*Sci., Tech. and Dev.*, 32 (2): 110-119, 2013

# INCIDENCE OF AFLATOXINS IN EXPORT QUALITY BASMATI RICE COLLECTED FROM DIFFERENT AREAS OF PAKISTAN

**MUHAMMAD ASIF ASGHAR, JAVED IQBAL, AFTAB AHMED, ZUZZER ALI SHAMSUDDIN AND MOBEEN AHMED KHAN***

***Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Laboratories Complex, Shahrah–e–Salimuzzaman Siddiqui, Off University Road, Karachi–75280, Pakistan.***

**Abstract**

A survey on the level of aflatoxins (AFs) in export–quality Pakistani basmati rice was performed. In this regard, 2047 basmati rice samples were collected from various rice vendors at different spans of time, during 2006–2011. Samples were analysed by thin layer chromatography (TLC) for the presence of aflatoxin $B_1$ ($AFB_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_1$ ($AFG_1$) and aflatoxin $G_2$ ($AFG_2$). Among 2047 samples, about 73.3% samples were found contaminated with $AFB_1$ ranging from 1.17 to 6.91µg/kg and a mean of 1.15µg/kg. Moreover, in 70.6% samples, $AFB_1$ level was found lower than maximum tolerated level (MTL = 2µg/kg) as recommended by European Union. Furthermore, 2.3% samples contained $AFB_1$ level ranging from 2.05 to 3.36µg/kg. However, only 0.44% samples exhibit $AFB_1$ contamination ranged between 4.07 to 6.91µg/kg. Remaining 26.7% samples does not contain $AFB_1$ level within detectable limit (≥1µg/kg). Moreover, all the samples were found below than MTL of 20µg/kg as assigned by the authorities in United State (FDA and FAO) and Pakistan (PSQCA). During the whole study, AFs contamination in basmati rice seems to differ monthly due to variation in climatic conditions. Highly contaminated samples with $AFB_1$ were found during the months of August (6.91µg/kg), September (4.89µg/kg) and December (4.88µg/kg). On the basis of achieved results, it can be concluded that the level of AFs in Pakistani basmati rice was found safe for human consumption. Thus, Pakistani rice could be exported to the other countries. Though, the small quantities of AFs warrant performing further investigation, monitoring and routine analysis on regular basis.

**Keywords:** Aflatoxins, Basmati rice, Thin layer chromatography (TLC), Food safety, Pakistan.

## 1. Introduction

Rice *(Oryza sativa* Linn.) is one of the most staple foods in the world. Rice and corn are the second most produced cereals in the world, wheat being the first. Pakistan is one among Asian countries where basmati rice is cultivated in *kharif* or wet season. The total cultivation area is 10% (2.963 million hectares) while the production during 2008–2009 was 6.952 million tonnes. This accounts for 5.5% of the total value added in the agriculture and has 1.6% share in the GDP of Pakistan (Zafar, 2009).

Internationally, Pakistan ranks 14$^{th}$ in the rice production and is the 6$^{th}$ largest exporter, holding 6% share for the rice export of the world. Rice industries contribute 21.75% in the country's economy with a production of 29.5%. However, the total quantity of rice as per economy and production in Pakistan are lost a bit during post-harvest operation. The quality of seed, unfortunately, does not meet the international standard, so it could affect on marketing of rice and might decline the per acre yield as the technologies are out-dated (Junejo et al., 2007). Various varieties of rice are cultivated in different parts of the world. Some varieties are restricted to specific geographical regions such as basmati rice in Pakistan and India or jasmine rice in Thailand (Bhattacharjee et al., 2002). Basmati rice has a typical *pandan*–like (*Pandanusfascicularisleaf*) flavor which is caused by the aroma compound 2–acetyl–1–pyrroline (Wongpornchai et al., 2003).

***Author for correspondence E-mail: mobeenaklivdetox@yahoo.com**

Rice kernel can be contaminated by moulds during harvest, improper drying, handling, packaging, storage and transportation. By virtue of these, fungal growth could be facilitated which results in the increased risk of aflatoxins production. Hygroscopicity of rice is also an important factor that aids in the establishment and development of fugal species, especially toxigenic fungi that produces mycotoxins, like AFs. Post–harvest contamination can occur if the drying is delayed and moisture is allowed to exceed. Prevailing sub-tropical conditions in Pakistan, such as, temperature, humidity and the nearness to sea also play a vital role.

*Aspergillus*, *Penicillium* and *Fusarium* are the toxigenic fungi that frequently invade foodstuffs. These deleterious hepatotoxic, immune-suppressive, mutagenic and teratogenic toxic fungi are responsible for reproductive and fetal toxicity. The reason behind is the lack of adequate food consumption data, inadequate knowledge about relative health risks associated with specifically proposed limits and the possibility of synergism with other mycotoxins present in the same food commodities (Creppy, 2002). Among *Aspergillus*, *A. flavus* and *A. parasiticus* are the two species of most concern in agriculture and predominant saprotrophs with limited parasitic ability (Payne, 1998).

Aflatoxins (AFs) are a group of fairly distributed mycotoxins that are produced by fungi of the genus *Aspergillus*. AFs are reported as hepatotoxic, mutagenic, immunosuppressive and neoplastic. According to the quantity ingested, frequency of intake and the age of individual, result could be cirrhosis, necrosis of the liver, encephalopathy and increased susceptibility to hepatitis B (Lereau et al., 2012).

