

Emerging Fungal Pathogens and Mycotoxins: Epidemiology and Extraction

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Abstract

Emerging fungal infections and mycotoxins pose a severe threat to food safety, agriculture, and global health, necessitating rapid action and innovative solutions. Secondary metabolites of filamentous fungi, known as mycotoxins, have the potential to impair both human and animal health. The bulk of filamentous fungi that produce these mycotoxins fall into four genera: Alternaria, Penicillium, Fusarium, and Aspergillus. In addition to their capacity to contaminate crops and ruin food, mycotoxicogenic fungi offer a long-term and major hazard to agriculture, including the risk of malnutrition when nutritional quality is reduced. Because these diseases and toxins are so complex, detecting and identifying them can be extremely difficult; sophisticated molecular and analytical procedures are frequently necessary. The chemical diversity and great stability of these toxins complicate matters, enabling them to endure the usual processing, storage and transportation conditions. Their prevalence in significant commodities including cereals, fruits, and animal feed leads to both acute and chronic health issue, including hepatotoxicity, carcinogenesis, immunological suppression, and gastrointestinal disorders. The chapter also highlights the significant economic burden resulting from crop rejection, livestock losses, and the heightened demand for surveillance and mitigation strategies. Recent advancements in analytical technologies, such as chromatographic techniques, ELISA, biosensors, and molecular assays, have significantly improved detection accuracy, however they remain difficult to implement in resource constrained environments. Additionally, the extraction methodologies covered QuEChERS, SLE, LLE, ASE, SFE, MAE and VALDS-ME are essential for enhancing sample preparation and lowering matrix interference. Therefore, reducing mycotoxin exposure and ensuring global food security require integrated approaches that combine pre-harvest management, post-harvest control, and effective extraction and detection techniques.

Keywords

Fungal Pathogens, Mycotoxicogenic Fungi, Mycotoxin Determination, Chromatography

1. Introduction

Invasive mycosis caused by new opportunistic fungal infections and mycotoxins has expanded dramatically over the previous two decades. Mycotoxins are toxic secondary metabolites produced by filamentous fungi in the genera Aspergillus, Penicillium, Fusarium, Alternaria, Claviceps, Mucor, and Rhizopus under specific conditions such as high humidity, poor agricultural practices, crop storage conditions, and inadequate transportation facilities during international trade [1]. Mycotoxins are widespread and can contaminate a wide range of foods and agricultural commodities at any stage of the food production process, exposing humans directly to mycotoxin-contaminated food products or indirectly through feeds. Mycotoxins consumed through food produce severe intoxication in both people and animals, known as mycotoxicosis. Mycotoxin, which is mutagenic, teratogenic, cytotoxic, neurotoxic, and carcinogenic, as well as capable of inhibiting DNA and RNA synthesis via interaction with nucleic acids at the cellular level, can cause a variety of health problems, including central nervous system disorders, hepatotoxicity, cardiotoxicity, nephrotoxicity, gastrointestinal tract damage, cancer, and even death in the worst-case scenario [2]. This rise in diseases and disorders has also resulted in an increase in morbidity and mortality rates, particularly among people undergoing or who have previously undergone solid-organ transplantation, blood and bone marrow transplantation, major surgery, AIDS patients, and premature babies, which is a major concern in public health and welfare around the world. The most prevalent and significant mycotoxins include aflatoxin, ochratoxin, ergot alkaloids, fumonisins, deoxynivalenol, zearalenone, enniatins, patulin, trichothecenes, Alternaria toxins, and T-2 and H-2 toxins [3]. Various mycotoxins are very resistant to chemical, physical, and biological treatments, as well as heat procedures, making them extremely difficult to remove from food during processing. Detecting and identifying certain fungal infections and mycotoxins is highly difficult due to their complexity; sophisticated molecular and analytical approaches are frequently required. This chapter offers numerous integrated methodologies and approaches to addressing these difficulties, which are built on two pillars: current pre-harvest contamination prevention tactics and pathogen detection and removal after harvest [4]. Innovative approaches include molecular assays such as the enzyme-linked immunosorbent assay (ELISA), chromatographic methods such as high-performance liquid chromatography (HPLC), gas chromatography, high-

resolution mass spectrometry, biosensors, portable detection devices, and immunochemical-based testing. The study of natural antifungals and biocontrol agents provides hope for safer and longer-lasting therapeutics [5].

