



Original Research Article

Survey of market samples of food grains and grain flour for Aflatoxin B₁ contamination

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ABSTRACT

Keywords

Food grains;
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Aflatoxin B₁;
thin layer chromatography;
high performance thin layer chromatography.

A study was conducted on market samples of food grains and grain flour, procured locally from Chennai, Tamil Nadu, India, to assess aflatoxin contamination. Food grains (Bengal gram (2), bajra / cumbu (6), maize (10) and jowar / sorghum (1) and grain flour (3) were analysed for aflatoxin B₁, as per AOAC method with screening by thin layer chromatography and quantified by high performance thin layer chromatography using reference standards. The recovery percentage for aflatoxin B₁ was 90%. The contamination of aflatoxin B₁ was found to be 68.18% in food grains whereas 100% in grain flour, which might be due to improper post harvest technology and storage condition. The present study warrants for the further improvement of Good Agricultural Practices and the need for accredited laboratory as per regulatory norms to assess the residue contamination.

Introduction

Mycotoxins are chemically and biologically active by-products produced by fungal (mold) growth naturally in a range of plant products. Mycotoxins have received considerable attention due to their significance in agricultural loss and livestock / human health. Amongst the mycotoxins that are known to cause human diseases, aflatoxins have been studied most. Aflatoxin came to relevance four decades ago in England following a poisoning outbreak causing 100,000 turkey deaths. Aflatoxins are by-products produced during the growth of the fungi *Apergillus flavus* and *Aspergillus*

parasiticus. These moulds are common contaminants of foodstuff, particularly in the tropical regions. Unlike *Aspergillus*, which often look greenish to the naked eyes, aflatoxins are odourless, tasteless and colourless. Chemically, they are stable in foods and resistant to degradation under normal cooking procedures. It is difficult to eliminate aflatoxin once produced (Risk Assessment Studies, 2001). Aflatoxin contamination of food results in some toxic effects such as liver cancer and immunosuppression in various animals and humans.

Aflatoxin B₁ is classified by the International Agency for Research on Cancer (IARC) as a class-1 human carcinogen (IARC – International Agency for Research on Cancer, 1993). Aflatoxin is classified into a number of subtypes. However, the most important ones are B₁, B₂, G₁ and G₂, distinguished by their fluorescent colour blue or green under ultraviolet light. In addition, aflatoxin, M₁ and M₂ are hydroxylated metabolites of aflatoxin B₁ and B₂. Aflatoxin B₁, the most potent one, is metabolized into a variety of hydroxylated derivatives (aflatoxin P₁, M₁, B₂) which are less toxic than the parent compound, although their presence in food is still a threat to human health.

Fungi produce aflatoxins in the presence of higher moisture, temperature and adequate substratum. Synthesis is highest when humidity is above 13% and temperature is between 24°C and 37°C. That is why warm and wet geographic regions are the most favorable environments for aflatoxins and usually are affected. Before harvest, the risk for the development of aflatoxin is greatest during major droughts. When soil moisture is below normal and temperatures are high, the number of *Aspergillus* spores in the air increases. These spores infect crops through areas of damage caused by insects, and inclement weather. Once infected, plant stress occurs; the production of aflatoxin is favoured. During post-harvest stage, proliferation of aflatoxin can be exacerbated in susceptible commodities under storage conditions such as hot and humid storage environment (Risk Assessment Studies, 2001). Hence, prolonged storage of cereal in warm humid condition should be avoided to minimize the risk of aflatoxin contamination (Saleemullah *et al.*, 2006).

Aflatoxin contamination has been frequently encountered in food grains especially maize, rice, groundnuts and other oily products. Aflatoxicoses both in humans and animals as well as primary liver cancer have been reported in many Asian countries where the above mentioned products have been a constituent of the diet. As aflatoxin is epidemiologically implicated as carcinogen in humans and an environmental contaminant, which is widespread in nature, its possible chronic toxicity is therefore of greater concern than acute toxicity (Risk Assessment Studies, 2001)

Thin layer chromatography (TLC) techniques were extensively used for aflatoxin analysis, although recently an increase in the use of high performance thin layer chromatography (HPTLC) has been noted. The accuracy of TLC is less than that of high performance thin layer chromatography (HPTLC) but the results obtained using HPTLC are similar to that of HPLC and more consistent than enzyme-linked immunosorbent assay (ELISA) data (Jaimez *et al.*, 2000). The present study was conducted to assess aflatoxin B₁ contamination in market samples of food grains using TLC for screening and HPTLC for quantification.

