

Aflatoxins in Rice Imported to Bushehr, A Southern Port of Iran

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Abstract: Aflatoxins (B₁, B₂, G₁ and G₂) levels were surveyed in rice samples imported to Iran through a southern port in Bushehr. Aflatoxin analysis was performed by solvent extraction, immunoaffinity clean-up and determination using high performance liquid chromatography equipped with post-column photochemical reactor and fluorescence detector. Among 152 samples analyzed, 75 % showed levels of aflatoxin B₁ (AFB₁) contamination. However, there was no sample with AFB₁ above maximum tolerated level (MTL) of 5 ng/g assigned by Institute of Standard and Industrial Research of Iran (ISIRI). AFB₁ concentrations in the samples was 0.09-3.3 ng/g. Out of 152 samples analyzed, about 76.97% were contaminated with total aflatoxin (AFT) with the mean of 0.671 ng/g which is lower than MTL of AFT in rice (30 ng/g) assigned by (ISIRI). AFT concentration ranged from 0.15-4.27 ng/g. Contamination of aflatoxins in imported rice was dissimilar between different months. The highest levels of AFB₁ and AFT were detected in rice samples imported in September, while the lowest levels were in rice imported during November.

Key words: Aflatoxins • Food Safety • Rice • Iran

INTRODUCTION

Rapid population growth in developing countries leads to an ever increasing demand for food. In the production of food crops losses occur during the growth cycle in the field, harvest and storage where up to 5% of grain weight can reduce the agricultural output [1]. The risk of contamination by mycotoxins is an important food safety concern for grains and other field crops [2-4]. The genus *Aspergillus* is distributed worldwide and contains over 180 species. It is one of the most ubiquitous and abundant of all groups of fungi and one of the most studied fungal groups [5-7]. *A. flavus* and *A. parasiticus*, the two *Aspergillus* species of most concern in agriculture, are predominant saprotrophs with limited parasitic ability [8].

Toxins produced by some *Aspergillus* spp. are called aflatoxins. Aflatoxins were first discovered in Europe in animal feed. AFs are found as contaminants in various agricultural commodities such as maize, rice, sorghum, wheat, oats, spices (black pepper, ginger) and chilli and are considered to be of greater significance for human beings [10]. The four major AFs that occur in crops are B₁, B₂, G₁ and G₂. *A. flavus* produces aflatoxins B₁ and B₂,

while *A. parasiticus* produces all four aflatoxins; aflatoxin B₁ is the most toxic and best studied of the aflatoxins [8].

Afs are the most potent carcinogens in animal and human populations [8, 9-11].

The International Agency for Research on Cancer has designated Afs as a human liver carcinogen [12]. They also interfere with the function of the immune system [13]. A wide variety of animals including fish, rodents, waterfowl, poultry, swine and cattle can be affected by aflatoxins [14-16]. The knowledge that AFs effect on humans and animals has led many countries to establish maximum tolerated level (MTL) on aflatoxins levels allowed in food and feed in the last decades to safeguard the health of humans, as well as the economical interests of producers and traders. Currently, worldwide range of limits for AFB₁ and total AF (AFT) are 1-20 and 0-35 ng/g, respectively [12-17, 18-20].

Rice (*Oryza sativa* L.) is the most important staple food crop in Iran and is cultivated in different areas that have sultry and rainy climate. Since the amount of rice cultivation is not enough for domestic consumption, the country imports rice from other regions such as India, Pakistan, Bangladesh and Thailand which are the largest producers of rice in the World. However some of those

countries have frequent and heavy rainfall and floods in coastal areas particularly near harvest, which under this climate the development of fungi, especially species of the *Aspergillus* and *Penicillium*, is a common and unresolved problem [21, 22]. Thus, when the rice imported to Iran port, it is usually examined for aflatoxins contamination. The Institute of Standards and Industrial Research of Iran (ISIRI) has set minimum levels of 5 and 30 ng/g for aflatoxin B₁ and the total aflatoxins, respectively [24]. Bushehr is the major port for importing rice (*Oryza sativa*) in south west of Iran. Therefore, the aim of this investigation was to identify the levels of aflatoxin B₁ and the total aflatoxins in imported rice to Bushehr port.

MATERIALS AND METHODS

Samples: One hundred and fifty two samples were collected randomly by inspectors of Food Control Offices in Bushehr port from May 2008 to February 2009. Samples were taken according to method of the Iranian National Standard of Sampling for aflatoxins analysis in agricultural products [23]. Then, all the samples were transferred to Toxicology Labs in Food and Drug Control Laboratory. A minimum size of 2 Kg from each sample was used for analysis. Samples were kept at 20°C in PE (Poly ethylene) bags until analysis.

