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REVIEW

Detection and Biodetoxification of Aflatoxins in Food

B. AISHWARYA†, G.S. VAIDYANATHAN†, AVAYA MANIVANNAN, M. JASWANTH SHA ALLAN, SEEMA A. KULKARNI, S. PERIYAR SELVAM* and M. MAHESH KUMAR

Department of Food Process Engineering, Postharvest Research Lab, School of Bioengineering, SRM Institute of Science and Technology, Potheri, Kattankulathur-603203, India
†These authors contributed equally to this work

*Corresponding author: E-mail: periyar.india@gmail.com; periyars@srmist.edu.in

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Harmful agents such as toxins, chemicals and pollution are causing public health hazards around the world. The food and agriculture sectors in particular are highly sensitive to exposure to toxic waste. Among the various toxic products of microbial extraction, aflatoxin is a deadly mycotoxin produced by the species *Aspergillus*. Aflatoxin contamination is common in commercial foodstuffs, veterinary foods as well as in cosmetics. However, some viable strategies related to the screening and detection are considered an important response by the scientific community to prevent early-stage contamination, followed by detection or screening approaches. This article presents current study that emphasizes the effectiveness of biosensors as a good indicator of aflatoxin detection strategies and also the detoxification of the identified aflatoxins, which causes food spoilage and industrial losses by physical, chemical and biological methods.

Keywords: Aflatoxin, *Aspergillus*, Food, Biosensors, Detection, Detoxification.

INTRODUCTION

Mycotoxins are fungal metabolism-related toxic compounds that vary from animal food to human food. Ingestion can pose significant health threats to both animals and humans when swallowed and therefore have larger economic and public health effects as shown in Fig. 1 [1,2]. The mycotoxin infectivity can be severe and attributed to several environmental factors such as excessive moisture in and around the storage areas, together with tropical and temperate climates and pest infestations. Almost all species are affected by mycotoxin toxicity in the animal feed, but it is more common in dairy animals, pigs, and poultry. Mycotoxins decrease the capacity to produce, hinder resistance to infection and endanger reproduction in these species [3].

As reported by FAO, as a result of mycotoxins, over 1/4th of the global crop yield is affected [4,5]. Amongst the several mycotoxins, aflatoxins are reported with a greater degree of toxicity and known for their broader degree of contaminating food and other byproducts, for example, dehydrated fruits, maize, groundnuts, meat, milk-based products [6,7]. Aflatoxin producing species are *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus* [8,9]. Similarly, it is also produced by a certain other species known as *Aspergillus astellatus* [10]. The environment like humid and semitropical areas seemed to be highly desirable for growth of such fungi [11]. Owing to the heat resilient nature of the aflatoxins, existing food production practices are not sufficient for eradicating the infected food and feed [12].

Extreme health complications can occur to humans, when aflatoxin enters the body through contaminated food or feed [13,14]. Thus, to maintain good human health, several countries have introduced stern rules regarding the risk of contaminating and preventing aflatoxin in various foods and animal feed [15]. The maximum limit of aflatoxin in any food, estimated somewhere between 4-30 µg/kg is regarded safe for human consumption. A stern standard level with aflatoxin B1 is being maintained by the European Union (EU). It also upholds the overall level of aflatoxin B1 in any food product intended for direct consumption by humans, not exceed 2 µg/kg and the total

