Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 2267-2271

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.259

Quantitation of AFB1 by Spectrophotometric Method

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Keywords

Spectrophotometric, *Aspergillus flavus*

Article Info

Accepted:
20 March 2018
Available Online:
10 April 2018

A B S T R A C T

Aspergillus flavus produces potent aflatoxin and contaminates cereals, oil seeds and nuts, due to the climatic change the aflatoxin infestation is wide spread to various crops Viz: corn, soya, wheat, rice and cottonseeds, and nuts (peanut, almonds, Brazil nuts, hazelnuts, walnuts, cashew nuts, pecans, and pistachio nuts. Aflatoxin diet exposure has been observed through the consumption of foodstuffs, the contamination of maize, peanuts, and oilseeds. Aflatoxin causes deleterious health effects *viz.*, human and animal health such as causing cancer, liver damage, diarrhoea, dizziness and fever are the common symptoms of aflatoxin contaminates food consumption. The aflatoxin contamination is observed pre and post-harvest, factors such as temperature, moisture, oxygen, and carbon dioxide, insect and rodent's infestation, incidence of broken grains or nuts and the cleaning of the product will increase the incidence of toxigenic fungal load by transport, waiting time for drying, frying system and storage condition effect. Some countries established maximum concentration for aflatoxin in specific products ranging from (ppb to µg levels based on products, consumption pattern and country specific). The detection methods includes thin layer chromatography (TLC), high performance liquid chromatography (HPCL), mass spectroscopy, enzyme linked immuno sorbent assay (ELISA), and electrochemical immune sensor have been used for quantifying aflatoxins in foods and food commodities. These methods are costly, difficulty in performing assay and required technical knowhow. Hence, we have evaluated the inhibition of Acetyl choliesterase by aflatoxin (AFB1) for utility in assessing the presence of aflatoxine.AFB1 at132ng the inhibition obtained was 38.18%.Hence can be used for assessing aflatoxin contamination.

Introduction

Aflatoxins are a group of secondary fungal metabolites so far known to be produced by nine different species of *Aspergillus* and two different *Emericella* species (Frisvad *et al.*, 2006). Due to their high toxicity and carcinogenic potential they are of high concern for the safety of food worldwide (Ellis *et al.*, 1991). Based on chromatographic and fluorescence characteristics, all aflatoxins known to date can be classified into 18 different types. The major ones are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), as well as M1 (AFM1) and M2 (AFM2) (Fig. 1) (Lerda *et al.*, 2010). Other aflatoxins have less commonly been found in nature since they are metabolic derivatives

mostly found in pure cultures (Franco *et al.,* 1998). The order of acute and chronic toxicity is AFB1 > AFG1 > AFB2 > AFG2, reflecting the role played by epoxidation of the 8,9-double bond and also the greater potency associated with the cyclopentenone ring of the B series, when compared with the six-membered lactone ring of the G series. Among these compounds, AFB1 is normally predominant in concentrations in cultures as well as in food products (Oliveira and Germano, 1997). AFM1 and AFM2 are hydroxylated forms of AFB1 and AFB2 (Dors, 2011). When AFB1 in contaminated feed or foodstuffs is ingested by domestic animals, such as dairy cows, the toxin undergoes liver biotransformation and is converted into aflatoxin M1 (AFM1), becoming the hydroxylated form of AFB1. AFM1 is excreted in milk, tissues and biological fluids of these animals (Oatley *et al.,* 2000; Peltonem *et al.,* 2001; Murphy *et al.,* 2006) and in this form can be taken up by consumers. A linear relationship between the concentration of AFM1 in milk and the concentration of AFB1 in contaminated feeds consumed by the animals has been reported. It was found that about 0.3% to 6.2% of AFB1 ingested with feed is transformed into AFM1 in milk (Creppy *et al.,* 2002; Bakirci *et al.,* 2001)

Consumption of Aflatoxins by human beings can lead to liver cancer. A person's chances of contracting cancer are compounded significantly if he/she carries the hepatitis B virus, as do an estimated 20 million people in India. Aflatoxins do their damage by suppressing the immune response. They affect poultry and, when present in cattle fodder (groundnut cake and haulms), the yield and quality of milk.