Materials and Methods

Food grains analysed for aflatoxin B₁ were Bengal gram (2), bajra / cumbu (6), maize (10) and jowar/ sorghum (1) and grian flour (3) with regards to Maximum Permissible Level (MPL) in European Union, USFDA and Indian Standards.

Sampling

Moulds and aflatoxins occur in an

extremely heterogeneous fashion in food commodities. Food grains were randomly collected from the supermarkets and traditional bazaars of Chennai city, Tamil Nadu, India. Sampling was carried out in a way that ensured the analytical sample truly represent the consignment. Samples were taken from each food grains, totaling 19 samples for analysis of aflatoxin B₁.

Method

As per AOAC method by HPTLC and quantified with reference standards. The technique is screening by TLC and quantified by HPTLC.

Results and Discussion

The recovery percentage for aflatoxin B₁ was 90%. The contamination of aflatoxin B₁ was found to be 63.16% in food grains whereas 100% in grain flour and 68.18% in total food grains and grain flour with mean concentration (ppb) of 75.18, 60.4 and 72.23 respectively (Table. 1), which might be due to improper post harvest technology and storage condition.

In the present study, among the food grains analysed for aflatoxins, except maize, were within the safe limit of Indian and USFDA standards but higher than the European Union standards. Saleemullah *et al.*, (2006) reported aflatoxin contents of cereals, collected from local markets of North-West Frontier Province in Pakistan determined by thin layer chromatography (TLC). Aflatoxin content of cereals (wheat, maize and rice) ranged from 14 to 45 ppb, found to be within the safe limit (50 ppb) recommended by FAO. They concluded that aflatoxin content of food should be monitored to ensure food safety.

Liu *et al.*, (2006) reported that aflatoxin B₁

contamination was detected in 37 samples of milled rice grains and found that 92% of the samples showed positive to aflatoxin B₁. In another study, Toteja *et al.*, (2006) reported the presence of aflatoxin B₁ in parboiled rice collected from 11 states in India and found 38.5% of the samples were positive to aflatoxin B₁. Reddy *et al.*, (2009) reported that out of 675 paddy samples, 70.7% showed aflatoxin B₁ positive. Of the 525 milled rice samples obtained from commercial market, aflatoxin B₁ was detected in 64.1% of samples. Aflatoxin B₁ contamination was recorded below the permissible limits from New Delhi, Tamil Nadu, Andhra Pradesh and Kerala states of India. A high proportion of stored samples (67.8%) showed positive to aflatoxin B₁ contamination. Eighty-two percent of samples from open storage that were exposed to rain showed aflatoxin B₁ contamination followed by one-year-old seed.

Mohammadi *et al.*, (2012) from their work in Iran reported that among 152 samples of rice analyzed, 75% showed levels of aflatoxin B₁ contamination with the mean of 0.671 ppb, which was lower than maximum tolerated level of 5 ppb for aflatoxin B₁ in rice assigned by Institute of Standard and Industrial Research of Iran. Contamination of aflatoxin in imported rice was dissimilar between different months. The highest levels of aflatoxin B₁ were detected in rice samples imported in September, while the lowest levels were in rice imported during November.

Ahsan *et al.*, (2010) has reported from their study in Pakistan that samples of maize grain and processed maize (flour) were collected in kharif (rainy season) and rabi (winter season) and overall

Table.1 Aflatoxin B₁ contamination in food grain and grain flour samples

Type of sample		% of samples contaminated	Concentration (ppb) Mean ± S.E.
Bengal Gram		0	Not detected
Bajra / Cumbu		33.33	2.19±1.46
Maize		100	88.91±62.02
Sorghum / Jowar		0	Not detected
Grain flour		100	60.41±29.54
Total	Food grains	63.16	75.18±52.04
	Grain flour	100	60.41±29.54
	Food grains and Grain flour	68.18	72.23±41.59

frequency of aflatoxins was very high among samples (85%). A few samples (15%) were found free from aflatoxins contamination. The percentage of aflatoxins contamination in maize samples was 80%, 87% and 90% with their respective mean values of 45 ppb, 54 ppb and 62 ppb in urban, semi-urban and rural areas, respectively.