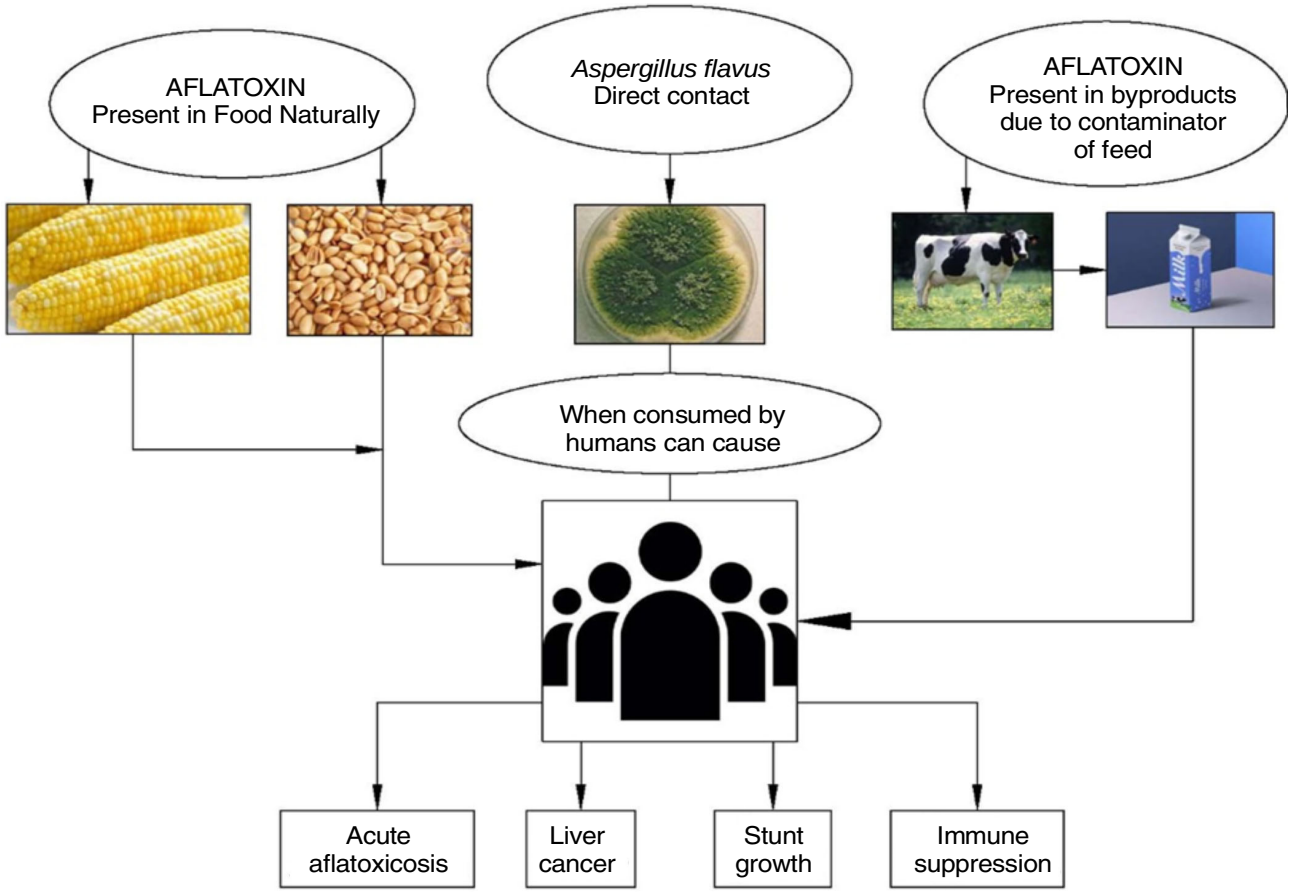

Fig. 1. Aflatoxin transmission and harmful effects on humans

aflatoxin not exceeding 4 µg/kg [16]. Likewise, in the United States, the highest tolerable limit fixed for aflatoxin is 20 µg/kg [17,18]. For the improvement in the horticulture yield, proper harvest management procedures followed by numerous ground breaking technologies plus numerous control strategies are also implemented [19]. In this review article, innovative approaches established for detecting aflatoxins in food products, with particular emphasis on concealed aflatoxin has been discussed in detail.

Aflatoxin infestation and biological action: The economic damage incurred by mycotoxicosis is a direct consequence of animal feed polluted with morbidity, death and pollution. About 25% of the earth's crops were estimated to be mycotoxin infected each year. In addition to the drawbacks mentioned above, attention must also be given to the expense of controlling mycotoxin levels.

