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DETERMINATION OF AFLATOXINS IN SUPER KERNEL RICE TYPES CONSUMED IN DIFFERENT REGIONS OF PUNJAB, PAKISTAN

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ABSTRACT

A study was conducted to check the aflatoxins (B_1, B_2, G_1 & G_2) in super kernel basmati rice types consumed in different regions (Lahore, Narowal, Faisalabad and Multan) of Punjab, Pakistan. For that purpose, 48 samples of super kernel basmati rice types (white, brown, parboiled and broken) were collected from local markets of Lahore, Narowal, Faisalabad and Multan. Moisture contents and aflatoxin levels of the collected samples were determined. Aflatoxins detection in rice samples were done by HPLC technique. The results showed the presence of aflatoxins in rice samples. For the total (48) rice samples, 58% were contaminated with total aflatoxins out of which 56% were contaminated with aflatoxin B_1 and 33% with aflatoxin B_2. Out of 58% of the contaminated rice samples with total aflatoxins, 7% samples had aflatoxin level lower than the maximum tolerable limit of 4µg/kg and 93% samples were contaminated with total aflatoxin above the maximum tolerable limit. The 56% contaminated samples with aflatoxin B_1, showed 100% contamination above the maximum tolerable limit (4µg/kg). A positive relationship was also seen between the moisture content and presence of aflatoxins (B_1 and B_2). The study showed significant ($P<0.05$) results and it could add to various approaches leading to aflatoxin management in rice types.

Key words: Rice, Aflatoxins, Moisture content, HPLC, Food safety.

INTRODUCTION

In Pakistan, rice (*Oryza sativa*) is an important cereal crop used as staple food after wheat. Basmati variety of rice is well known globally for its delicious taste and aroma (Ahmad *et al*. 2008). Area wise, it is the 3rd largest cultivated cereal crop of Pakistan after wheat and cotton. Pakistan is the chief exporter of rice as; it is the major cash crop contributing 6.10% of total value added in agriculture and 1.3% to the GDP (Iqbal. 2012). Provincial involvement in production of rice in percentage is; Punjab 56%, Sindh 34%, Baluchistan 8% and NWFP 2% wherein Punjab is at the leading position (Khan & Khan. 2010).

Rice is the primary source of carbohydrate, protein, fiber, some vitamins like the B complex & E and minerals. The 75% of the weight of rice grain comprises of starch, the major carbohydrate (Vaughan and Geissler. 2009). Although the nutritional value of rice is very good but despite all this the problem is the toxin production due to fungal attack on crops or during storage. Rice is not a favorable commodity for growth of Aspergillus and aflatoxin contamination under normal conditions, but high humidity and heavy rains could enhance the capacity of rice grains for risk to aflatoxin contamination (Siruguri *et al*. 2012). Aflatoxins are produced as by product during the growth of fungi, *Apergillus flavus* and *Aspergillus parasiticus*. They are toxic substances produced by fungi of the genus Aspergillus which grow on cereals and other agricultural crops (National Toxicology Program. 2011). When aflatoxins once produced it is very difficult to get rid of them because they are chemically stable in nature and extremely resistant to degradation procedure by normal cooking methods (Ramesh. 2013). Fungal proliferation depends upon environmental favorable conditions like, high humidity and temperature (Choudhary and Kumari. 2010). The permitted limits in cereal grains set by European Union Commission Regulation (EC) No. 1881/2006 for total aflatoxins are 4.0 µg/kg and for aflatoxin B_1 is 2.0 µg/kg (Journal of the European Union. 2006).

Aflatoxins adversely affect the human health. Aflatoxicosis is a disease caused by the aflatoxins. Sudden death occurs as a result of acute aflatoxicosis and in chronic aflatoxicosis the prolonged pathological changes like cancer and immune suppression happens (Magnussen and Parsi. 2013). Aflatoxin is well known agent for its hepatocarcinogenic properties. The risk of liver cancer is almost 30 times higher in the subjects exposed to aflatoxin than unexposed ones (Liu and Wu. 2010).

