

## Comparison of Prevalence of Aflatoxins in Different Varieties of Rice on the Basis of Post Harvesting Activities

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### Abstract

This research was conducted to monitor the existence of aflatoxins types (B type, G Type) in different rice varieties. A number of samples of rice were collected from different regions or from different sites of the Punjab district of Gujrat, Lahore, Sialkot, and Mandibahudin. These samples were analyzed using Thin Layer Chromatography (TLC) and Enzyme-Linked Immunosorbant Assay (ELISA) technical methods. And mostly Basmati brown rice samples were found affected with aflatoxin B1 especially, rest of the affected samples include sella Rice, white rice etc. These affected samples can be detoxify by using natural compounds such as the detoxification promised by specified natural compounds such as sodium bicarbonate, citric acid, *Allium Sativum* and black seed oil was (88, 100, 63 and 80) percent respectively. Foods contaminated with aflatoxins are toxic or harmful to human health, with aflatoxins ranging from 1 to 20 ppb. High doses of aflatoxins can be a major cause of liver disease, such as cirrhosis, liver cancer, mental disorder and death in humans and animals. These toxins are also produced by autoimmune diseases such as malaria. Aflatoxins also damage the immune system, growth factors, kidney and liver function. It has been observed that there is a need for continuous and strict national measures to avoid its prevalence in future.

**Keywords:** Rice sampling; Detection methods; Detoxification; Health effects

### Introduction

Aflatoxin is the name of group of mycotoxin which includes *Aspergillus Flavus* and *Aspergillus Parasiticus*. Actually the word aflatoxin is the combination of various letters A and F. Moulds play an important role in the creation of cheese, vitamins, enzyme and other nutritional parameters which are very important for human body when consumed and grow up in a smooth way. On the other hand, many moulds are also harmful because of their injurious effect to health and cause spoilage to food. Similarly, mycotoxins are chemical substances which are harmful for the human health [1]. These compound are the main source of poisonous and eventually show out toxic symptom

when such affected food is consumed. Currently, there are 18 various types of aflatoxins are produced but the very common are B1, B2, G1, M1 and M2. Aflatoxins B1 m G1 exit most abundantly, with B1 being the most important [2]. The aflatoxins types B and G represents to fluorescent colours taken into consideration under UV light and such pattern can also be verified through TLC plates. Dead animals and plants are the main source of prevalence of aflatoxins. Aflatoxins mostly found in food and agricultural storage commodities because storage conditions are very important. Aflatoxins producing species like *aspergillus* can be grown on different substrates and under various environmental conditions. Therefore, at early stages of production like transportation, processing, and storage mould growth can happen and can be the major source of aflatoxins and then damage to the complete food item. Mostly, aflatoxin growth is being observed in during unfavorable storage conditions, improper transport facility and during processing and somehow human error is also involved in causing of aflatoxins [3]. The most dependent factors affecting growing of aflatoxin production by, *Aspergillus flavus* and of production of *Aspergillus parasiticus* are RH% surrounding the substrate, which in most cases is between around 85% to 93% and storage temperature of room temperature is 25°C.

### Materials and Methods

#### The methods designed in detection of the aflatoxins

Are available and complex in nature. This is due the more. Complexity in the contaminated food items or any other contaminated item. Development of a detection method depends upon the severity of the nature of the contaminated food item. These methods are of various types physio chemical method, biological method as well as analytical method [4].

#### General procedure of TLC

Preparation of the sample to be tested as per the directions of AOAC.

For spotting purpose, dark spots are applied at the end of plate by using syringe or any other source.

Pouring of mobile phase into the TLC tank to proceed the process.

Closing of lid after placing a TLC plate into the TLC chamber.

Immersion of the TLC plate with spotting into the mobile phase for development.

Drying of TLC plates after development and observing the spots under UV light [5].

### Detection of aflatoxin by ELISA

(Enzyme Linked Immunosorbent Assays) ELISA is a plate type assay technique which is made for the identification and quantification of the peptides, anti-bodies, hormones. ELISA is an advanced technique to detect contamination of rice and other food products, particularly aflatoxins [6].

### Principle of ELISA

This method depends upon the method of binding of antibody to antigen and this binding detection is shown through a detection device. If ELISA containing plate is carefully coated then precision of the sample tested will be high [7].

### General procedure of ELISA

Coating of the plate and the binding of the antigen and antibody.

UN bounded antigen and antibody are get bounded and washed away to the rest.

After washing out with the washing agent, substrate is added at the end [8].

