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Detection of aflatoxins B_1 & B_2 in food grains from Mumbai local market

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Abstract
Fungus *Aspergillus flavus* produces aflatoxin whose potency & toxicity makes them highly carcinogenic causing severe contamination to the food sources & making them primary health hazards even at low concentration. During pre & post-harvest conditions, aflatoxin contamination due to fungal infection has been reported in food grains such as groundnuts, jowar, bajra, rice, maize & wheat. Thus detection of aflatoxin concentrations in food becomes necessary. However, due to low concentration, the analytical methods applied required to be specific sensitive& simple to carry out. High- performance liquid chromatography (HPLC), was applied in detection & quantification of aflatoxin.

Keywords: aflatoxin, *Aspergillus flavus*, food grains, food grains, HPLC

Introduction

The secondary metabolites produced by fungi are natural mycotoxins, developed during the storage of food grains under a wide range of climatic conditions. For ages, the mycotoxins have been well known for quality degradation of food crops that causes changes in their characteristics such as texture, colour& flavour. According to the Food & Agriculture Organisation (FAO), at least 1/4th of the world's crop is affected by mycotoxins. Out of the various fungi species that have been identified, several types of mycotoxins such as Aflatoxin, ochratoxin, patulin, zearalenone etc have been discovered that show diverse structure that results in different chemical & physicochemical properties. Amongst all these, aflatoxin has gained vast attention due to their toxic & potent carcinogenic nature even in small quantities. (FAO, 1979, Mirocha and Christensen, 1982, and Campos, 1987) [10]. Aflatoxin (AFs) are known to be highly toxic & can contaminate a wide range of food grains such as maize, wheat, rice, Jowar, Bajra etc. (Mutegi *et al.*, 2009; Perrone *et al*., 2014' Iqbal *et al.*, 2015) [11]. Production of aflatoxin can occur in food grains during pre-harvesting or after harvesting. Due to improper treatment of postharvest of commodity i.e improper storage conditions, insufficient drying can result in increased levels of aflatoxins. Aflatoxin formation may also increase during the growing stages of crop due to environmental factors i.e drought or excess rains, insect infestation.

Species of *Aspergillus* is known to produce aflatoxins. These fungi require warm & humid conditions to grow. Hence, tropical & subtropical regions become suitable for them to grow & nurture. (Payne and Brown, 1998) [13], (Magan and Aldred, 2007; Battilani *et al.*, 2011) [7]. Due to their heat resistant nature, it becomes insufficient to eliminate aflatoxins from contaminating food & feed using the basic food processing techniques. (Medina *et al*., 2017b) [9]. Consumption of aflatoxin from contaminated food can cause serious health complications in human beings & animals. Therefore strict regulations for aflatoxins have been applied to maintain the health of individuals (Fung and Clark, 2004; Binder *et al*., 2007; Sherif *et al*., 2009). Juan *et al*., 2012). For human consumption, the safe limit for aflatoxins lies within the range of 4-30. (EC, 2007, 2010). Aflatoxins are derivatives of difuranocoumarin. Depending upon the type of aflatoxin, they are made of bifuran group which is attached to a pentanone ring or lactone ring & coumarin nucleus (in case of AFBs. (Schuda, 1980) [17]. There are four main types of aflatoxins, AFB1 (fig1), AFB2 (fig 2), AFG1 (fig 3), AFG2 (fig4). *Aspergillus flavus* produces B-types while *A. parasiticus* produces G-type (Kumar *et al*., 2017) [6].