In order to control aflatoxins in rice, there is a need to determine and quantify aflatoxin levels for making a comparison with the permissible levels set by the food regulatory authorities in a pursuit to ensure safe food supply. The present study was therefore planned to determine the aflatoxins levels in different types of super kernel basmati rice consumed in different regions of the Punjab, Pakistan. Furthermore, it was aimed to evaluate the frequency of occurrence of various types of aflatoxins

in rice species/varieties grown in various regions of the Punjab, Pakistan.

MATERIALS AND METHODS

Sample Collection: Samples of super kernel basmati rice grains were procured by simple random sampling technique from local market of Lahore, Narowal, Faisalabad and Multan districts of Punjab. Total 48 samples of super kernel basmati rice (white, brown, parboiled and broken), 12 samples of each type were included in the study. These samples were stored in appropriate plastic bags at room temperature to prevent from moisture and other impurities.

Moisture Analysis: Samples of rice were analyzed for Moisture by AOAC (2000) procedure.

Aflatoxins determination by HPLC:

Chemicals and regents: Standard solutions of 50µg/ml of aflatoxins were purchased from Sigma-Aldrich (St. Louis, Mo., USA). MycoSep column 226 (AflaZone) was purchased from Romer Labs (Union, Mo., USA). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich (St. Louis, Mo., USA). All other chemicals and organic solvents were of analytical grade.

Extraction and purification: Extraction and purification of samples were carried out using a slightly modified method of Iqbal *et al*. (2010). The rice samples were ground to uniform consistency. Samples (25g) were extracted with 100 ml of acetonitrile/water (80:20 v/v) and 5 gram of sodium chloride (NaCl) by shaking for 60 min at 50 rpm at room temperature in 250 ml glass flasks fitted with a stoppers. The solutions were filtered through Whatman No.5 papers. To 9 ml portions of the filtrates, 70µl acetic acid was added; the mixture was then transferred to MycoSep columns (product code 226) and passed through at a flow rate of 2 ml/min. The aflatoxins passed through the column. 2 ml portion of each eluate was taken and evaporated to dryness by nitrogen evaporator in a centrifuge glass tube. After drying the mixture 200µl n-hexane was added and vortex mix the sample for 1-2 min. Then 50µl trifluoro acetic acid was added and mixed by vortex mixer. 1.95 ml of water and acetonitrile (1:0.95) were added and mixed again. 1 ml of sample filtrate obtained from syringe filter was collected in HPLC vials. A 20µl portion of the sample was subjected to HPLC analysis.

Mobile Phase: The mobile phase was acetonitrile/ methanol/ water (20:20:60 v/v/v), which was degassed by sonication. The HPLC (Shimadzu, Kyoto, Japan) was fitted with a Supelco C18 Column (Discovery HS) with a fluorescence detector (RF-530). Excitation and emission wavelengths were 360 nm and 440 nm, respectively. The flow rate was 1 ml/min and the column was maintained at 40°C. The injection volume was 20µl.

Statistical analysis: Data obtained by estimating aflatoxins analysis was analyzed by using SPSS software version 16. For comparing groups two way Analysis of variance (ANOVA) was used. Correlation analysis was done to see the relationship between parameters. Completely randomized design (CRD) was applied on the data to assess the significance level. P-value < 0.05 was taken as significant.

RESULTS

Moisture Content: Mean moisture content (percentages) of the rice samples obtained from four Districts of the Punjab ranged from 9.5±0.92 to 10.2±0.72, 9.8±0.27 to 10.5±0.98, 9.7±0.20 to 9.8±0.26 and 10.1±0.38 to 10.7±1.16 for white, brown, parboiled and broken rice respectively (Table No.1).

