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Review Article *Bacteriology*

Types of Mycotoxins and different approaches used for their detection in foodstuffs

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ABSTRACT

Mycotoxins are toxic complexes generated by various genera of filamentous fungi and pose severe public health hazards due to their carcinogenic and mutagenic properties. Toxigenic fungi produce mycotoxins on various foodstuffs when favorable conditions for their production are exist. The most important mycotoxin-producing fungal genera are Aspergillus, Fusarium and Penicillium. The major mycotoxin of concern, including aflatoxins, trichothecenes, ochratoxins, patulin, fumonisins, and zearalenone, are expressively contaminate foodstuffs, with implications for human and animal health. There are various environmental factors influencing mycotoxin production involving temperature, water activity, animal type, aeration, pH, light, and nature of substrate. The temperature and relative moistness range for ideal mycotoxin generation may shift from that steady fungal development. The high temperature and moistness stress primarily have a potential issue. A full understanding of the existence of mycotoxins in addition the implementation measures to overcome their contamination of foodstuffs is crucial. There are different important analytical techniques to precisely screen the levels of mycotoxins contamination and give a wider overview about what is the most common, pathogenic; along with further strategies to control measures. Thus, in this review sampling, sample preparation and different analytical methods for determination of mycotoxins have been discussed.

Keywords: **:** Mycotoxins in foodstuffs

1. INTRODUCTION

Mycotoxins are secondary metabolites of low molecular weight, created by number of micromycets mostly related to the genera *Aspergillus, Fusarium,* and *Penicillium*, which ubiquitously colonize foodstuffs. Mycotoxin contamination occurs either directly by consumption of the contaminated food or indirectly through animals fed on contaminated feed, particularly milk. Mycotoxins are harmful to humans and animals causing a variety of syndromes from gastroenteritis to cancer [1]. The identification and chemical characterization of several mycotoxins have been documented. Somewhat small number of mycotoxins can progress on foodstuffs for instance cereals, dried fruits, nuts and spices. Among these mycotoxins, aflatoxins, trichothecenes, ochratoxins, patulin, fumonisins, and zearalenone, significantly contaminate foodstuffs, with existent implications for human and animal health and thus are considered a public health concern [2]. Mycotoxins are recognized to be either carcinogenic (aflatoxin B1, ochratoxin and fumonisins B1); estrogenic (zearalenone), neurotoxic (fumonisins B1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes); or immunosuppressive (aflatoxin B1 and ochratoxin A) **[3, 4]**. The existence of fungi does not essentially indicate the production of mycotoxins, and not all fungal development results in mycotoxin development not all fungal growth consequences in mycotoxin construction [5].

It is vital to notice that the circumstances encouraging for fungal growth and improvement in foodstuffs and stored grains are not continuously helpful for mycotoxin production. Many foodstuffs and cereals are liable to fungal contamination either in the field or throughout storage. There are many factors affecting mycotoxin production on foodstuffs and stored grains, including environmental factors (temperature, water activity, pH, light, and nature of substrate, primarily control the mycotoxin production). Temperature and relative humidity range are the two environmental factors; vary for optimal mycotoxin production and fungal growth. Overall, mycotoxins are optimally produced at 24-28°C. Increasing humidity and temperature in the tropical and subtropical districts than in the temperate areas increase the vulnerability of crops to mycotoxin contamination [6]. Therefore, the effectiveness of climatic variations could be an issue on mycotoxigenic fungal contamination of crops. Consequently, it is hard to designate a single set of optimum circumstances for fungal growth and mycotoxin production [7]. Additionally, poor harvesting practices, improper storage, marketing and processing could favor the fungal growth and raise the dangerous of mycotoxin production. All of these interrelating factors warranted the need for production of mycotoxin free grains and foodstuffs in the last decade.