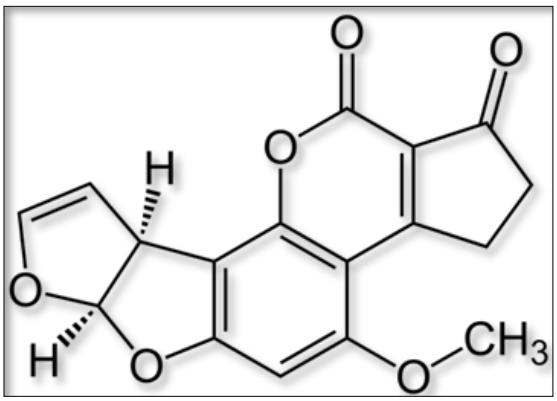

Fig 1: Aflatoxin B1

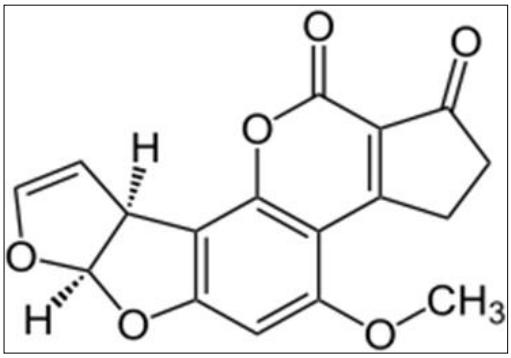

Fig 2: Aflatoxin B2

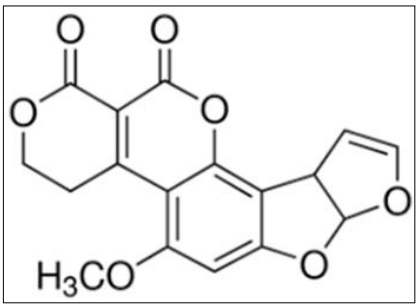

Fig 3: Aflatoxin G1

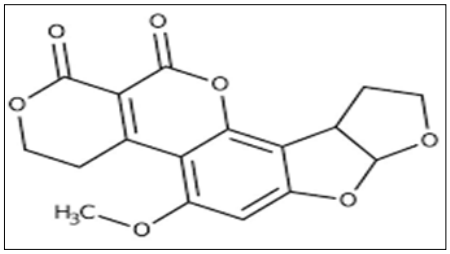

Fig 4: Aflatoxin G2

Determination of aflatoxin in cereal & cereal base products becomes necessary to properly assess the relevant risk of exposure & toxicological risk for the living being. Hence, the analytical method required to be rapid, sensitive, & accurate for determination. The general principle behind these methods involves toxin extraction from the matrix with appropriate extraction solvent, a cleanup step that can eliminate interferences from the extract and subsequently detection or determination of the toxins by a suitable technique. (Michelangelo N. Pascale., 2009) [12]

The chromatographic method which is commonly used for the quantitative determination of mycotoxins includes High-Performance Liquid Chromatography (HPLC) & Gas chromatography (GC) that are coupled with different detectors. In the following paperwork was carried out using different cereals such as wheat, rice, jawar, bajra, maize & groundnut, as the main source of aflatoxin & the technique applied was HPLC.

Materials and Method

Sampling

The amount of mycotoxin is non-uniform in distribution throughout the foodstuff because of mould contamination id non-homogenous in nature. Mycotoxin contamination occurs in pockets of high concentration particularly in grains & nuts which may not be randomly distributed. Hence, sample preparation is carried out keeping this factor into consideration. 100g of rice, wheat, maize, jawar, groundnut, bajra grains were procured from different Mumbai local markets.

Sample Preparation

25g of the sample (grains) was weighed & placed in a plastic conical flask. 5g of NaCl was introduced along with 125ml of the diluent (Methanol + water 70:30 ratio). This flask was then placed on a shaker for 25 minutes. After 25 minutes 50ml of the supernatant was taken for further procedure. 50ml of the solution was centrifuged at 5000rpm for 5 minutes. Later, 15 ml of this solution was pipetted out & further diluted with 30ml distilled water. This solution underwent vortex & then again centrifuged at 5000 rpm for 5 minutes. 15ml of supernatant is introduced into the cartridge. The immuno-affinity column (cartridge) has a capacity of 200nanograms. It is present with antibodies while the sample contains antigens. This two bind with each other in the cartridge. When methanol & distilled water 1ml each is introduced in the cartridge the antigens & antibodies break down which results in reconstitution of antigens. The solution is then filtered & injected into HPLC.