The most important AFs are aflatoxin $B_1$ ($AFB_1$), $B_2$($AFB_2$), $G_1$ ($AFG_1$) and $G_2$($AFG_2$). However, $AFB_1$ is the most frequently occurring among all of AFs. Moreover, the presence of $AFB_1$ owing to its extreme toxicity is often associated with severe hepatotoxigenicity and hepatocarcinogenicity. Biotransformation of $AFB_1$ occurs mainly in the liver through cytochrome P450 enzymes that can act in detoxification (Wang et al., 1998). The optimal temperature for AFs production ranged between 20–35°C. Elevation of temperature upto 40°C or decline upto $10^0$C could result in reduced toxins production. The high temperature within the optimal range favours the production of aflatoxin B (AFB). On the other hand, low temperature favours the production of aflatoxin G (AFG) (Schroeder, and Hein, 1967). Several countries, including Pakistan, have set forth the guidelines and acceptance level for AFs due to their frequent occurrence, toxicity and potential health hazards to humans. For instance, European Union (EU) has set maximum level of 4µg/kg for total AFs ($AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$) in the cereal and 2µg/kg for $AFB_1$ alone (ECR., 2010). As per USA (FDA and FAO) and Pakistan (PSQCA), the acceptance level for total AFs in rice is 20µg/kg (FDA, 2000; PSQCA., 2009).

Pakistani basmati rice has been a favourite among international community. Punjab and Sindh provinces are the major basmati producing areas in the country. Major basmati rice export markets for Pakistan are U.A.E, Saudi Arabia, UK, Yemen, Qatar, Bahrain, Kuwait, Malaysia and USA. Rice is usually examined for AFs contamination as per requirement of phytosanitary certificate as it is widely consumed within the country and exported to other countries as well. On the basis of above mentioned facts, this study was designed to determine the presence and level of AFs in rice. As the comprehensive study on AFs in Pakistani basmati rice was limited, therefore, in the present study, the level of AFs was determined in large number of Pakistani basmati rice samples.

## 2. Materials and Methods

### 2.1 Reagents and Apparatus

Aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ standards (crystalline powder) were purchased from Sigma–Aldrich (St. Louis–MO, USA). Precoated TLC plates of Silica gel 60 (layer thickness 0.25mm, 20cm x 20cm) on glass or aluminum, without fluorescent indicator were purchased from E. Merck (Dramstadt, Germany). Analytical grade acetone, acetonitrile, benzene, chloroform, cupric carbonate, ferric chloride, potassium chloride, potassium hydroxide, sodium hydroxide, sodium sulfate, sulfuric acid, xylene and other solvents procured from BDH (Poole, England).

### 2.2 Sampling

During 2006–2011, a total of 2047 samples of Pakistani basmati rice were collected across the country from various local vendors after some

period of storage in the warehouses. They included conventional unpacked rice, iconic labels and store brands, as well. Sindh and Punjab provinces are the major producing areas of basmati rice in Pakistan. Therefore, maximum samples were from these two regions. It is well documented that AFs are heterogeneously distributed throughout most food and feed commodities. For this reason, sampling procedure was based on the method as described in AOAC official method no. 977.16 for the accurate estimation of AFs (Trucksess, 2005). Briefly, a minimum sample size of 500-1000g was taken and thoroughly mixed for 10 minutes. Samples were pulverised into particles ≤ 1mm by passing through sieve number 20 in a sample grinder (Cyclotec 1093 mill, Sweden) to obtain a homogeneous and representative sample. Finally, pulverised samples were kept in air tight polyethylene bags stored at -20°C till further analysis.

**2.3 Preparation of Aflatoxins Standards**

The standard stock solutions (10µg/ml) of each $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ were prepared by dissolving 1mg crystalline powder in 100ml benzene : acetonitrile (98 : 2; v/v). Final concentration of each AFs stock solution was thereafter determined by measuring the absorbance at 350nm using Jenway Genova MK3 spectrophotometer (Dunmow–Essex, England). Working standard solution of each $AFB_1$ and $AFG_1$ (1µg/ml) was individually prepared by combining 100µl of each stock solution (10µg/ml) and 900µl of benzene: acetonitrile (98:2; v/v), whereas, working standard solutions of each $AFB_2$ and $AFG_2$ (0.5µg/ml) were separately prepared by adding 50µl of each stock solution (10µg/ml) in 950µl of benzene : acetonitrile (98:2; v/v).

**2.4 Aflatoxins Analysis**

A modified Romer method based on bi–directional thin layer chromatography (TLC) was used for the detection of AFs as described in the AOAC official method no. 975.36/968.22 (Trucksess, 2005). The whole analytical procedure could be sub–divided into two major steps as mentioned below:

1. Sample purification and aflatoxins extraction
2. Thin layer chromatography

**2.4.1 Sample purification and aflatoxins extraction**

50g of each grinded basmati rice sample was weighed accurately and dispersed in 250ml of acetone : water (85:15; v/v). Sample suspension was blended for 3 minutes at 5000rpm, using Eberbach 8017 **e**xplosion–proof blender (Haverhill–Massachusetts, USA). The blended extract was filtered through Whatman number 4 filter paper. An aliquot of 150ml of sample extract was taken and mixed with 3g of cupric carbonate for 20s. The sample mixture was then added into a conical flask already containing 170ml sodium hydroxide (0.2M) and 30ml ferric chloride (0.41M), mixed well and filtered through Whatman number 4 filter paper. An aliquot of 250ml filtrate was taken into a separating funnel, vigorously shaken in the presence of 150ml sulfuric acid (0.03%; v/v) and 10ml chloroform and allowed to settle–down for 2 minutes. The lower layer of chloroform was transferred into another separating funnel already containing 1g potassium chloride and 100ml potassium hydroxide (0.02M) solution. After a gentle swirling of 30s, the lower chloroform layer was separated and re–collected into a graduated cylinder after passing through a bed of anhydrous sodium sulfate (1g). Finally, 8ml of chloroform extract was evaporated to dryness at 45$^{o}$C under gentle stream of nitrogen and stored at -20°C till the further quantification by TLC.