The common mycotoxins contaminants that are present in animal nourishment are aflatoxins, ochratoxins, *Fusarium* toxins (zearalenone, fumonisin, trichothecenes, such as T-2 toxin, deoxynivalenol) [20]. Aflatoxin is a toxic and carcinogenic compound produced by the *Aspergillus* fungus with a maximum temperature of 25-32 ºC and moisture content of 12 to 16% [21]. Wheat, peanut cake, cotton-seed cake are the most commonly contaminated feeds for pigs, chickens, goats and sheep. Animal intake of aflatoxin contaminated feed can lead to carcinoma, stunted growth, aflatoxicosis, loss in weight, reduced milk production, liver failure and other type of infections. Aflatoxin M1 is metabolized by the liver and released in milk and egg/meat metabolites [22].

Aflatoxin B1 epoxide derivative binds to DNA and prevents the activities of transcription and translation, thereby inducing carcinogenesis. Free radicals and cells are impaired by the oxidative nature of toxic derivatives. Advances in nuclear technology have come to explain the precise mode of action of aflatoxins, such as microarrays and PCR [23]. Modern studies in gene expression demonstrated that significant reduction in mitochondrial carnitine palmitoyltransferase (MCPT) and decrease in various metabolic processes involving closely related lipids or fatty acid could minimize the number of B cells, thereby leading to increased cell death, reduced body weight and a weaker immune system with greater risks of fatty liver and carcinoma in livestock due to the feed [24].

Sampling and some highlights for sample preparation: The sampling of aflatoxins is especially complex since the growth of mold and the distribution of toxins in the feed and grain can be irregular. A clear "heat zone" and single sampling exposes a reasonable event that normally removes toxin from an entire batch of feed. For feed farmers or importers/exporters, this is expensive [25]. A statistically derived risk-based sampling method for tracking chemical and biological hazard sample allocations using the distribution of binomial probabilities was developed by Lee *et al.* [26]. Berthiller *et al.* [27] elaborated about few advances in the techniques of sampling and analysis of mycotoxins related to LC/MS. Multiple agencies have

released sample guidelines for testing mycotoxin in feed. The American Association of Feed Control Officials (AAFCO) have also laid out processes and procedures used to conduct an animal feed safety inspection in their Feed Inspector's Manual [28]. The Food and Agriculture Organization has recently invented a mycotoxin screening system for effective screening of the samples [29].

Commercially available aflatoxin detection devices: So many analytical methods that can be used to estimate aflatoxins in agricultural products have been developed. The methods used for identification of aflatoxin, with some evidences from literatures are mentioned below in Table-1.

Biosensors and their implication in aflatoxin: Biosensors are characterized by a high level of accuracy provided by bio-component that reacts uniquely to the analyte or substrate. Biosensor can be defined as claimed by IUPAC's concept, "A device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals". Combining this feature with a sensitive transducer device, numerous analysts had discovered unique features of biosensors, even when they are found in complex matrixes. This summarizes the benefits of biosensing approaches over certain conventional analytical techniques; thus reducing the processing time, allowing monitoring of a large number of sample separation and analysis [47]. The different types of biosensors used in the detection of aflatoxins are given in Table-2.

Detoxification of aflatoxins: Aflatoxins present in food as well as feed can be reduced, inactivated or detoxified by chemical,