Table 1. Moisture content of different types of super kernel basmati rice

	Moisture Mean (%)			
	White Rice	**Brown Rice**	**Parboiled Rice**	**Broken Rice**
Lahore	9.5 ± 0.92	10.5± 0.98	9.8±0.26	10.7±1.16
Narowal	9.9 ± 0.61	9.9± 0.60	9.8±0.20	10.1±0.38
Faisalabad	10.2 ± 0.72	10.0±0.45	9.8±0.26	10.4±0.68
Multan	9.6 ± 0.15	9.8±0.27	9.7±0.20	10.5±0.50

Aflatoxins Content: Aflatoxins (B_1, B_2, G_1 and G_2) in different types of super kernel basmati rice were determined by using high performance liquid chromatography (HPLC) separation technique. Aflatoxin B_1 was the most commonly occurring toxin in all types of super kernel basmati rice followed by aflatoxin B_2 which was the second common toxin present in super kernel basmati rice types examined under this study.

Out of 48 samples of different types of super kernel basmati rice 28 (58%) were contaminated with total aflatoxins, of these 27 (56%) were contaminated with aflatoxin B_1 and 16 (33%) were contaminated with aflatoxin B_2. Out of 28 (58%) of the contaminated rice

samples with total aflatoxins the 2 (7%) samples had aflatoxin level lower than the maximum tolerable limit of 4µg/kg and 26 (93%) samples were contaminated with total aflatoxin above the maximum tolerable limit. Similarly, out of 27 (56%) contaminated samples with aflatoxin B_1; all samples were contaminated with aflatoxin B_1 above the maximum limit as it shown in Table No. 2.

Most of the samples contaminated by aflatoxins were found to fall in 0-20 µg/kg range, some in 21-40 µg/kg, 41-60 µg/kg and 3 samples of each were contaminated by total aflatoxins whereas aflatoxin B_1 was found in the range >60 µg/kg range (Table. No. 3).

Table 2. Contamination of Aflatoxins detected in various rice samples

Aflatoxins	No. of samples	Positive samples	Contamination detected (%)	Upper limit	Lower limit
Total Aflatoxin	48	28	58	26	2
B_1	48	27	56	27	0
B_2	48	16	33	-	-
G_1, G_2	48	ND	-	-	-

ND-not determined

Table 3. Aflatoxins range in different types of super kernel basmati rice

	Aflatoxins range (µg/kg)			
	0-20	**21-40**	**41-60**	**>60**
Both Aflatoxins (B1+B2)	18	4	2	3
Aflatoxin B1	18	5	1	3
Aflatoxin B2	16	ND	ND	ND

ND-not determined

Total contamination of aflatoxins in white rice from different regions (Lahore, Narowal, Faisalabad and Multan) of Punjab was found 41.7%, in brown, parboiled and broken rice the aflatoxin presence was 75%, 25% and 100% respectively. The mean for total aflatoxins from all the four regions of Punjab for white rice was 8.24%, for brown, parboiled and broken rice; it was 17.8%, 2.2% and 27.42% respectively (Table no. 4).

In super kernel basmati white rice from the regions of Lahore, Narowal, Faisalabad and Multan, the aflatoxin B_1 was detected in a range of 32.16µg/kg to 8.0µg/kg. Almost equal proportion achieved from each region. The aflatoxin B_2 was present only in one sample from Faisalabad at a level of 20µg/kg (Figure No. 1). Aflatoxin B_1 presence was seen in super kernel basmati brown rice in a range of 80.04µg/kg to 8.0µg/kg equally from all the regions. The aflatoxin B_2 contamination was present in a range of 2µg/kg to 8µg/kg (Figure No. 2)

Table 4. Total Aflatoxins contamination in rice samples from different regions.

Rice type	Region	No. of samples	Contaminated samples (%)	Mean ± SD
White	Lahore	3	41.7	8.24 ± 7.0
	Narowal	3		
	Faisalabad	3		
	Multan	3		
Brown	Lahore	3	75	17.8 ± 10.3
	Narowal	3		
	Faisalabad	3		
	Multan	3		
Parboiled	Lahore	3	25	2.2 ± 1.4
	Narowal	3		
	Faisalabad	3		
	Multan	3		
Broken	Lahore	3	100	27.42 ± 19.1
	Narowal	3		
	Faisalabad	3		
	Multan	3		

The most important type of super kernel basmati rice contaminated with aflatoxins was broken rice from the four regions of Lahore, Narowal, Faisalabad and Multan where the contamination ranged from 120.6µg/kg to 8.0µg/kg. The highest value was detected in broken rice from the Lahore region.

