# Studies on Contamination Level of Aflatoxins in Pakistani Rice

ARSHAD HUSSAIN *, JAVED ALI, SHAFQATULLAH
*Food Technology Center, PCSIR Labs Complex, Jamrud Road, Peshawar*

(Received on 6th April 2010, accepted in revised form 15th October 2010)

**Summary**: Aflatoxins (AF) are highly toxic and carcinogenic secondary fungal metabolites and have been detected in various food commodities including cereals. Rice samples collected during 2008–2009 were analyzed for aflatoxin $B_1$ ($AFB_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_1$ ($AFG_1$) and aflatoxin $G_2$ ($AFG_2$) by thin layer chromatographic (TLC) technique. In total, 40 rice samples were collected and after dividing samples to sub-samples, AF analyses were carried out. $AFB_1$ was detected in 28 samples (70 % of the total). The mean of $AFB_1$ was 3.7ng/g for all samples. Total AF (AFT) was detected in 28 samples (70 % of the total). The mean of AFT was 4.9ng/g for all samples. $AFB_1$ levels in 20 samples were below the maximum tolerated level (MTL) of $AFB_1$ (2ng/g). Regarding AFT, the mean contamination level (4.9ng/g) was higher than the EU maximum permissible level for AFT (4ng/g).

## Introduction

The aflatoxins (AF) are a group of toxic and carcinogenic polyketide secondary metabolites, which are produced by strains of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* and *Aspergillus* pseudotamarii [1]. The International Agency for Research on Cancer (IARC) has classified aflatoxin $B_1$ ($AFB_1$) as a group I carcinogen, primarily affecting liver [2]. AF is found as contaminants in various agricultural commodities such as cereals, tree nuts, groundnut and cottonseed [3]. Among 18 different types of aflatoxins identified, major members are aflatoxins $B_1$, $B_2$, $G_1$, $G_2$, $M_1$ and $M_2$. Aflatoxin $B_1$ is produced most abundantly and is also most toxic followed by $G_1$, $B_2$ and $G_2$ [4]. Basmati rice *(Oryza sativa* Linn.*)* together with corn, are the second most produced cereals in the world, wheat being the first. The annual world production of these two cereals is estimated at 500 million tons and Pakistan is one of the Asian countries wherein rice is cultivated and used as a basic food item. China heads this list with an annual production of 180 million tons [5]. The occurrence of mycotoxins in rice has been reported from several countries [6-8].

AFs are found as contaminants in various agricultural commodities such as cereals, tree nuts, groundnut and cottonseed. The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish maximum tolerated level (MTL) on mycotoxins in foodstuffs and feedstuffs 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 $AFB_1$ and total (AFT) are 1–20ng/g and 0–35ng/g, respectively [9].

Rice (*Oryza sativa* Linn.) is the second major cash crop and is also one of the main exports of Pakistan. It accounts for 6.10 % of the total value added in the agriculture and 1.3 % to the GDP [10]. The nutritive value of rice (as an energy source i.e. 2345 kcal/kg) and its high hygroscopicity make it an ideal substrate for the establishment and growth of fungal species, especially toxigenic fungi that produce mycotoxins like aflatoxins. Factors like moisture content, water activity, temperature, period of storage, initial level of contamination, toxigenic potential of fungal strains and nature of substrate influence the production of mycotoxins [11]. In addition, during storage fungi causes organoleptic changes that result in grain discoloration, mould color, mustiness and reduced germinability by destroying the embryo [12].

## Results and Discussion

The average recoveries and relative standard deviation of the analytical method applied for $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ in rice were investigated. The results are shown in Table-1 and 2. Both recoveries and SD of AF were in the acceptable range (70 – 110%). Among 40 samples analyzed, 12 samples (30 %) were not contaminated with $AFB_1$ (<LOD) (Fig.1). Considering the EU limit for $AFB_1$ in rice (2ng/g), 8 samples had levels above the maximum tolerable limit (MTL) were in the range of 3.2-18ng/g, while 20 samples in the range of 1.2-1.8ng/g were below MTL. 8 samples (20 %) were contaminated with $AFB_2$, representing 4 samples in the range of 0.8-1.5ng/g were above the maximum tolerable limit (MTL) and 4 samples in the range of 2.6-10.4ng/g were below the MTL. 3 samples (7.5 %)

were contaminated with $AFG_1$ in the range of 2.8-8.4ng/g was above MTL and 4 samples (10 %) were contaminated with $AFG_2$, representing 3 samples in the range of 2.5-12.0ng/g were above MTL. $AFB_1$ was detected in 28 samples (70 %) and its levels in 8 samples in the range of 3.2-18.0ng/g were above the MTL in rice (2ng/g), which has been set by European Commission (EC) No. 856/2005. The mean of $AFB_1$ in the samples were 3.4ng/g.