**2.4.2 Thin layer chromatography**

Dried extracts were solublised in 100µl benzene–acetonitrile (98:2; v/v) and vortexed. Finally, spots of 2, 5 and 10µl of samples and standards were individually applied on the TLC plate. Chromatographic plates were developed in unlined tank containing 20ml chloroform : xylene : acetone (6 : 3 : 1; v/v). TLC Plates were dried and observed under long wavelength UV light ($\lambda$ = 254 and 366*nm*) in an enclosed Camag 2930 UV visualiser (Germany). The retention factor ($R_f$) of each AFs ($B_1$, $B_2$, $G_1$ and $G_2$) was calculated in accordance with the equation (i).

$R_f$ = distance moved by compound/distance moved by solvent …. (i)

Furthermore, the concentration of individual $AFB_1$, $AFB_2$, $AFG_1$ or $AFG_2$ was calculated according to the following:

Concentration of $AFB_1$, $AFB_2$, $AFG_1$ or $AFG_2$ in µg/kg = $S \times Y \times V / X \times W$ …. (ii)

where;

S = Volume (µl) of $AFB_1$, $AFB_2$, $AFG_1$ or $AFG_2$ standard equal to unknown.

Y = Concentration (µg/ml) of $AFB_1$, $AFB_2$, $AFG_1$ or $AFG_2$ standard

V = Volume (µl) of final dilution of sample extract

X = Volume (µl) of sample extract spotted to give fluorescent intensity equal to S

W = Weight (g) of sample contained in final extract.

The concentration of Total AFs ($B_1$, $B_2$, $G_1$ and $G_2$) was calculated using

Total AFs = Concentration of $AFB_1$ + $AFB_2$ + $AFG_1$ + $AFG_2$ ……. (iii)

All positive findings of AFs, naturally present in rice samples, were confirmed by spraying the TLC plates with $H_2SO_4$ (50%; v/v) and making the derivative with trifluoroacetic acid.

### 2.5 Method Validation and Analytical Quality Assurance

The accuracy of the analytical procedure was verified by performing the analysis in triplicate. Furthermore, method validation was done through the analysis of control samples. Briefly, homogenised control rice samples (n = 20) were analysed five times per day for four successive days. Results were incorporated in a control chart with an upper and lower warning (±2σ; 95% confidence limit) and control limits (±3σ; 90% confidence limit). Additionally, at least two control samples were tested with each series of routine samples (n = 25) and results were compared with the previous control measurements. Results of control samples were found within ±2σ range. The accuracy of procedure was determined by the recoveries of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ from spiked samples. Furthermore, the recovery studies were performed by the analysis of uncontaminated (blank) rice samples before and after the addition of 2, 5 and 10µg/kg of each AFs standard. The average recoveries rate for the four AFs was from 83.3% to 91.8%. Results from the recovery study are presented in Table 1.

**Table 1. Recovery of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ from spiked samples of rice. All measurements were done in triplicate**

| Concentration added (µg/kg) | $AFB_1$ | | $AFB_2$ | | $AFG_1$ | | $AFG_2$ | |
|---|---|---|---|---|---|---|---|---|
| | Recovery concentration (µg/kg) | Mean recovery (%) | Recovery concentration (µg/kg) | Mean recovery (%) | Recovery concentration (µg/kg) | Mean recovery (%) | Recovery concentration (µg/kg) | Mean recovery (%) |
| 2 | 1.83 ± 0.11 | 91.8 | 1.70 ± 0.11 | 85.2 | 1.70 ± 0.11 | 85.22 | 1.77 ± 0.20 | 88.5 |
| 5 | 4.39 ± 0.45 | 87.8 | 4.16 ± 0.49 | 83.3 | 4.16 ± 0.25 | 83.26 | 4.52 ± 0.34 | 90.5 |
| 10 | 8.85 ± 0.59 | 88.5 | 8.65 ± 0.34 | 86.5 | 8.85± 0.59 | 88.5 | 8.46 ± 0.34 | 84.6 |

The limit of detection (LOD) for $AFB_1$ and $AFG_1$ was 1µg/kg and for $AFB_2$ and $AFG_2$ was 0.50µg/kg. General laboratory performance (GLP) was verified by participation in the FAPAS® proficiency testing program via test number 0488/2006, 0490/2006, 0493/2006, 04109/2007, 04113/2007, 04132/2008 and 04169/2011. Results were found within the range (±2 Z score). Food and feed safety laboratory has an ISO–17025 accreditation status with Pakistan National Accreditation Council.