TABLE-1
VARIOUS METHODS FOR IDENTIFICATION OF AFLATOXIN

Methods	Description	Ref.
Conventional methods	The introduction of HPLC is a costly technology, primarily due to the expense of facilities and the cost of employing technical operators. This is a widely known procedure for milk aflatoxin analysis. It became a tool of the Organization of Analytical Communities (AOAC) in 1986.	[30]
	For AFM_1 assessment, several others have introduced immunoaffinity columns. In all the techniques, fluorescence was the method of detection. Recently, HPLC has been used for AFM_1 identification with detailed analysis. One of the most common separation methods in aflatoxin analysis is TLC, otherwise called the planar chromatography.	[31,32]
	Since 1990, it has been the official AOAC tool and is considered the safest way to identify and measure 1 ng/g of low aflatoxin levels. This is a recognized procedure, unlike HPLC, it might have more benefits such as being cost effective over the HPLC system. Though, trained professionals aren't required for operation, it might be less sensitive than the HPLC system.	[33]
Advanced techniques	The advanced method for aflatoxin detection involves prophylactic methods. Sampling preparations for immunoassay (*e.g.* ELISA) are generally easy, inexpensive, rapid and usually derivative-free. An immunoassay is a particular type of biochemical test that measures the presence or concentration of substances (known as "analyte") that typically contain complex mixtures of substances.	[34]
	For immunoaffinity column assays (IAC), IACs are widely used to model mycotoxin assay. IAC contains anti-mycotoxin antibodies, which are immobilized on a solid support like phosphate buffer agarose gel, all of which are stored in a small plastic cartridge. Usually, the approach to LC-MS and LC-MS-MS requires chemical-based solid-phase extraction, although there is a use of immunoaffinity methods is attractive since several structurally distinct analytes are simultaneously examined. Maize silage using Oasis® HLB pellets are used to evaluate about 11 mycotoxins, including AFB1, to elute/methanol mixture.	[35]
	Quantitative methods for evaluation of aflatoxin, reduction stage is minimal for complete assessment. Liquid chromatography, for instance, is combined with thorough refining prior to injection of pure compounds. MS-based has an intrinsic advantage over conventional methods. Even when chromatographic signals overlap, the detector may distinguish between two distinct mass/charge components, so less costly and straightforward sample preparation techniques, such as QuEChERS (quick, simple, inexpensive, reliable, robust) are used in even microextraction of liquid-liquid or diluted samples. A QuEChERS system for evaluating 56 mycotoxins of *Penicillium*, *Fusarium*, *Alternaria*, *Aspergillus* and *Claviceps* that are present in feed of livestock was optimized by UHPLC-MS/MS by Dzuman and coworkers.	[36]
	A HPCL-MS/ESI+ system as early as 2006 was developed. Special attention is given to this matrix since many ruminants' diets are focused on silage and feed. Finally, adsorbents for solid-phase extraction focused upon polymers imprinted molecularly (AFFINIMIP®) that are accessible abundantly for most mycotoxin estimation. Two separate approaches that boost aflatoxin immunity are effectively supported by Pickering laboratories. The derivatized Pinnacle PCX, the second pulse pump, as well as response device could also be linked with an LCD in front of the fluorescence detector (FLD). Computer pump reagents such as perbromide solution for iodine salt or pyridinium hydrobromide perbromide (PBPB) was installed (at fixed flow rate). And the photochemical reactor was fitted with a lamp of 254 nm or a coil reactor (UV TM) that converts aflatoxins to solid fluorescent hydroxylated analogues, *e.g.* AFB1 to AFB2. Above-mentioned method has been done with several RP-HPLC grain contaminant tests using photodiodes, fluorescence detectors and a photochemical reactor. Investigators noticed that the boundaries for identification were 0.025 and 0.012 ng g^{-1}, respectively, for AFB1/AFG1 and AFB2/AFG2.	[37]
	Likewise, only a few techniques have been reported elsewhere, providing detailed information by LC for the identification of aflatoxin in multifunctioning column cleaning feeds as well as the components. Biotage Isolute® Multimode Columns have been used to test aflatoxins successfully. There are three modes of action in these basic columns: heavy cation exchange ($R\text{-}SO_3^- H^+$), weak anion-exchange [$(CH_2)_3N + (CH_3)_3Cl^-$] and hydrophobic retention [$-(CH_2)_{17}CH_3$]. The structural assessment for aflatoxin, such pathways perform the part throughout processing. Researchers record outstanding performance for AFG1/AFG2 and AFB1/AFB2, respectively *i.e.* 0.10 and 0.06 ng g^{-1} sensitivity. The mixture of acetonitrile: water (9:1) provided sufficient recovery for all aflatoxin (> 85%).	[38]