A number of mycotoxins are immunosuppressive and likely could be involved in human diseases. Humans are exposed to mycotoxins through several routes such as ingestion (the most prominent means of exposure), contact and inhalation [13]. Aflatoxins are storage mycotoxins and known for causing acute aflatoxicoses in human but chronic forms of aflatoxicoses, especially carcinomas are the more problematic. Aflatoxins have been declared by IARC (International Agency for Research on Cancer) as class 1 carcinogen [14-15]. All the samples were analyzed for AFT, among which 12 samples (30 %) did not show any contamination (Fig. 2). However, AFT was detected in 28 samples (70 % of the total) with the mean of 4.9ng/g. The mean contamination level (4.9ng/g) was higher than maximum tolerable limit (MTL) of AFT in rice (4ng/g).

Rice as an important cereal is cultivated in several countries including India, Pakistan, Thailand, and Uruguay. Many countries have developed MTL for mycotoxins in foodstuffs and feedstuffs [9]. Rice is our major export items and is being exported to various countries of Europe and Middle East. So far in Pakistan no safety regulations have been established for aflatoxins in food grains for use as a staple diet and for export.

Table-1: Mean, standard deviation, maximum of aflatoxin $B_1$, $B_2$, $G_1$, $G_2$ and total aflatoxins (ng/g) in rice samples.

| AF | $B_1$ | $B_2$ | $G_1$ | $G_2$ | Total |
|---|---|---|---|---|---|
| Mean | 3.7 | 3.4 | 5.1 | 5.4 | 4.9 |
| Max | 18.0 | 10.4 | 8.40 | 12.0 | 14.0 |
| STD | 0.15 | 0.10 | 0.35 | 0.20 | 0.30 |

## Experimental

*Materials*

*Collection of Samples*

A total of 40 (1-2) Kg samples were collected from the rice producing areas and various major stores of government / private sectors in Pakistan. These samples were ground and sub sampled (100g) by grinding mill for the purpose of obtaining a homogeneous and representative sample. All the samples were transferred to Mycotoxins Laboratory, Food Technology Centre, PCSIR Laboratories Complex Peshawar and were analyzed.

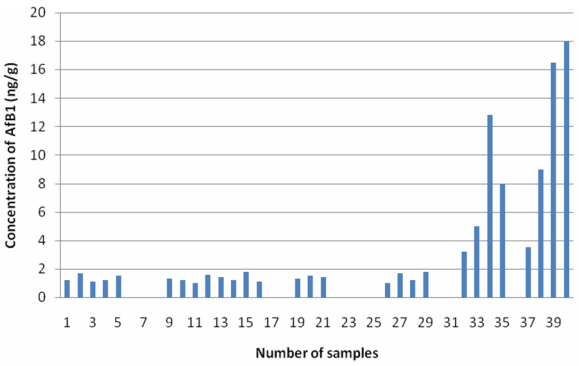


Fig. 1: Incidence of $AFB_1$ in rice samples.

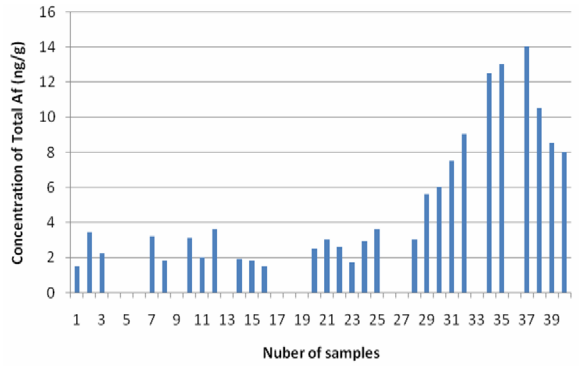


Fig. 2: Incidence of AFT in rice samples.

Table-2: Contamination level of aflatoxins in rice samples.

| Toxins | No. of samples | Positive samples | Contamination detected (%) | Samples with aflatoxins | | Limits (EC) No. 856/2005 (maximum) |
|---|---|---|---|---|---|---|
| | | | | Below limit | Above limit | |
| Aflatoxin $B_1$ | 40 | 28 | 70 | 20 (1.2 -1.8)* | 8 (3.2-18.0) | 2 ng/g for $AFB_1$ |
| Aflatoxin $B_2$ | 40 | 8 | 20 | 4 (0.8 -1.5) | 4 (2.6-10.4) | 4 ng/g for AFT ($B_1$, $B_2$, $G_1$ & $G_2$) |
| Aflatoxin $G_1$ | 40 | 3 | 7.5 | - | 3 (2.8-8.4) | |
| Aflatoxin $G_2$ | 40 | 4 | 10 | 1 (1.7) | 3 (2.5-12.0) | |
| Aflatoxin Total | 40 | 28 | 70 | 18 (1.5-3.6) | 10 (5.6-13.9) | |

* Values in bracket represent aflatoxins range in ng/g

Limit of Detection (LOD): 1ng/g for $AFB_1$ and $AFG_1$; 0.5ng/g for $AFB_2$ and $AFG_2$

*Chemicals*

All the chemicals of analytical grade used in the present study were procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (ST. Louis, USA). Standards of aflatoxin $B_1$ (2.02 µg/ ml), aflatoxin $B_2$ (0.500 µg/ ml), aflatoxin $G_1$ (2.01 µg/ ml) and aflatoxin $G_2$ (0.500 µg/ ml) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ of concentrations 1 µg/ ml each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were then stored at 4 °C in refrigerator, wrapped in aluminum foil due to that aflatoxins gradually breakdown under UV light.

