

# Aflatoxin Contamination and Detection

Niroj Nandi\* ,Shikha\*\*

\* Department of Forensic Science, School of Bioengineering and Biosciences,Lovely Professional University

\*\* Assistant Professor, Department of Forensic Science, School of Bioengineering and Biosciences,Lovely Professional University

## Abstract

Mycotoxin is common defilement in agriculture food commodities which affect both humans and animals and decreases their commercial esteem.. Mycotoxin carcinogenic class Aflatoxins produced by *Aspergillus* fungi and known to contaminate an expansive portion of the world's agriculture and food supply. The four major Aflatoxins among the identified 20 are AFB1, AFB2, AFG1 and AFG2. The B-types are majorly harmful and are produced by *A. flavus* while G-types are produced by *A. parasiticus*. Methods like in vitro digestion and hydrolysis, as applied in the case of masked fumonisins, can be carried out for masked AFs in food and feed followed by detection with LC/MS/MS and confirmation by other methods like ELISA to ensure the food and feed safety. Therefore, biocontrol measures in synchrony with other physical and chemical methods along side improved packaging materials *ought to be actualized to accomplish* to attain food safety and security

## Introduction

Different fungi produces secondary metabolites called mycotoxins. The label secondary metabolites was coined to differentiate components like flavonoids, terpenes, alkaloids and products that are considered to be non-essential in the development of plants. Inversely fatty acids, nucleic acids, amino acids and proteins and other useful components were coined primary metabolites [1]. Mycotoxins have following features (A) They have a confined apportion in microorganisms (B) They are individualistic to species and genera (C) They are produced by specialized pathways from primary metabolites [2]. There are total 300 approx. various known mycotoxins [3] out of which only 20 mycotoxins generally contaminate food and feed which pose a health risk to human beings and animals and among them aflatoxins poses the most serious threat due to its occurrence and deleterious effects.

.Aflatoxins are toxic secondary metabolites produced by *Aspergillus flavus* [4,5,6], *Aspergillus Nomius* [7,8], *Aspergillus parasiticus* [9,10,11]. There are other fungi as well that produces aflatoxins but are less recurring such as *Aspergillus bombycis*, *Aspergillus pseudotamarii* and *Aspergillus ochraceoroseus* [12,13,14].

### **Types of Aflatoxins**

Aflatoxins have about 20 secondary metabolites out of which Aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 are found in foods of plant origin whereas aflatoxicol, aflatoxin M1, aflatoxin M2, aflatoxin Q1, aflatoxin P1, aflatoxin B2a and aflatoxin G2a are found in food of animal origin due to formation of hydroxylates by incorporation of OH negative group [15] i.e. aflatoxins are passed on to animal food such as eggs, milk, meat and other milk products in the form of hydroxylates due to consumption of aflatoxin contaminated feed by animals.

The tags 'B' and 'G' indicates blue and yellowish green fluorescence under 366nm mercury lamp on TLC plates whereas the numbers 1 and 2 denote minor and major compound [16].

### **Occurrence**

The botheration of contamination of food and feed by aflatoxins is a mundane issue in tropical and subtropical regions of the world [17]. Poor harvesting (pre-harvesting and post-harvesting) and storing practices along with humid climatic conditions aid to the growth of aflatoxin producing fungi. However the highest levels of contamination occurs during post-harvest growth of *Aspergillus* fungi in improperly stored food items.