Pre harvest infection and aflatoxin contamination often occur when the plant is exposed to moisture and heat stress during pod development, when pods are damaged by insects or nematodes or when they are mechanically damaged during cultural operations. Due to the reliance on rainfall for watering crops and the recent variations experienced with weather patterns, these conditions commonly occur. Postharvest infection in groundnuts is influenced by shelling methodology, relative humidity, temperature, and insect damage. Some strains of *A. flavus* also produce cyclopiazonic acid (CPA), a harmful mycotoxin that is currently not regulated (Abbas *et al.,* 2005). In most developing countries the level of aflatoxin contamination is extremely high. For example, results of recent studies in Mali have shown levels of contamination in groundnuts in excess of 3,000 parts per billion (ppb) with a mean contamination of 164 ppb (Thirumala *et al.,* 2002 forthcoming). These levels are much higher than international standards allow for human consumption (4 ppb in the EU and 20 ppb in the United States). Results from Mali have revealed that only 30–55 percent of all groundnut products are safe to eat by EU standards (Thirumala *et al.,* 2002 forthcoming). Further, results from our studies in Mali show that granaries have a significantly higher aflatoxin load during the storage period (October to June) due to high moisture and temperatures recorded during this time of year (IFPRI, 2012). It is thus imperative to improve management of aflatoxins in groundnuts for food, health, and nutritional security.

The recently immunochemical methods such as enzyme linked immune sorbent assay (ELISA) have been developed by ICRISAT is commercially available analytical and chromatographic techniques to researchers and traders are expensive. ELISA methods are limited by matrix effect or matrix interference commonly occurs in ELISA which can give rise to underestimates or overestimates in mycotoxin concentrations in commodity

samples. Additionally, insufficient validation in ELISA methods causes the methods to be limited in the range of matrices examined. Hence development of simple and cost-effective methods for detecting Aflatoxins in various Grains, foods and feed commodities is a pre-requisite.

Materials and Methods

Sample preparation

Aflatoxin B1 was purchased from Sigma-Aldrich (St. Louis, MO) and further diluted in methanol to 1 mg/ml solution of B1. Different aliquots ranging from 16.66 to 132.6ng were used for assaying acetyle cholinesterase inhibition for 0 to 9min.

Eelacetyle cholinesterase was purchased from Sigma- Aldrich (St. Louis, MO) and the concentration equivalent to 40units was used in all the replicates for the assay of acetyl cholinesterase.

Measurement of acetyl cholinesterase inhibitory activity

The inhibition of AChE activity by Aflatoxine (AFB1) obtained from sigma Aldrich was incubated with electric eel acetyl cholinesterase in 50mM Tris buffer (pH 7.4) for 3, 6 and 9 min and the spectrophotometric assay is measured by addition of 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB) and acetyl thiocholine iodide (ATCI). The contents are rapidly read at maximum absorbance peak of 412 nm (Ellman *et al.,* 1961). The absorbance is converted to percent inhibition of electric eel acetyl cholinesterase by AFB1 and is proportional to the concentration is calculated.

