

Aflatoxin Concentrations in Various Rice Varieties of Punjab, Pakistan

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ABSTRACT

Background: Mycotoxins are secondary fungal metabolites that are produced in rice, corn, nuts and several other cereals. The contamination of food stuff by mycotoxins is a cause of numerous diseases throughout the world. Aflatoxins are potent mycotoxins that have chronic and acute toxicity.

Objectives: To evaluate the presence of aflatoxins B1, B2, G1 and G2 in branded and non-branded rice varieties available in Punjab, Pakistan.

Methodology: Total 150 rice samples were collected in May 2019 from various cities (Islamabad, Rawalpindi, Gujar Khan, Jehlum, Kharian, Gujrat, Gujranwala, Lahore, Kasur, Okara, Sahiwal, Mian Channu, Khanewal, Multan, Bahawalpur and Rahim Yar Khan) of Pakistan. All the rice samples were subjected to physical testing methods, followed by extraction (using chloroform extraction method) and qualitative and quantitative detection of aflatoxin using Thin layer Chromatography (TLC) Technique and Enzyme linked Immunosorbent assay (ELISA).

Results: No aflatoxins was detected in branded rice varieties which were stored in good packaging. The aflatoxin B1 (AFB1) was detected in 65% of poorly stored rice varieties, with maximum contamination of 8.92ppb. The lowest moisture 9.4% content was found in (branded samples) and 10.9% (non-branded samples) while highest moisture content 10.8% found in (branded samples) and 16% found in (non-branded samples). The 54 out of 100 non-branded samples found to have more than 13% moisture contents which is alarming.

Conclusion: With the implementation of effective strategies and special precautions during storage, harvesting, transportation and drying, we can prevent the contamination of rice product with fungi and stop aflatoxin production which is a major threat to country economy.

Keywords

Aflatoxins, Branded, Nonbranded, Punjab, Pakistan, Rice.

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Article info.

Received: May 09, 2021

Accepted: June 14, 2021

Cite this article Tahir NI, Hussain S, Kalim I, Asghar A, Syed SK, Idrees A, Ahmad Aflatoxin Concentrations in Various Rice Varieties of Punjab, Pakistan. RADS J Biol Res Appl Sci. 2021; 12(1):39-53.

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INTRODUCTION

The contamination of foodstuffs by mycotoxins is a cause of numerous diseases throughout the world¹. Mycotoxins are secondary fungal metabolites² that are produced in rice, corn, nuts and several other cereals³. Aflatoxins are potent mycotoxins that have chronic and acute toxicity⁴ comprised of closely related compounds e.g., aflatoxins B1, B2, G1, G2, M1 and M2⁵⁻⁶. They exist ubiquitously in nature and may lead to several harmful and toxic effects on living beings⁵. They are produced in food commodities and animal feeds by the fungal strains (mainly by *Aspergillus parasiticus* and *Aspergillus flavus*; rarely by *Emericella venezuelensis*, *Emericella astellata*, *Aspergillus tamarii*, *Aspergillus pseudotamari*, *Aspergillus ochraceoroseus* and *Aspergillus bombycis*)^{5, 7}. When animals consume aflatoxin-contaminated feed, then their meat, eggs, and milk are also contaminated^{8, 9}.

Aflatoxins have mutagenic, carcinogenic and immunosuppressive effects^{10, 11}. The presence of aflatoxins in diet severely affect kidneys, liver, growth rate, and reproduction, and can cause serious illness and even death. This problem is more common in developing countries⁵. Uganda has higher cases of liver cancer due to aflatoxin-contaminated foods¹². In Kenya in 1981, twenty (20) people complained about stomach trouble and mild fever due to consumption of aflatoxin-contaminated food later they were diagnosed of livers damaged and 12 individuals were reported died¹³. Aflatoxins gain entry into food products when proper storage conditions (e.g., little moisture content and low temperature) are not applied^{14, 15}. The moisture and a hot climatic conditions are important factors contributing to toxin production^{16, 17}.

Plants are most valuable sources of food and food products throughout the world¹⁸⁻²⁰. Rice is one of the most important food crops²¹ having an excellent nutritional value²². It is the most popular staple food and an important source of vitamins, minerals, energy, fiber, and other biomolecules²³. Rice exports are the second highest source of income in Pakistan²⁴. Rice is consumed worldwide as a food and food additive due to its unique fragrance, cooking advantages and taste. The diverse rice varieties include kernel, basmati, superkernel, super basmati, super shaheen, long-grain white and brown, guard supreme basmati, guard super kernal basmati, zainab basmati rice, falak premium

basmati rice, mughal badshah rice, aroma super basmati rice, aroma world longest basmati rice, falak daily basmati rice, fine life super kernal basmati rice, kalaar basmati steam rice, zarafa super kernal basmati rice, ream awami basmati rice, hashmi super kernal basmati 1121, honeymoon basmati rice, shan shahi jalwa awami long grain rice family super kernel basmati rice, hassan 1121 basmati rice, khyber basmati rice, kausar basmati rice, naubhar nayab silla basmati rice, mehran basmati daily rice, Jazaa premium basmati rice, motidana steamed 1121 basmati rice, al badar classic super kernal basmati and anmol super kernal basmati rice etc. These varieties are commonly used in Asian dishes^{2, 5}.

