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Aflatoxin and Its Different Methods of Detection

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Aflatoxins are ubiquitous toxic contaminants of food in developing world. AFB1, AFB2, AFG1 and AFG2 are major aflatoxins where as M1 and M2 are additional metabolic products (Samuel *et al*., 2013). IARC classified AFB1 as potent carcinogenic compounds (Tavakoli *et al*., 2013). *Aspergillus flavus* is one of the most predominant fungal species distributed across tropical environment and causes severe aspergillosis in human beings. Besides this, it causes insect diseases (Campbell, 1994) as well as develops diseases in agricultural crops such as maize, rice, peanuts etc. Agricultural commodities if contaminated by aflatoxigenic *A. flavus*, it pose a severe health risk to humans as well as animals. The characterization of mycotoxic fungi is pre-requisite for management (Dawlatana *et al*., 2008).

Methods of aflatoxin detection

Choosing the best rapid, sensitive, efficient and reliable detection method is the primary step to detect and quantify aflatoxin concentrations level in agricultural commodities. Aflatoxin detection and quantification methods are majorly classified into two categories:
(A) Cultural method (B) Analytical method

A. Cultural Method: This method is most inexpensive, requires small equipments or no equipments but exhibit poor sensitivity, accuracy and detection limits **(Table 1)**. Sometimes this method is non-specific to reactants based on simple color change assay. One can detect and quantify the aflatoxin in the different commodities in limited time. The amount of aflatoxin produced by fungus in the commodities need not to be correlated with amount of infestation level in the seed kernel.

In the presence of enhancer p-cyclodextrin in the culture media emitted blue fluorescence (Ordaz *et al*., 2003). Yellow pigmentation turned into plum-red when culture media exposed to ammonium hydroxide vapour (Gupta and Gopal, 2002). Many developing nations had problem to detect and quantify aflatoxin level in their food and feed lot. To screen large number of commodities in a limited time is difficult because of lack of expertise to prepare sample and analysis in analytical methods. In this context cultural methods are more user friendly, inexpensive and less time consuming. Pre-screening large number of commodities in a limited time to identify the best candidate commodities for export purpose is possible through employing cultural method.

Table 1 Different cultural methods for aflatoxin detection

S. No.	Aflatoxin types	Detection methods	Key features/ Property	References
1.	Aflatoxin B1 and B2	Blue Fluorescence	UV Photography used to screen aflatoxin shows Blue Fluorescence	Gupta and Gopal, 2002

2.	Aflatoxin B1 and G1	Cyclodextrin-Enhanced Blue Fluorescence	Cyclodextrins (CDs) used as enhancer agent for emitting Blue Fluorescence	Lemke *et al.*, 1988
3.	Aflatoxin B1	Yellow Pigment	Production of yellow to orange pigments	Davis *et al.*, 1987
4.	Aflatoxin B1	Vapor-induced color change due to ammonium hydroxide	Concentrated ammonium hydroxide solution shows plum-red color in presence of B1 aflatoxin	Bhatnagar *et al.*, 2003
5.	Aflatoxin B1	Cyclodextrin-Enhanced Blue Fluorescence Combined with Ammonium Hydroxide Vapor-Induced Color Change	Bright-yellow pigmentation observed under natural light and blue fluorescence observed under UV light (365 nm)	Abbas *et al.*, 2004

B. Analytical Method: This method measures the toxin at lower concentration but requires extraction with solvents such as chloroform, methanol and ethanol etc. Methods are sensitive, reliable and reproducible. For more precise limit of detection, sampling, preparation of samples, method of extraction and method of detection are very important to detect and quantification of more precise level of toxin (Nilufer and Boyacioglu, 2002) **(Table 2)**.