Reagents and Apparatus: All reagents (potassium chloride, phosphoric acid, hydrochloric acid) and solvents (methanol, acetonitrile, propanol-2-ol, n-hexane, chloroform) used were of HPLC grade. AF standards were purchased from Sigma Chemical Company, USA. Afla test immunoaffinity columns (IAC) were purchased from VICAM Company, Watertown, MA, USA.

Apparatus characteristics were WATERS 1525 binary HPLC pump and 2475 Multi λ fluorescence detector. HPLC column (C₁₈ 250 × 4.6 mm: 4 μ m) was purchased from Waters, USA.

Sample Preparation: To avoid the sub-sampling error due to highly heterogeneous nature of fungal distribution in AF analysis, every sample was grinded with the miller and collected in plastic bag and finally 50 g of test portion from the ground samples were taken for analysis.

Extraction and Clean Up: Samples were analyzed using a high performance liquid chromatography (HPLC) following AOAC [24] and ISIRI method [25]. Samples were extracted with methanol: water: hexane (240:60:100, v/v/v). The mixture was shaken for 30 minutes on a mechanical shaker. The solution was left to sediment and filtered through a Whatmann filter No.1. After filtration, the extract was diluted with water and filtered through glass

micro fiber filter. Afla test was used for samples clean up. First, 10 ml phosphate buffer saline (PBS) was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at a flow rate of 1 ml/min. The column was washed with water and dried using vacuum. Finally, AF was eluted with methanol using the following procedure. First, 0.5 ml methanol was applied on the column which passed through by gravity. After 1 min, the second portion of 0.75 ml methanol was applied and collected. The elute was diluted with water and analyzed using HPLC.

AF Standards: After preparation of standard solutions of AFs, the concentration of each one was determined using UV spectrophotometer. These standards were used to prepare mixed working standards for HPLC analysis.

Recovery and Limit of Detection (LOD): The effectiveness of the extraction procedure was confirmed by sample fortification. The recovery of extraction method was determined by fifty grams of milled rice fortified with a solution of AF in methanol at 5 μ g/ml (for B₁ and G₁) and 1 μ g/ml (for B₂ and G₂) 1 h before extraction. The AF fortification solution was prepared in methanol and used for quantification of the analytic recovered after extraction. Sample were fortified with 0.25 ml of this solution in order to have 5 ng/g of AFB₁ in rice, which is the maximum permitted limit in cereals by the National Standard of Iran [26]. LOD were 0.07, 0.08, 0.1, 0.07 and 0.32 ng/g for AFB₁, AFB₂, AFG₁, AFG₂ and total AFs, respectively.

Analysis of Af Using HPLC: AF was quantified by reverse-phase HPLC and 2475 Multi λ fluorescence detector with post column derivatization (PCD) involving bromination [27]. The waters HPLC system was applied with a Kobra cell and addition of bromide to the mobile phase. After dilution of AF elute with water, 100 μ L was injected into HPLC. Mobile phase was water: methanol: acetonitrile (600:300: 200, v/v/v) and 350 μ L of nitric acid 4M and 120 mg of potassium bromide with a flow rate of 1ml/min. The fluorescence detector was operated at an excitation wavelength of 365 nm and emission wavelength of 435 nm [27,28]. The calibration curve for each individual AF including AFB₁, AFB₂, AFG₁ and AFG₂ was used to check for the linearity and quantification of AF in rice samples.

RESULTS AND DISCUSSION

Among 152 samples analyzed, 38 samples (25 %) were not contaminated with AFB1 (<LOD). A high proportion of samples (75 %) were positive to AFB1 contamination

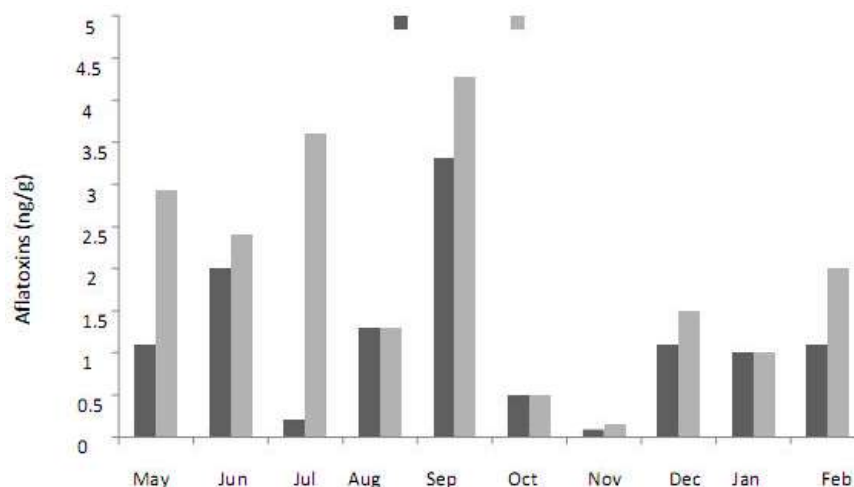


Fig. 1: Maximum levels (ng/g) of aflatoxin B₁ (AFB₁) and total aflatoxins AFT detected in the examined rice samples in different months