Accurate diagnosis by using satisfactory laboratory methods to differentiate mycotoxicosis from other infections and to identify the causal mycotoxin is very important to reduce economic losses and reducing public health hazards. Identification of mycotoxicosis is essential to distinguish affected animals and know how to deal with them. In addition, diagnosis is essential to choose and test the food consumed by affected animals and to manage such contaminated food either by thrown away or dilute with good food and to control the transportation, storage and handling of the similar type of food. As well, the diagnosis assists in dealing with the products originated from the affected animals. Mycotoxicosis diagnosis, allow treatment plans to be considered. Not only these contribute has reduced economic losses but also prohibited public health hazards. This article provides an insight on the most predominant types of mycotoxins, factors affecting their production and methods used for extraction and cleanup of mycotoxins from foodstuffs. In addition, the traditional and new analytical approaches for the determination of mycotoxins were reported.

2. TYPES OF MYCOTOXINS

2.1. Aflatoxins

Aflatoxins are one of the furthermost toxic mycotoxins and formed by *Aspergillus flavus* and *Aspergillus parasiticus* that are found in soil, decaying vegetation, hay, and grains [8, 9]. The production of aflatoxins is connected to spore formation by various *Aspergillus* species [10]. As well, aflatoxigenic fungi disseminate in storage zones, processing services and in the distribution schemes for manufactured products. Other less frequently aflatoxin-producing species are *Aspergillus bombycis, Aspergillus ochraceoroseus, Aspergillus nomius,* and *Aspergillus pseudotamari* [11]. From the mycological viewpoint, excessive qualitative and quantitative variances in the toxigenic aptitudes are exhibited by diverse strains in each aflatoxigenic species [12]. There are four main types of aflatoxins: B1, B2, G1, and G2 depend on their fluorescence underneath UV light and relative chromatographic mobility throughout thin-layer chromatography. Aflatoxin B1 is considered as the furthermost effective natural carcinogenic toxin [13]. During processing of contaminated food, the entrance of aflatoxins to the general food source in addition to feedstocks for raising livestock, aflatoxin has been detected somewhere in both pet and human feed. somewhere aflatoxin has been present in both pet and human feed, in addition to feedstocks for farming animals. Aflatoxin transformation products in animals could be released into eggs, milk products, and meat. All cereal crops are frequently affected by *Aspergillus* species producing aflatoxins [14]. Even more challenging is the fate of crops kept underneath circumstances which stimulate mold growth because the furthermost significant variables are the moisture content of the substrate and the relative humidity of the surrounds during storage [15]. Aflatoxins have been detected in the milk of animals which consumed contaminated feed, these animals metabolically biotransform aflatoxin B1 into a hydroxylated form called aflatoxin M1 [16]. Such milk is the main cause of aflatoxin outbreaks for humans [17]. Also, aflatoxin contaminated poultry diets reduced the poultry efficiency and are causing huge economic losses through poor body performance, in form of retarding bird growth, increasing feed consumption, and reducing meat production or through sequels of aflatoxin [18]. The toxicity in chickens is characterized by mortality, lethargy, anorexia, reduced growth rates, reduced efficiency of feed conversion, fatty liver, declined egg production, deprived pigmentation and raise sensitivity to other diseases **[9]**. High concentrations of aflatoxins could result in acute poisoning (aflatoxicosis) and so associated with high mortality in farm animals [19]. Aflatoxins have been shown to be life threatening through damage to the liver, DNA and result in animals and human liver cancer (carcinogens).

2.2. Trichothecene

Several fungal genera, involving *Fusarium, Stachybotrys, Myrothecium, Trichothecium, Trichoderma, Cephalosporium, Cylindrocarpon, Verticimonosporium,* and *Phomopsis*, are the trichothecene-producer [20, 21]. Trichothecenes are categorized as macrocyclic or non-macrocyclic, dependent on the existence of a macrocyclic ester or an ester-ether bridge between C-4 and C-15 [22]. *Fusarium* molds are the most economically important source of the non-macrocyclic trichothecenes. The genus *Fusarium* comprises numerous field fungi adapt to infect plants including wheat, corn, barley, oats, and forages. Fusarium is mostly communal in temperate climates areas, where foodstuffs contamination is widespread. Trichothecenes are potent inhibitors of protein construction and have toxicity to molds, bacteria, plants, and animals [23]. Trichothecene are usually established as food and feed contaminants. The consumption of trichothecene results in hasty irritation to intestinal mucosa leading to alimentary hemorrhage, vomiting and diarrhea, while direct contact leads to dermatitis [24]. Also, chronic exposure in animals leads to suppression of the immune system.