2. Epidemiology and Effect on Economy

According to the Food and Agriculture Organisation (FAO), one-fourth of global food products and raw materials are infected with mycotoxin after pre-harvest or post-harvest strategies each year, resulting in an estimated loss of 1.3 billion metric tonnes of global food and food products, wasting 1.47-1.96 Gha of arable land, 0.75-1.25 trillion cubic litres of water, and 1-1.5% of total global energy. Approximately 50% of global cereal products are infected with mycotoxin, with almost 10% permanent [6]. According to several studies, more than 5 billion people are exposed to mycotoxins on a daily basis from unknown sources and even consume mycotoxin-infected food. In 1960, 100,000 turkeys in the United Kingdom perished after being fed mouldy and aflatoxin-contaminated food. Severe aflatoxicosis outbreaks caused by aflatoxin-contaminated maize have killed hundreds of people in India, Malaysia, China, and Kenya, in addition to causing hepatocellular carcinoma or liver cancer, vomiting, oedema, and stomach pain [7,8]. Mycotoxin-contaminated maize caused long-term health and economic problems in the United States. Mycotoxicogenic fungi and mycotoxin production not only pose a threat to human health and welfare, but also cause a number of economic losses. A) Reduced crop output due to increased plant diseases and the removal or discarding of afflicted plants and products. B) Reduced quality and nutritional content, lowering the product's economic worth. C) Increased animal health problems caused by mycotoxin-contaminated feed, lowering productivity. D) Increase investment in the use of chosen resistant cultivars and fungicides to reduce mycotoxicogenic fungus contamination, maintain optimal sterile storage and transportation conditions, and establish suitable surveillance networks to assure animal and human health. Thus, in addition to being a potential concern to human health, it is also a serious threat to the world economy and must be addressed as soon as possible utilising precise detection and purification techniques [9,10].

3. Types of Mycotoxins

The major mycotoxins produced by different fungal genera, which are the organism source are summarized in Figure 1.

3.1 Aflatoxins

Aflatoxins (AFTs) are the most significant mycotoxins in the world in terms of occurrence, toxicity, and economic impact. Aflatoxin B (AfB), the primary toxin produced, is one of the main forms of aflatoxin (AFT). Farm animals metabolize these forms to produce the less toxic aflatoxin M1 (AfM1) and M2 (AfM2), commonly found in meat, milk, and its derivatives. As per Varga et al. (2011), the primary producers of *Aspergillus flavus* (AfB1 and AfB2) and *A. parasiticus* (AfB1, AfB2, AfG1 and AfG2) are those fungi that cause AFTs. Even though the following *Aspergillus* species are also aflatoxigenic: *A. nomius*, *A. sergii*, *A. bombycis*, *A. minisclerotigenes*, *A. parvisclerotigenus*, *A. pseudocaelatus*, *A. pseudotamari*, and *A. ochraceoroseus*, their relevance to the occurrence of mycotoxin is minimal. *Aspergillus* species are widely recognized as saprophytes because they can grow on a wide range of substrates. Certain species can colonize crops in the field and show some limited parasitic ability under the right circumstances. Xerophyte species such as *Aspergillus flavus* and *Aspergillus parasiticus* can thrive and multiply in low-water environments (aw < 0.85), as well as during dry spells. AFTs are thought to be the most potent mycotoxin currently known to science because of their interactions with proteins, enzymes, RNA, and DNA, which can result in acute or chronic liver diseases as well as teratogenic, mutagenic, and chronic hepatocarcinogenic effects [11].