Most common aflatoxin contaminated food items include chillies, spices, cotton seeds, peanuts, oil seeds, figs, nuts, corn, barley, oats etc. [1] but peanuts and corn are found to be the most contaminated food items all over the world [18]

### **Effects of Aflatoxins**

At high dosage aflatoxins causes acute toxicity or death in fish, birds, mammals and human beings [19]. Main target organ is the liver but aflatoxins at higher dosage have also been detected in lungs, hearts, brains and kidneys. The B1 variant of aflatoxin is carcinogenic and mutagenic which causes cancer in liver. Low dosage of aflatoxin over a long period of time is associated with liver cancer, jaundice, chronic hepatitis, cirrhosis [20]. Aflatoxin exposure may also play a role in Kwashiorkor and Reye's Syndrome [21,22]

**Major historical outbreaks**

The first outbreak occurred in the early 1960s in England where 100000 turkeys died due to consumption of aflatoxin contaminated peanut meal [23]. Again in 1974 a mammoth outbreak of hepatitis due to aflatoxin contaminated maize that lasted for almost 2 months was reported in about 200 villages of Rajasthan and Gujarat that killed around 106 people. Studies of contaminated maize showed consumption of 2000-6000 µg/kg each day by the affected individuals [24,25]. Again an outbreak occurred in Kenya during January to June 2004 which resulted in 125 deaths out of 317 cases occurred due to consumption of aflatoxin contaminated maize [26] and a recent outbreak occurred during the May to 14<sup>th</sup> November 2016 in Tanzania that affected a cluster of individuals where out of 68 cases occurred 20 people died because of consumption of aflatoxin contaminated maize. [27]

**Regulations**

At least in 199 countries there is a regulation level set for aflatoxins [28,29] out of which 77 countries have regulations minimising mycotoxin levels where 48 countries have specific regulation level in foodstuffs and more 21 countries have on feed stuffs. The United States Food and Drug Administration (USFDA) have set the lower limit at 200ppb for food and 0.5ppb for milk. In India lower limit for aflatoxin in all food stuffs is set at 30ppb and in the European Union limit is set at 4ppb [30] which is same in Germany as well. Taiwan have set the maximum limit at 50ppb in food stuff whereas Kenya and Guatemala have set it at 20ppb. Australia have set the regulatory limit in food at 5ppb and Ireland have set it at 30ppb in food for human consumption[31].

Aflatoxin B1 has been classified as a Group 1 carcinogen in humans by the International Agency For The Research Of Cancer (IARC) [32] and the World Health Organization (WHO) also classifies aflatoxin B1 as Class 1 carcinogen.[33]

**Different methods for the analysis of aflatoxins**

At present there are many methods applied for analysis of aflatoxins ranging from pretty basic to highly sophisticated methods such as thin layer chromatography to multi toxin liquid chromatography tandem mass spectrometry as well as different rapid immunological methods[34]. Analysis of aflatoxins could be grouped into (a) biological process (b) analytical process and (c) immunological process. Biological processes were basically used for screening general toxicity because biological assays were time taking, nonspecific and less

sensitive. Test such as reaction of amplex with hydrochloric acid or trifluoroacetic acid were developed for detection of aflatoxins [35]. On the other hand analytical methods were developed for aflatoxin analysis which were far better than that of biological ones due to analytical methods being more rapid, repeatable, reproducible and had lower limit of detection [36] methods included High performance thin layer chromatography, Thin layer chromatography, High pressure liquid chromatography etc. Mostly chromatographic techniques like TLC, HPLC are used for detection and quantification of aflatoxins but there are different developed techniques such as a portable kit using drip strip technique for analysis of aflatoxin was created for different public health laboratories, poultry industries and environment monitoring agencies[37] but with the arrival of monoclonal and polyclonal antibodies against aflatoxins ELISA was developed for analysis of aflatoxins[38] because it was more rapid, simple and specific.

### **Discussion**

Aflatoxins have already gained a global impact due to its major effects on both human and animal health. Though there have been many extensive research done from a food safety point of view but much remains to be explored in the field of forensic science as aflatoxins more specifically the B1 variant may be used as a desired biological weapon of choice for biological warfare or bioterrorism. There have been many instances where terrorist opt for biological weapons instead of conventional ones as it gives an upper hand to terrorists to swiftly perform their task of terrorism without been coming into the radar of government suspicion and investigations. A major reason about biological weapons that allures terrorists is the ability of using the weapon to affect a large pool of population and it also being easily feasible to produce and administer. Aflatoxin B1 may be used to commit crimes such as mass murders, homicide, and killing livestock as it is carcinogenic and supports both acute and chronic mode of poisoning. Therefore accessibility of Aflatoxins in wrong hands can prove to be disastrous for global population.