2.3. Ochratoxins

Ochratoxin A is produced by numerous species of *Aspergillus*, involving *Aspergillus alliaceus, Aspergillus auricomus, Aspergillus carbonarius, Aspergillus glaucus, Aspergillus melleus,* and *Aspergillus niger* [25] as well as *Penicillium verrucosum* [26]. It is a common food-contaminants defined as a potent nephrotoxin to all animal species. contamination of food supplies with ochratoxin A; for examples cereals and cereal products, coffee beans, wheat, dry vine fruits, wine and grape juice, spices and other plant products, is widespread [27, 28]. Ochratoxin A is produced throughout the storage of crops and is responsible for several toxic effects in various animal breeds. The furthermost sensitive and

noteworthy effect is kidney impairment, but also it could negatively affect fetal expansion and the immune system. Opposing to the distinct indication of kidney toxicity and cancer commonly associated with ochratoxin A in animal species, such suggestion in humans is unclear, however its impact on kidney have been established [29]. Besides being a nephrotoxin, ochratoxin A is proved to be a liver toxin, an immune suppressor, a powerful teratogen, and a carcinogen in animal species [30]. Ochratoxin had been found in blood and other animal tissues, in milk [27] and pork prepared for human ingestion [31]. Furthermore, ochratoxin is related to disease and mortalities in poultry [32].

2.4. Patulin

Patulin is a toxin formed by a variability of molds, particularly *Aspergillus*, *Penicillium (P. griseofulvum;* formerly *Penicillium patulum* and *P. expansum)* and *Byssochlamys*. It is frequently present in rotting apples and apple products. As well, patulin occurs in several moldy fruits, grains and other foods. Main human nutritional sources of patulin are apples and apple juice produced from contaminated fruits. Although patulin has antibacterial, antiviral, and antiprotozoal effects, it was toxic to both plants and animals [33, 34]. Thus, patulin is discontinued from its clinical uses as antibiotics and classified as mycotoxins [35]. Patulin is genotoxic and associated with the following acute symptoms in animal species; liver, spleen and kidney damage and the immune system toxicity, while in humans it is responsible for nausea, gastrointestinal disorders and vomition.

2.5. Fumonisins

Fumonisins are formed by several *Fusarium* species, especially *Fusarium verticillioides*, *Fusarium proliferatum,* and *Fusarium nygamai,* besides *Alternaria alternata* f. sp. *lycopersici* [36]. Fumonisin B1 is the furthermost abundantly produced by member of the family. *Fusarium verticillioides,* the main species of economic importance and exist in all corn [36]. Fumonisins have showed its effects on animal species through interfering with sphingolipid metabolism [37, 38]. The most common syndromes associated with fumonisins are leukoencephalomalacia in horses (39); pulmonary edema and hydrothorax in pigs [40]; and hepatotoxicity, carcinogenicity [41] and apoptosis in the liver of rats [42]. An opportunity of esophageal cancer may occur in humans as well[43].

2.6. Zearalenone

Zearalenone is one of the mycotoxin formed by numerous *Fusarium* species, principally *F. graminearum* and further species as *Fusarium crookwellense, Fusarium culmorum,* and *Fusarium equiseti*, that are regularly contaminating cereal crops [44]. The biological effectiveness of such mycotoxins is significant, but the real toxicity is slight. Higher concentrations of zearalenone consumption (zearalenone toxicosis) increase estrogenic activity and result in disturbed conception, abortion, and other reproductive problems such as infertility, vulval edema, and feminization of males in many animal species; particularly swine, cattle and sheep [45, 46].