3.2 Ochratoxin A

Ochratoxins are mycotoxins generated by various *Aspergillus* and *Penicillium* species. Ochratoxins are divided into three categories: ochratoxin A (OTA), ochratoxin B (OTB), and ochratoxin C (OTC), with OTA having the highest prevalence, toxicity, and significance for human and animal health compared to OTB and OTC. OTA was first found as a metabolite of *Aspergillus ochraceus* and then isolated from numerous other *Aspergillus* species. However, the first report of OTA occurring naturally came from a strain of *Penicillium viridicatum*, which was eventually called *P. verrucosum*. The "black aspergilli," or *Aspergillus* Section Nigri, *A. carbonarius*, and *A. niger*, which are commonly found in grapes, wine, and coffee, are the primary producers of OTA. It is less common for *Aspergillus ochraceus* and such of its kinds to generate such toxin in coffee and occasionally in stocked grains. Only *P. verrucosum* and the closely related *P. nordicum* are known to produce OTA. *Penicillium verrucosum* is typically found in cereals grown in moderately cold climatic condition, while *P. nordicum* is occasionally noticed in preserved meats [12].

Trichothecenes: Trichothecenes, a large class of fungus sesquiterpenoid secondary metabolites, are produced by species including *Fusarium*, *Myrothecium*, *Spicellum*, *Stachybotrys*, *Cephalosporium*, *Trichoderma*, and *Trichothecium*. There are currently about 200 identified trichothecenes, but only a limited number of them are commercially and environmentally significant since they have the ability to infect key commodities, resulting in mycotoxicoses in both people and animals. These chemicals originate from *Fusarium* spp. Trichothecenes are classified into four types: Type A contains toxins such as T-2, HT-2, diacetoxyscirpenol [DAS], and neosolaniol [NEO], Type B contains toxins such as nivalenol [NIV], fusarenon-X [FX], and deoxynivalenol [DON], and so on. According to Ferrigo, Raiola, and Causin (2016), type A analogues are considered more hazardous than type B equivalents. Type A includes toxins like T-2, HT-2, diacetoxyscirpenol [DAS], and neosolaniol [NEO], type B includes toxins like nivalenol [NIV], fusarenon-X [FX], and deoxynivalenol [DON]," and so on. As per (Ferrigo, Raiola, and Causin 2016), type A analogues are thought to be

more toxic than type B analogues. Type A primarily causes bleeding, diarrhoea, and vomiting, but it can also promote other toxicity effects like necrosis, myelosuppression, hematotoxicity, and retarded growth. TCTs are mostly found in cereals, such as rice, rye, corn, wheat, barley, and oats, besides being detected in some cereal-derived products, such as noodles, bread, beer, and breakfast cereals, as well as sunflower seeds, bananas, soybeans, potatoes, and peanuts [13,14].

Zearalenone: Zearalenone (ZEN) is a macrocyclic β -resorcyclic acid lactone that is primarily produced by a number of *Fusarium* species, including *F. equiseti*, *F. culmorum*, and fungal species of *F. graminearum*, *F. semitecum*, and *F. crookwellense*. Highly humid, extremely moist and temperate climatic conditions for storage account for typical environment required for the growth of ZEN producing fungi. ZEN has been considered an oestrogenic mycotoxin because of its structural resemblance to natural oestrogens. This can have a very noticeable oestrogenic effect on both humans and animals. In girls, for example, exposure to ZEN may have an oestrogenic effect that causes early puberty. Furthermore, according to El Golli Bennour, Bouaziz, Ladjimi, Renaud, & Bacha (2009), ZEN may cause toxicity by causing the production of reactive oxygen species (ROS). Additionally, ZEN can be partially eliminated in high-temperature conditions, but it has good stability at normal cooking temperatures. As of right now, numerous nations have reported experiencing ZEN, including Egypt, Iran, Thailand, Germany, China, the Philippines, Japan, Croatia, and Egypt. ZEN is commonly present in barley, corn, rye, sorghum, and wheat. Maize exhibits the highest ZEN infection rate among these cereals. In Canada and the US, wheat and maize are the primary sources of ZEN contamination. However, ZEN frequently contaminates wheat, rye, and oats in European nations. Remarkably, it has been discovered that ZEN contamination occurs concurrently with OTA, AFB1, FB1, and deoxynivalenol (DON) [15,16,17].