Table 2 Advantages and disadvantages of different methods used for aflatoxin detection

S. No.	Name of aflatoxins (AFs)	Method of detection	Advantages	Disadvantages	References
1.	Determining four major AFs (B1, B2, G1, and G2)	Gas chromatography	Aflatoxins analysis is done in real time, sensitivity is good	Require expertise to handle equipments and are expensive	Pascale 2009
2.	Four major AFs (B1, B2, G1, and G2) as well as AFM1, AFQ1, and AFP1	Liquid chromatography	Provide good sensitivity, high dynamic range, and high versatility	Traditionally, it is considered as a slower aflatoxin technique	Gurban *et al.*, 2017
3.	Typically used for screening of AFM1 in milk	Thin-layer chromatography	Reliable quantification method when combined with densitometry, quick method to identify aflatoxins at a level as low as 1 ng/g	Require outdated equipment, destructive working sample preparation	Fuchs *et al.*, 2011
4.	Very sensitive and reproducible for analysis of aflatoxins M2, M1, G2, G1, B1, and B2	High-performance liquid chromatography	Standard method for detection, sensitivity and selectivity is good, repeatability is good, requires short analysis times	Expensive equipment, destructive sample preparation, may require derivatization.	Zheng Y 2016
5.	Detection and quantification of aflatoxins B1, B2, G1, G2 and M1 and M2	Liquid chromatography–mass spectrometry (LC–MS)	Real time detection, Confirmation of Aflatoxin, low limit of detection (LC/MS/MS), no derivatization required.	Very expensive and required expertise, sensitivity relies on ionization, matrix assisted calibration curve (for quantitative analysis), lack of internal standards for aflatoxin compound.	Aiko and Mehta, 2015

6.	AFB1 in groundnut, corn, wheat, and chilli. AFM1 in milk	Enzyme-linked immunosorbent assay (ELISA)	Specific, rapid and relatively easy to use, sample preparation is easy, inexpensive equipment, low limit of detection, simultaneous analysis of multiple samples for toxin presence , suitable for screening, semiquantitative or quantitative analysis possible	Cross reactivity with related mycotoxins and sample protein, possible false positives/negatives, matrix interference problems, narrow detection range	Adanyi *et al.*, 2007
7.	Specifically used for the qualitative and quantitative determination of AFB1 and AFM1 toxin	Radioimmunoassay (RIA)	Radioactive marker have vital role in determining the sensitivity of RIA. RIAs provide the scope to conduct multiple analyses simultaneously with improved sensitivity and specificity	Use of radioactive materials are unsafe for storage as well as handling	Waliyar *et al.*, 2009
8.	Detecting AFB1 at levels down to 20 ng	Lateral flow devices (immunodipsticks)	Rapid method, no expensive and sophisticated equipment needed, no specific training required	Semi-quantitative (visual assessment) method, cross-reactivity with related mycotoxins is seen	Goh *et al.*, 2014
9.	All types of aflatoxins have a maximum absorption of around 360nm	Fluorescence and spectrometric methods	Easy to operate	Precision is required	Akbas and Ozdemir, 2006
10.	Detection of aflatoxins	Hyperspectral imaging	high spectral and spatial resolution, rapid, non-destructive method	Calibration model must be validated, Require knowledge of statistical methods	Hruska *et al.*, 2013
11.	Detection of aflatoxins molecule	Laser-induced fluorescence (LIF) screening method	Appropriate aflatoxin detection technique, LIF spectroscopy can employed in industrial purpose for aflatoxins measurement	High cost of lasers, Precision required for handling	Simeon *et al.*, 2001
12.	To measure AFM1 in milk	Green immunoassay	Feasible, safe, and reliable for detecting AFM1 in milk	Precision needed	Guan *et al.*, 2011

High throughput technique for aflatoxin detection: Internal transcribed spacer region (ITS 2) of rRNA gene used to develop specific primer and probe for the quantitative real-time PCR analysis to detect and quantify aflatoxin level in grapes produced by several species of Aspergillus (Gonzalez-Salgado *et al*., 2009). Rodriguez *et al.* (2012) developed two protocols of real-time PCR (qPCR) based on SYBR Green and TaqMan; their sensitivity and specificity were evaluated. Primers and probes were designed from the o-methyltransferase gene (omt-1) involved in aflatoxin biosynthesis.

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