### **References**

- [1] Sydenham E.W., Shephard G.S. Chromatographic and allied methods of analysis for selected mycotoxins. In: Gilbert J. (eds) Progress in Food Contaminant Analysis. Springer, Boston, MA, 1996

- [2] Steyn PS The biosynthesis of mycotoxins. *Vue M´ed V´et* 149: 469-478.1998
- [3] Cole RJ, Cox RH Handbook of toxic fungal metabolites. Academic Press, New York.1981
- [4] Link HF Observationes in Ordines plantarum naturales. Dissertatio prima, complectens Anandrarum ordines Epiphytas, Mucedines Gastomycos et Fungos Der Gesellschaft Naturforschender Freunde zu Berlin. *Magazin für die neuesten Entdeckungen in der gesamten Naturkunde* 3: 1–42.1809
- [5] Payne GA Process of contamination by aflatoxin producing fungi and their impacts on crops. In: *Mycotoxins in Agriculture and Food Safety*. Sinha KK and Bhatnagar D. Marcel (eds). Dekker, Inc. New York. USA.1998.
- [6] Scheidegger KA, Payne GA Unlocking the secrets behind secondary metabolism: A review of *Aspergillus flavus* from pathogenicity to functional genomics. *J Toxicol-Toxin Rev* 22: 423-459.2003
- [7] Speare AT Fungi parasitic upon insects injurious to sugar cane. *Pathology and Physiological Series, Bulletin* 12. Honolulu: Hawaiian Sugar Planters' Assoc Exp Stat. Path Phys. Series Bull 12:1-62.1912.
- [8] McAlpin CE, Wicklow DT, Platis CE Genotypic diversity of *Aspergillus parasiticus* in an Illinois corn field. *Plant Dis* 82: 1132–1136.1998.
- [9] Klich MA, Pitt JI Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Trans Brit Mycol Soc* 91:99–108.1988.
- [10] Kurtzman CP, Horn BW, Hesseltine CW *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamaris*. *Antonie van Leeuwenhoek* 53: 147-158.1987.

- [11] Horn BW, Moore GG, Carbone I Sexual reproduction in aflatoxinproducing *Aspergillus nomius*. *Mycologia* 103: 174-183.2011.
- [12] Goto T, Wicklow DT, Ito Y Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamaris* strain. *Appl Environ Microb* 62: 4036–4038.1996.
- [13] Klich MA, Mullaney EJ, Daly CB, Cary JW Molecular and physiological aspects of aflatoxin and sterigmatocystin biosynthesis by *A. tamaris* and *A. ochraceoroseus*. *Appl Microbiol Biotechnol* 53: 605–609.2000.
- [14] Peterson SW, Ito Y, Horn BW, Goto T *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. *Mycologia* 93: 689–703.2001.
- [15] Heathcote JG, Hibbert JR Aflatoxins: chemical and biological aspects. Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.1978
- [16] Menza NC, Margaret MW, Lucy KM, et al Incidence, types and levels of aflatoxin in different peanuts varieties produced in Busia and Kisii Central Districts, Kenya. *Open J Med. Microbiol*; 5:209.2015.
- [17] Zhang C, Selvaraj JN, Yang Q, et al. A survey of aflatoxin-producing *Aspergillus* sp. from peanut field soils in four agroecological zones of China. *Toxins*; 9:40.2017.
- [18] World Health Organization World health statistics quarterly- food safety and food borne diseases .50:143-147.1997
- [19] Sahoo PK, Mukherji SC, Nayak SK, et al. Acute and subchronic toxicity of aflatoxin B1 to Rohu, *Labeo rohita* (Hamilton). *Indian J Exp Biol*.39:453-8.2001.
- [20] Wild CP, Montesano R. A model of interaction: Aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. *Cancer Lett*.286:22-8.2009.