3.3 Ergot Alkaloids

Many species of pathogens that infect grasses and cereals in the genus *Claviceps* produce mycotoxins known as ergot alkaloids (EAs). EAs can be divided into four main groups: ergo peptides, lysergic acid amides, clavines, and lysergic acids. The most significant substance is ergotamine, which is a member of the ergopeptide family and a precursor to the psychoactive drug lysergic acid diethylamide (LSD). EAs are more common in rye and rye processing products due to *C. purpurea* but are also found in different types of cereals including wheat, rye, triticale, oats, barley, and millet. A serious disease that causes male sterile sorghum seed is caused by *Claviceps africana*, which is also significant in terms of mycotoxins. EAs demonstrate toxicity in both humans and animals, resulting in convulsions, hallucinations, false acuity, agalactia, fever, and acute burn in humans and hypersensitivity, convulsions, decreased productivity, internal haemorrhage, suppressed lactation, abortion, diarrhoea, and gangrene in animals [18].

3.4 Patulin

Patulin (PAT), a polyketide mycotoxin, is primarily produced by several *Aspergillus* and *Penicillium* species, including *A. clavatus*, *P. patulum*, *P. griseofulvum*, *P. urticae*, *P. expansum*, *P. leucopus* and *Penicillium crustosum*. Worldwide reports of PAT have been made in a wide range of fruits and vegetables, with apples and apple-processing products being the most commonly affected. Other fruits that have been reported to contain PAT include oranges, grapes, pears, and their processing products. During fruit juice processing, PAT can be transferred to juices without having to be removed from rotten fruits. Furthermore, research revealed that in apple juices from all over the world, about half of the analytical samples had a comparatively higher level of PAT. Moreover, Chinese law, the US Food and Drug Administration, and the EU have established a 50 $\mu\text{g/L/kg}$ upper limit for PAT in apple and fruit juices [19].

3.5 Citrinin

A secondary benzopyran metabolite, citrinin (CIT) is primarily generated by *Penicillium expansum* and *Aspergillus* and *Monascus* species. It has been reported that pig nephropathy and yellow rice disease are connected to CIT. Furthermore, CIT and OTA can occur simultaneously in order to inhibit the mechanism of RNA synthesis in the kidneys of murine. Moreover, humans are susceptible to cancer from CIT exposure. The fungus responsible for producing CIT primarily grows on stored grains, and research has shown that barley is a suitable substrate for this growth [20].

3.6 Fumonisins

Fumonisins (FUMs) are a class of mycotoxins primarily produced by *Fusarium proliferatum* and *F. verticillioides*, with a small amount of other *Fusarium* species also producing them. Recent data suggests that *A. niger* produces FUM on a fairly regular basis. It was found that FUMs caused by *A. niger* are more common than previously thought, as the bacteria are commonly found on onions and in some fresh fruits, especially berries. There are currently 28 distinct forms of FUM that are divided into four groups: FUMs A, B, C, and P. The most prevalent and commercially significant form is fumonisin B1 (FB1), which is followed by fumonisins B2 and B3. Several studies, FB1 which is thought to be the primary driver for cancer in human is the very commonly found protein having highest concentration level. Animals exposed to FUMs may develop equine leukoencephalomalacia, while pigs may experience hydrothorax, thorax swelling, and porcine pulmonary edema syndrome. Furthermore, reports have stated that eating maize kernels contaminated with FUMs increases the risk of esophageal cancer, neural tube defects, and esophageal cancer incidence in humans. FUMs have been found to contaminate soybeans, black tea, grapes, asparagus spears, raisins, sorghum, barley, medicinal plants, wheat, milk, and figs, in addition to corn grains and corn-based products [21].