*Determination of aflatoxins*

Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ were determined according to the method described by Soares and Rodrı´giez–Amaya [16]. Briefly, 50 g of each sample of hulls and kernels was extracted with 270 ml methanol and 30 ml 4% potassium chloride. Samples were blended at moderate speed for 30 min and filtered, and 150 ml of the filtrate was collected into a graduated cylinder. Next, 150 ml 10% copper sulfate and 50 ml diatomaceous earth were added, followed by moderate stirring and filtration. The filtrate was again recovered up to 150 ml and transferred to a separation funnel, and toxins were extracted three times with 10 ml chloroform. The chloroform extracts were collected into a beaker and submitted to solvent evaporation in a water bath at 60 °C. Extracts were re dissolved in 500 µl chloroform and immediately submitted to thin-layer chromatography (TLC).

Final identification and quantification of aflatoxins were performed by one-dimensional thin-layer chromatography on pre coated silica gel plates (Merck). The plates were developed in a saturated chamber with chloroform/acetone (9:1, v/v). Aflatoxins spots were observed under long-wave ultraviolet light (k = 366 nm) and determined by visual comparison with $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ standards prepared. Confirmatory tests for aflatoxins were carried out using trifluoroacetic acid [17].

*Recoveries Study*

The recovery (percentage of standard added to sample that is recovered after extraction and clean up) of extraction method was determined by sample fortification. 50 grams of milled rice was fortified one hour before extraction with a solution of AF in benzene: acetonitrile (98:1 v/v) at 5 µg/ml (for $B_1$ and $G_1$) and 1µg/ml (for $B_2$ and $G_2$). The AF fortification solution was prepared in benzene: acetonitrile and used for quantification of analyte recovered after extraction.

*Note*

AFs are carcinogens and care should be exercised to avoid personal exposure and potential risk of contamination. All handling of pure compounds were done in the fume hood with protective gear such as safety glasses, gloves, laboratory coat and a disposable face mask. The glassware were washed with hypochlorite and dilute acid before re-using and the waste materials treated with hypochlorite before disposal.

**References**


1 G. A. Payne, Process of contamination by aflatoxin-producing fungi and their impact on crops. In: K.K.S. Sinha and D. Bhatnagar, Editors, Mycotoxins in Agriculture and Food Safety, Marcel Dekker, Inc., New York, pp. 279-306 (1998).

2 I. A. R. C (International Agency for Research on Cancer), IARC Monograph on the evaluation of carcinogenic risk to humans, vol. 56. IARC, Lyon, France (1993).

3 A. Pittet, *Revue Medical Veterinary,* **149**, 479 (1998).

4 Y. L. Krishnamurthy and J. Shashikala, *Letters in Applied Microbiolgy*, **43,** 469 (2006).

5 O. Sindarroz, *Acesso Em,* **20**, (2003). http://www.sindarrozsc.com.br

6 S. Tabata, H. Kamimura, A. Ibe, H. Hashimoto, M. Iida, Y. Tamura and T. Nishima, *Journal of Association of Official Analytical Chemists International*, **76**, 32 (1993).

7 A. H. El-Gohary, *Indian Journal of Animal Sciences*, **66**, 468 (1996).

8 S. Patel, C. M. Hazel, A. G. M. Winterton and E. Mortby, *Food Additives and Contaminants*, **13**, 833 (1996).

9 F. A. O. (Food and Agriculture Organization of the United Nations). World wide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper, No. 81, Rome (2004).

10 G. O. P (Government of Pakistan). Economic survey of Pakistan. Government of Pakistan, Ministry of finance, Islamabad (2006).

11 Q. H. Nafeesa and K. Salma, *Pakistan Journal of Food Science*, **16** (1-4), 44 (2006).
12 H. K. Taligoola, M. A. Ismail and S. K. Chebon, *Journal of Biological Sciences*, **4**, 271 (2004).
13 C. A. S. T. Mycotoxins, Risks in plants, animals and humans. Task Force Report No. 139. Council for Agricultural Science and Technology (CAST), Ames, lowa, USA (2003).
14 K. Painter. Puberty signs evident in 7 and 8 year old girls. USA Today, Washington, D.C. April 8, pp. 1-2 (1997).
15 C. A. Saenz de Rodriguez, A. M. Bongiovanni and L. Conde de Borrego, *Journal Pediatrician*, **107**, 393 (1985).
16 L. M. V Soares and D. B. Rodrı´giez-Amaya, *Journal of the Association of Official Analytical Chemists*, **72**, 22 (1989).
17 P. M. Scott, Natural poisons. In K. Helrich (Ed.), Official methods of analysis (16th ed.), Arlington, VA, Association of Official Analytical Chemists (1990).