ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 4 Issue 1 January 2021

Research Article

Estimation of Aflatoxins (B1, B2, G1 and G2) in Rice Grain Collected from Different Division of Punjab Pakistan

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Received: November 30, 2020

Published: December 22, 2020

Abstract

Aflatoxins are bioactive fungi or specialized metabolite or acutely toxic or cancer-causing substances and substances that alter DNA that are derived from polyketides produced by certain fungi that cause severe contamination in agricultural crops. Fungi are primarily responsible for producing aflatoxins in white rice in the presence of high moisture. Mostly, the rice samples were contaminated with aflatoxins due to unsuitable storage conditions. Furthermore, the food and feed contamination with mycotoxins is a major issue for food safety and for human health. The toxic effects of aflatoxin can cause chronic disease, poor productivity, severe life-threatening toxicity and damage to the immune system. A total of 70 samples of rice were collected from the Capital and different selected divisions of Central, North and South Punjab, Pakistan. The rice is harvested with 18 to 24 percent natural water content and the paddy moisture for storage is dried at 12 to 14 percent. In this study, in Central Punjab (Lahore), the moisture content of 10 samples of rice was 13.47%, in Gujranwala 11 samples were 13.50% and in Sahiwal it was 12.50% respectively. In addition, the thin layer chromatography (TLC) method was used to detect aflatoxin in rice. Aflatoxins B_1, B_2, G_1, G_2 were detected in 32 samples (45.7%). The maximum B_2 aflatoxin (32%) was detected in the central Punjab samples. Similarly, B_1 (7.5%) and G_1 (7.5%) aflatoxins were also detected in the samples of central Punjab. Moreover, 20 samples were collected from south Punjab in which 5 samples were infected with B_2 aflatoxin (25%) and 2 samples were detected with G_1 aflatoxin (10%) and B_1 (5%). Aflatoxin G_2 was not detected in all samples. The result was showed that aflatoxin has been assessed more in samples that contain high moisture contents.

Keywords: Aflatoxin; Rice; Moisture Contents; Thin Layer Chromatography

Introduction

Food security is usually safe and nutritive food for all people, at all times, have physical, social, and economic access to sufficient that meets their food preferences and dietary needs for an active and healthy life. Food safety is a necessary part of food security [1]. Today safety and quality of food and food products are needed because of a number of health problems creating. Globally, mycotoxin contamination is the major cause of food insecurity [2]. Aflatoxins are basically grown on stable food at the time of storage. Different diseases such as cancer, malaise, impaired immune function occur due to the consumption of aflatoxin contaminated foods [3].

Oryza sativa or *Oryza glaberrima* is botanically called Asia rice and Africa rice respectively. The seed of Rice is the grass species which considered a staple food in a large part of the world [4]. Especially in many countries of Asia and Africa, rice is a vital food for populations [5]. The rice is composed of moisture, protein, fat, carbohydrates and minerals. The protein, carbohydrate and fat are present in rice in the form of amino acids, starch and fatty acids respectively. The minerals like Ca, Zn, Fe, Na, K are present in rice [6]. Due to adequate level of nutrients, rice is well for human health, it's also aids in improved and regulate body function [7].

Plant base food are affected by climate changes. The role of climate in agricultural productivity is creditable [8]. The rice production is influence due to climate which is major problem like food security [9]. Asia is produced and consumed about 90% of the world's rice [10]. According to staple food crops, in Pakistan the level of rice is 2nd after wheat. The rice played an important role in improve economy and it is considered vital export crop. Pakistani rice is high quality that exported to several countries such as Saudi Arabia, and Afghanistan, United Arab Emirates and Iran [11].

The toxic family called aflatoxins is the secondary metabolites produced from Aspergillus species (mainly *A. flavus* and *A. parasiticus*) that can contaminate agricultural crops like wheat, rice, maize and groundnuts [12]. Cereals' exposure to aflatoxins is a public health concern due to their carcinogenic, acute and chronic effects [13]. In developing countries, where the climates remain hot and humid, and where food storage conditions are poor and lack of regulatory limits enforcement. at that place chances of aflatoxins are increased [14]. AFB1, AFB2, AFG1 and AFG2 are the major types of aflatoxins, its produce hepatocarcinogen effects on humans at any stage of life [15].

This study was conducted on rice grain collected from different markets. The moisture content of rice was evaluated that may be associated with aflatoxins contamination. The TLC technique was used for the determination of aflatoxins in rice grains.