## 3. Results and Discussion

Aflatoxins (AFs) contamination in food commodities is concerned to public health because of the severe toxicity associated with AFs. In the present study (2006–2011), a total of 2047 samples of basmati rice were analysed for the presence of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$. Relevant data regarding contamination of AFs is summarised in Table 2.

The results showed that 1501 (73.3%) samples were found positive for $AFB_1$ with an average of 1.15µg/kg which was considerably lower than European Union (EU) Maximum Tolerated Level (MTL=2µg/kg). The $AFB_1$ contamination ranged from 1.17–6.91µg/kg. Furthermore, in 546 (26.7%) samples, $AFB_1$ was not found within detectable limit (≥1µg/kg). In

1445 (70.6%) samples, $AFB_1$ level was found between 1.17–1.98µg/kg. Moreover, in 47 (2.3%) samples, $AFB_1$ ranged between 2.05–3.36µg/kg. However, only in 9 (0.44%) samples, $AFB_1$ ranged between 4.07–6.91µg/kg. It was also examined that AFs concentration in 1991 (97.3%) samples was found below than EU MTL (4µg/kg). However, none of the sample contains AFs contamination more than MTL of 20µg/kg as assigned by food authorities of USA (FDA and FAO) and Pakistan (PSQCA). Moreover, it has been observed that after 2007, unscheduled rain and flood continuity in Pakistan had affected the rice crop. The ripple effect can be seen upto the year 2011 (Table 2).

**Table 2. Distribution of $AFB_1$ in basmati rice during 2006–2011**

| Year | TN (n)[a] | % Positive samples | Number of samples in concentration range, *µg/kg* | | | | Average | Range |
|---|---|---|---|---|---|---|---|---|
| | | | NF[b] | 1.17–1.98[c] | 2.05–3.36[d] | 4.07–6.91[e] | | |
| 2006 | 87 | 0 | 87 | 0 | 0 | 0 | 0 | 0 |
| 2007 | 300 | 7.00 | 279 | 6 | 10 | 5 | 0.21 | 1.65–6.56 |
| 2008 | 115 | 17.39 | 95 | 2 | 18 | 0 | 0.44 | 1.94–3.31 |
| 2009 | 728 | 93.96 | 44 | 674 | 8 | 2 | 1.41 | 1.17–4.88 |
| 2010 | 612 | 96.08 | 24 | 575 | 11 | 2 | 1.51 | 1.17–6.91 |
| 2011 | 205 | 91.71 | 17 | 188 | 0 | 0 | 1.41 | 1.18–1.97 |
| Total | 2047 | 73.3 | 546 (26.7%) | 1445 (70.6%) | 47 (2.3%) | 9 (0.44%) | 1.15 | 1.17–6.91 |

[a] Total number of samples, [b] Not found within detectable limit (≤ 1*µg/kg*), [c] Below EU MTL, [d–e] Below FDA, FAO and PSQCA MTL

The level of AFs in rice differs from one place to another. This is due to various factors like temperature, relative humidity and agricultural practices. In general, hot and humid conditions are supposed to be favourable for the growth of toxigenic fungi and mycotoxin production in agricultural products (Reddy et al., 2008). The occurrence of AFs in crops is supposed to be strongly influenced by weather, during and after the growing season. A climate change is likely to lead to an increase in hot and dry spells and increased risk of AFs contamination.

Climatic information, regarding average temperature (T) and average relative humidity (RH) during the study, was gathered from Climatemps.com, 2012. It has been observed that AFs contamination in rice varied among different months due to variation in climatic conditions. Highly contaminated samples were found during the months of August ($AFB_1$ = 6.91µg/kg), September ($AFB_1$ = 4.89µg/kg) and December ($AFB_1$ = 4.88 µg/kg) (Table 3, Fig. 1) but contamination level was found within the range and does not potential risk to the human health.

**Table 3. Distribution of $AFB_1$ in basmati rice in different months and average temperature and relative humidity in Pakistan**

| Month | Number of samples | Positive samples (%) | Mean (µg/kg) | Range (µg/kg) | Average temperature ($^0$C) | Average relative humidity of the cultivation area (%) |
|---|---|---|---|---|---|---|
| Jan | 242 | 156 (64.4) | 1.00 ± 0.56 | 1.17–4.07 | 12.5 | 46 |
| Feb | 208 | 157 (75.5) | 1.17 ± 0.49 | 1.17–2.73 | 14.5 | 41 |
| Mar | 148 | 127 (85.8) | 1.24 ± 0.45 | 1.17–2.28 | 20 | 29 |
| Apr | 170 | 127 (74.7) | 1.05 ± 0.73 | 1.17–3.31 | 26 | 23 |
| May | 141 | 113 (80.1) | 1.17 ± 0.46 | 1.17–2.83 | 31 | 20 |
| Jun | 141 | 111 (78.7) | 1.35 ± 0.53 | 1.17–1.95 | 34 | 22 |
| Jul | 141 | 116 (82.3) | 1.24 ± 0.47 | 1.17–4.2 | 33 | 50 |
| Aug | 184 | 173 (94.0) | 1.47 ± 0.74 | 1.19–6.91 | 31 | 58 |
| Sep | 169 | 88 (52.1) | 0.97 ± 0.74 | 1.28–4.89 | 30 | 43 |
| Oct | 202 | 135 (66.8) | 1.10 ± 0.70 | 1.17–2.73 | 25 | 33 |
| Nov | 152 | 86 (56.6) | 0.92 ± 0.64 | 1.27–3.11 | 19 | 41 |
| Dec | 149 | 112 (75.1) | 1.23 ± 0.61 | 1.27–4.88 | 13.5 | 49 |