After wheat, the rice family is Pakistan's second-largest food²⁶. It is the third most cultivated crop in Pakistan after wheat and cotton²⁷. Rice is a major export of Pakistan and a main cash crop and a source of income for the Pakistan, accounting for approximately 5.9% of total agricultural value-added and 1.3% of GDP^{27, 28}. The contamination in this costly crop create a huge impact on Pakistan economy. In addition the existence of Aflatoxins in rice-based products may cause severe toxicity especially in human genetic material i.e. DNA and RNA²⁹. The impact of aflatoxins on human health is an important challenge today^{30, 31}; that is why the aflatoxin related research is catching attraction world wide³².

The present study was conducted to evaluate the presence of aflatoxins B1, B2, G1 and G2 in branded and non-branded rice varieties of Punjab, Pakistan.

MATERIAL AND METHODS

All the experimental work was performed in PCSIR laboratories complex, Lahore, Pakistan.

AFB1, AFB2, AFG1 and AFG2 standards were imported from the Trilogy Analytical Laboratory (870 Vossbrink Dr, Washington, MO 63090, USA), Acetone (Fisher Scientific, USA), Anhydrous Ether (Merck USA), Chloroform (Sigma-Aldrich UK) and benzene acetonitrile (Sigma-Aldrich UK). TLC plates were procured from Merck (290 Concord Road Billerica Massachusetts USA).

Elisa kit (Model 680) BIO-RAD USA, Reader grinding mill (Romer Series Mill) Romer Lab Singapore, Wrist action shaker (Model 75) Burrell Scientific Singapore, Hot Plate (Hot Plate 180 PCSIR Pakistan), Water bath (water bath

AL/16, PCSIR Pakistan) and UV spectrophotometer (UV reader/280) Redicom Austria, were used.

COLLECTION OF RICE SAMPLES

Total 150 rice samples were collected in May 2019 from various cities (Islamabad, Rawalpindi, Gujjar Khan, Jehlum, Kharian, Gujrat, Gujranwala, Lahore, Kasur, Okara, Sahiwal, Mian Channu, Khanewal, Multan, Bahawalpur and Rahim Yar Khan) of Punjab, Pakistan. It is worth mentioning that rice crop planted from May-June and harvested in October-December in Pakistan.

SAMPLE PREPARATION AND TESTING

Physical Testing

Homogenous rice samples (100g each) were collected from each rice variety, marked and stored for analysis. The defective, broken/smashed, other contaminations and rice weevils were removed before sample collection.

Grinding

A total of 50g of each sample was carefully mixed for 10min. It was ground in a sample grinder (ILP, FBRC/AL/05) and then passed through 20 mesh sieve to obtain a uniform (1mm particle size) and descriptive sample. The ground samples were stored in a sealed plastic pack to keep them safe before the experimental use. These samples were finally used for aflatoxins assessment.

Extraction of Aflatoxins

Various methods of extraction and analysis are utilized for diverse types of raw materials because of their different chemical behaviors. Researchers follow numerous extraction procedures depending upon their resources. In current studies, we have chosen the chloroform extraction method which has been considered an appropriate method for the aflatoxin extractions. Extraction technique was applied by utilizing 50g of milled sample of rice in a 500ml Erlenmeyer flask. Twenty five (25ml) of water and 150ml of chloroform was added in the allocated flask. The Erlenmeyer flask was mounted on a wrist shaker and shaken for 30min. The sample solution was then filtered by Whatman Grade No. 1 filter paper. Extract (50ml) was positioned on a hot plate for evaporation³³. It was finally subjected to analysis for the presence of aflatoxins B1, B2, G1 and G2.

Aflatoxin Determinations by Thin Layer Chromatography Technique (TLC)

Dilutions were done up to per microliters for TLC spot applications. Then 25µl of the test solution was applied on a thin layer chromatography plate, using a microscope syringe. Total 5µl and 10µl concentrations of AFB1, AFB2, AFG1 and AFG2 were also applied as internal standards for aflatoxins on this plate. The TLC plate was placed in "First Mobile Phase" (Anhydrous Ether) in TLC container No. 1, until the solvent was reached on an average height. Then TLC plate was taken out and dried before it was placed in the "Second Mobile Phase" tank containing acetone and chloroform (2:1). When the mobile phase was reached to the required height, the TLC plate was taken out from tank 2 and then dried. The test plate was carefully observed under 365nm ultraviolet light for the presence/absence of aflatoxins. Aflatoxins were evaluated by careful observation of fluorescence brightness and comparing the results with those of the aflatoxin standards applied on the TLC plates along with the samples³⁴.

The test solution was completely dried for quantitative determination. Benzene and acetonitrile (98:2) were mixed to the test solution. Then 3.5, 5.0 and 6.5µl spots (all of the same size) of the test solutions were put on the TLC plates. Spots of the identical size of the standards were also put on the TLC plate. The tested and standard solutions were compared to identify the similarities between the two. fluorescent light used to examine the spots. When the sample spot and standard spot were superimposed, this indicates the presence of aflatoxin in the sample. In such situation, the color of the sample and Rf values of the samples were corresponded to the aflatoxin standards³⁵.