Table 1: Mean and standard deviation of aflatoxin B₁ (AFB₁) and total aflatoxins (AFT) (ng/g) in the examined rice samples

Month	N	ND	AFB ₁ (ng/g)	AFT(ng/g)
May	4	1	0.633±0.404	1.057±1.27
Jun	8	0	0.875±0.851	0.806±0.03
Jul	10	1	0.155±0.052	0.935±1.087
Aug	20	1	0.426±0.424	0.626±0.509
Sep	23	4	0.579±1.010	0.882±1.294
Oct	13	4	0.144±0.133	0.155±0.133
Nov	19	6	0.584±0.288	0.646±0.371
Dec	2	1	0.09	0.15
Jan	30	11	0.426±0.202	0.526±0.202
Feb	23	9	0.685±0.377	0.927±0.581

(Table1). Mean of AFB₁ in the samples was 0.46 ng/g. However, there was no sample with AFB₁ above MTL of 5 ng/g assigned by Institute of Standard and Industrial Research of Iran (ISIRI) [26] (Figure 1). Maximum level of AFB₁ in rice samples was 3.3 ng/g.

Among 152 samples analyzed, 35 samples (23.03 %) did not show any AFT contamination. However, 76.97% of samples were contaminated with AFT with the mean of 0.671 ng/g which were lower than MTL of AFT in rice in Iran (30 ng/g) [26] (Table1). Maximum level of AFT in rice samples was 4.27ng/g (Figure 1). Levels of aflatoxins in rice samples were different between different months. The highest levels of AFB₁ and AFT detected were in rice samples imported in September 2009. The lowest levels were detected in rice imported during November 2009 (Figure 1).

In a similar study from Iran by Mazaheri [28] among 71 rice samples analyzed, AFB₁ was detected in 59 samples (83% of the total). The mean of AFB₁ was 1.89

ng/g for all samples. Total AF was detected in 83% of samples. Mean of AFT was 2.09 ng/g. AFB₁ levels in two samples (2.8%) were above the maximum tolerable level (MTL) of AFB₁ in Iran (5ng/g). Another survey conducted by Food Standards Agency, UK in 2002 [29] to determine the levels of mycotoxins in rice showed that their levels ranged from 0.2 to 1.8 mg/kg. All levels found were below the EC (European Commission) legislative limits of 2 mg/kg aflatoxin B₁ and 4 mg/kg total aflatoxin in cereal products for direct human consumption [30, 31].

Sales and Yoshizawa [32] reported that the incidence of AFB₁ in rice from the Philippines ranged from 0.025 to 11.0 mg/kg. In another study the AFB₁ contamination was detected in 37 samples of rice grains from China. They found that 92% of the samples were positive to AFB₁ [33]. Toteja *et al.* [34] reported the presence of AFB₁ in parboiled rice collected from 11 states in India; 38.5% of the samples were positive to AFB. It has been reported that from 1200 samples from India, 67.8% were positive to AFB₁. The highest level of AFB₁ found in the samples was 38.5 mg/kg [35, 36].

Prasad *et al.* [37] tested 56 samples of stored rice and found that 12 were positive for aflatoxin. Levels of aflatoxins ranged from 184 to 2830 mg/kg. Jayaraman and Kalyansundaram [38] reported that 35% of the samples of raw rice bran and parboiled rice bran showed the presence of aflatoxin B₁. It was shown that bran of parboiled rice supports higher aflatoxin production than bran of raw rice. In a study carried by Bandara *et al.* [39] who reported that in almost all the samples of parboiled rice, AFB₁ and AFG₁ contents were significantly higher than the raw milled rice. Cultivar differences in the amount of

aflatoxin B₁ and G₁ were showed by Sinha and Dubey [40]. A survey on the prevalence of aflatoxin B₁ (AFB₁) in rice bran in coastal and interior districts of Tamil Nadu and Andhra Pradesh, India revealed that 62% of the samples contained AFB₁ and the levels far exceeded the permissible limit of 50 mg/kg [41].

Our investigation demonstrated that market rice represents a significant source of exposure to aflatoxin. These data suggest that public health efforts to interrupt aflatoxin exposure must include both an assessment of aflatoxin contamination and the replacement of contaminated rice in accordance with the strict laws on permissible limits of aflatoxin levels in food and feed products.

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