- [21] Kocabas CN, Coşkun T, Yurdakök M, et al. The effects of aflatoxin B1 on the development of kwashiorkor in mice. *Hum Exp Toxicol*.22:155-8.2003.
- [22] Rogan WJ, Yang GC, Kimbrough RD. Aflatoxin and Reye's syndrome: A study of livers from deceased cases. *Arch Environ Health*.40:91-5.1985.
- [23] Richard J.L. Discovery of aflatoxins and significant historical features. *Toxin Reviews*, 27(3-7),171-201.2008.
- [24] Krishnamachari KAVR, Bhat RV, Nagarjan V and TBG Tilak Hepatitis due to aflatoxicosis – An outbreak in western India. *Lancet*;1:p1061-1063.1975.
- [25] Krishnamachari KAVR, Bhat RV, Nagarjan V and TBG Tilak Investigations into an outbreak of hepatitis in part of western India. *Ind. J. Med. Res.* 63: p1036-1048.1975.
- [26] Aziz Baumgartner E, Lindblade K, Giesecker K, Rogers HS, Kierak S, Njapau H, Schleicher R, McCoy LF, Misore A, De Cock K, Rubin C, Slutsker L and The aflatoxin investigative group Case control study of an acute aflatoxicosis outbreak, Kenya, *Environmental health perspectives*; 113: p1779-1783.2005.
- [27] A. Kamala, C Shirima, B Jain, M Bakari, H. Sillo, N. Rusibamayila, S. De Saeger, M Kimanya, Y.Y. Gong, A. Simba, the investigation team. Outbreak of an acute aflatoxicosis in Tanzania during 2016, *World Mycotoxin Journal*.2018.
- [28] H. P. Van Egmond, M. A. Jonker, Worldwide regulations on aflatoxins. In H.K. Abbas (ed.) *Aflatoxin and Food Safety*, Boca Raton, FL: Taylor and Francis, p77-93.2005.
- [29] F. Wu, Mycotoxin risk assessment for the purpose of setting international regulatory standards, *Environmental Science and Technology*, 38(15): p4049-4055.2010.
- [30] M. A. Klich, *Aspergillus flavus*: The major producer of aflatoxin, *Molecular Plant Pathology*, 8(6): 713-722.2007.
- [31] J. Ajani, D.V. Sudhur Chakravarthy, P. TAnuja, K. Vali Pasha, *Aflatoxins*, Indian

Journal of Advances in Chemical Science 3: p49-60.2014.

[32] IARC, International Agency for the Research of Cancer Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the evaluation of carcinogenic risks to humans 82: 171.2002.

[33] Loper C, Ramos L, Bulacio L et. al. Aflatoxin B1 content in patients with hepatic diseases. Medicina- Buenos Aires.2002.

[34] G. G. Shepard, Aflatoxin analysis at the beginning of the twenty-first century, Analytical Bioanalytical Chemistry, 395(5): p1215-1224.2009.

[35] S. Nesheim, W. C. Brumley, Confirmation of identity of aflatoxins, Journal of American oil Chemists Society, 64: p945A-949A.1981.

[36] H. P. Van Egmond, W. E. Paulsch, Mycotoxins in milk and milk products, Netherlands Milk and Dairy Journal, 40: p175-188.1986.

[37] R. B. Sashidhar, Dip-strip method for monitoring environmental contamination of aflatoxin in food and feed: Use of a portable aflatoxin detection kit, Environmental Health Perspectives, 101 Suppl 3: p43-46.1993.

[38] J. J. Pestka, P. K. Gaur, F. S. Chu, Quantitation of aflatoxin B1 and B1 antibody by an enzyme linked immunosorbent microassay, Applied and Environmental Microbiology, 40(6): p1027-1031.1980.