Calculations

The concentration of aflatoxins in a sample can be calculated by the following equation³⁶:

$$\text{Concentration of aflatoxins (mg/kg)} = \frac{S \times V \times Y}{W \times Z}$$

S: Volume (ml) of aflatoxin standard of equivalent intensity to Z = ml of sample

V: Volume (ml) of solvents required for dilution of the final extract

Y: Concentration (mg/ml) of aflatoxin standard

W: Weight (g) of the original sample contained in the final extract

Z: Volume (ml) of sample extract required to produce fluorescence intensity comparable to that of S = ml of aflatoxin standard.

Enzyme linked Immuno Sorbent Assay (ELISA) Method

Sample preparation was performed according to the instructions of the test kit manual of RIDASCREEN Aflatoxin B1 30/15 (R-Biopharm GmbH, 2010)³⁷. Twenty-five milliliter (25ml) mixture of methanol: water (70:30) was added to 5g of rice followed by its vigorous stirring for 3min manually. The obtained extract was filtered through a filter paper and diluted with distilled water (1:1). At last, 50ml of the diluted filtrate was used for each well in a test.

Aflatoxin level was determined quantitatively by an ELISA test procedure (RIDASCREEN Aflatoxin B1 30/15 (Art No.: 1211) test kit manual) reported earlier³⁸. To each well of a microtiter plate, 50ul of a solution (test solution and a standard solution) was added in duplicate followed by addition of 50ul each of the enzyme conjugate and the anti-aflatoxin antibody to every well. The resultant solution was mixed gently and incubated at 20-25°C for 30min. Then the wells were tapped upside down vigorously for the removal of their liquids into an absorbent paper. Each empty well was then washed twice by 250ul washing buffer. Subsequently, there was addition of 100ul of substrate/chromogenic solution to each well followed by its gentle mixing and incubation at 20-25°C for 15min in a dark place. Then there was addition of 100ul of 1N H₂SO₄ (stop solution) to each well. Finally, the absorbance was noted in ELISA plate reader at 450nm³⁸.

Moisture Analysis

A known amount of fine and ground sample was taken in a well dried (by oven) petri dish. The petri dish was then heated in the oven at 100°C overnight to ensure the effective removal of all the moisture. The dish was placed in a desiccator and cooled; it was heated again in an oven for 2hours again and weighed was taken to ensure the maximum removal of moisture from the sample. Heating and weighing was continued until the constant reading of petri dish and sample was achieved³⁹.

$$\% \text{ Moisture} = \frac{\text{Weight of the dried rice sample}}{\text{Weight of the original sample}} \times 100$$

RESULTS AND DISCUSSION

The rice samples were collected and analyzed (qualitatively and quantitatively) for the aflatoxin contents by Thin Layer Chromatography (TLC) and Enzyme Linked Immunosorbent Assay (ELISA). The prepared samples were run through TLC plates and finally observed with a UV spectrophotometer. The results for TLC tests are compared in **Fig. 1** and **2** for branded (packed) and non-branded (open or poorly stored) samples, respectively.

AFLATOXIN CONCENTRATION IN OPEN RICE SAMPLES

No aflatoxins were detected in all 50 branded rice samples which had their packaging according to the standard requirements. The corresponding TLC results are shown in **Fig. 1**. The obtained data are displayed in **Table 1** and summarized in **Table 2**.

Table 1. Aflatoxin Concentration in Branded Rice Varieties Available in Local Market (Each Rice Sample = 500g).

S. No.	City and Location from where the rice sample was picked	Company name & Rice Variety	Packaging conditions	Time in shelf (Months)	H ₂ O Content (%age)	Aflatoxin conc. (ppb)
1	Kechery Chowk, Rawalpindi	Guard supreme basmati	Good	2	10	Not Detected
2	Kechery Chowk, Rawalpindi	Guard Super Kernal Basmati	Good	4	9.8	Not Detected
3	Tulsa Rd, opposite Lalazar, Rawalpindi	Zainab Basmati rice	Good	5	10.2	Not Detected

Contd...

4	Tulsa Rd, opposite Lalazar, Rawalpindi	Falak Premium Basmati rice	Good	7	10.5	Not Detected
5	GT Road, Kotha Kalan Morgah, Rawalpindi	Mughal Badshah rice	Good	3	9.5	Not Detected
6	GT Road, Kotha Kalan Morgah, Rawalpindi	Guard Super KernalBasamati rice	Good	8	9.8	Not Detected
7	Islamabad, Islamabad Capital Territory, Pakistan	Aroma Super Basmati rice	Good	6	10.1	Not Detected
8	Islamabad, Islamabad Capital Territory, Pakistan	Aroma world Longest Basmati rice	Good	2	10.5	Not Detected
9	G.T. Rd, Islamabad, Islamabad Capital Territory	Falak Daily Basmati rice	Good	3	10.7	Not Detected
10	G.T. Rd, Islamabad, Islamabad Capital Territory	Fine Life Super Kernal Basmati rice	Good	5	9.4	Not Detected
11	National Hwy 5, Sector F DHA Phase II, Islamabad.	Kalaar Basmati Steam rice	Good	6	10.1	Not Detected
12	National Hwy 5, Sector F DHA Phase II, Islamabad	Zarafa Super Kernal Basmati rice	Good	3	9.5	Not Detected
13	G.T. Rd, Islamabad	Guard Super Kernal Basmati	Good	6	10.8	Not Detected
14	G.T. Rd, Islamabad	Guard Super KernalBasamati rice	Good	4	10	Not Detected
15	G.T. Rd, Gujar Khan, Rawalpindi	Ream Awami Basmati rice	Good	7	9.9	Not Detected
16	G.T. Rd, Gujar Khan, Rawalpindi	Mughal Pure Basmati rice	Good	3	10.8	Not Detected
17	Grand Trunk Rd, Jada, Jhelum	Hashmi Super Kernal basmati 1121	Good	8	9.7	Not Detected
18	Grand Trunk Rd, Jada, Jhelum	Hashmi Super Kernal basmati 1121	Good	4	10.5	Not Detected
19	Grand Trunk Rd, Panjan Kasana Kharian	Honymoon Basmati rice	Good	8	10.8	Not Detected
20	Grand Trunk Rd, Panjan Kasana Kharian	Honymoon Basmati rice	Good	4	9.9	Not Detected
21	Grand Trunk Rd, Ali Chak, Gujrat	Shanshahi Jalwa Awami long grain rice	Good	6	10.2	Not Detected