Results and Discussion

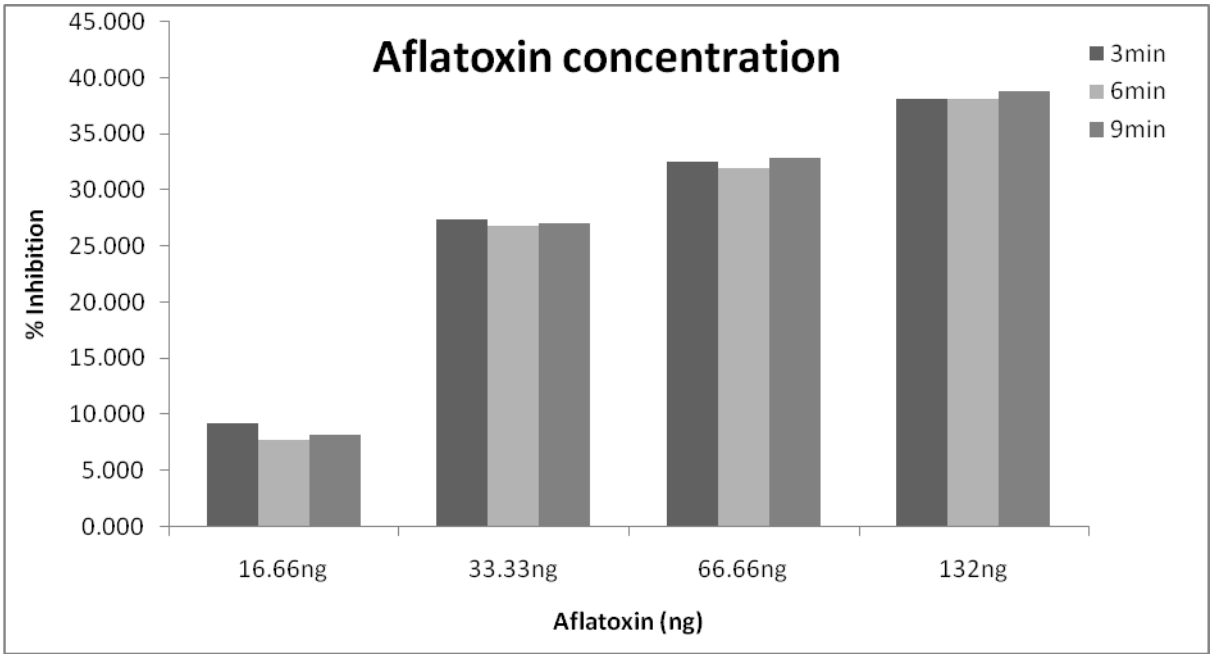
The AChE from electric eel was the most sensitive as reported by (Arduini *et al.,* 2007).and they have shown the inhibition pattern IC_{50} (IC_{50} equal to amounts of inhibitor that gave 50% enzyme inhibition) obtained with 60 ng mL^{-1} AFB1.The degree of inhibition obtained with AFB1 at 60 ng mL^{-1} using AChE wild type, B24, and B394 were 20, 17, and 21%, respectively. In our study the 38.18% inhibition was obtained at 132 ng of AFB1 and the result demonstrated that the electrical eel AChE can be used as inhibitory to understand the AFB1 quantitatively at nano gram levels. Similarly results of (Arduini *et al.,* 2007) demonstrated that the AChE from electrical had the highest sensitivity to AFB1 among the cholinesterases previously tested and was also considerably more sensitive than the unpurified AChE from mouse brain tested by (Cometa *et al.,* 2002). Which showed IC50 at 10 mg mL^{-1}.The degree of inhibition at a fixed concentration of AFB1 (60ng mL-1) using various concentrations of AChE was determined BY (Arduini *et al.,* 2007). The enzyme concentrations used for this experiment were 70, 40, and 7mU mL-1, and the degrees of inhibition obtained were 45%. These results essentially no change in degree of inhibition) seem to support the hypothesis that the inhibition of AChE by AFB1 follows a reversible mechanism. In order to develop an effective assay for AFB1 determination based on the decrease in enzyme activity, the spectrophotometric Ellman's method for the determination of AChE activity had to be adapted and optimized, taking into consideration parameters such as substrate concentration, solvent, pH, etc.

As noted above, the characteristics of the inhibition made it possible to adjust the enzyme and substrate concentration of the assay mixture to achieve suitable assay times and levels of inhibition. An enzyme concentration of 40 mU mL^{-1} was chosen because it permitted the achievement of reasonable optical densities from Ellman's reaction in only 3 min (Table 1).