Materials and Methods

Sample collection and sampling technique

Moisture content and quantification of aflatoxins like B_1, B_2, G_1, and G_2 in this study was performed for rice grain collected from local markets in different selected areas of Punjab and capital. A total of 70 rice grains sample was collected from Capital and from different selected divisions of Central, North and South Punjab, Pakistan. In the collection of rice samples from local markets, following random sampling method three different bags of same variety were selected. From each bag sample was drawn from different locations and made composite sample of 1 kg. All samples were free of insect infestation and with no chemical preservative treatment applied for storage. These collected samples were packed in plastic bags and stored at ambient temperature which prevents them from further contamination.

Moisture content analysis

The moisture content of the rice grains was calculated as percentage moisture of the stored rice grains [16]. Samples of ground rice grain (5 g) were placed in hot air oven and temperature was set at 105 ± 5 °C according to AACC Method No. 44-01. After 24 hours' hot air oven showed result as per following relationship.

$$\text{Moisture (\%)} = \frac{\text{Wt. of dried sample (g) x 100}}{\text{Wt. of original sample (g)}}$$

Determination of aflatoxin by thin layer chromatography

Aflatoxins were determined in present study according to Sherma and Fried, [17]. In the present study used chemicals were of analytical grade and purchased from different well-known companies. These chemicals include acetonitrile, chloroform commercial grade, chloroform analytical grade was purchased from Merck (Darmstadt, Germany), distill Water, anhydrous diethyl ether, acetone extra pure, and sulfuric acid was acquired from Sigma-Aldrich (St Lois-MO, USA). Mixture of aflatoxins standard [AFB_1 (2.03µg/ml), AFB_2 (0.503 µg/ml), AFG_1 (2.02 µg/ml) and AFG_2 (0.504 µg/mL)] were purchased from Romer Labs Inc. Singapore. These solutions of standards were stored in refrigerator at -4 ºC and also wrapped in an aluminum foil to prevent them from sunlight because UV light causes gradual breakdown of AFs. Graduated cylinder 100mL, funnels 100mm top diameter, conical flasks 250 mL, beakers 500 mL, whatman No. 4 filter paper, TLC tank, pre-coated silica gel plates of TLC on aluminum sheets with a layer thickness of 0.25mm, 20x20cm (E. Merck, Darmstadt, Germany), Micro syringe 25 µL (Hamilton, USA) and UV lamp were used in this research. Samples were crushed to small particles of size less than 2 mm by using Romer series II milling machine (Romer LABS Inc. USA).

Extraction and purification of AFs from rice grains

Chloroform extraction

From each of the ground rice grain, sample (50 g) was added separately in 500 mL conical flasks. Distilled water (25 mL) add-

ed into conical flask. The flasks were shaken manually to moist the sample properly and for proper dispersion of water. Chloroform (commercial grade) was added after proper moistening of sample and made total volume up to 250mL. Then, aluminum foil was placed on conical flasks and shaken vigorously on wrist action shaker for 30 minutes at 25-30 rpm. The mixture was filtered through Whatman no 4 filter paper in a beaker and filtrate (50 mL) was taken while residue was discarded.

Concentration of sample extract

The extract of rice samples was dried on hot plate in fume hood at 100 ºC until all chloroform evaporated and only sample remained. The concentrated sample was sealed with aluminum foil and kept in cool place for the prevention of re-absorption of moisture.

Spotting of the TLC aluminum plate

Analytical grade chloroform (0.5mL) were added and dissolved in each concentrated extract and vortexed properly. Plates were cut into required size (10Cm×10Cm) on cutter and base line about 1.5cm from lower side was drawn with help of scale and lead pencil. Various volumes of the samples extract 10, 15 and 25µL were spotted on the base line of the plate for all the samples while placing TLC plate on hot plate. The spotting was done using 25µL microsyringe.

Plates development and viewing

In first mobile phase anhydrous diethyl ether was added in TLC tank. The plates were placed vertically along the wall side in tank containing anhydrous diethyl ether. The plates were placed with the upper edge leaning against the back side of the tank. Then cover was closed and plates were allowed to develop until complete development of spots. It took 25 to 30 min for the solvent to reach the stop line (10 Cm) from the base line. The plates after development were removed carefully and allowed to dry on hot plate for few seconds.