Contd...

22	Grand Trunk Rd, Ali Chak, Gujrat	Shanshahi Jalwa Awami long grain rice	Good	2	10.5	Not Detected
23	Askari Homes Rahwali Cantonment, Gujranwala	Falak Easy cook Daily Basmati rice	Good	7	10.6	Not Detected
24	Askari Homes Rahwali Cantonment, Gujranwala	Falak Easy cook Daily Basmati rice	Good	3	9.9	Not Detected
25	Hussain Chowk, Block B3, Gulberg III, Lahore	Aroma World Longest Basmati rice	Good	9	10.4	Not Detected
26	Hussain Chowk, Block B3, Gulberg III, Lahore	Aroma World Longest Basmati rice	Good	7	10.6	Not Detected
27	Fortress Stadium, Lahore	Family Super kernel basmati rice	Good	8	10.1	Not Detected
28	Fortress Stadium, Lahore	Family Super kernel basmati rice	Good	4	10.4	Not Detected
29	Aziz Bhatti Road, 4 st, Cantt, Lahore	Falak select Super Kernal basmati rice	Good	3	9.4	Not Detected
30	Aziz Bhatti Road, 4 st, Cantt, Lahore	Guard Super Kernal Basmati	Good	4	9.5	Not Detected
31	PAF Market, Cantt, Lahore	Hassan 1121 Basmati rice	Good	5	9.9	Not Detected
32	Ahmed Bukhsh Rd, R A Bazaar Cantt, Lahore	Zainab Basmati rice	Good	4	10.4	Not Detected
33	Walton Road, Super Town Lahore	Falak Daily Basmati rice	Good	8	10.6	Not Detected
34	Walton Road, Super Town Lahore	Fine Old basmati rice	Good	11	10.3	Not Detected
35	Walton Road, Madina Colony, Lahore	Hassan 1121 Basmati rice	Good	5	10.2	Not Detected
36	Walton Road, Madina Colony, Lahore	Mughal Super Sella Basmati rice	Good	4	9.8	Not Detected
37	Walton Road, Pir Colony, Lahore	Guard Extreme Basamati rice	Good	4	10.4	Not Detected
38	Model Town Circular Road, Model Town, Lahore	Khyber Basmati rice	Good	7	10.7	Not Detected
39	Model Town Circular Road, Model Town, Lahore	Kausar basmati rice	Good	6	9.4	Not Detected
40	Model Town Link Rd, Lahore	Guard Super Kernal Basamati rice	Good	4	10.2	Not Detected

Contd...

41	Model Town Link Rd, Lahore	Falak Extreme Basmati rice	Good	8	9.5	Not Detected
42	Cash & Carry, Airport, Lahore	Kalaar Basmati Steam rice	Good	6	10.4	Not Detected
43	Cash & Carry, Airport, Lahore	Mughal Pure Basmati rice	Good	8	9.8	Not Detected
44	Cash & Carry, Airport, Lahore	Naubhar Nayab Silla basmati rice	Good	9	9.4	Not Detected
45	Cash & Carry, Airport, Lahore	Mehran Basmati daily rice	Good	3	9.9	Not Detected
46	Y-Block, DHA Phase 3, Lahore	Jazaa Premium Basmati rice	Good	4	9.6	Not Detected
47	Y-Block, DHA Phase 3, Lahore	Motidana Steamed 1121 Basamti rice	Good	7	9.8	Not Detected
48	Street 5, H-Block, DHA Phase 4, Lahore	Al badar Classic Super Kernal basmati	Good	6	10.4	Not Detected
49	Abdul Haque Rd, Johar Town, Lahore	Anmol Super Kernal Basmati rice	Good	4	10.4	Not Detected
50	Abdul Haque Rd, Johar Town, Lahore	Falak Easy cook Sella	Good	8	10.6	Not Detected

Table 2. Summary of Results for the Branded Rice Varieties.

