Determination of Aflatoxins in Different Varieties of Chillies Collected from Lahore, Pakistan

Naseem Zahra¹*, Nayyab Naeem², Aaliya Iqbal Butt², Muhammad Khalid Saeed¹, Jannat Akram², Esha Gulzar¹

¹Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Ferozepur road, Lahore-54600, Pakistan.  
²Govt. Postgraduate College for Women Gulberg, Lahore, Pakistan.

How to cite this paper: Naseem Zahra, Nayyab Naeem, Aaliya Iqbal Butt, Muhammad Khalid Saeed, Jannat Akram, Esha Gulzar. (2022) Determination of Aflatoxins in Different Varieties of Chillies Collected from Lahore, Pakistan. International Journal of Food Science and Agriculture, 6(3), 349-354. DOI: 10.26855/ijfsa.2022.09.017

Received: July 29, 2022  
Accepted: August 25, 2022  
Published: September 28, 2022

*Corresponding author: Naseem Zahra, Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Ferozepur road, Lahore-54600, Pakistan.  
Email: drnaseemzahra@gmail.com

Abstract

Aflatoxin is a potent carcinogenic, curious metabolite mainly produced by many fungal species such as A. flavus and A. parasiticus. AFs are very toxic food contaminants that can acutely damage human health. There are four main groups of aflatoxins which include B1, B2, G1, and G2. A survey of red chili for aflatoxin contamination was performed in different varieties of chilies including Capsicum annum, L. Piper nigrum, L. C. frutescence, and fresh green chilies. In the present study detection of aflatoxin contamination in red chilies was analyzed through TLC (Thin Layer Chromatographic) technique. A total of 20 various red chili samples were collected from different local regions of Lahore, Pakistan. The occurrence of aflatoxin B1 was detected in 9 samples of chilies. In the case of red powdered chilies, 2 samples were contaminated with aflatoxin B1 ranging between 11.88ppb-11.89ppb. These two contaminated samples are unfit for human health and beyond the permissible limits. In the case of red round chilies, 3 samples were contaminated with aflatoxin B1 and its concentration ranged between 4.68ppb-13.37ppb. These two contaminated samples are unfit for consumption and outside the satisfactory limits. One contaminated sample is fit and below the permissible limit. While in P. nigrum four samples were contaminated with aflatoxin B1 ranging between 2.21ppb-13.47ppb. In which two contaminated samples were unfit for human health and outside the satisfactory limits. The other two contaminated samples were fit for consumption and below the satisfactory limits. In fresh green chilies, all samples were found to be free from contamination. While other types of aflatoxins B2, G1, and G2 were absent in all the samples. The relative percentage of aflatoxin contamination in C. annum (red chili) was 40%, C. frutescence (round red chilli) was 60%, P. nigrum (black pepper) was 80% and fresh green chili was found to be free from contamination. The supply of red chilies in Lahore is extremely dangerous to human health because when exceeded beyond permissible level, aflatoxin can cause cancer. The main reason for aflatoxin contamination in chilies samples is the lack of implementation of qualitative methods.

Keywords

Aspergillus Flavus, Aflatoxin, Red Chili, Thin Layer Chromatography

1. Introduction

Many species of fungi like Aspergillus flavus and Aspergillus parasiticus produced toxic compounds called aflatoxins [1]. Aflatoxins are known as secondary metabolites that are produced by fungi during severe invasive conditions. These uncomplimentary conditions for aflatoxin may be geographical, climate, or even a suitable and hygienic storage setting [2]. There are four major types of aflatoxin B1, B2, G1, and G2. All these types are based on comparative chro-
matographic mobility and thin-layer chromatography. These all types can be analyzed under UV light (blue or green).

They are produced by a polypeptide pathway by various strains of *A. flavus* and *A. parasiticus*. *A. flavus* is a very common ingredient in agriculture. *A. bombycis*, *A. ochraceoroseus*, *A. pseudotamari*, and *A. nomius* are also aflatoxin-producing species but in fewer amounts [3-5]. According to International Agency for Research on cancer purposed that Aflatoxin can also cause liver cancer in humans [6].