In second mobile phase, in 2nd TLC tank chloroform analytical grade (45 mL) and acetone extra pure (5 mL) was added and plates were placed and allowed to develop until solvents reached on stop line. It also took 25 to 30 minutes. Removed the plates carefully and dried on hot plate before further investigation.

Interpretation of TLC plates

Observed under a long wavelength UV lamp at 365 nm (λ= 365nm) in dark room specially designed for aflatoxin detection to prevent photo-degradation of aflatoxin. This process was repeated for all the samples.

Quantification of aflatoxins

Formula for estimation of quantity of Aflatoxins

Concentration of AFB_1:

$$AFB_1\ \mu g/\ Kg\ (ppb) = \frac{S \times Y \times V}{Z \times W}$$

S = Volume of standard matched with sample concentration to give fluorescence

Y = Concentration of standard

V = Volume of the Sample used

Z = Volume of Sample required to give fluorescence

W = Effective weight of Sample taken

Same procedure to calculate concentrations were followed or the AFB_2, AFG_1 and AFG_2 spots.

The concentrations of total aflatoxins were calculated by using underlying equation.

Total AFs (TAFs) = Concentration of AFB_1+AFB_2+AFG_1+AFG_2

Statistical analysis

Data was statistically analyzed by using SPSS (One-way ANOVA) [18].

Results and Discussion

Moisture contents

The pure water content is naturally present in foods called moisture or water activity or natural water content or natural moisture content. The moisture content of rice is extremely important due to the management and marketing of paddy and rice. It affects the physical properties of a substance and acts primarily on quality control in most food products. It is the most important component in food products that affects the food stability, food quality and food shelf life. Temperature is one of the most important factors affecting the value of present water during the measurement of moisture content [19]. The rice is harvested with 18 to 24 percent natural water content and the paddy moisture for storage is dried at 12 to 14 percent. In this study, in Central Punjab (Lahore), the moisture content of 10 samples of rice was 13.47%, in Gujranwala 11 samples were 13.50% and in Sahiwal it was 12.50% respectively.

Furthermore, in the South Punjab samples, the value of moisture of 8 samples of Bahawalpur was measured 8 12.85%. In addition, Rawalpindi's 5 samples of moisture content were 12.67% respectively as shown in the table 1. According to Zheng., *et al.* [20] different drying temperatures were used to test the effect of treatment on the quality of rice and moisture content. The moisture content of rice was directly affected on the quality of rice. The results indicated that the value of moisture content has been reduced to 15% and the quality of rice also reduced. In addition, the moisture content of two different types of rice was measured using the oven method. The moisture content of Junjuangan rice was 10 to 27%. Similarly, the moisture in Mundam was 14 to 27%. The moisture content of the Junjuangan sample was higher than that of the Mundam sample [21]. The average of moisture content was measured using the standard oven method. The moisture contents during the storage were reduced from 22.8% to 16.3% [22]. Another study by Muhammad., *et al*. [23] on physicochemical analysis of white and brown rice types. Moisture content was estimated range from 6.84% to 9.08% in ten different types of rice. In the study of Mohd., *et al*. [24] to determine changes in lipid profile, rabbits were given different doses of processed rice. White rice, brown rice, and germinated brown rice were used in the experimental diet. Raw material chemical analysis show that the moisture content of white rice was 7.73% which is significantly lower than that of brown rice, and germinated brown rice. Chemical analysis of Nigerian rice carried out by Oko., *et al.* [25] indicate that the presence of moisture contents was 7.68%.

Division	Total Samples	Moisture ± SD
Central Punjab		
Lahore	10	13.47 ± 0.12
Gujranwala	11	13.50 ± 0.07
Sargodha	5	12.91 ± 0.15
Faisalabad	8	12.98 ± 0.09
Sahiwal	6	12.50 ± 0.04
South Punjab		
Multan	7	12.75 ± 0.19
Bahawalpur	8	12.85 ± 0.50
Dera Ghazi Khan	5	10.45 ± 0.31
North Punjab		
Islamabad	5	12.79 ± 0.71
Rawalpindi	5	12.67 ± 0.84

Table 1: Moisture Contents of Different Division of Punjab.

