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# Detection of Aflatoxins in Various Samples of Red Chilli

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**Abstract.** In this study 183 samples of red chilli were screened out for aflatoxin determination, 48 samples were positive for aflatoxins $B_1$ with the range from 1.2 ppb to 968.3 ppb. Aflatoxin $B_2$ was detected only in 3 samples with the range of 0.3 ppb to 159.8 ppb. Aflatoxin $G_1$ and $G_2$ were absent in all chilli samples.

**Keywords:** aflatoxin, red chilli, thin layer chromatography

## Introduction

Aflatoxins are the potent carcinogenic, mutagenic metabolites produced primarily by many fungal species such as *Aspergillus flavus* and *A. parasiticus*. The food and feed stuff especially in warm climates are susceptible to invasion by aflatoxigenic *Aspergillus* species and the subsequent production of aflatoxins during preharvesting, processing, transportation and storage conditions. Foods of plant origin are normally contaminated more frequently and with higher concentrations than food of animal origin (Bauer, 2004; Shapira *et al*., 1996; Cullen and Newberne, 1993; Eaton *et al*., 1993). Many agricultural commodities such as cereals, oilseeds, dry fruits and spices have been reported to be contaminated with toxigenic moulds and aflatoxin $B_1$ under improper storage conditions (Aquino *et al*., 2005; Reddy *et al*., 2001; Jelinek *et al*., 1989). Chillies are reported to be contaminated with moulds and their toxic metabolites and *A. flavus* is the predominant mould on chilli samples in several cases (Patel *et al*., 1996; Seenappa *et al*., 1980; Flannigan and Hui, 1976; Scott and Kennedy, 1973; Christensen *et al*., 1967). Powdered red pepper is often added in variety of Indian snack foods without any processing or raw processed, thus presence of toxic metabolites and moulds will have serious implications on nutrition and health of human population (Banerjee and Sarkar, 2003). The toxic limit for total aflatoxins (AFs) given by WHO is above 30 ppb. Red (chilli) peppers are native for Central and South America. Portuguese traders introduced them to Europe, Middle East, India, Indonesia and other parts of Asia around 450-500 years ago (Berke, 2002). The world's largest producer of red (chilli) peppers was China at 8.1 million metric tons in 2000. Turkey is one of the major red pepper producing countries together with India, Mexico, Spain and USA (Kithu, 2002; FAO-UN, 2000). *A. flavus* was reported in red chilli samples obtained from Pakistan (Shamshad *et al*., 1985). Reddy *et al*. (2001) analyzed the pods and powders of chillies from India and found high levels of $AFB_1$, particularly in lowest grade pods. In addition, a survey of chillies was undertaken where high levels were reported in a few cases. There is no information on aflatoxins concentrations in chillies from Pakistan (UK Food Standards Agency, 2005). The present study was conducted in Food and Biotechnology Research Centre, PCSIR Labs. Complex Lahore for the detection of aflatoxin presence in red chillies imported from India.

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## Materials and Methods

The chilli samples imported from India were received for test and analysis for aflatoxins presence. Samples were prepared for aflatoxin analysis (Begum *et al*., 1985). Aflatoxins were detected by method of Romers (1976) and its estimation in toxic extracts was made by comparison with standard technique (AOAC, 2005). In this study Thin Layer Chromatographic (TLC) technique was used for the determination of aflatoxin in red chilli samples. 183 samples were screened out for aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ (Table 1).

**Preparation of sample.** ***Extract.*** The half laboratory sample was grinded through Romer grinding mill. The other half sample was kept for reference. Grinded sample was mixed properly and test portion was taken from this mixture. 50 g of ground sample was taken into 500 mL conical flask. 25 mL of water, 25 g diatomaceous earth