3. FACTORS AFFECTING MYCOTOXIN PRODUCTION

3.1. *Temperature and water activity*

The temperature and water activity are the most important parameters influencing thegrowth of fungi producing mycotoxin and mycotoxin synthesis. Furthermost fungal species make a maximum level of mycotoxins at their optimum growth temperature. Generally, mycotoxins are optimally produced at 24-28°C, though some exceptions may be found. The lowest and optimum temperatures and water activity for growth and mycotoxin production of the foremost mycotoxin-producing fungal species are various [47, 48]. The warm conditions are favorable for aflatoxin producer so, the tropical and subtropical areas demonstrate high existence of mycotoxins [49]. The optimum temperature and water activity for aflatoxin producing were 33°C and 0.99, successively [50]. Although toxin formation still obvious at 32°C, temperatures overhead 33°C evidently interfere aflatoxin producing even supposed enhancement of fungal growth was occurred [51]. The toxins are not produced at low temperature (8°C). A higher toxin production by *Fusarium* species occurs after incubation at a low temperature of 12–14°C [52].

Depending on the moisture necessities, mycotoxin-producing fungi have been classified into three sets: field fungi, storage fungi, and advanced decay fungi. The field fungi include species of the genera *Alternaria* and *Fusarium*. The storage fungi primarily comprise species of the genera *Aspergillus* and *Penicillium* [53]. The advanced decay fungi contain species of the genera *Cladosporium*, *Fusarium*, and *Trichoderma* [54]. The field fungi producing mycotoxin need a grain moisture contented of 22-25%, whereas storage mycotoxin producing frequently require a grain moisture of 13-18% (equivalent to 70% to 90% relative humidity), and advanced decay fungi grow in more than 18% moisture [55]. Grains and stored crops underneath these environments could simply be affected by fungi mycotoxin-producers [56]. Consequently, the dominant mycotoxin contamination of grains and crops in tropical and subtropical areas is associated with a great health hazard.

3.2. Atmosphere

The adapted atmosphere with raised up carbon dioxide and diminished oxygen levels has inhibitory effect on the growth and mycotoxin producing of toxic fungal species [57].

3*.3. Nature of substrate (foodstuffs)*

There are a lot of foodstuffs, including cereal grains (wheat, rice, corn, maize, and sorghum), oilseeds (sunflower, peanut, cottonseed, and soybean), spices (black pepper, chillies, and

ginger), tree nuts (pistachio, coconut, and walnut), which may be affected with mycotoxins, whereas peanuts and maize are the furthermost liable foodstuffs [58] due to peanuts growth in the soil, numerous fungal species affect peanut shell, testa, and seed. Also, any mechanical impairment throughout harvest, drying, and storage rises the accidental fungal infection, fungal growth and mycotoxin creation (Ref.). Milk of animal and human and milk products as cheese, and butter could be a base of mycotoxin contamination upon consumption[49].

3.*4. Animal species*

Monogastric farm animals as chickens and pigs are more vulnerable to mycotoxins, particularly aflatoxins. There are two reasons for such high susceptibility: firstly, they consumed a large part of cereals in their food. Secondly, these animals are deficient in the ruminal reservoir of a multitude of microorganisms. However, ruminants appear to be less vulnerable to mycotoxins rather than other animals because their rumenal flora have the capacity to transform some mycotoxins into less carcinogenic metabolites or biologically inactive compounds [59].

3.5. pH

The synthesis of mycotoxins is observed on acidic pH [60]. For example, the pH level for *Aspergillus niger* growth and its mycotoxin production is from 4 to 6.5 [61].

3.6. Light

UV and fluorescent light have the capacity to detoxify mycotoxins or hinder the synthesis of mycotoxins to diminish their hazardous effects. For example, some mycotoxins produced by *Aspergillus parasiticus, Fusarium verticillioides, Scopulariopsis fusca* and *Verticillium lecanii* were completely eliminated when they exposed to fluorescent light, short and long UV and kept for three weeks underneath diverse relative humidity (50-80%) at room temperature [62].

3.*7. Other factors*

More mycotoxin contamination can be detected in subtropical and tropical countries with poor infrastructures such as processing facilities, transportation, storage, and skilled human resources [63].

4. DETECTION OF MYCOTOXINS

4.1. Sampling

It is very important to obtain a representative samples for determining mycotoxins**.** Mycotoxins usually are not evenly distributed in stored commodities and tend to generated in isolated pockets. In addition, due to its heterogeneity there are difficulties in collecting representative samples**.** The inconsistency connected with mycotoxins analyses decreases by intensifying the sample size, degree crushing, subsample size, and the amount of aliquots quantified **[64].** For liquid dairy products s milk, there is no doubt in the detection results. However, in solid products as cheese, it is imperative to get a homogenous sample. Therefore, the whole sample is necessity to be crushed and mixed carefully before starting analysis to have the same concentration of toxin. The European Commission (EC) has defined necessities for collecting samples and performance criteria for analytical techniques to obtain comparable data **[65]**. Therefore, sampling, extraction and clean up and determining methods used have to subject to a validation procedure to meets all performance criteria.