Saleemullah *et al.*, 2006, studied three hundred and forty-nine breakfast and infant cereal samples collected at retail level across Canada from 2002 to 2005. They included rice-, soy-, barley-based and mixed-grain infant cereals, corn, wheat, rice-based and mixed-grain breakfast cereals, and were analysed for aflatoxins B₁, B₂, G₁ and G₂ using a modified AOAC International official method. Results indicated that 50% of both breakfast and infant cereals had detectable levels (limit of detection = 0.002 ppb) of aflatoxin B₁, which is the most toxic of the four toxins. The levels found varied from 0.002 to 1.00 ppb for aflatoxin B₁, from 0.002 to 0.14 ppb for aflatoxin B₂, from 0.008 to 0.27 ppb for aflatoxin G₁, and from 0.008 to 0.048 ppb for aflatoxin G₂. Only 4% of the breakfast cereals and 1% of the infant cereals had aflatoxin B₁ levels exceeding 0.1 ppb, which is the European Union maximum

limit for aflatoxin B₁ in baby foods and processed cereal-based foods for infants and young children (Saleemullah *et al.*, 2006). Contrary to the above findings, in the present study, the contamination of grain flour, with aflatoxin B₁ was 100% and with high mean levels of 60.41 ppb.

In the present study aflatoxin B₁ in food grains and grain flour were analyzed. In food grains and grain flour, 68.18% of the samples found contaminated with aflatoxin B₁ with a mean concentration of 72.23 ppb, which is above the Maximum Permissible Level (MPL) in European Union, USFDA and Indian Standards. The contamination of grain flour with aflatoxin B₁ was higher than the food grains, except maize which might be due to improper post harvest technology and storage condition. The study warrants for the further improvement of Good Agricultural Practice (GAP) and the need for accredited laboratory as per regulatory norms to assess the aflatoxin contamination.

The present study necessitates the periodical monitoring of post harvest surveillance of mycotoxin in processed foods, studies in compliance with regulatory norms. The results of the present study demands strengthening

and further enhancement of post harvest technology in crops by implementing Hazard Analysis Critical Control Point (HACCP) 'from farm to fork' to provide quality and safe food for enhancing food safety and global security. Mycotoxin-contaminated products cause significant economic and trade problems at almost every stage of production and marketing. Many of our crops are affected by these mycotoxins, and standards are becoming progressively stricter. It is therefore critical to undertake research that will help small-scale farmers to meet international quality standards and continue to profitably market their crops. To attain this, quality control and food safety in food production areas are necessary.

Competing interests

Authors declare that they have no competing interest.

References

- Ahsan, S., I.A. Bhatti, M.R. Asi, H.N. Bhatti and Sheikh, M.A. 2010. Occurrence of aflatoxins in maize grains from central areas of Punjab, Pakistan. *Int. J. Agric. Biol.* 12(4): 571–575.
- IARC – International Agency for Research on Cancer., 1993. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 56: 489–521.
- Jaimez, J., C.A. Fente, B.I. Vazquez, C.M. Franco, A. Cepeda, G. Mahuzier and Prognon, P. 2000. Application of the assay of aflatoxins by liquid chromatography with fluorescence detection in food analysis. *J. Chromatogr. A.* 882: 1–10.
- Liu, Z., J. Gao and Yu, J. 2006. Aflatoxins in stored maize and rice grains in Liaoning Province, China. *J. Stored Prod. Res.* 42: 468–479.
- Mohammadi, M., G.H. Mohebbi, S. Akbarzadeh and Shojaee, I. 2012. Detection of *Aspergillus* spp. and determination of the levels of aflatoxin B₁ in rice imported to Bushehr, Iran. *Afr. J. Biotechnol.* 11(38): 9230-9234.
- Reddy, K.R.N., C.S. Reddy and Muralidharan, K. 2009. Detection of *Aspergillus* spp. and aflatoxin B₁ in rice in India. *Food Microbiol.* 26: 27–31.
- Risk Assessment Studies., 2001. Report No. 5. An Evaluation of Aflatoxin Surveillance Findings in Hong Kong 1998 – 2000. Chemical Hazards Evaluation; Aflatoxin in Foods, Food and Environmental Hygiene Department, HKSAR http://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_fc_01_16_report.html.
- Saleemullah, A. Iqbal, I.A. Khalil and Shah, H. 2006. Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chem.* 98(4):699-703.
- Toteja, G.S., A. Mukherjee, S. Diwakar, P. Singh, B.N. Saxena, K.K. Sinha, A.K. Sinha, N. Kumar, K.V. Nagaraja, Bai, C.A. Girija, Krishna Prasad, S. Vanchinathan, Roi, Rajat, and Sarkar, S. 2006. Aflatoxin B₁ contamination of parboiled rice samples collected from different states of India: a multi center study. *Food Addit. Contam.* 23 (4): 411–414.