Area	Rice Brand	Time in Shelf	Moisture Content	Aflatoxin Level
Islamabad, Rawalpindi, Gujar Khan, Jehlum, Kharian, Gujrat, Gujranwala, Lahore	Guard supreme basmati, Gurad Super Kernal Basmati, Zainab Basmati Rice, Falak Premium Basmati rice, Mughal Badshah Rice, Aroma Super Basmati Rice, Aroma world Longest Basmati Rice, Falak Daily Basmati rice, Fine Life Super Kernal Basmati Rice, Kalaar Basmati Steam rice, Zarafa Super Kernal Basmati rice, Ream Awami Basmati Rice, Hashmi Super Kernal basmati 1121, Honymoon Basmati Rice. Shanshahi Jalwa Awami long grain rice. Faimly Super kernel basmati Rice, Hassan 1121 Basmati Rice, Khyber Basmati Rice, Kausar basmati Rice, Naubhar Nayab Silla basmati rice, Mehran Basmati daily Rice, Jazaa Premium Basmati rice, Motidana Steamed 1121 Basamti rice, Al badar Classic Super Kernal Basmati, Anmol Super Kernal Basmati Rice	2-11 Months	9.4-10.8	Not Detected

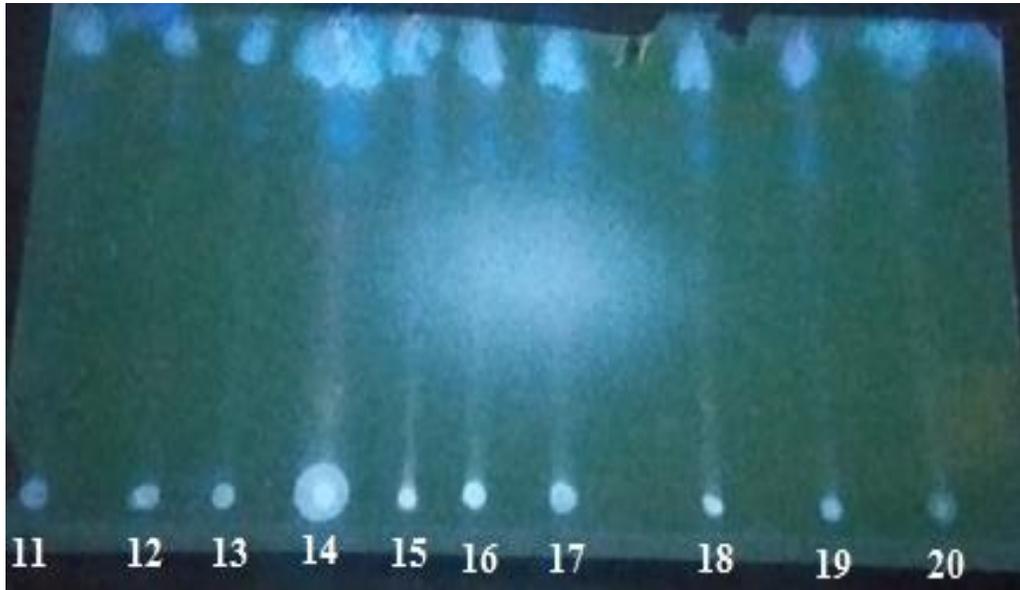


Figure 1. TLC Results for the branded rice Varieties.

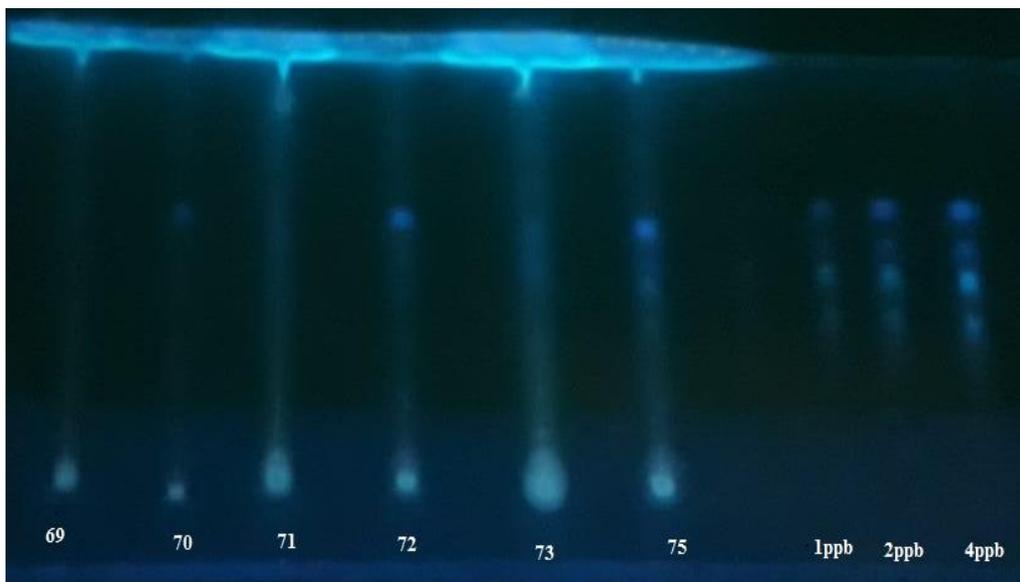


Figure 2. TLC results of open rice (poorly stored samples) (Left lanes-**Samples:** 69-73, 75; Right lanes: 1ppb, 2ppb, 4ppb-**Standards**).