### Detoxification contaminated test samples

Detoxification process of the rice samples for AFB<sub>1</sub> was carried out on the 50 g of the rice products that are naturally contaminated. For more clarity and accurate study, 100 g of rice samples were spiked, to contaminate the rice samples. After that, both, uncontaminated and contaminated rice samples are mixed together so that to enhance the homogeneity of the aflatoxin in rice samples and then undergone through the TLC method for further procedure [9].

**Table 1:** Samples of rice shown negative results.

No	Sample description	No of samples shown negative results	Results (ppb)
1	Brown rice	56	Not detected
2	White rice	50	Not detected
3	Basmati white rice	15	Not detected
4	Basmati brown rice	15	Not detected
5	Pakistani rice	10	Not detected

### *Allium sativum* (Garlic) treatment

Garlic, a best anti-fungal natural substance used for the detoxification purpose specially type B<sub>1</sub>, 50 g sample was collected from the local super market of the Ravi Road, Lahore and then it was grinder to prepare an aqueous extract. Then blending was done by taking 10ml of the distilled water and 10 g of the garlic extract and after proper blending filtrate was filtered out with the help of muslin cloth. By using this filtrate, 50 g of the contaminated rice samples were treated and kept on room temperature and incubated for 30 minutes under hood of fumes to get the better results [10].

### *Nigella sativa* (Kalonji) oil treatment

This plant seeds oil was collected from the local super market of Ravi Road, Lahore. 10 ml of the oil of black seeds, was added into the 50g of rice samples which were naturally contaminated, and sample placed in the same environmental condition and incubate under hood of fumes to get the better results [11].

### Citric acid treatment

Lemons, collected from the same market as mentioned above, were squeezed with hands and collected 10 ml of the lemon extract and mixed with 50 ml of the distilled water to make a homogenized solution and then mixed up with the 50 g of the contaminated rice samples to contaminate the sample and again placed in the same conditions as mentioned above. Sodium Bicarbonate Treatment. This chemical was purchased from the local market. 5 g of the chemical was mixed up with the 10 ml of distilled water to make a solution which was later on sprinkled over the 50 g of the naturally contaminated rice samples [12].

## Results and Discussion

### By TLC method

With the application of the TLC procedure, many samples were tested. Most of them were brown and white rice samples, red chilies and walnuts samples. Some other samples of random food groups were also tested. Out of tested samples, majority of the samples resulted negative (**Table 1**) [13].

6	Parboiled white rice	4	Not detected
7	Parboiled brown rice	5	Not detected

Most of the samples shown negative results because those samples were supposed to be exported to other countries [14]. The packaging conditions were according to export standards. The environmental conditions were suitable, and all the tests

were carried out in hygienic and controlled atmosphere. Among the negative results, there was a countable number of positive samples containing different types of aflatoxins. B<sub>1</sub> was most found aflatoxin (**Table 2**) [15].

**Table 2:** Sample of rice shown positive results.

No	Sample description	No of samples shown positive results	Results (ppb)
1	Super basmati brown rice	8	3.79- 4.75
2	Brown rice (001)	1	2.21
3	Brown rice (002)	1	2.32
4	Brown rice (003)	1	4.52
5	Parboiled rice	1	4.75
6	Sella rice	2	1.8

**By elisa method:** Same results were found in case of ELLISA testing when rice samples were get tested. B<sub>1</sub> aflotoxin prevalence is observed very high during the same procedures that is ELISA and HPLC [16].

## Conclusion

From above mentioned experimental data, it is very easy and important to draw a conclusion that if infected food especially rice is taken in large amount at a time or keep on eating for so long will be very injurious to the health and eventually may cause serious health effects. Rice and other staple foods are important with respect to aflatoxin and will get affected if remain in unfavorable conditions. In that study, 12 samples of rice of different kind showed the aflatoxins contamination, ranging from the 1 to 5 ppb in samples of rice. Statuary and regulatory bodies in the Pakistan must monitor and verify the samples of food for the presence of the aflatoxins on the regular basis, aflatoxins may not be present at the range where it can cause the harmful effects to public health. Meanwhile, taking into account the prevalence of aflatoxins in Pakistan rice and other food commodities must be examine at every stage from field to the end of processing. Aflatoxins also can also be controlled at the early stages by taking over the mold growth at the farm level in specific agricultural types of commodities. Proper measures and precautions must be taken for the inactivation of aflatoxins and reduction of the aflatoxins content in specific post-harvesting types of commodities. It is recommended for consumers to buy products of rice from the authentic sellers. It is also advised that, the food items must be

stored in dry and cool conditions and any damage; spoiled opened packaging of the items must be rejected.

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