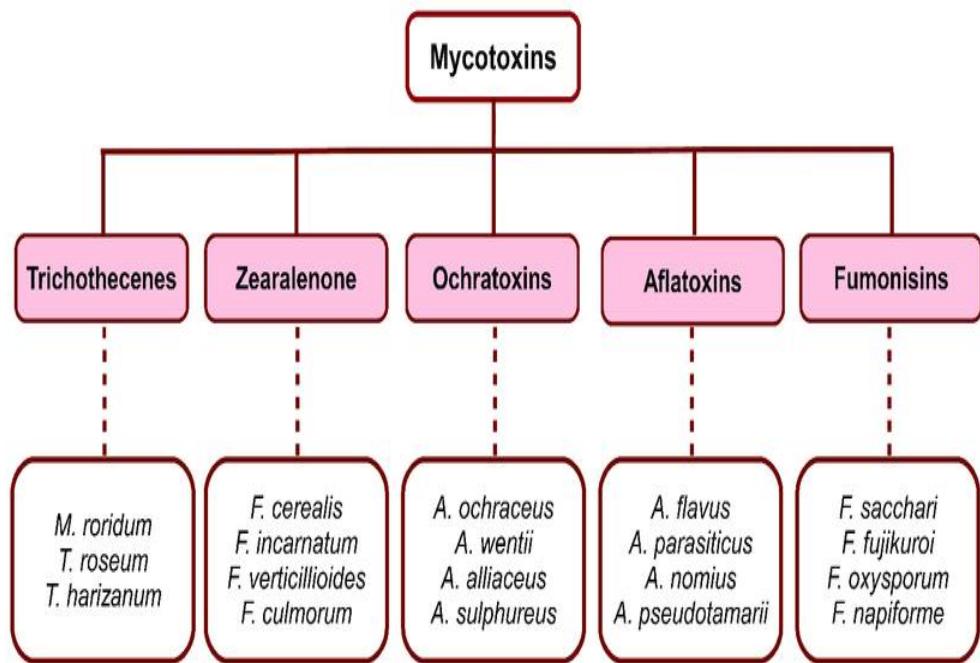


Figure 1. Types of Mycotoxins and it's organism source

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4. Extraction Methodology and Solution

Using the appropriate solvents, mycotoxins must first be extracted and purified from food samples in order to prepare the samples. Extraction is required to release the mycotoxins from the matrix and remove any materials that could make it difficult to identify the mycotoxins. The primary objectives of sample preparation include obtaining quick findings, optimising the process, and identifying environmentally appropriate solvents. Methods for extraction and cleansing are the most important before mycotoxin analysis. In order to remove mycotoxins from the material, the tainted food and feed samples are extracted using the appropriate solvents. To greatly improve the success of the extraction process, the most important factors are the appropriate extraction method and solvent selection. It lessens the matrix impact, is affordable, and safe for the user. The two most commonly utilised extraction solvents in mycotoxin analysis are methanol and water mixture, as well as acetonitrile and water in differing ratios. Several extraction solvents, such as a combination of ethyl acetate and formic acid, must be used due to the presence of pigments, essential oils, and fatty acids in the samples, which make extraction challenging. Mycotoxins have also been extracted using different extraction solvents, such as 1-octanol and toluene, dichloromethane, acetone, and chloroform. While polar mycotoxins, such as FBs, are soluble in water, hydrophobic mycotoxins are readily dissolved in all of the previously mentioned solvents [22].

Table 1 shows the types of extraction methods and it's advantages and disadvantages, the extraction methods including QuEChERS, SLE, LLE, PLE or ASE, SFE, MAE and VALDS-ME.

QuEChERS which stands for - Quick, Easy, Cheap, Effective, Rugged, and Safe method was earlier introduced to perform pesticides analysis. To identify mycotoxins in various grids, QuEChERS is the technique employed. Using acetonitrile and water, the toxin is initially extracted, and mycotoxins are subsequently transferred to the organic phase by adding inorganic salts to induce liquid-liquid partitioning. It generates high quality results of mycotoxin analysis and is pretty fast-paced. QuEChERS has been utilized in jam and juice made from berries to analyze mycotoxins like OTA and Afs [23].

SLE stands for liquid-solid extraction. It is a technique that involves weighing a homogenized sample in an extraction solvent and shaking it. SLE mostly employs ultrasonic extraction, homogenization, and shaking for mycotoxin analysis in agricultural goods with solid structures [24].

The liquid-liquid extraction (LLE) approach relies on the fact that toxins are soluble in distinct aqueous and organic phases. Moreover, LLE has been used to analyze OTA and AFs. Mycotoxins have varying solubilities in the two immiscible phases that are employed in LLE from separate solvents. The nontarget materials are eliminated in one step, and the mycotoxins are eliminated in the subsequent phase [25].