Aflatoxin Concentration in Open Rice Samples with Poor Storage Conditions

Rice varieties which were collected from different cities and towns of Punjab had shown the presence of aflatoxins. The corresponding TLC results are shown in **Fig. 2**. Out of 100 non-branded samples, the presence of aflatoxins was detected in 65 samples while the remaining 35 samples did not show the presence of aflatoxins. Out of 65 total contaminated samples, 23 rice varieties had shown the

existence of aflatoxins below the limits recommended by the European authorities (2-4ppb) whereas 24 samples had shown aflatoxins in the recommended limits of 2-4ppb. 18 rice samples showed aflatoxin concentration above the recommended limits. The highest value of aflatoxin (8.92ppb) for observed for the rice sample 40 (NIAB IR-9). Table 3 displays the aflatoxin concentration in the poorly stored rice samples whereas the final data are summarized in Table 4 and **Fig. 3**.

Table 3. Aflatoxins and Moisture content analysis of Local Rice Varieties.

S. No.	City and Location from where the Rice Samples Picked (Minimum 500g each)	Variety of Rice	Time in a Shelf (Months)	H ₂ O Content (%age)	Aflatoxin Concentration (ppb)
1	Kechery Chowk, Rawalpindi	Basmati 370	4	12	Not Detected
2	Bilal Street, Morgah, Rawalpindi	Basmati Pak	9	12.4	Not Detected
3	Adamjee Street, Rawalpindi	KS-282	12	14	1.86
4	G.T Road, Rawalpindi	Basmati 385	11	14.2	2.96
5	Soan Garden Extension, Islamabad	Basmati 2000	15	15	3.56
6	Shalimar Rd, Block E Soan Garden, Islamabad	Basmati Pak	6	11.8	Not Detected
7	Phase 1 Jinnah Garden, Islamabad	Super Basmati	2	12	Not Detected
8	GT Rd, Rawat, Islamabad	Basmati 515	11	15.5	4.53
9	Chamber More Rawat, Islamabad	Shaeen Basmati	6	11.5	Not Detected
10	Service Rd, Rawat, Islamabad	NIAB IR-9	9	15	2.62
11	Bagga Shaikhan, Rawalpindi, Islamabad	KSK-133	6	15.1	1.73
12	Banth, Rawalpindi, Punjab, Pakistan	Basmati 2000	5	12	Not Detected
13	Grand Trunk Rd, Mandra, Rawalpindi	Basmati 370	12	14.6	2.55
14	Tharjiyal Rd, Mandra, Rawalpindi	Super Basmati	10	15.4	1.79
15	Tharjiyal Rd, Rawalpindi	KS-282	6	10.9	Not Detected
16	ChehariKalyal, Rawalpindi	Basmati 515	8	12.4	Not Detected
17	Gujar Khan, Rawalpindi	Basmati 370	4	11.8	Not Detected
18	Sandal Road Ward 14, Gujar Khan, Rawalpindi	Super Basmati	10	15.4	8.78
19	Housing Scheme 2, Gujar Khan, Rawalpindi	KSK-133	4	15.5	3.64
20	W7 Gujar Khan, Pakistan	Basmati 2000	6	15.2	1.62
21	Bhai Khan, Gujar Khan, Rawalpindi	NIAB IR-9	10	11	Not Detected
22	Sohawa City, Jhelum	KS-282	10	11.5	Not Detected
23	Ehsan Butt St, DomeliMohallah, Dina	Basmati 515	9	14.9	2.48
24	Mangla Rd, Dina, Jhelum	Basmati 370	8	15.1	2.93

Contd...

25	Main Bazaar, Dina, Jhelum	KS-282	6	16	3.73
26	Grand Trunk Rd, Burha Jungle, Dina, Jhelum	NIAB IR-9	9	14.7	1.97
27	Kala Gujran, Jhelum, Punjab, Pakistan	Basmati 370	5	12.3	Not Detected
28	G.T Road, Opp Fauji Mill Ground, Kala Gujran, Jhelum	KSK-133	10	15.4	3.47
29	Nawab Colony, Jhelum	KS-282	6	11.6	Not Detected
30	Sultan St, Jada, Jhelum	NIAB IR-9	10	14.6	1.21
31	Chak Jamal Rd, Jada, Jhelum	Basmati 2000	8	15.7	4.63
32	Tufail Rd, Jhelum Cantt, Jhelum	Super Basmati	6	12.4	Not Detected
33	Jhelum Cantt, Jhelum		8	11.8	Not Detected
34	Arain Street, Eidgah, Jhelum	Basmati 2000	8	14.3	0.92
35	Pinddadan Khan - Jhelum Rd, Jhelum Cantt, Jhelum	Basmati 515	6	16	3.85
36	Arsal Town Jhelum Cantt, Pinddadan Khan - Jhelum Rd	KSK-133	10	15.4	6.43
37	Bagga, Jhelum Pinddadan Khan - Jhelum Rd	KS-282	8	12.9	Not Detected
38	Nougran Link Rd, Pinddadan Khan - Jhelum Rd, Jhelum	Basmati 370	6	14.7	1.42
39	Nougran, Jhelum Pinddadan Khan - Jhelum Rd	Super Basmati	14	13.2	2.76
40	Ghous Plaza, Grand Trunk Rd, Sarai Alamgir, Gujrat	NIAB IR-9	12	15.7	8.92
41	Jalil Pura, Sarai Alamgir, Gujrat	Basmati 2000	6	11.7	Not Detected
42	Main Bazaar, Mohalla Shaheedan, Sarai Alamgir, Gujrat	Basmati 370	10	12.5	Not Detected
43	N5, Mohalla Shaheedan, Sarai Alamgir, Gujrat	Super Basmati	4	14.7	2.96
44	G.T. Road, Sarai Alamgir, Gujrat	KS-282	5	14.4	5.32
45	Bani Mohalla Kharian, Guliana Road, Gujrat	KSK-133	7	12.6	Not Detected
46	Panjan Kasana Village, Gujrat	NIAB IR-9	2	14.5	1.62
47	Grand Trunk Rd, Thikrian, Lala Musa, Gujrat	Basmati 370	4	14.7	6.12

Contd...