Linearity of HPLC system was checked to inject different concentrations of reference standards. The system was calibrated using the working solution of aflatoxins in the range of 0.5-10 µg/ml in acetronitrile. HPLC method was validated by testing linearity, recovery, limit of detection (LOD) and limit of quantification (LOQ). Results for recovery are given in Table No.5. LOD and LOQ were 0.5 and 1µg/kg for AFB1 and AFG1, respectively, and 0.05 and 0.1 µg/kg for AFB2 and AFG2, respectively (data not shown).

Table 5. Recovery (%) of aflatoxins in rice samples (n = 6).

Aflatoxins	Spike level (µg/kg)	Recovery (%)	RSD (%)
AFB1	10	90 ± 0.17	0.17
AFB2	5	88 ± 0.12	0.14
AFG1	10	89 ± 0.17	0.19
AFG2	5	86 ± 0.12	0.15

AFB1= Aflatoxin B1, AFB2 = Aflatoxin B2, AFG1 = Aflatoxin G1, AFG2= Aflatoxin G2

Table 6. Parameters of linear regression for aflatoxins.

Aflatoxins	Concentration (µg/kg)	Slope	Intercept	R^2
AFB1	0.5 – 10	1.0907	0.042	0.9996
AFB2	0.05 – 5	2.6638	0.0388	0.9993
AFG1	0.5 – 10	1.0907	0.042	0.9996
AFG2	0.05 – 5	2.6638	0.0388	0.9993

AFB1= Aflatoxin B1, AFB2 = Aflatoxin B2, AFG1 = Aflatoxin G1, AFG2= Aflatoxin G2

It was also observed that aflatoxin contamination is directly related to the moisture level present in the rice grains. The rice types with higher moisture content have aflatoxin B_1 and B_2 contamination (Figure No. 1 and 2).

It is further confirmed by finding out correlation analysis between moisture content and aflatoxins (B_1 & B_2) contaminations that correlation was significant at the 0.01 level for aflatoxin B_1 and for aflatoxin B_2 it was significant at the 0.05 level. This showed the strong relationship among higher moisture content and aflatoxins contamination in different types of super kernel basmati rice.

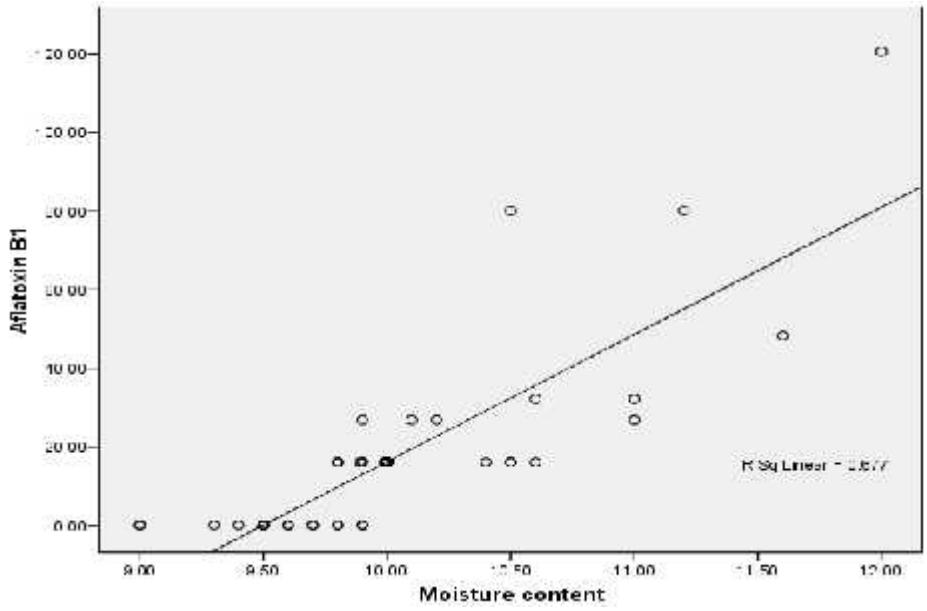

Figure No. 1 Correlation between moisture content and aflatoxin B_1 presence in different types of super kernel rice