4.2. Extraction and cleanup

Analytical methods based on chromatography necessitate using appropriate solvents to liberate the mycotoxin from the sample matrix. Therefore, samples preparation is very important which involves two important steps of extraction and clean-up. Extraction methods are strongly affected the recovery of the specific compounds and therefore the accuracy of the results. A suitable extraction solvent should be efficient to remove mycotoxins, inexpensive, safe to the user, and reduces matrix effect. The extraction techniques should be fast and effective and of low costs. Additionally, environmental pollution due to using a chlorinated solvent should be taken in consideration. Various combinations of solvents are used for extraction. Organic solvents are frequently used in the extraction protocols such as methanol, acetone, based on the physical properties of mycotoxins on interest, matrix of samples and the subsequent type of clean-up performance [66]. While, water can be used for extraction of polar mycotoxins, as well as non-polar organic solvents used for other compounds. Sample pretreatment methods include many techniques for examples liquid–liquid extraction (LLE), supercritical fluid extraction (SFE), solid phase extraction (SPE).

4.2.1. Liquid–liquid extraction (LLE)

The liquid-liquid partitioning or extraction (LLE) is depended on the dissemination of analytes in two nonmiscible stages, wherever the analyte is transmitted from one stage into the other. The compound is extracted into one solvent separating from the rest of the matrix in the other. The phases are frequently an aqueous solvent (hydrophilic) and a hydrophobic organic solvent [67]. Therefore, solvents as cyclohexane and hexane are useful to eliminate non-polar contaminants, e.g. lipids and cholesterol [68]. Though, it is time consuming, and is dependent on which matrix is useful, and which complexes are been detected. Difficulties of this strategy are with probable loss of sample by adsorption onto the glassware, the elevated amounts of applied organic and previously chlorinated solvents, and formation of emulsions. New establishments in LLE comprise the usage of aqueous two-phase polymeric systems, aqueous two [69].

4.2.2. Supercritical fluid extraction (SFE) usages

SFE practices a supercritical fluid as carbon dioxide for extraction of the essential complex from the matrix. Such technique is properly performed because of the elevated solvating power, and density of the solvating liquid, on the other hand it is not an effective technique due to the complications linked to SFE. As well as, such technique is unsuitable for repetitive analysis because of its high costs **[70]**.

4.2.3. Solid phase extraction (SPE)

Solid phase extraction is dependent on the difference of chromatographic techniques using one-use cartridges full with silica gel. In solid phase extraction, the sample is overloaded in one solvent, commonly underneath reduced pressure followed by rinsing to eliminate the contaminants and then eluted in another solvent [71].The cartridges can bind of small molecules. Overall, SPE is more effective than LLE, easily to achieve, quickly and could be certainly automated. Furthermore, there is no requirement for usage of chlorinated solvents; in addition a lesser amount of organic solvent is needed and less timewasting.

4.3. Analytical techniques

4.3.1. Conventional analytical techniques

There are different common conventional chemical analytical methods applied for detection of mycotoxins from various samples which refer to chromatographic separation combined with a suitable detection system. High performance liquid chromatography (HPLC) with diverse detectors is used commonly for routine analyses and as confirmatory technique for the modern techniques [72]. Mass spectrometer is the detector of choice rather than tandem mass spectrometer [73]. Fluorometric detector for HPLC is common because of its sensitivity, low cost and simplicity, though derivatization is required for most mycotoxins. Also, other detectors for HPLC are applied, particularly Ultra Violet-spectrometric. Gas chromatography (GC) is frequently used for detection of some mycotoxins such as volatile mycotoxins, followed by electrophoretic methods, modern thin-layer chromatography and others [74].