Aflatoxin is naturally present in the form of seed in some food products in producing fungi and when conditions are favorable they can produce more aflatoxin. These foods include sorghum, rice, wheat, maize, pearl millet, groundnuts, sunflower seeds, chilies, coriander, turmeric, soybeans, and ginger. Tree nuts, including pistachio, almonds, coconut, and walnut are also being attacked. In addition to aflatoxin M1, animal milk products are found as B1 metabolites in powdered milk and are also directly attacked by aflatoxin-producing molds [7]. It is important to integrate safety assurance systems to avoid contagion. There should be an appropriate distribution of resources to deal with such kinds of threats and disasters for the improvement of food quality [8-9]. Aflatoxin are health disaster and has impact on economy because of their ability to contaminate animal and human food, especially in grains, nuts and oilseeds [10-12].

The economic impact of aflatoxin directly derives from the cost of regulatory programs devised to overcome crop and livestock losses and threats to animal and human health due to aflatoxin [13]. Food and Agricultural Organization (FAO) evaluate that the world’s 25% of crops are being infected by mycotoxins, which is one of the most famous aflatoxins. Aflatoxin also damages livestock and poultry producers due to contaminated feeds that have an impact on the immune system, lowering growth rate and damaging feed efficiency [14]. Aflatoxin B1 is more toxic and generally dominant [15-16].

Pakistan is the sixth-largest exporter of red chilies. Red chilies are being exported all over the globe. Unfortunately, Japan and European Union have banned the import of red chilies from Pakistan due to huge number of contamination by aflatoxins in Chilies. In food, the level of aflatoxin is 20 ppb in both Turkey and USA. In European countries, the total aflatoxin was 10 ppb, and the maximum level of aflatoxin was determined as 5 ppb [17-18].

2. Materials and Methods

Aflatoxins were examined in those different samples of red chilies that are collected from different regions of Lahore. Aflatoxins were analyzed in almost all samples by using a technique, Thin Layer Chromatography.

2.1. Collection of Different Varieties of Chillies Samples

Total 20 samples were collected which included 5 samples of each; red chilli powder, round red chillies, fresh chilli and black pepper were taken from shops, hawker and peddlers of different areas of Lahore, Pakistan.

2.2. Apparatus and Chemicals

Pestle and mortar, Pipette, Burette, Beakers, Micro Syringe, Filter Paper, Conical Flask, Distilled water, Chloroform, TLC plates, Hard hot plate, Acetone, Diethyl ether, wrist action shaker, TLC tanks.

2.3. Methodology

Aflatoxins were determined by the Thin Layer Chromatographic technique and detected under a UV detector. Suitable sampling plans were adopted to attain an additional representative portion of samples [19].

1) Overall 500g of chilies samples were collected in polyethylene bags that were collected from different areas.

2) Placed through a sample divider and reduced to 50g for analysis purposes. As a result homogeneity of the contaminated ratio of samples was obtained.

3) Each sample was grounded with the help of a pestle and mortar and made into powder form.

The glassware was washed with tap water and rinsed with distilled water and then sterilized in the oven at 121°C for 24 hours. Glassware was placed in a desiccator for drying purposes.

First of all, the flasks were taken and labeled according to the code of the sample and then 50g of sample was weighed with the help of a weighing machine and taken in a conical flask. After taking the 50g of ground sample in the flask, 25ml of distilled water was measured in the measuring cylinder and added into the flask having the sample and then 250ml of chloroform was added into the flask. The flasks were capped by using aluminum foil to prevent the evaporation of chloroform. The flasks were attached to the wrist action shaker and allowed to shake for 30 min. After shaking, all the samples were filtered using filter paper and 50 ml of filtrate was taken in the beaker. The collected filtrate was placed on the hot plate for the evaporation of chloroform. As a result, the crude extract was obtained. That crude sample was treated with 0.5ml of chloroform to convert the sample into liquid form so it can be used for spotting. By using a micro syringe, spotting on the TLC plate was done on a hot plate for better absorbance for the sample on the TLC plate [20].
2.4. Thin Layer Chromatography