Latest techniques	A dispersive liquid-liquid micro extraction to detect aflatoxins in grains such as maize, barley and wheat was deployed. The transition solvent was chloroform, while methanol: water (8: 2) extract was selected and an enrichment factor of 2.5 was recorded.	[39]
	A device was developed to increase the ultraviolet volume of AFB1, AFB2, AFG1 and AFG2 using different propagation liquid microcircuits. The authors validate various parameters, with solution for processing chloroform, dispenser sample (acetonitrile) pH and centrifugation length and ultimately compromised the two-step process.	[40]
	In rice samples with a mixture of neutralization acid as a solvent for characterization and chloroform as a distributor, the microextraction process was concentrated 1.25 times of mycotoxins such as aflatoxin B1, B2 and ochratoxin A. It should be noted that comparing output outcomes with immunophobic column extraction or between methods as an additional step is normal for these micro transformation techniques.	[41]
	A dispersed solution micro-leachate and also cleaning technique until 72% and 58% occurrence of aflatoxin in eggs and poultry was achieved which typically deals with miniaturized analytical pretreatment options with an emphasis on green chemistry (*e.g.*, drop micro-purification, liquid-phase hollow fiber microextraction, liquid-liquid dispersive micro-processing).	[42]
	Acetonitrile was even used as processing agent for the "dilute and burn" process for evaluating AF of stimulation calibration for livestock foods.	[43]
	Clean loading of low complex models is very enticing for fast response on a comparatively low budget, but typically due to high limits of matrix interference (when injecting raw extracts). New techniques such as direct extract injection into a computerized uneven flow cleaning system fixed with LC-HRMS (high-resolution mass spectrometry [Orbitrap]) screening are constantly being developed and 600 fungal metabolites have been used to produced feed contamination profiles	[44]
	At the same time, the use of multiple external calibrations to adapt for sample was performed. Samples such as illustrating the powerful effects of the feed matrix, to identify internal standards labeled by isotopes, a single internal isotopic calibration and isotopic norm deck evolution.	[45]
	The technique for evaluating thirty types of mycotoxins (*e.g.*, aflatoxins, ochratoxin A, citrinin, trichothecenes, zearalenone, fumonisins) of livestock as well as their by-products were established. This was compared with several separation formulations, various SPE cartridges like Oasis HLB®, amino cartridges, Oasis MAX® and MycoSep® 226 multipurpose cartridges and hydrogels like C18, chitin and nanoparticles.	[46]

biological and mechanical methods. Processed products should be chemically shielded from contaminants and their vital nutrient content should not be damaged.

Physical techniques: Aflatoxin-contaminated plants, which are labor-intensive and expensive, may be manually removed by hand-picking or photoelectric detectors. Nearly 70% of aflatoxins can be inactivated when heated and boiled under pressure. About 50-70% of aflatoxin can be lowered by dry roasting and drying feed polluted with aflatoxin in the sun can decrease toxin levels up to 70%. The application with binders may reduce the biocompatibility of these compounds present in livestock, which reduce the occurrence of residual toxins within processed foods. Throughout the event of aflatoxin B1 (AFB1), phyllosilicates and regular zeolites of hydrated sodium and calcium aluminosilicate (HSCAS) are widely used for *in vivo* studies [59,60]. Zeolites, which are hydrated inorganic materials of alkaline cation, are able to absorb AFB1. Bentonites are efficient for absorbing AFB1 [61].

While clays are effective against aflatoxins, their levels of composition are not very high and without contaminants such as dioxin, they should be avoided. These compounds are likely to bind to antibiotics such as minerals and monensin when the synthesis level is so high that it is practically essential for their efficacy. Some binders can cause environmental problems and are not biodegradable [62]. Though some nutrients were lost from the feed, certain approaches like roasting as well as cooking at higher pressures were also used to detoxify aflatoxins [63,64]. Other methods like exposing the feed contaminated with aflatoxins, under direct sunlight and by using various substrates such as treatment with activated charcoal, propionic acid and monoprop also have resulted in the detoxification of aflatoxins to a greater extent [65]. Further when exposed to γ-irradiation with a dose of 10 kGy, 65% reductions in the total aflatoxins were observed [66].