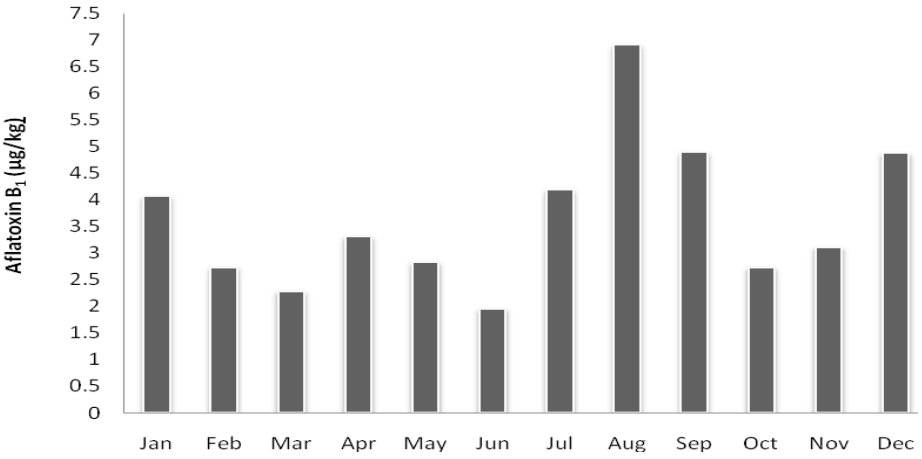


**Fig. 1. Distribution of $AFB_1$ in basmati rice in different months**

The maximum frequencies of positive samples were also found during the month of August. During this month, the average temperature and RH was 31°C and 58%, respectively (Figs. 2 and 3), which are the favourable conditions for the AFs producing fungi (Pitt and Hocking, 1997). Pitt and Hocking (2000) also described that the suitable temperatures for growth of *A. flavus* vary from a minimum of 10.0–12.8°C to a maximum of 43.0–48.8°C, with an optimum of approximately 33.8°C. According to Paterson and Lima (2009), AFs production is permitted at 28°C and completely inhibited at 37°C, which is close to the growth optimum. The results of present study were found in good agreement with the above mentioned previous studies.

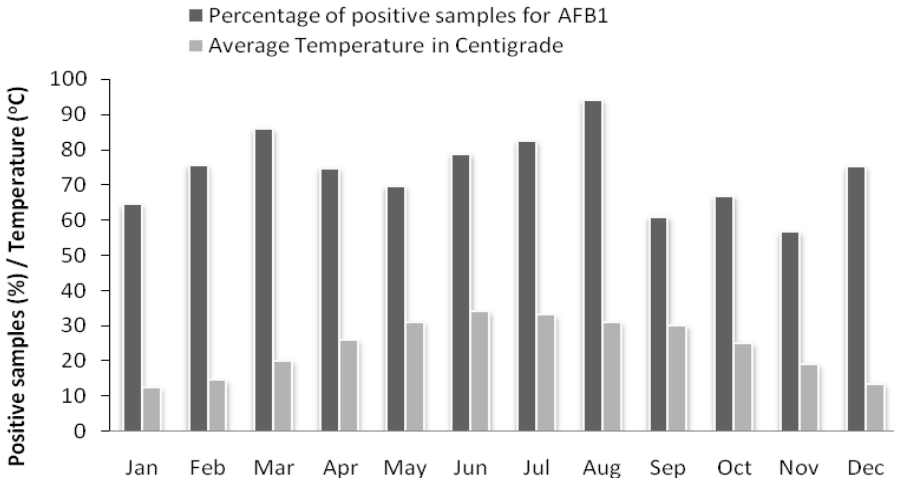


**Fig. 2. Percentage of positive samples for $AFB_1$ in basmati rice in different months and correlation with average temperature in Pakistan**

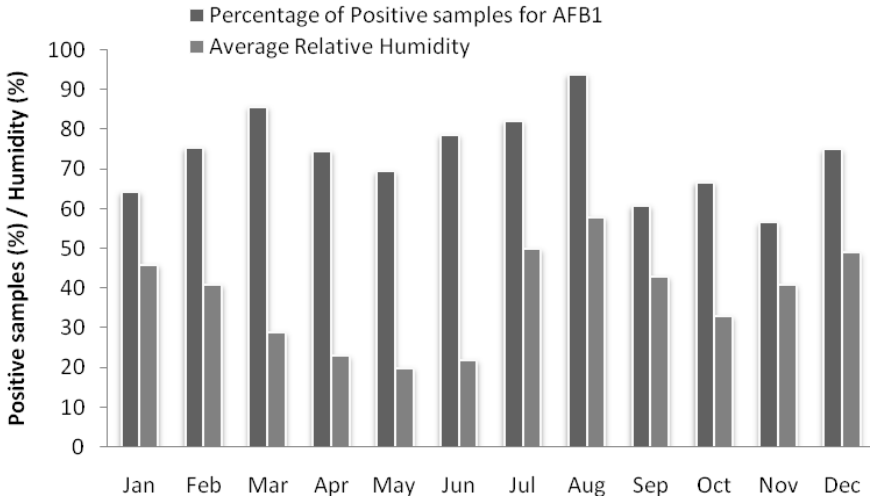


**Fig. 3. Percentage of positive samples for $AFB_1$ in basmati rice in different months and correlation with average relative humidity in Pakistan**

AFs contamination in rice is concerned to public health because of the severe toxicity associated with AFs. Several studies reported that the detectable level of AFs in rice from Pakistan (Table 4).