Pressurized Liquid Extraction (PLE) or Accelerated Solvent Extraction (ASE) is a technique that enhances the extraction of mycotoxins by operating at higher temperatures (100–180°C) and pressures (1,500–2,000 psi) in a vessel.

Using this technique, mycotoxins in tomato samples were found. Utilizing conventional solvents and temperatures above the boiling point, ASE is a quick, easy, and environmentally friendly extraction method [26].

The extraction of non-polar chemical compounds, like ZEA found in maize flour, is accomplished by the use of supercritical fluid extraction (SFE). The benefit of the SFE approach is that supercritical CO₂ reduces and eliminates the need for organic solvents [27].

MAE is considered an environmentally friendly method that needs less time for extraction and few organic solvents. This method involves quickly heating organic solvents using microwave radiation in order to segregate mycotoxins inside the solution. Water, an extraction solvent, and a dispersive solvent are the three main solvents used in this technique of VALDS-ME [28].

Table 1. Types of extraction methods and it's advantages and disadvantages

Methods of Extraction	Extraction Solvents	Advantages	Disadvantages	Reference
QuEChERS	Organic solvents and mixtures (CH ₃ CN, MeOH, CH ₃ CN/MeOH)	Low ppb level detection that is affordable, quick, easy to use, and has improved repeatability and accuracy	Alterations to the first process and the requirement for a further enrichment phase	Gonzalez-Jartin et al., 2019
SLE	Combination of diluted acids or water and organic solvents	Reduced solvent volumes	Matrix effects	Rubert et al. (2012)
LLE	Combination of diluted acids or water with organic solvents (cyclohexane and hexane)	Efficient when carrying out small-scale preparations	Time-consuming, based on the matrix and identified substances, the sample may be absorbed by the glass apparatus.	Turner et al., 2015
PLE or ASE	Organic solvent mixtures (MeOH/CH ₃ CN, CH ₃ CN/water)	Higher extraction efficiency, less solvent use, fully automated, and quicker extraction than traditional methods	Expensive equipment and heavily coextracted matrix elements	Rico-Yuste et al., 2018
SFE	Supercritical fluids (CO ₂), acetone, MeOH, ethanol	Quick method, tiny solvent quantities, preconcentration impact, and temperature-sensitive analyte extraction	Requires costly and specialized instruments, unsuitable for routine analysis	Xie et al., 2016
MAE	Aqueous solution	Shorter extraction times and requiring less solvent	Useful only for thermally stable substances and requires expensive equipment	Somsubsin et al. (2018)
VALDS-ME	Combination of water, dispersive solvent, and organic solvents	Utilizing low density solvents is easy, quick, and efficient.	Optimization following extensive parameter control	Somsubsin et al. (2018)

5. Conclusion

Global food safety, agriculture, and public health are seriously threatened by emerging fungal pathogens and mycotoxins. These mycotoxins, which are produced by different filamentous fungi, are difficult to detect and manage because of their chemical complexity and resistance to environmental changes. They are also persistent in contaminating foods. The most common mycotoxins are highlighted in this chapter, including fumonisins, ochratoxins, aflatoxins, trichothecenes, and zearalenone. These toxins can have a wide range of harmful effects on both humans and animals, including immunosuppressive, neurotoxic, mutagenic, and carcinogenic effects.

In this chapter, we have covered a number of cutting-edge detection strategies that can help with these problems. These strategies include biosensors, chromatographic techniques, and sophisticated molecular assays that provide accurate and timely detection. Moreover, pre- and post-harvest interventions must be combined in integrated strategies to minimise the effects of these pathogens and mycotoxins. Utilising different extraction and purification techniques to lower contamination levels is one of these tactics, along with improving storage conditions and using biological control agents.

The persistent and widespread nature of these pathogens requires continuous research and development of more efficient, sustainable, and financially viable methods to ensure food safety and public health, even with the advancements in detection and control measures. Developing comprehensive strategies to manage and mitigate the risks associated with fungal pathogens and mycotoxins requires a multidisciplinary approach involving molecular biology, chemistry, agriculture, and biotechnology.

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