Aflatoxins assessment

TLC is an innovative technique used for the separation of compounds or non-volatile compounds or a mixture of compounds from a solution or a product or an ingredient. Fungi are primarily responsible for producing aflatoxins in rice in the presence of high moisture. Mostly, the rice samples were contaminated with aflatoxins due to unsuitable storage conditions. The standard of aflatoxin in food according to the recommendations of the Codex, some countries apply different limits for aflatoxin B1 in food which does not exceed 9 μg/kg [26]. In this study, the TLC method was used to detect aflatoxin in white rice. Aflatoxins B_1, B_2, G_1, G_2 were detected in 32 samples (45.7%) as shown in the table 2. The maximum B_2 aflatoxin (32%) was detected in the central Punjab samples. Similarly, B_1 (7.5%) and G_1 (7.5%) aflatoxins were also detected in the samples of central Punjab. Moreover, 20 samples were collected from south Punjab in which 5 samples were infected with B_2 aflatoxin (25%) and 2 samples were detected with G_1 aflatoxin (10%) and B_1 (5%). From north Punjab, 10 samples were collected from different divisions of which 3 samples were also infected with B_2 aflatoxin (30%) and G_1 (20%). In the all samples, G_2 aflatoxin was not detected. Nisa., *et al.* [27] research work used the TLC method to detect aflatoxins in different types of rice samples. B_1 Aflatoxin (13.31%) significantly presented in the white rice. High performance liquid chromatography can be used to detect the aflatoxins B_1, B_2, G_1, G_2. The results showed that aflatoxin B_1 was present in 40% of white rice [28]. Aflatoxins were detected from white rice using the different analytical methods such as enzyme-linked immunosorbent assay and HPLC. The results show that the B_1 aflatoxin range was between 17-90%, which was higher than the European commission standard values [29]. A study carried out by Mukhtar., *et al.* [30], 56% of rice was contaminated by aflatoxins B_1 and white rice also contained 33% of B_2 aflatoxin. Similarly, white rice was collected from different cities of Pakistan to detect aflatoxin using HPLC method. Final results show that 42% samples of white rice were contaminated with aflatoxin B_1. In a study by Sultana., *et al.* [31] B_1 aflatoxins 76.58% was present in white rice due to preferable conditions for fungi. Furthermore, 18.46% of aflatoxins B_2, G_1, G_2 were also detected. The previous study conducted on wheat flour in which all samples were free from aflatoxins because the moisture level of flour remained below the standard [32].

		Central Punjab			
	Aflatoxins				
Division	Total Samples	B1	B2	G1	G2
Lahore	10	1	3	1	0
Gujranwala	11	2	4	1	0
Sargodha	5	0	2	0	0
Faisalabad	8	0	2	1	0
Sahiwal	6	0	1	0	0
		South Punjab			
	Aflatoxins				
Division	Total Samples	B1	B2	G1	G2
Multan	7	0	2	1	0
Bahawalpur	8	1	3	1	0
Dera Ghazi Khan	5	0	0	0	0
		North Punjab			
	Aflatoxins				
Division	Total Samples	B1	B2	G1	G2
Islamabad	5	1	2	1	0
Rawalpindi	5	0	1	1	0

Table 2: Aflatoxins (B1, B2, G1 and G2) Assessment of Different Division.

Conclusion

The current survey concluded that Pakistani rice is not free from aflatoxins. The results showed that these aflatoxins contamination in rice is due to high moisture contents. The high moisture level of rice is due to poor storage conditions and the climate effect. Central Punjab samples were contained high aflatoxins contamination because their moisture level recorded as high. Furthermore, for the determination of aflatoxins in rice, an advanced technique like HPLC is required.

Bibliography

1. Porter JR., *et al*. "Food security and food production systems". Cambridge University Press (2014): 485-533.

2. Udomkun P., *et al*. "Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application–A review". *Food Control* 76 (2017): 127-138.

3. Agag BI. "Mycotoxins in foods and feeds: 1-aflatoxins". *Assiut University Bulletin for Environmental* 7.1 (2004): 173-205.

4. Mokuwa A., *et al*. "Robustness and strategies of adaptation among farmer varieties of African rice (Oryza glaberrima) and Asian rice (Oryza sativa) across West Africa". *PLoS One* 8.3 (2013): e34801.

5. Seck PA., *et al*. "Crops that feed the world 7: Rice". *Food Security* 4.1 (2012): 7-24.

6. Verma DK and Prem PS. "Proximate composition, mineral content and fatty acids analyses of aromatic and non-aromatic Indian rice". *Rice Science* 24.1 (2017): 21-31.

7. Bhullar NK and Wilhelm G. "Nutritional enhancement of rice for human health: the contribution of biotechnology". *Biotechnology Advances* 31.1 (2013): 50-57.