4.3.1.1. Thin layer chromatography (TLC) Method

TLC is a prevalent technique applied for mycotoxin analysis, due to its capability to investigate great numbers of samples, low operating cost and simplicity of determination of target compounds [75]. TLC techniques practice percentage has declined to reach 7% in 2002, but this method is still recommended for quantitative and semi-quantitative purposes [76]. Such method is applied as a qualitative process, after coupled with densitometry it becomes used more for quantitative analysis. However, TLC is essentially requires sample preparation and clean up protocol based on the physical characteristics of the matrix and the kind of the toxin. Silica gel columns used for purification of mycotoxins. Nowadays, TLC methods are still used for the recognition of aflatoxin (AF) in some plant material. Due to its low costs and less equipment required, TLC techniques are occasionally performed for the detecting mycotoxins in raw herbal drug substances. Numerous progressive techniques have been applied throughout the last years for the mycotoxin quantitative assessment in food and cereals.

4.3.1.2. High Performance Liquid Chromatography (HPLC)

It is quantitate method used as a reference technique for mycotoxins detection. It is expensive and needs qualified persons. Its separating is frequently attained on C_{18} reversed phase columns with methanol/water mixtures as mobile phase [77]. Current analysis of mycotoxins deeply depends on HPLC using numerous adsorbents dependent on the physical and chemical structure of the mycotoxin. Normal and reversed-phase columns are applied for separating and purifying toxins basing on their polarity. Small mini columns are performed for sample pretreatment and large scale preparative columns are applied for preparing mycotoxin standards [78].

High-performance liquid chromatography (HPLC) with a FLD detector

It is the furthermost generally used approach for Aflatoxins (AF) detection in herbal medicine mediums**.** AFB_1 and AFG_1 fluorescence is expressively quantified by aqueous solvent mixtures in reverse-phase chromatography. Consequently, a derivatization reaction is naturally applied for identification. Over the previous years, both pre- and post-column derivatization protocols have been recommended.

Ultra-high performance liquid chromatography (UPLC) is lately carried out to detect mycotoxins in herbal medicines [79]. UPLC improve chromatographic resolution compared to traditional HPLC and UPLC, in addition, it is more sensitive and less time consuming which is more appropriate for high-throughput determination of trace complex mixtures.

4.3.2. Rapid Screening Technologies for Mycotoxin Analysis

Immunological techniques are rapid qualitative analyses carried out for detecting mycotoxins in herbal medicines. Immunological methods mostly used for rapid screening. These techniques characterized by simplicity of sample preparation, low costs. Conversely, it sometime gives false-positive results

4.3.2.1. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is immune response between antigen and specific antibody in presence of catalytic enzyme [80]. ELISA is commonly used because it is rabid, simple and somewhat inexpensive technique. There are some commercial kits used in ELISA technique used for detection on common mycotoxins such as AFs, OTA, trichothecenes, FBs, and CIT [81]. Most of these kits applied for determination of mycotoxins in foodstuff. Because of the complex co-extract of herbal samples, leads to unspecific reactions of antibodies, results in mis-calculation [82]**.**

Conclusion

Mycotoxins are toxic metabolic substances formed due to improper feed storage and may result in massive outbreak of diseases in humans and animals. The most important types of mycotoxins posing potential hazards comprise aflatoxins, trichothecene, ochratoxin, patulin, fumonisins, and zearalenone. Environmental factors, such as temperature, water activity, atmosphere, pH, and light, may considerably affect fungal growth and mycotoxin formation in foodstuffs. Thoughtful the influences of these factors are useful to develop the efficient strategies for controlling mycotoxigenic fungi and mycotoxin production. In addition, the recent diagnostic methods of mycotoxigenic fungi and mycotoxins detection assist the developing of control measures to reduce mycotoxin formation. Consequently, this review helps in understanding different types of mycotoxins, factors affecting mycotoxin production, and recent methods for their diagnosis. Further research studies are necessary to give a better understanding of fungal control approaches, involving environmental and biological measures.

Conflict of interest statement

No conflict of interest.

Authors'contribution

Rasha M. Elkenany and *Amal Awad* shared in writing and revision the paper and took the responsibility of correspondence to the journal. All authors approved the final version of the manuscript for publication.

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