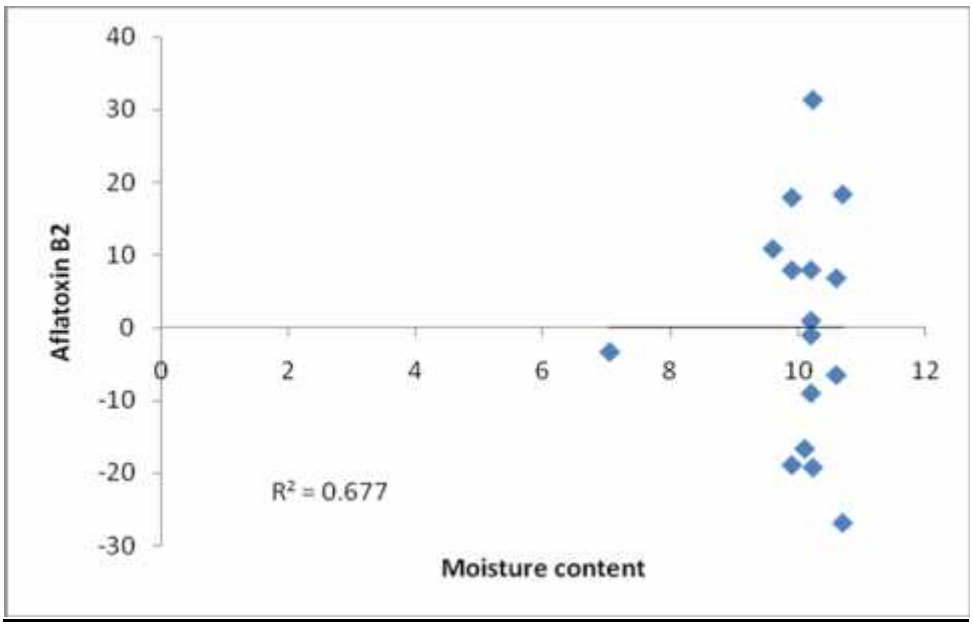

Figure 2. Correlation between moisture content and aflatoxin B_2 present in different types of super kernel rice

DISCUSSION

The moisture content for some of the samples of white, brown and broken rice was higher than others. If the moisture content is not maintained properly during storage of rice grains it results in ultimate growth of fungi and production of aflatoxins (Reddy *et al*. 2009). Reddy *et al.* investigated rice samples collected from India. These samples were assessed for *Aspergillus spp.* and aflatoxin B_1. Aflatoxin B_1 contamination was observed by two percent (2%) of samples from open storage that were exposed to rain. The most important parameters which influence the production of aflatoxins are temperature, storage conditions, moisture level, and insect infestation (Babu *et al*. 2011, Siruguri *et al*. 2012). The mean moisture percentage for four districts ranged from 9.5±0.92 to 10.7±1.16 (Table No. 1).