48	Asghar Town Haji Asghar Town, Lalamusa, Gujrat	Basmati 2000	6	12.8	Not Detected
49	Karimpura, Lalamusa, Gujrat	KSK-133	6	15.5	3.76
50	Chota Bazar Road, Lalamusa, Gujrat	KS-282	10	15.2	1.62
51	Service Rd W, MeherMohalla, Lalamusa, Gujrat	Basmati 385	5	15.3	5.74
52	Grand Trunk Rd, Ellahi Colony, Gujrat	Super Basmati	11	15.4	3.63
53	Kalra Khasa, G.T Road, Gujrat	NIAB IR-9	6	14.6	0.75
54	Muslim Town, Rahwali Cantonments, Gujranwala	Basmati 2000	10	15.1	8.75
55	Meher Colony, Rahwali Cantonments, Gujranwala	Basmati 370	5	12.1	Not Detected
56	Service Rd, Shaheenabad, Gujranwala	KS-282	4	15.4	1.52
57	Safdar & Asif Rice Trader, Mohalla Raitanwala Krishan Nagar, Gujranwala	Basmati 515	9	12.5	Not Detected
58	Civil Lines, Gujranwala	Super Basmati	6	14.9	5.73
59	G.T. Rd, Near Jama Masjid Bilal, Eminabad, Gujranwala	KSK-133	5	15.4	1.55
60	Mandiala Rd, Kamoke, Gujranwala	NIAB IR-9	10	15.5	2.73
61	Canal Road, Sadhoke, Gujranwala	Basmati 2000	6	14.9	1.7
62	Mohalla Faiz-e-Madina, Muridke, Sheikhpura	Super Basmati	5	12.4	Not Detected
63	Ravi Rayon, Kala Shah Kaku, Sheikhpura	KS-282	4	14.4	1.22
64	Main Bazar, Rana Town Ferozewala, Sheikhpura	Basmati 370	6	15.7	6.86
65	G.T. Road, Shahdara Town, Shahdara, Lahore	Basmati 2000	9	15.4	3.76
66	Ahmed Bukhsh Rd, R A Bazaar Cantt, Lahore	KSK-133	6	11.6	Not Detected
67	Akram Park Gurumangat Gulberg III, Lahore	KS-282	3	14.4	1.65
68	Main Bazar Canal Park, Block O Gulberg 2, Lahore	Basmati 515	5	12.5	Not Detected
69	Chahgallanwala, Imran St, Rasool Park, Lahore	Basmati 370	4	12.6	Not Detected
70	Link Ferozepur Rd, New Islamia Park Islamia Park, Lahore	Super Basmati	6	14.8	1.76

Contd...

71	Multan Rd, Maraka Village, Lahore	NIAB IR-9	10	11.4	Not Detected
72	Canal Bank Rd, Chimbran wali Jhuggi, Lahore	Basmati 2000	6	15.1	4.93
73	Hospital Road, Manga - Raiwind Road, Manga Mandi, Lahore	KSK-133	2	12.4	Not Detected
74	Sherpur Road, Phool Nagar, Kasur	KS-282	6	14.4	1.92
75	Malik Colony, Phool Nagar, Kasur	Super Basmati	7	15.4	5.76
76	Jamber, Lahore Multan Road, Kasur	NIAB IR-9	5	11.8	Not Detected
77	Megha Rd, Pattoki, Bilal Colony, Kasur	Basmati 2000	4	15.2	3.72
78	Habibabad, Habibabad Road, Okara	Basmati 370	3	15.7	1.47
79	G.T. Rd, RenalaKhurd, Okara	Basmati 2000	1	15.4	5.92
80	Multan Okara Rd, Sheikh Basti, Okara	KSK-133	5	11.6	Not Detected
81	Kot Liaquat Hayat, Okara	Basmati 2000	2	12.4	Not Detected
82	Wan Bazar, Sahiwal, Sahiwal	NIAB IR-9	6	15.1	3.65
83	Liaquat Chowk, Sadman Town Sahiwal, Sahiwal	KS-282	6	15.4	2.73
84	Block 5, Chichawatni, Sahiwal	Basmati 370	4	15.1	6.57
85	Multan Road, Iqbal Nagar	Super Basmati	6	14.8	5.7
86	Multan-Mian Channu Road, Mian Channu	Basmati 370	5	12.6	Not Detected
87	Ayoub Chowk, Ayoub Road, Khanewal	Basmati 2000	6	15.4	3.72
88	Old Kachehri Rd, Main Bazar, Kabirwala	Basmati 515	4	15.1	1.75
89	T Block, New Multan Colony, Multan	KS-282	6	11.4	Not Detected
90	Gulshan Town, Multan	NIAB IR-9	7	12.4	Not Detected
91	Chak Raas, Multan	Basmati 370	5	16	3.75
92	Raiway Road, Shujaabād, Multan	KSK-133	4	15	3.42
93	Permit Road, Jalalpur Pirwala, Multan	Super Basmati	6	15.4	1.77
94	Alipur Road, Uch Sharif, Bahawalpur	Basmati 2000	4	14.6	2.63
95	Liaquat Pur Rd, Janpur, Rahim Yar Khan	KS-282	7	15.1	6.83
96	Khan Bela, Rahim Yar Khan	Basmati 370	5	14.4	1.65

Contd...