Table.1 Acetyle cholinesterase absorbance at 415nm incubation with AFB1

AFB1 Concentration	Control	16.66ng	33.33ng	66.66ng	132ng
3min	0.4335 ± 0.0440	0.3935 ± 0.0013	0.3145 ± 0.0126	0.2925 ± 0.0018	0.268 ± 0.0026
6min	0.4335 ± 0.0497	0.4± 0.0040	0.317 ± 0.0138	0.295 ± 0.0023	0.268 ± 0.0023
9min	0.442 ± 0.0523	0.4055 ± 0.0055	0.3225 ± 0.0124	0.2965 ± 0.0026	0.2705 ± 0.0032

Fig.1 % Inhibition of acetyle cholinesterase by AFB1



Furthermore, at a constant concentration of AFB1 of 60ng mL^{-1}, with different concentrations of substrate (0.1-1.6 mM), the degree of inhibition (47 to 50%) was found to be constant. Finally, a substrate concentration of 0.4mM was chosen for the rest of their work. Similarly we have used 40 mU Ml as a enzyme source for our studies and obtained an inhibition pattern of In our study the inhibition obtained were as 16.66ng AFB1 varied from 7.72 to 9.227%, 33.33ng AFB126.874 to 27.451%, 66.66ng AFB131.949 - 32.919% and 132ngAFB1 for 38.801%.

References

Abbas, H. K. (2005). Aflatoxin and food safety. Boca Raton, CRC Press.

Arduini, F., Ricci, F., Bourais, I., Amine, A., Moscone, D., Palleschi, G. Anal. Lett. 2005, 38, 1703-1719.

Bakirci, I. (2001). A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. Food Control 12, 47-51.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of

mycotoxins in Europe. Toxicology Letters, 127-19.

Ellis, W.O., Smith, J.P., Simpson, B.K. (1991). Aflatoxins in food-occurrence, biosynthesis, effects on organisms, detection and methods of control. Critical Reviews in Food Science and Nutrition 30, 403-439.

Ellman, G. L. Arch. Biochem. Biophys. 1959, 82, 70-77.

Franco, C. M., Fente, C. A., Vazquez, B. I., Cepeda, A., Mahuzier, G., Prognon, P. (1998). Interaction between cyclodextrins and aflatoxins Q1, M1 and P1: Fluorescence and chromatographic studies. Journal of Chromatography A 815, 21-29.

Frisvad, J.C., Thrane, U., Samson, R.A., Pitt, J.I. (2006). Important mycotoxins and the fungi which produce them. In: Hocking, A.D., Pitt. J.I., Samson, R.A., Thrane, U. (eds.). Advances in Food Mycology 57. Advances in Experimental Medicine and Biology, New York, Springer Science and Business Media, 3-3.

Lerda, D. (2010). Mycotoxin fact sheet. 3rd edition. JRC technical notes. IRRM, Geel Belgium.

Luzi, A. Cometa, M. F. Palmery, A. Toxicol. in Vitro 2002, 16, 525-529.

Murphy, P. A., Hendrich, S., Landgren, C., Bryant, C. M. (2006). Food Mycotoxins: An Update. Journal of Food Science 71, 51-65.

Oatley, J. T., Rarick, M. D., Ji, G. E., Linz, J. E. (2000). Binding of aflatoxin B1 to bifidobacteria in vitro. Journal of Food Protection 63, 1133-1136.

Oliveira, C. A. F., Germano, P. M. L. (1997). Aflatoxinas: conceitos sobre mecanismos de toxicidade e seu envolvimento na etiologia do câncer hepático celular. Revista de Saúde Pública 31, 417-424.

Peltonem, K., El-Nezami, H., Haskard, C., Ahokas, J., Salminen, S. (2001). Aflatoxin B1 binding by dairy strains of lactic acid bacteria and bifidobacteria. Journal of Dairy Science 84, 2152-2156.

Thirumala-Devi, K., M. A. Mayo, A. J. Hall, P. Q. Craufurd, T. R. Wheeler, F. Waliyar, A. Subrahmanyam, and D. V. R. Reddy Development and Application of an Indirect Competitive Enzyme-Linked Immunoassay for Aflatoxin M1 in Milk and Milk-Based Confectionery J. Agric. Food Chem., 2002, 50 (4), pp 933–937.

How to cite this article:

Kisan, B., K. Shruthi Nagaral, A.S. Halepyati and Shankergoud, I. 2018. Quantitation of AFB1 by Spectrophotometric Method. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 2267-2271.
doi: https://doi.org/10.20546/ijcmas.2018.704.259