistachio Nuts. *Food Chem. Toxicol.* 2007; 45: 812 - 6.

12. Ghiasian SA, Rezayat SM, Kord-Bacheh P, Maghsood AH, Yazdanpanah H, Shephard GS, Westhuizen L, Vismer HF, Marasas WFO. Fumonisin production by Fusarium species isolated from freshly harvested corn in Iran. *Mycopathologia* 2005; 159: 31 – 40.

13. Ghiasian SA, Maghsood AH, Yazdanpanah H, Shephard GS, Westhuizen LV, Vismer HF, Rheeder JP, Marasas WFO. Incidence of *Fusarium verticillioides* and Levels of Fumonisins in Corn from Main Production Areas in Iran. *J. Agric. Food Chem*. 2006; 54: 6118 - 22.

14. Khosrokhavar R, Rahimifard N, Shoeibi Sh, Pirali-Hamedani M, Hosseini MJ. Effects of zearalenone and α-zearalenol in comparison with Raloxifene on T47D cells. *Toxicol. mechanisms and methods* 2009; 19: 246 - 50.

15. Trucksess MW. Natural Toxins, In: Official Methods of Analysis: AOAC INTERNATIONAL. Ed. W. Horwitz, 17th edn. Gaithersburg, *MD: AOAC INTERNATIONAL*; 2000; chapter 49, pp: 4 - 5 and 34 - 7.

16. European Commission. Commission Regulation (EC) No 401/2006 of 23 February, 2006, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Off J. Eur. Union* 2006; L70, 12 - 34.

17. Yazdanpanah H, Gheidari PE, Homami SNS, Mirkarimi SK, Zarghi A, Shephard GS, Marasas WFO, Shafaati AR, Rasekh HR. Natural occurrence of fumonisin B_1 in maize and its risk in Iran. New Horizon of Micotoxicology for Assuring Food Safety. *Japanese Associat Micotoxicol*; 2004.

18. Vargas EA, Preis RA, Castro L, Silva CM. Co-occurrence of aflatoxins B_1, B_2, G_1, G_2, zeaalenone and fumonisin B_1 in Brazilian corn. *Food Addit Contam* 2001; 18: 981 - 6.

[DOR: 20.1001.1.2717204.2009.8.29.36.7]

19. Ono EY, Ono MA, Funo FY, Medinal AE, Oliveira TC, Kawamura O, Ueno Y, Hirroka EY. Evaluation of fumonisin- aflatoxin co-occurrence in Brazilian corn hybrids by ELISA. *Food Addit Contam* 2001; 18: 719 - 29.

20. Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G. The occurrence of mycotoxins in key commodities in Bangladesh: surveillance results from 1993 to 1995. *J. Nat. Toxins* 2002; 11: 379 - 86.

21. Pietri A, Bertuzzi T, Pallaroni L, Piva G. Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northern Italy. *Food Addit Contam* 2004; 21: 479 - 87.

22. Yoshizawa T, Yamashita A, Chokethaworn N. Occurrence of fumonisins and aflatoxins in corn from Thailand. *Food Addit Contam* 1996; 13: 163 - 8.

[DOR: 20.1001.1.2717204.2009.8.29.36.7]