**Table 4. The level of total AFs in Rice in different countries**

| Country | Rice type | Sample numbers | Toxin types | Number of positive samples (%) | Maximum (µg/kg) | Range (µg/kg) | Average | Year of survey | Authors |
|---|---|---|---|---|---|---|---|---|---|
| Pakistan | Mixed Quality Rice | 40 | $AFB_1$ | 28 (70) | 18 | 1.2–18.0 | 3.7 | 2008-2009 | Hussain et al. (2011) |
| | | | Total AFs | 28 (70) | 13.9 | 1.5–13.9 | 4.9 | | |
| Pakistan | Brown Rice | 599 | $AFB_1$ | 293 (49) | 16.65 | 0.1–16.65 | 0.56 | 2010 | Firdous et al. (2010) |
| | White Rice | | | | | | 0.49 | | |
| | Sella Rice | | | | | | 0.73 | | |
| Iran | Mixed Quality Rice | 256 | $AFB_1$ | 256 (100) | 5.8 | 0.0–5.8 | 1.4 | 2007-2008 | Rahmani et al. (2011) |
| | | | Total AFs | 256 (100) | 6.3 | 0.1–6.3 | 1.6 | | |
| Iran | Mixed Quality Rice | 261 | $AFB_1$ | 180 (68.9) | 4.3 | 0.20–4.3 | 0.72 | 2010 | Feizy et al. (2010) |
| Canada | Mixed Quality Rice | 100 | $AFB_1$ | 56 (56) | 7.14 | 1.44–7.14 | 0.19 | 2008 | Bansal et al. (2011) |
| | | 100 | $AFB_1$ | 43 (43) | 3.48 | 1.45–3.48 | 0.17 | 2009 | |
| Swedish | Mixed Quality Rice | 99 | $AFB_1$ | 58 (59) | 46.2 | 0.1–46.2 | 18.1 | – | Fredlund et al. (2009) |
| | | | Total AFs | 58 (59) | 50.7 | 0.1–50.7 | 19.8 | | |
| UAE | Long Grain Rice | 500 | $AFB_1$ | 160 (64) | 16.5 | 1.2 to 16.5 | – | 1992-1994 | Osman, et al. (1999) |
| | Short Grain Rice | | $AFB_1$ | 81 (32) | | | | | |
| India | Paddy Rice | 1200 | $AFB_1$ | 814 (67.8) | 308 | 01–308 | – | – | Reddy et al. (2009) |
| | Milled Rice | | $AFB_1$ | | 3.5 | 0.5–3.5 | | | |
| Pakistan | Basmati Rice | 2047 | $AFB_1$ | 1501 (73.3) | 6.91 | 1.17–6.91 | 1.15 | 2006-2011 | Present Study |
| | | | Total AFs | 1501 (73.3) | 6.91 | 1.17–6.91 | 1.15 | | |

Earlier observations of Hussain et al. (2011) reported from Pakistan that out of 40 samples of rice collected from the local market, 70% samples were found contaminated with AFs. The average

of $AFB_1$ and total AFs was found 3.7µg/kg and 4.9µg/kg, respectively. In another survey from Pakistan, a total of 599 samples of rice were collected and analysed for AFs contamination. The average concentrations of $AFB_1$ and $AFB_2$ in brown, white and sella rice were found to be 0.56, 0.49, 0.73µg/kg and 0.03, 0.03, 0.02µg/kg, respectively. In the month of August (2010), the level for $AFB_1$ and $AFB_2$ were found 16.65µg/kg and 2.64µg/kg, respectively. The climatic conditions during March, July and August (2010) seems to be related to favourable environmental conditions (Firdous et al., 2012). During the present study, it was observed that the highly contaminated sample was found in the month of August showing 6.91µg/kg of AFs, as the temperature and RH were more favourable in comparison to other months.

The results of present study revealed that the quality of Pakistani rice is far better than rice produced in other countries of the same region, such as, Sri Lanka and India (Table 4). For instance, Bandara et al. (1991) from Sri Lanka reported that the contamination of $AFB_1$ and $AFG_1$ in parboiled rice was higher than same AFs level in raw milled rice. The highest concentration of $AFB_1$ was 185µg/kg and $AFG_1$ was 963µg/kg, respectively. During the collection of samples, the average humidity and average temperature was 78% and 27°C respectively, which was the highest amongst the rice growing areas in Sri Lanka. Reddy et al (2009) reported from India that out of 1200 rice samples, 67.8% samples contaminated with $AFB_1$ ranged from 01–308µg/kg. $AFB_1$ in the milled rice was ranged 0.5–3.5µg/kg. The highest levels (>30µg/kg) of $AFB_1$ were found only in 2% samples. In another study from India by Toteja et al. (2006), a total of 1511 samples of parboiled rice were tested. In 38.5% samples, $AFB_1$ was found at levels ≥ 5µg/kg, whereas in 17% samples, $AFB_1$ was found more than 30µg/kg (Indian regulatory limit). In Assam, Bihar and Tripura States of India, mean $AFB_1$ was found as 15µg/kg while in other Indian States, the mean value was <5µg/kg.