In Pakistan contamination of agricultural crops with fungal and aflatoxins hazards due to environmental conditions are a major problem. In the second part of the study aflatoxins (B1, B2, G1 & G2) were determined in order to see the level of contamination in different rice samples collected from different areas of Punjab. Aflatoxin B_1 was most common in different types of super kernel basmati rice samples collected from different areas of Punjab. Nearly 56% of different rice samples were contaminated by aflatoxin B_1 and 33% were found to be contaminated with aflatoxin B_2 (Table No. 2). From health safety point of view aflatoxin B_1 is the most important toxin because it is commonly present in food and at the same time is the most toxic for human health (Deng *et al*. 2010). Out of 58% contaminated rice samples with aflatoxin B1, 64.3% samples had aflatoxin B1 level lower than the maximum tolerable limit of 20 ppb and 35.7% samples were contaminated by aflatoxin B1 above the maximum tolerable limit. Similarly, out of 33% contaminated samples with aflatoxin B2, 87.5% samples were above than maximum limit and 12.5% samples were contaminated by aflatoxin B2 above the maximum limit as it shown in Table No. 2. Almost same results were seen in Uganda where Pakistani rice samples were examined, 80% of samples contaminated with total aflatoxins ranging between 20-50 ppb. Aflatoxin B1 was found to be in a range of 16.08 ppb to 120.06 ppb. The level of aflatoxin B2 was present in a range of 4ppb to 20 ppb (Taligoola *et al*. 2011). Bansala *et al*., (2011) found that mostly basmati rice from India and Pakistan and black and red rice from Thailand were contaminated with aflatoxins.

It is obvious from the results that the aflatoxins B1 and B2 was present in highest amount in broken rice from each four areas of Punjab and then its presence was in descending order from brown rice to white rice to parboiled rice from all the four regions of Punjab. Same results were shown by the Lutfullah and Hussain in 2012. In a study conducted by Iqbal *et al*., (2012), 50% of broken rice was contaminated by aflatoxins that hardly give a clue which rice part is more prone to aflatoxin contamination. Castells *et al*., (2007) detected that aflatoxins were found throughout all fractions, but higher contamination levels were detected in hull and bran fractions. Regardless of the rice variety, the aflatoxin distribution pattern depended on the initial contamination level and type of milled fraction but not on the duration of polishing.

After manipulating the mean and standard deviation of all parameters used in this study, two way

ANOVA (Analysis of Variance) was used to compare the means differences between the four areas and four types of super kernel basmati rice and presence of aflatoxins B1 and B2. The result showed that for aflatoxin B1 it was observed that the p value for difference in means for different regions was > 0.05 which is non-significant. However, the mean difference between the different rice types for aflatoxin B_1 was < 0.05 which was significant. Similarly, for aflatoxin B2, it was observed that the p value for difference in means for different regions and between the different rice types was < 0.05 which was also non significant.

In Pakistan sub-tropical conditions such as, temperature and humidity also play a key role in aflatoxins production. Post-harvest contamination of aflatoxins can occur if the drying is delayed and moisture is allowed to go beyond the limits (Asghar *et al*. 2013). In the present study it is observed that the rice samples with higher moisture content are prone to aflatoxins contamination. It is further confirmed by using statistically significant technique of calculating correlation analysis between moisture content and aflatoxins presence in super kernel basmati rice types. The p value is less than 0.05 which was significant.

Conclusion: All this discussion concluded that the super kernel basmati rice types available in Lahore, Narowal, Faisalabad and Multan have good quality indeed but the main issue is the aflatoxins B_1 and B_2 presence in the different types of rice from the four areas under study. As the moisture level directly correlates with the aflatoxins contamination so it is important from food safety point of view to control or monitor the temperature during storage conditions so there is less chances of fungal attack during storage and it will help in reduction of contamination by aflatoxins.

REFERENCES

Ahmad, S., M. Zia-ul-Haq, M. Imran, S. Iqbal, J. Iqbal and M. Ahmad (2008). Determination of residual contents of pesticides in rice (*Oryza Sativa L.*) crop from different regions of Pakistan. Pakistan J Bot. 40(3): 1253-1257.

AOAC (2000). Natural Toxins. Official Methods of Analysis. 17th edn. Association of Official Analytical Chemists. Arrington, Virginia, USA. Official method no. 998.03: 11-12, 16-18.

Asghar, M.A., J. Iqbal, A. Ahmed, A.Z. Shamsuddi and A.M. Khan (2013). Incidence of aflatoxins in export quality basmati rice collected from different areas of Pakistan. Sci Tech and Dev. 32 (2): 110-119.