97	N5, Fateh Pur Kamal, Rahim Yar Khan	KSK-133	6	15	2.45
98	Jamia Masjid Chowk Zahir Pir, nearby, Khanpur Rd, Rahim Yar Khan	NIAB IR-9	5	15.4	1.42
99	Sardargarh, Rahim Yar Khan	KS-282	4	15.1	6.94
100	Main Road, Gulshan e Usman, Rahim Yar Khan	Super Basmati	6	12.4	Not Detected

Table 4. Summary of Open Rice Samples with Poor Storage.

Aflatoxin	Total No. of Samples	Contaminated Samples	Uncontaminated Samples	Moisture Content %age	Max ppb	EU limits for contamination
B1 in Rice sample with poor Storage	100	65	35	10.9 - 16	8.92	2-4ppb

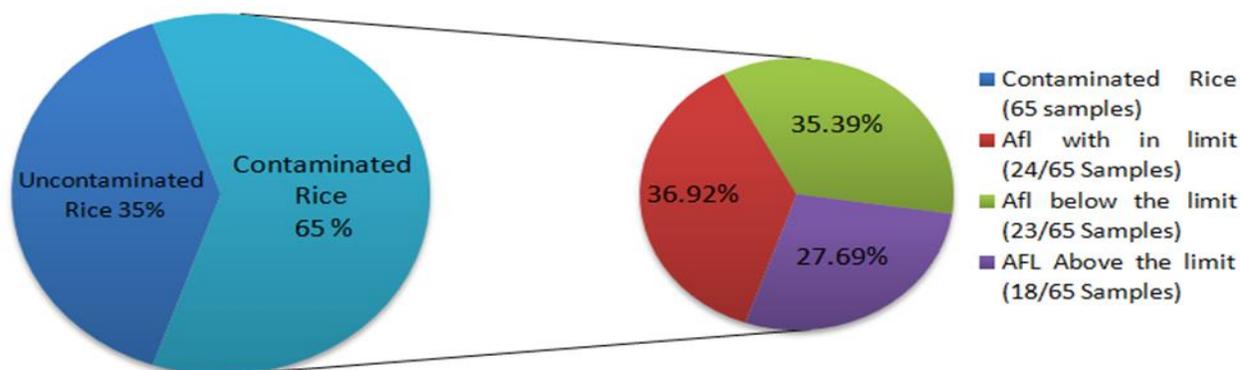


Figure 3. Comparison of aflatoxin concentration in open rice samples.

Aflatoxin B1 (AFB1) which is most common aflatoxin, was detected (0.75-8.92 ppb) in all the 65 rice samples having poor storage. AFB2, AFG1 and AFG2 were not detected in anyone of the tested samples. The recommended limit of aflatoxin B1 by European Union (EU) is 2ppb for rice and other edible foods for humans; it is 4ppb for total aflatoxins. However, according to the Pakistan Standards and Quality Control Authority (PSQCA), Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), the allowed concentration of AFB1 is 10ppb. So the observed AFB1 concentration in the rice samples was found within the recommended limits of PSQCA, FAO and FDA. However, it was found beyond the limits of EU indicating a dire need to overcome aflatoxin contamination in rice.

CONCLUSION

In the current study, detailed analysis has been conducted to monitor the presence of aflatoxins B1, B2, G1 and G2 in

branded and non-branded rice varieties from various cities of Punjab, Pakistan. Interestingly, all the branded rice samples were totally free from aflatoxins, however, 65% of non-branded and poorly stored rice samples had shown the aflatoxin contamination (0.75 to 8.92 ppb). Moreover, in the remaining 35% samples, aflatoxins were found below the limits recommended by the European authorities (2-4ppb). Highest concentration of aflatoxin was observed 8.92 ppb in few rice sample. In the branded rice samples the lowest moisture content detected were 9.4 and highest moisture content was 10.8%. Non-branded (54%) rice samples have shown the moisture contents more than 13%, which is alarming. It is recommended that Pakistani authorities should review periodically and analyze rice samples to ensure the absence of aflatoxins in foods. Special precautions must be taken during storage, harvesting, transportation, drying and harvesting to stop aflatoxin production in cereals. If aflatoxins are present in

food, their concentration should be minimized or eradicated by recommended de-toxifying technologies.

CONFLICTS OF INTEREST

None.

FUNDING SOURCE

None.

ACKNOWLEDGMENTS

Author is thankful to Pakistan Council of Scientific and Industrial Research (PCSIR) for providing necessary research facilities in their labs.

LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
EU	European Union
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization
Rf	Retention Factor
TLC	Thin Layer Chromatography Technique

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