However, several studies from other countries reported detectable levels of AFs in rice as well (Table 4). For instance, as per survey from Iran (2007-2008), all the collected samples (n = 256) were found contaminated with $AFB_1$. The range of $AFB_1$ and total AFs found was 0.0–5.8µg/kg (mean 1.4µg/kg) and 0.1–6.3µg/kg (mean 1.6µg/kg) respectively. In 75.8% samples, $AFB_1$ contamination was found below 2µg/kg. In 21.5% samples $AFB_I$ was found above 2µg/kg, while remaining 2.7% samples contained more than 4µg/kg of total AFs (Rahmani et al., 2011). In another study, Feizy et al. (2010) from Iran analysed 261 rice samples, using HPLC. It was found that 68.9% of the rice samples were contaminated with $AFB_1$ at a level greater than 0.2µg/kg. Bansal et al. (2011) reported a survey from Canada. During this study, almost 200 samples belonging to different types of rice were tested. The mean concentrations for $AFB_I$ were 0.19 and 0.17µg/kg with respective positive incidence of 56% and 43% (LOD 0.002µg/kg). The five most contaminated samples in each year contained 1.44–7.14µg/kg of $AFB_1$ (year 1) and 1.45–3.48µg/kg of $AFB_1$ (year 2). Fredlund et al. (2009) tested 99 rice samples collected from the Swedish retail market. Majority of these samples were found in accordance with the European Commission Regulation 401/2006. The majority of basmati rice (71%) and jasmine rice samples (20%) contained detectable level of $AFB_1$ (quantification level = 0.1µg/kg). Two samples of jasmine rice and 10 of basmati rice samples contained AFs level over the regulated EU maximum limits. The presence of *Aspergillus flavus* in 21% of the samples indicated that incorrect management of rice during production and storage implies a risk of mould growth and subsequent production of AFs.

In the United Arab Emirates, during summer 1992–1994, 500 samples of rice were collected from households in Al–Ain city. The levels of $AFB_1$ in 160 (64%) of long grain rice and 81 (32%) of short grain rice ranged from 1.2 to 16.5µg/kg. The moisture content among the samples varied between 5.7% and 15.3%. The discoloured, broken and insect damaged grain was found to be contaminated with the species of *Aspergillus* and *Penicillium* (including *A. flavus* and *A. parasiticus*). At least two of the isolates of *A. flavus* were aflatoxigenic (Osman et al., 1999).

The current study showed that rice from Pakistan, especially, the basmati rice was found to be contaminated with low levels of AFs. Therefore, the present status of the AFs level in Pakistani basmati rice does not concurrently present a potential risk to the human health. The

frequency of positive samples, having small quantities of $AFB_1$, indicated that there is need for further investigations, regular monitoring and performing routine analysis as per food quality control measure.

The concentration of $AFB_1$ can be further reduced by good processing and storage practices. In the first instance, there is a need to take precaution and proper action, such as, better harvesting practices, handling, packaging, storage, and as well as transportation. AFs which have a high impact in human health and storage conditions should be controlled and widespread surveillance programmes should be initiated.

Reddy et al. (2008) comprehensively studied different procedures, including, botanicals, microbiologicals, and cooking, regarding the reduction of mycotoxins contamination in rice. In the light of above mentioned facts, a comprehensive plan is required to deliver the food and feed safely that engages to fulfill the requirement regarding the issues of not only rice but also all other foods, feed and their ingredients. On the other hand, substantial work has been put forth on methodologies for the detection and quantification of AFs. However, sampling is of prime importance to have a representative sample of the total lot under assessment, and then sample handling and, finally, the analysis. It is very unfortunate that sampling, sample handling and analysis is not standardised on the part of growers, farmers, producers and ultimately the consumers who are at a great risk. Care must be taken in the elucidation and evaluation of results. As per Institute of Food Science (2012) regarding Technology Information Statement, mycotoxins can enter at any stage in the food chain via contaminated food that is consumed or via food chain from animals who consume the contaminated feed. For effective control, there is a need to enforce good agricultural practices, controlled storage conditions and surveillance at every stage from farm to fork. These practices will be difficult to achieve, if the climatic condition favour mold growth. Preventive measures, like, good agricultural practices during harvest, drying and storage of the products must be applied. Educational measures regarding the prevention and control of aflatoxicosis must be made. Industry and government should continue to take steps and bound the farmers, growers and stake holders to keep AFs levels in food and animal feed as low as possible. For instance, growers and processors must follow voluntary "good manufacturing practices" that include monitoring of mold growth and testing of the samples for AFs.

## 4. Conclusion

On the basis of achieved results, it is concluded that the present status of the aflatoxins (AFs) level in Pakistani basmati rice does not concurrently present a potential risk to the human health. Highly AFs contaminated samples were found during the months of August, September and December. However, the AFs contamination levels were found within the range and do not pose a risk and is not a serious public health issue. Nevertheless, the detection of small quantities of $AFB_1$ warrants further investigation, regular monitoring and performing routine analysis, as per food quality control measure. The initial approach to control AFs is to take precaution and proper action, such as, better harvesting practices, handling, packaging, storage and as well as transportation.

## References

Bandara, J.M., A.K. Vithanege and G.A. Bean. 1991. Occurrence of aflatoxins in parboiled rice in Sri Lanka. *Mycopathologia*, 116(2): 65-70.