Aflatoxin quantity of standards & samples were determined using HPLC, fluorescence detection. The HPLC system consisted of a pump & fluorescence detector. Aflatoxins were separated in HPLC column with a mobile phase of Methanol: Water (45:55 v/v). Fluorescence detection was at an excitation wavelength of 365nm & emission wavelength of 440nm.

Result & Discussion

Standard – Aflatoxin (10ppb)

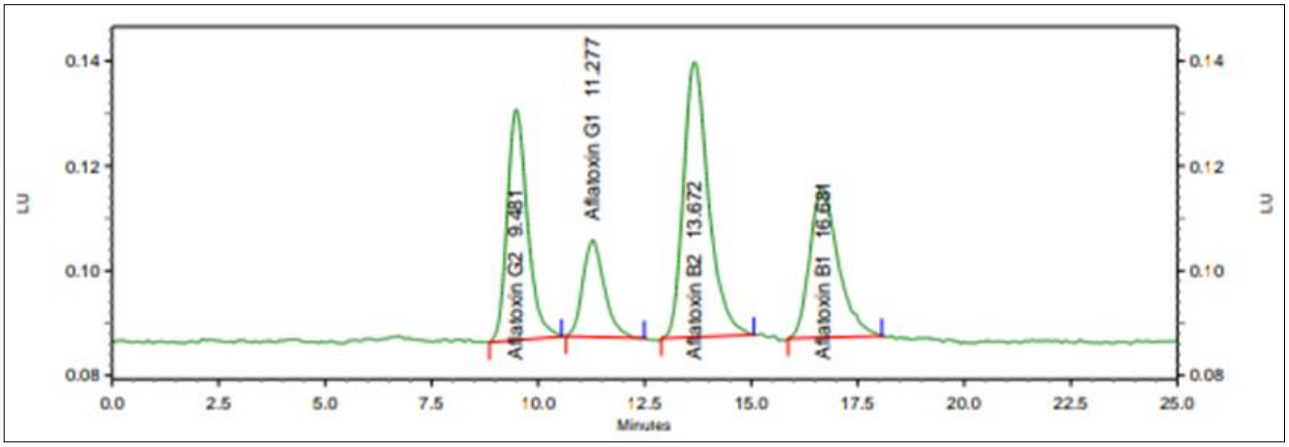

Fig 5: Standard of Aflatoxin (B1, B2, G1, G2)

1. Wheat

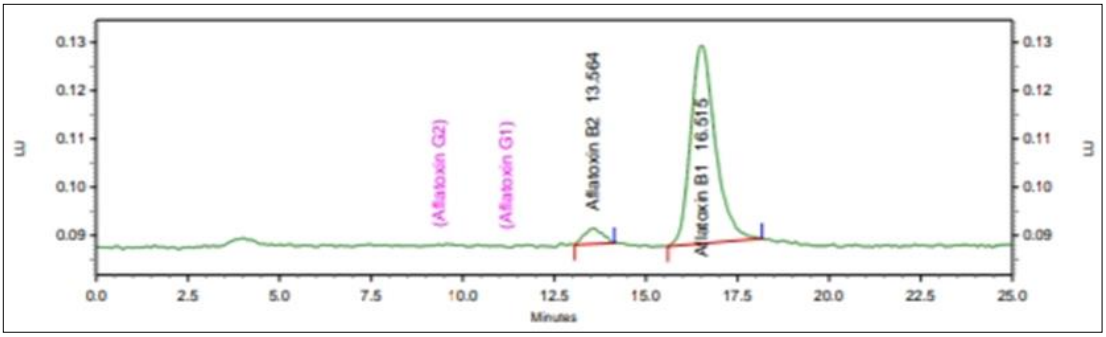

Fig 6: HPLC chromatogram showing presence of Aflatoxin B_1 & B_2

On running the HPLC for the sample of wheat, the retention time was found to be 16.51. The area was 324840 while the ESTD concentration was 58.831 for aflatoxin B_1. In the case of aflatoxin B_2, retention time was 13.56 & area was 18803 but the ESDT concentration was 0.00. (Fig 6).