Chemical techniques: Multiple compounds such as sodium hydroxide, sodium hydrogen sulphate, sodium hypochlorite, sodium sulphite, hydrogen peroxide and ammonia were studied in comparison to detoxify aflatoxins in the samples [67]. Detoxifying the aflatoxin contaminations in maize, with the help of neutral electrolyzed oxidizing water (NEW) has also been recorded [68]. Various chemical treatments including the use of oxidizing agents, ozone treatment, ammoniating agents, sulphites, dihydro sulphate, sodium bisulphide, chlorinating compounds, hypochlorite salts, formaldehyde, *etc*. were also utilized for the detoxification of aflatoxins. However, these methods are not entirely safe, time-consuming, expensive and undesirable to customers [69].

Biological/microbiological techniques: The biodegradation for mycotoxins utilizing yeast, fungal species and bacteria (LAB) has gained more attention. Fermentation of lactic acid bacteria (LAB) are believed to attach to the toxin complex to the surface of the cell wall. These will greatly reduce the risk of mycotoxins significantly and it is ultimately possible to use successful binding strains of these microorganisms to decrease aflatoxin toxicity and enhance overall animal health.

Certain yeast was examined to bind aflatoxin to cell walls to cope with soil fertility interaction. There is ample evidence to date that aflatoxins may be bound by β-glucans, fungus around the internal cell wall, unique sugars are present. Yeast-based binder's inclusion amount is much smaller than that of soil-based binders. There is a clay-like absorption potential of around 500 g of glucomannan in the yeast cell wall. At conc. of 0.05% of dehydrated mass by feeding contaminated food with AFB1, this limitation decreases the content of AFM1 in

TABLE-2
DIFFERENT TYPES OF BIOSENSORS USED IN THE DETECTION OF AFLATOXIN

Methods	Description	Ref.
Electrochemical biosensor	**i) Amperometric biosensors:** The electrochemical setup is composed of an electrode material (Ag/AgCl), as well as an auxiliary electrode (carbon, Pt, Au). The biological element on the working electrode and the constant potential applied by the potentiostat can be directly immobilized. An electrochemically active species comes into contact with the electrode during the reaction and the response is received by the detector. Mycotoxin analysis was directed to a large number of research efforts based on amperometric techniques.	[48]
	ii) Potentiometric biosensors: In this technique, the potential difference is measured under zero current conditions. This method is not very suitable for mycotoxin due to its small size.	[49]
	iii) Conductometric biosensors: In the conductometric method, the biocomponent is immobilized between two closely spaced electrodes. Here, the transducer evaluates the electrical conductivity of the cell solution.	[50]
Optical biosensor	**SPR biosensor for analysis of aflatoxins:** The surface plasmon resonance biosensor is a revolutionary technology of fast aflatoxin detection. The theory of surface plasmon resonance is focused on the identifying a distinction there in the optical properties for a material if the analyst adds an antibody receptor towards the immobilized recipient. Category related to refractometric sensors is composed of optical sensors based on surface plasmon excitation and typically SPR sensors. Production of SPR sensors used in the chemical and biological organism recognition is increased considerably. The amount of literature has increased, covering SPR biosensor applications for analytical detection during medical diagnosis, environmental control and surveillance. The protection of food has rapidly improved.	[51]
	The theory of the SPR biosensor is that surface plasmon is excited at a certain angle by the polarized laser beam and the light intensity reflected is estimated. The information including the identification of aflatoxin in wheat samples are gathered utilizing SPR biosensor.	[52]
Receptor based biosensor	**i) Labeled sensor:** Biologically active molecules like antibodies, DNA and enzymes or Artificial chemical elements like aptamers, MIP, mimotopes, *etc*. that are capable of a particular reaction with the analyte are receptor molecules used for mycotoxin quantification. A mark refers to a category of products that may be used for signal enhancement, such as electroactive species, horseradish peroxidase (HRP), α-galactosidase, alkaline phosphatase, redox material like fluorescent or antacid, electroluminescent materials, *etc*. The label's function is to amplify the signal produced by the interaction of mycotoxins with the biomolecules of the receptor.	[53]
	Horseradish peroxidase (HRP) labeled secondary antibody with a gold screen-printed working electrode were used to obtain a range between 0.1 to 10 μg L^{-1} and LOD of 0.5 μg L^{-1} were also utilized during the detection of OTA using the competitive sandwich format.	[54]
	ii) Label-free sensor: The use of a label for the detection of small size mycotoxins is a convenient way. The label has limitations such as the involvement of multi-steps and costly chemicals and the generation of a false or delayed signal. Therefore, it is always desirable to directly measure the interaction of antibody/aptamer with the target or mycotoxin by the transducer. The transmitter plays a major role for obtaining an improved sensitivity for each biosensor.	[55]
	To overcome the background current in an electrochemical sensor, polyethylene–aptamer macromolecules-based sensor was designed for the detection of OTA while macromolecules act as a tunnel for the electron transfer through medium to electrode surface.	[56]
	Although the process of aflatoxin detection plays a major role, the alteration of the matrix containing either a metal or a metal oxide nanoparticle, *etc*. has been found to boost the overall efficiency of the label-free sensing platform	[57,58]