8. Vaghefi N., *et al*. "Impact of climate change on food security in Malaysia: economic and policy adjustments for rice industry". *Journal of Integrative Environmental Sciences* 13.1 (2016): 19-35.

9. Ali A and Olaf E. "Assessing farmer use of climate change adaptation practices and impacts on food security and poverty in Pakistan". *Climate Risk Management* 16 (2017): 183-194.

10. Wassmann R., *et al*. "Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies". *Advances in Agronomy* 101 (2009): 59-122.

11. Chandio AA and Yuansheng JIANG. "Determinants of adoption of improved rice varieties in northern Sindh, Pakistan". *Rice Science* 25.2 (2018): 103-110.

12. Ali N. "Aflatoxins in rice: Worldwide occurrence and public health perspectives". *Toxicology Reports* 6 (2019): 1188-1197.

13. Wild CP and Yun YG. "Mycotoxins and human disease: a largely ignored global health issue". *Carcinogenesis* 31.1 (2010): 71-82.

14. Marina Goumenou D., *et al* "Aflatoxins: Food Sources, Occurrence and Toxicological Effects". *Nova Publications* (2014): 191-232.

15. Elzupir AO Alamer AS and Dutton MF. "The occurrence of aflatoxin in rice worldwide: a review". *Toxin Reviews* 34.1 (2015): 37-42.

16. AACC. Approved Methods of Analyses. 10th Ed. American Association of Cereals Chemists. St. Paul, Minnesota. USA. (2000)

17. Sherma J and Bernard Fried, eds. Handbook of thin-layer chromatography. CRC press 89 (2003).

18. Steel RG. Pinciples and procedures of statistics a biometrical approach (No. 519.5 S8) (1997)

19. Muga FC Marenya MO and Workneh TS. "Effect of temperature, relative humidity and moisture on aflatoxin contamination of stored maize kernels". *Bulgarian Journal of Agricultural Science* 25.2 (2019): 271-277.

20. Zheng XIANZHE and Lan YUBIN. "Effects of drying temperature and moisture content on rice taste quality". *The Proceedings of the 5th Asia-Pacific Drying Conference* 2 (2007).

21. Makky M., *et al*. "Determination of moisture content in rice using non-destructive short-wave near infrared spectroscopy". *AIP Conference Proceedings* 2155.1 (2019).

22. Ilieva V., *et al*. "Effect of rice moisture at harvest and rough rice storage time on milling yield and grain breakage". (2014).

23. Muhammad Z., *et al*. "Proximate composition and minerals profile of selected rice (Oryza sativa L.) varieties of Pakistan". *Asian Journal of Chemistry* 24.1 (2012): 417-421.

24. Mohd Esa N., *et al*. "Improving the lipid profile in hypercholesterolemia-induced rabbit by supplementation of germinated brown rice". *Journal of Agricultural and Food Chemistry* 59.14 (2011): 7985-7991.

25. Oko AO., *et al*. "Comparative analysis of the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State of Nigeria". *International Journal of Agriculture and Forestry* 2.2 (2012): 16-23.

26. Bugang W and Woolsey M. "National Food Safety Standard-Maximum Levels of Contaminants in Foods (GB 2762-2010)". Global Agricultural Information Network, USDA. Washington DC, (2010): 2-7.

27. Nisa A Zahra N and Hina S. "Detection of aflatoxins in rice samples". *Bangladesh Journal of Scientific and Industrial Research* 49.3 (2014): 189-194.

28. Firdous S *et al*. "Occurrence of aflatoxins in export-quality Pakistani rice". *Food Additives and Contaminants: Part B* 5.2 (2012): 121-125.

29. Morrison D. "The Determination of aflatoxins in paddy and milled fractions of rice in Guyana–Preliminary results". (2016).

30. Mukhtar H Farooq Z and Manzoor M. "Determination of aflatoxins in super kernel rice types consumed indifferent regions of Punjab, Pakistan". *Journal of Animal and Plant Sciences* 26.2 (2016): 542-548.

31. Sultana N., *et al*. "Dietary exposure and natural occurrence of total aflatoxins in basmati rice of pakistan". *Journal of Food Protection* 80.2 (2017): 331-337.

32. Waseem K., *et al*. "Assessment of aflatoxins in wheat flour from different areas of Punjab in Pakistan" *International Journal of Biosciences* 17.3 (2020): 230-240.