Bansal, J., P. Pantazopoulos and J. Tam. 2011. Surveys of rice sold in Canada for aflatoxins, ochratoxin A and fumonisins. *Food Additive and Contaminants Part A*. 28(6):767-774.

Bhattacharjee, P., R.S. Singhal and P.R Kulkarni. 2002. Basmati rice: a review. *International Journal of Food Science and Technology*, 37(1): 1–12.

Climatemps.com http://www.pakistan.climatemps.com.

Creppy, E.E. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*, 127(1–3): 19–28.

European Commission Regulation (ECR). 2010. (EC No. 165/2010 of 26 February 2010) Amending regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union,* 50: 8-12.

FDA, U.S. Food and Drug Administration. 2000. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. http://www.fda.gov.

Feizy, J., H.R. Beheshti, N.K., Fahim, S.S.F. Janati and G. Davari. 2010. Survey of aflatoxins in rice from Iran using immunoaffinity column clean–up and HPLC with fluorescence detection. *Food Additive and Contaminants Part B,* 3(4): 263–267.

Firdous, S., N. Ejaz, T. Aman and N. Khan. 2012. Occurrence of aflatoxins in export–quality Pakistani rice. *Food Additive and Contaminants. Part B*. Surveillance. 5(2): 121–125.

Fredlund, E*.,* A.M. Thim, A. Gidlund, S. Brostedt, M. Nyberg and M. Olsen. 2009. Moulds and mycotoxins in rice from the Swedish retail market. *Food Additive and Contaminants*, 26(4):527–533.

Hussain, A., J. Ali and Shafqatullah. 2011. Studies on Contamination Level of Aflatoxins in Pakistani Rice. *Journal of Chemical Society of Pakistan*. 33(4): 481–484.

IFST. 2012. The Institute of Food Science & Technology. *Information Statement on Mycotoxins. **www.ifst.org/document.aspx?id=421***.

Junejo, M.A., C.L. Rohra and H.A. Kanasro. 2007. Analyzing the impact of Sindh Rice Industries on Economy of Pakistan. *Australian Journal of Basic Applied Science*, 1(4): 853–859.

Lereau, M., G. Doriane and S. Villar 2012. Interactions between hepatitis B virus and aflatoxin B1: effects on p.53 induction in Hepa RG cells. *Journal of General Virology*, 93: 640–650.

Osman, N.A., A.M. Abdelgadir, M.O. Moss and A. Bener. 1999. Aflatoxin contamination of rice in the United Arab Emirates. *Mycotoxin Research*, 15(1): 39-44.

Paterson, R.R.M. and N. Lima. 2009. Mutagens manufactured in fungal culture may affect DNA/RNA of producing fungi. *Journal of Applied Microbiology*, 106(4): 1070–1080.

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

Pakistan Standard & Quality Control Authority (PSQCA). 2009. Standard Development Centre, Agriculture and Food Division.

Pitt, J.I. and A.D. Hocking. 1997. *Fungi and Food Spoilage*, 2nd edition. Blackie Academic and Professional. London.

Pitt, J.I. and A.D. Hocking. 2000. *Fungi and Food Spoilage,* 3rd edition, Springer, Berlin, Germany.

Rahmani, A., F. Soleimany, H. Hosseini and L. Nateghi. 2011. Survey on the occurrence of aflatoxins in rice from different provinces of Iran. *Food Additive and Contaminants Part B. Surveillance*. 4(3): 185–190.

Reddy, K., C. Reddy, H.K. Abbas, C.A. Abel and K. Muralidharan. 2008. Mycotoxigenic Fungi, Mycotoxins, and Management of Rice Grains. *Journal of Toxicology Toxin Reviews*, 27(3-4): 287–317.

Reddy, K.R.N., C.S. Reddy and K. Muralidharan. 2009. Detection of Aspergillus spp. and aflatoxin B1 in rice in India. *Food Microbiology*, 26 (1): 27–31.

Schroeder, H.W. and H.Jr. Hein. 1967. Aflatoxins: production of the toxins *in vitro* in relation to temperature. *Applied Microbiology*, 15(2): 441–445.

Toteja, G.S., A. Mukherjee and S. Diwakar. 2006. Aflatoxin B(1) contamination of parboiled rice samples collected from different states of India: A multi–centre study. *Food Additive and Contaminants*, 23(4): 411–414.

Trucksess, M.W. 2005. Natural Toxins. In: W. Horwitz, G.W., Latimer (Eds.), *Official Methods of Analysis of AOAC International*, AOAC International, Gaithersburg, MD, USA, pp. 1–85.

Wang, H., R. Dick and H. Yin. 1998. Structure-function relationships of human liver cytochromes P450 3A: aflatoxin B1 metabolism as a probe. *Biochemistry*, 37(36): 12536–12545.

Wongpornchai, S., T. Sriseadka and S. Choonvisase. 2003. Identification and quantitation of the rice aroma compound, 2–acetyl–1–pyrroline, in bread flowers (*Vallarisglabra* Ktze). *Journal of Agricultural Food Chemistry,*51(2): 457–462.

Zafar, Y. 2009. Genetic Diversity of Rice in Pakistan. National Institute for Genomics and Advanced Biotechnology (NIGAB), Pakistan. *http://ec.europa.eu/agriculture/analysis/external/basmati/rice_pakistan_zafar_en.pdf*.