cow's milk by 58%. It has been shown that *Lactobacillus acidophilus* CU028 (probiotic strain) binds with aflatoxin. Fermented milk containing or in conjunction with *L. casei* and *L. rhamnosus* isolates has a defensive effect on aflatoxin B1 and causes liver damage. In intestinal settings, acid treated-(LAB) can bind with large doses of aflatoxin [21].

Bacterial species involved: Research statistics state more than 40 years ago, various bacteria are able to destroy aflatoxins [70]. Bacteria previously reported to have effectively degraded AFB1 include *Nocardia corynebacteroides* [71]. Liquid cultures of bacteria that have been shown to degrade aflatoxins include *Rhodococcus erythropolis*, *Bacillus licheniformis* [72], *Nocardia corynebacteroides* [73]. Aflatoxin was also degraded by *Pseudomonas putida* into less toxic AFD1, AFD2 and AFD3 [74]. Aflatoxin detoxification by lactic acid bacteria (LAB) by means of a binding mechanism has also been reported by several authors [75-77]. Peculiar to LAB and yeast; during the detoxification process, the organisms adsorb the aflatoxin into their cell wall [78].

Fungal species involved: While some fungal species develop aflatoxin, aflatoxin has also been seen to be detoxified by some species of fungi and strains. Other metabolites that lower the pH of the medium is produced by fungal organisms and post-acid conditions have been shown to lower aflatoxin levels. These fungi possess genes such as laccases and peroxidase that lead to aflatoxin-destroying enzymes [79].

Yeast and Protozoan species involved: Method of detoxification is bonding to aflatoxin, yeast has mechanisms of action similar to lactic acid bacteria. *Saccharomyces cerevisiae* was used to reduce the toxicity of aflatoxin *in vivo* [80]. Shetty *et al.* [81] and Gonçalves *et al.* [82] have reported similar changes in yeast binding and subsequent detoxification of aflatoxin. Few researches based on protozoa in aflatoxin destruction have been performed. Pyriformis was then discovered to reduce a carbonyl group present in pentamethylene of an aflatoxin enzyme to negatively charged anion group [83]. The cells of *Tetrahymena pyriformis* reduced aflatoxins B1 levels by 67% within 48 h, producing a fluorescent blue component [84].

Conclusion

Mycotoxins including aflatoxins are one of the best well-known and harmful food-related toxins. They are important pollutants that have a variety of effects on both human and animal health, as well as creating major economic problems in the agriculture food chain. The scientific community considers certain viable screening and detection methods to be an essential response to avoid early-stage contamination in food or feed, accompanied by detection or screening approaches. This review depicts the various detection methodologies categorized under computational, advanced and latest testing procedures' used in the detection of aflatoxin in various feed samples. It also explains about the different types of biosensors such as: Electrochemical, optical and receptor-based biosensors which work efficiently and furthermore explaining the strategies involved in the aflatoxin detection. Also, several detoxification techniques including the use of physical, chemical and biological techniques which are employed in detoxification/limiting the aflatoxins to a specific range are also specified in this paper. Thus, regular monitoring of the aflatoxin levels in the food/feed is important thereby preventing various problems during promotion, dispersion and ingestion of the foods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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