Babu, G.R., M.G. Prasad and T.N.V.K.V. Prasad (2011). Isolation and Quantification of Aflatoxin from Aspergillus Flavus Infected Rice. Int J Pure Appl Sci Technol. 5(1): 16-24.

Bansala, J., P. Pantazopoulosa, J. Tama, P. Cavlovica, K. Kwonga, A.M. Turcotteb, B.P.Y. Laub and P.M. Scottb (2011). Surveys of rice sold in Canada for aflatoxins, ochratoxin A and fumonisins. Food Addit and Contam. 28(6): 767–774.

Castells, M., A.J. Ramos, V. Sanchis and S. Marin (2007). Distribution of Total Aflatoxins in Milled Fractions of Hulled Rice. J. Agric. Food Chem. 55: 2760-2764.

Choudhary, A.K. and P. Kumari (2010). Management of Mycotoxin Contamination in Preharvest and Post-Harvest Crops: Present Status and Future Prospects. J of Phytol. 2(7): 37-52.

Deng, S.X., L.X. Tian, F.J. Liu, S.J. Jin, G.Y. Liang, H.J. Yang, Z.J. Du and Y.J. Liu (2010). Toxic effects and residue of aflatoxin B1 in Tilapia (Oreochromis miloticus x O. aureus) during long-term dietary exposure. Aquaculture. 307: 233-240.

Iqbal, S.Z., M.R. Asi, A. Arino, N. Akram and M. Zuber (2012). Aflatoxin contamination in different fractions of rice from Pakistan and estimation of dietary intakes. Mycotoxin Res. 28: 175–180.

Iqbal, S.Z., R. Russell, M. Paterson, M.I. Bhatti, M.R. Asi, M.A. Sheikh and H.N. Bhatti (2010). Aflatoxin B1 in chilies from the Punjab region, Pakistan. Mycotox Res. 26: 205–209.

Khan, M.A., S.L. Khan (2010). Trade Development Authority of Pakistan. Report on Potential Markets of Rice.

Liu, Y. and F. Wu (2010). Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment. Environmental Health Perspectives. 118:818-24.

Lutfullah, G. and A. Hussain (2012). Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. Food Cont. 23: 32-36.

Magnussen, A. and M.A. Parsi (2013). Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol. 19(10): 1508-1512.

National Toxicology Program, Department of Health and Human Services (2011). Report on carcinogens-Aflatoxins CAS No. 1402-68-2.

Official Journal of the European Union (EU) Commission Regulation (EC) No 1881/2006 of 19 December (2006). Setting maximum levels for certain contaminants in foodstuffs. Section 2: Mycotoxins. L 364/15.

Ramesh, J., G. Sarathchandra and V. Sureshkumar (2013). Survey of market samples of food grains and grain flour for Aflatoxin B1 contamination. Int J Curr Microbiol App Sci. 2(5): 184-188.

Reddy, K.R.N., H.K. Abbas, C.A. Abel, W.T. Shier, C.A.F. Oliveira and C.R. Raghavender (2009). Mycotoxin contamination of commercially

important agricultural commodities. Toxin Reviews. 28(2–3): 154–168.

Siruguri, V., P.U. Kumar, P. Raghu, M.V. Rao, B. Sesikeran, G.S. Toteja, P. Gupta, S. Rao, K. Satyanarayana, V. M. Katoch, T.S. Bharaj, G.S. Mangat, N. Sharma, J.S. Sandhu, V.K. Bhargav and S. Rani (2012). Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India. Indian J Med Res. 136(1): 89-97.

Taligoola, H.K., M.A. Ismail and S.K. Chebon (2011). Mycobiota and aflatoxins associated with imported rice grains stored in Uganda. Czech Mycol. 63(1): 93–107.

Vaughan, J.G. and C.A. Geissler (2009). Grain crop Rice. The New Oxford Book of Food Plants. Oxford University Press Inc., New York. pp 10.