Approximately 5, 10, and 15 mL of sample were spotted on the TLC plate, about 1.5 cm above the base by using a capillary tube/micro syringe. 5 mL of standard solution was also spotted as internal standard. To make a comparison one spot of the standard was also applied on the TLC plate. Two tanks were used for developing purposes i.e. first one consists of anhydrous ether and the second of acetone-chloroform. The plate was first settled in a developing tank with anhydrous ether. After expansion in ether, the plate was then removed from the tank and dried on a hot plate. When the TLC plate was fully dried, it was then transferred into the second tank. In the second tank, the TLC plate was redeveloped in the same way in the tank with acetone-chloroform (1:9) (v/v). The acetone-chloroform ratio was adjusted as per needed to modify the $R_f$ value of aflatoxins. The developed plate was then analyzed for the presence or absence of spots originating from the test solution and with the same $R_f$ and appearance of authentic aflatoxin. Observed the interior standard for any change in the $R_f$ or appearance of aflatoxins caused by the extract. The presence or absence of aflatoxins was observed by using 365nm UV light [21].

2.5. Qualitative Determination

The concentration of the Aflatoxin was calculated by applying given formula:

$$\text{Aflatoxins contents (µg/kg)} = S \times Y \times V / W \times Z$$

Where;
- $S$ = Volume in µL of Aflatoxin standard of equivalent intensity to $Z$ = mL of samples
- $Y$ = Concentration of Aflatoxin in reference standard in mg/mL
- $Z$ = Volume in µL of sample extract required to give fluorescence intensity (spotting) comparable to that of $S$ (µL of the Aflatoxin standard)
- $V$ = Volume in mL of solvents (chloroform) required to dilute final extract
- $W$ = Effective Weight, in grams of original sample contained in final extract

3. Results and Discussion

There are 20 samples that were randomly collected from the local areas of Lahore including Dharampura, Gulberg, Johar Town, Kahna, Tajpura, Thokar, and Gajjumata. Aflatoxin B1 was analyzed quantitatively from the samples by using TLC (Thin Layer Chromatography) method. Out of 20 local quality samples, nine samples were analyzed to contain aflatoxin B1 and 11 samples were free from aflatoxin contamination. According to the permissible limit of aflatoxin contamination, set by the European Commission (10ppb), 3 samples were contaminated below the acceptable limits and 6 samples were contaminated beyond the acceptable limits. Aflatoxin B2, G1, and G2 were absent in all samples. The TLC results of aflatoxins are given in the following Tables 1-4.

Total of 5 samples of red chilli powder were analysed quantitatively. In which 2 samples were contaminated with aflatoxins and 3 samples were free from contamination. Two contaminated samples were unfit for human health and beyond the permissible limits and three contaminated samples were fit for human use and its value below the satisfactory limits (Table 1).

Total 5 samples of round red chillies were analysed quantitatively. In which 3 samples were found to be contaminated with aflatoxin B1 and other 2 samples were free of contamination. Two contaminated samples were unfit for the consumption and contamination was above the satisfactory limits. One contaminated sample was fit and below the permissible limit as shown in Table 2. Aflatoxin contamination was higher in chillies collected in summer (38%) as compared to winter (36%). Thus, on the basis of achieved results, it could be concluded that winter chillies may provide a better-quality product with respect to aflatoxins contamination [22].

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Location</th>
<th>Aflatoxin (B1+B2+G1+G2) ppb</th>
<th>Total Aflatoxin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$B_1$ $B_2$ $G_1$ $G_2$</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Powder A</td>
<td>Dharampura</td>
<td>11.89 0 0 0</td>
<td>11.89</td>
</tr>
<tr>
<td>2</td>
<td>Powder B</td>
<td>Gulberg</td>
<td>0 0 0 0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>3</td>
<td>Powder C</td>
<td>Johar Town</td>
<td>0 0 0 0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>4</td>
<td>Powder D</td>
<td>Kahna</td>
<td>11.88 0 0 0</td>
<td>11.88</td>
</tr>
<tr>
<td>5</td>
<td>Powder E</td>
<td>Gajjumata</td>
<td>0 0 0 0</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>
Total 5 samples of black pepper were analyzed quantitatively. In which 4 samples were contaminated with aflatoxin B₁, among them 2 contaminated samples were unfit for human health and beyond the satisfactory limits. Other 2 contaminated samples were fit for consumption and below the satisfactory limits. 1 sample was found to be free from contamination (Table 3).