2. Rice

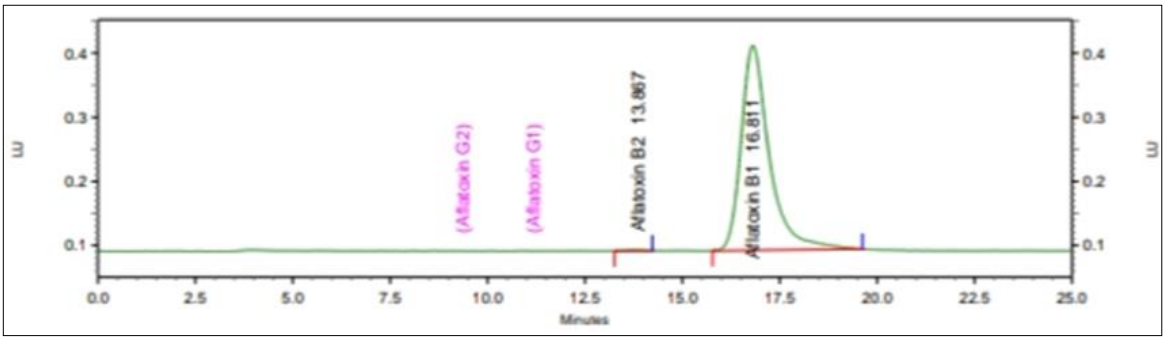

Fig 7: HPLC chromatogram showing aflatoxin B1

On running the HPLC for the sample of wheat, the retention time was found to be 16.905. The area was 15886 while the ESTD concentration was 2.075 for aflatoxin B_1. Aflatoxin B_2 was absent. (Fig 7).

3. Groundnut

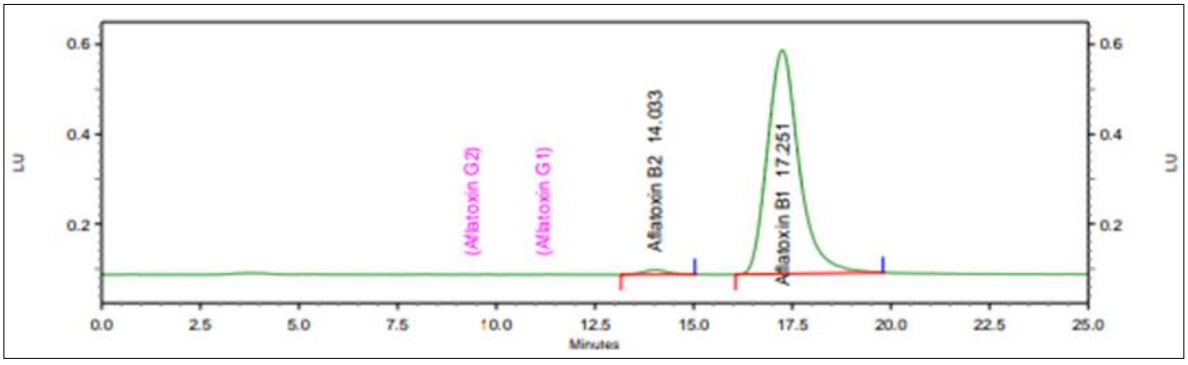

Fig 8: HPLC chromatogram showing aflatoxin B1 & B2

On running the HPLC for the sample of wheat, the retention time was found to be 17.251. The area was 4666848 while the ESTD concentration was 412.42 for aflatoxin B_1. In the case of aflatoxin B_2, retention time was 14.033 & area was 77990 but the ESDT concentration was 3.852. (Fig 8).