**Table 1.** Results of TLC for aflatoxin contamination level (ppb) in red chilli samples

| Sample no. | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ |
|---|---|---|---|---|
| 1. | 1.2 | 0.3 | N.D. | N.D. |
| 2. | 3.0 | N.D. | N.D. | N.D. |
| 3. | 2.0 | N.D. | N.D. | N.D. |
| 4. | 2.0 | N.D. | N.D. | N.D. |
| 5. | 4.4 | N.D. | N.D. | N.D. |
| 6. | 3.0 | N.D. | N.D. | N.D. |
| 7. | 189.2 | 30.7 | N.D. | N.D. |
| 8. | 908.0 | N.D. | N.D. | N.D. |
| 9. | 123.9 | N.D. | N.D. | N.D. |
| 10. | 82.7 | N.D. | N.D. | N.D. |
| 11. | 19.2 | N.D. | N.D. | N.D. |
| 12. | 968.3 | 159.8 | N.D. | N.D. |
| 13. | 180.9 | N.D. | N.D. | N.D. |
| 14. | 8.22 | N.D. | N.D. | N.D. |
| 15. | 15.9 | N.D. | N.D. | N.D. |
| 16. | 16.1 | N.D. | N.D. | N.D. |
| 17. | 9.7 | N.D. | N.D. | N.D. |
| 18. | 12.5 | N.D. | N.D. | N.D. |
| 19. | 12.5 | N.D. | N.D. | N.D. |
| 20. | 12.4 | N.D. | N.D. | N.D. |
| 21. | 12.4 | N.D. | N.D. | N.D. |
| 22. | 12.4 | N.D. | N.D. | N.D. |
| 23. | 12.5 | N.D. | N.D. | N.D. |
| 24. | 37.6 | N.D. | N.D. | N.D. |
| 25. | 25.0 | N.D. | N.D. | N.D. |
| 26. | 12.5 | N.D. | N.D. | N.D. |
| 27. | 25.0 | N.D. | N.D. | N.D. |
| 28. | 74.2 | N.D. | N.D. | N.D. |
| 29. | 74.6 | N.D. | N.D. | N.D. |
| 30. | 247.4 | N.D. | N.D. | N.D. |
| 31. | 1.2 | N.D. | N.D. | N.D. |
| 32. | 1.5 | N.D. | N.D. | N.D. |
| 33. | 3.0 | N.D. | N.D. | N.D. |
| 34. | 60.9 | N.D. | N.D. | N.D. |
| 35. | 121.0 | N.D. | N.D. | N.D. |
| 36. | 24.2 | N.D. | N.D. | N.D. |
| 37. | 318.7 | N.D. | N.D. | N.D. |
| 38. | 212.4 | N.D. | N.D. | N.D. |
| 39. | 18.9 | N.D. | N.D. | N.D. |
| 40. | 60.3 | N.D. | N.D. | N.D. |
| 41. | 60.2 | N.D. | N.D. | N.D. |
| 42. | 48.2 | N.D. | N.D. | N.D. |
| 43. | 90.4 | N.D. | N.D. | N.D. |
| 44. | 48.3 | N.D. | N.D. | N.D. |
| 45. | 60.5 | N.D. | N.D. | N.D. |
| 46. | 60.3 | N.D. | N.D. | N.D. |
| 47. | 12.6 | N.D. | N.D. | N.D. |
| 48. | 60.3 | N.D. | N.D. | N.D. |

*N.D. = not detected

and 150 mL chloroform was added, after shaking for 30 min, filtered through filter paper. Collected 2nd 50 mL portion $CHCl_3$ and evaporated on a steam bath.

**Thin layer chromatography.** ***Qualitative determination.*** Immediately spotted 5, 10 and 15 µL on TLC plate (Approximately 1.5 cm from the base). Spotted 5 µL standard on one spot in a duplicate as internal standard. The plate was developed with anhydrous ether in developing tank uptill half. After development in ether removed the plate from tank and let it to dry. Re-developed in same direction in TLC tank with acetone - chloroform (1:9) (v/v). Adjusted the acetone - chloroform ratio as needed to modify $R_f$ of aflatoxins. Finally presence or absence of aflatoxins in test solution spot was observed.

***Quantitative determination.*** For quantitative analysis 1, 2, 3, 4 and 5 µL of test solution was spotted on silica gel coated plates. Similarly on same plate 1, 2, 3, 4 and 5 µL of aflatoxin standard was spotted. The fluorescence intensities of the spots were compared and the concentration of aflatoxins was calculated by applying following formula:

$$\text{Aflatoxins } (\mu g/kg) = \frac{S \times Y \times V}{W \times Z}$$

where:

- Z = Volume in µL of sample extract required to give fluorescence intensity comparable to that of S (µL of aflatoxins standard)
- S = Volume in µL of aflatoxins standard of equivalent intensity to Z (µL of sample)
- Y = Concentration of aflatoxins standard in µg/mL
- V = Volume in µL of solvents required to dilute final extract
- W = Weight in g of original sample contained in final extract

## Results and Discussion

The results revealed that 48 out of 183 samples were contaminated with aflatoxins. Aflatoxins $G_1$ and $G_2$ were not detected in any of chilli samples. Aflatoxin $B_1$ was present in 48 samples out of all chilli samples. The contamination range was noted to be between 1.2 - 968.3 ppb for aflatoxin $B_1$. 22 chilli samples (i.e. sample no. 7, 8, 9, 10, 12, 13, 24, 28, 29, 30, 34, 35, 37, 38, 40, 41, 42, 43, 44, 45, 46 and 48) were contaminated with aflatoxins $B_1$ which exceeded the maximum level of WHO *i.e.* 30 ppb. 26 out of 48 contaminated chilli samples had aflatoxins $B_1$ level

within the permissible limit and were safe for human consumption. Aflatoxin $B_2$ was present in 3 samples out of all chilli samples. The contamination range was noted to be between 0.3 ppb - 159.8 ppb for aflatoxin $B_2$. Only 2 chilli samples (*i.e*. sample no. 7 and 12) were contaminated with aflatoxins $B_2$ which exceeded the maximum level of WHO *i.e*. 30 ppb. The concentrations of aflatoxins are presented in Table 1.

## Conclusion

Aflatoxin $B_1$ in 48/183 samples and aflatoxin $B_2$ in 3/183 samples were detected in red chillies imported from India may be due to poor drying and storage conditions as these conditions cause moisture and humidity which are main cause of aflatoxin growth. The excessive use of aflatoxin contaminated red chillies may be a source of liver cancer in human beings. So, extra care should be taken to avoid aflatoxin contamination of red chillies.

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