Total 5 samples of green chillies analyzed quantitatively. It was found that all samples were free from contamination and fit for the consumption (Table 4). According to European Commission the permissible level of Aflatoxin in green chillies is 10ppb [23]. Pakistan has not yet established permissible limit for this important spice [24]. If this is the general situation for Pakistan, then high aflatoxin B₁ represents a significant barrier to exports to countries such as the European Union. Similarly, the levels represent a threat to the health of the native population because AFB₁ is highly toxic.

### Table 2. Aflatoxin detection in round red chilli

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Aflatoxin (B₁+B₂+G₁+G₂) ppb</th>
<th>Total Aflatoxin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chilli A</td>
<td>Dharampura</td>
<td>13.37</td>
</tr>
<tr>
<td>2</td>
<td>Chilli B</td>
<td>Muslim Town</td>
<td>11.89</td>
</tr>
<tr>
<td>3</td>
<td>Chilli C</td>
<td>Thokar</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Chilli D</td>
<td>Iqbal Town</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Chilli E</td>
<td>Taj Pura</td>
<td>4.68</td>
</tr>
</tbody>
</table>

### Table 3. Aflatoxin detection in green chilli

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample</th>
<th>Location</th>
<th>Aflatoxin (B₁+B₂+G₁+G₂) ppb</th>
<th>Total Aflatoxin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Green Chilli A</td>
<td>Gulberg</td>
<td>0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>2</td>
<td>Green Chilli B</td>
<td>Samanabad</td>
<td>0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>3</td>
<td>Green Chilli C</td>
<td>Dharampura</td>
<td>0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>4</td>
<td>Green Chilli D</td>
<td>Qainchi</td>
<td>0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>5</td>
<td>Green Chilli E</td>
<td>Johar Town</td>
<td>0</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

### Table 4. Aflatoxin detection in black pepper

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample</th>
<th>Location</th>
<th>Aflatoxin (B₁+B₂+G₁+G₂) ppb</th>
<th>Total Aflatoxin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pepper A</td>
<td>Mughal Pura</td>
<td>2.32</td>
<td>2.32</td>
</tr>
<tr>
<td>2</td>
<td>Pepper B</td>
<td>Kahna</td>
<td>2.21</td>
<td>2.21</td>
</tr>
<tr>
<td>3</td>
<td>Pepper C</td>
<td>Johar Town</td>
<td>0</td>
<td>Not Detected</td>
</tr>
<tr>
<td>4</td>
<td>Pepper D</td>
<td>Qainchi</td>
<td>13.47</td>
<td>13.47</td>
</tr>
<tr>
<td>5</td>
<td>Pepper E</td>
<td>Kalma Chock</td>
<td>11.86</td>
<td>11.86</td>
</tr>
</tbody>
</table>
Figure 1. Percentage contamination of aflatoxins in all chillies.

From the above Figure 1, it is clear that green chilli was not contaminated with aflatoxins, while round red chilli, red chilli powder and black pepper were 60%, 40% and 80% contaminated respectively.

4. Conclusion

A survey of various types of chillies was performed to determine the presence of aflatoxin in these products. Aflatoxin B1 was detected in 9/20 samples of red chili powder, black pepper, round red chili, and fresh green chili due to poor storage conditions as these conditions cause humidity and moisture which are the main cause of the growth of mycotoxin producing fungi. Excessive consumption of aflatoxin-contaminated red chilies can cause liver cancer in humans. There is a need for more careful production of chilli products in Pakistan. To avoid Aflatoxin contamination, it is necessary for appropriate inspection and proper monitoring of red chilies.

References