4. Maize

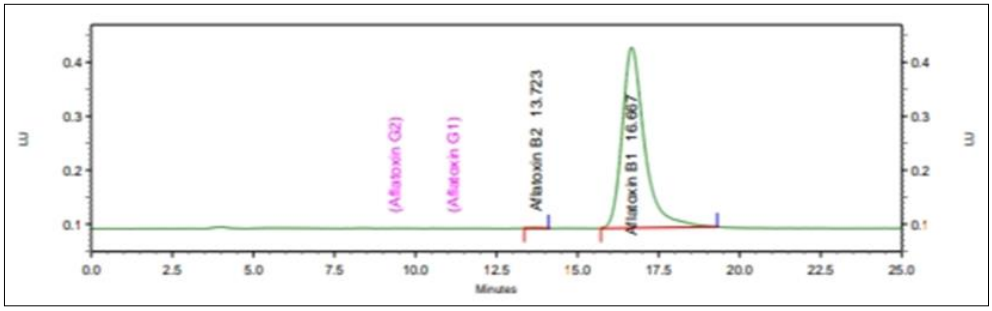

Fig 9: HPLC chromatogram showing aflatoxin B1

On running the HPLC for the sample of wheat, the retention time was found to be 16.667. The area was 2705266 while the ESTD concentration was 495.749 for aflatoxin B_1. In case of aflatoxin B_2, retention time was 13.723 & area was 5205 but the ESDT concentration was 0.00. (Fig9).

5. Jowar

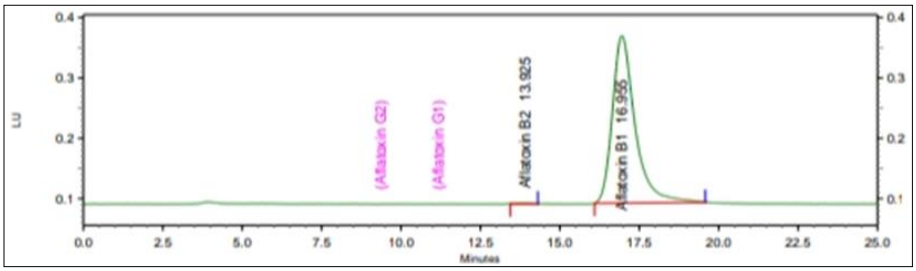

Fig 10: HPLC chromatogram showing aflatoxin B1

On running the HPLC for the sample of wheat, the retention time was found to be 16.995. The area was 2307971 while the ESTD concentration was 423.137 for aflatoxin B_1. In the case of aflatoxin B_2, retention time was 13.925 & area was 5474 but the ESDT concentration was 0.00. (Fig 10)

6. Bajra

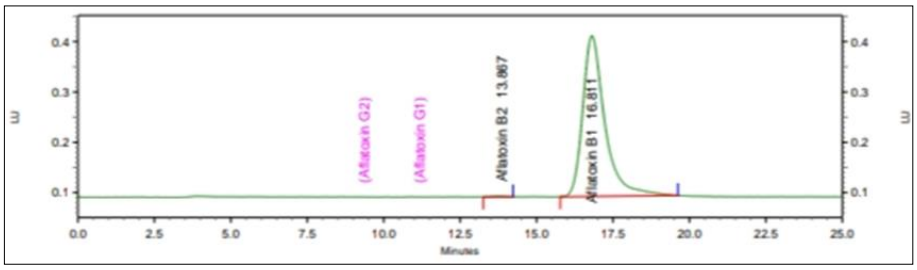

Fig 11: HPLC chromatogram showing aflatoxin B1

On running the HPLC for the sample of wheat, the retention time was found to be 16.8118. The area was 2700771 while the ESTD concentration was 494.515 for aflatoxin B_1. In the case of aflatoxin B_2, retention time was 13.867 & area was 8764 but the ESDT concentration was 0.00. (Fig 11)

In all of the above samples, aflatoxin G_1 and aflatoxin G_2 were absent.

Conclusion

Samples that were obtained from local Mumbai markets were analysed using HPLC technique on comparison of results. It showed that aflatoxin B_1 & B_2 were present more in all the food grains except for rice, which showed absence of aflatoxin B_2. Different analytical methods were employed in the analysis of aflatoxins in food grains. Chromatographic methods such as HPLC is considered as standard method & hence most widely used in aflatoxin analysis. Aflatoxin is the major sources of disease outbreak due to consumption of contaminated food & lack of knowledge. To ensure the safety & quality of food, application of genetic recombination in Aspergillus flavus & the other species is being invested for its potential to mitigate aflatoxin.

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