PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–16
https://doi.org/10.14719/pst.5816

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REVIEW ARTICLE

Extenuating mycotoxin contamination in spices: detection, regulatory frameworks and preventive strategies

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OPEN ACCESS

ARTICLE HISTORY
Received: 14 October 2024
Accepted: 03 November 2024
Available online
Version 1.0 : 30 December 2024
Version 2.0 : 31 January 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Saravanan VO, Theradimani M, Paranidharan V, Kavitha PA, Mohamed JJ, Juliet HS, Kokila DE. Extenuating mycotoxin contamination in spices: detection, regulatory frameworks and preventive strategies. Plant Science Today.2024;11(sp4):01-16. https:/doi.org/10.14719/

Abstract

Mycotoxins are secondary metabolites produced by fungi, primarily from the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*. The attention is on the existence of mycotoxin compounds in food substances that jeopardize public health and it is directed to systematic regulation to overcome these issues. Pathogenic fungi, including *Aspergillus*, *Penicillium* and *Fusarium* species, infiltrate spice crops during the pre-harvest, postharvest and storage stages. These fungi create toxic secondary metabolites called mycotoxin. The reviews' intend to examine the prevalence, types and levels of mycotoxins commonly found in spices, including aflatoxins, ochratoxin A and fumonisins. The study highlights the factors that influence mycotoxin contamination, such as environmental conditions, agricultural practices and storage methods. Analytical techniques for detecting mycotoxins, including chromatography and immunoassays, are evaluated for efficacy and sensitivity. It also discusses the regulatory frameworks and safety standards established by international bodies like the Codex Alimentarius Commission to mitigate mycotoxin risks. In addition to these regulatory measures, mycotoxin detection needs to be addressed before framing the standards. The preventive strategies and mitigation measures, including good agricultural practices (GAP), proper drying, storage conditions and biocontrol agents, were explored based on previous research conducted earlier. This comprehensive review underscores the critical importance of implementing integrated approaches combining advanced detection methods, harmonized regulatory standards and preventive strategies to ensure the safety and quality of spices in the global food supply chain.

Keywords

detection methods; fungal contaminants; mycotoxin; spices

Introduction

Spices refer to the parts of plants like leaves, bark, seeds, fruit, or roots that enhance colour and flavour and even preserve food (1). Spices are widely used in everyday cooking and are essential for improving the flavour of food in many different cuisines worldwide. However, there is a risk as these ingredients get contaminated by harmful fungi, leading to mycotoxins that remain unaffected by cooking processes (2). Commonly utilized worldwide and integral to Persian cuisine, it includes cinnamon, turmeric, black pepper and chilli powder in various dishes. The

most inexpensive and widely used spice in food is black pepper, which is grown in tropical climates. In addition to being used as a spice in meals, meat items, soups, vegetables and marinades, black pepper is also used as a preservative in the pharmaceutical and perfumery industries (3). Mycotoxin contamination in spices represents a significant global food safety concern, affecting food security and international trade. Spices, essential components of culinary traditions worldwide, are particularly susceptible to mycotoxin contamination due to their cultivation, harvesting, processing and storage conditions in predominantly tropical and subtropical regions (4). The presence of mycotoxins, primarily produced by *Aspergillus*, *Penicillium* and *Fusarium* species, poses substantial health risks to consumers, ranging from acute toxicity to chronic diseases, including carcinogenic, immunosuppressive and teratogenic effects (5).

Mycotoxins, toxins generated by fungi, are byproducts of toxigenic fungi. The presence of mycotoxins in food products is a primary worldwide health concern, affecting both economies and public health. These substances can pose severe risks to human and animal health, potentially leading to illnesses or even deaths (6). Due to the lack of a protein structure, mycotoxins resist heat and can persist through cooking processes (80-121 °C) (7). Specific types of mould fungi with toxigenic properties are responsible for producing these substances. Environmental factors such as temperature and moisture play a significant role in promoting fungal growth and, consequently, the production of mycotoxins in food items (8). Currently, over 300 identified mycotoxins, primarily focusing on those known to be carcinogenic and toxic. Ochratoxin A (OTA) and aflatoxins (AFTs) are among the numerous mycotoxins that have gained attention due to their harmful effects and economic significance. Mycotoxins cause significant economic losses globally in human and animal health and products every year (9).

Aflatoxins (AFTs) and ochratoxin A (OTA) are the most prevalent and hazardous mycotoxins. Aflatoxin, a significant mycotoxin, is primarily produced by species within the *Aspergillus* section Flavi, like *A. flavus*, *A. parasiticus* and *A. nomius,* under suitable environmental conditions. The main AFTs, including B_1, B_2, G_1 and G_2, are identifiable under fluorescent or violet light (appearing green or blue) (10). Aflatoxin B1, a well-known toxin within the AFTs group, is recognized for its harmful effects, such as mutagenicity and carcinogenicity in humans and animals (11). OTA is another mycotoxin produced by specific species of *Aspergillus* and *Penicillium*, such as *A. ochraceus*, *A. carbonarius* and *P. verrucosum* (12). The United Nations Food and Agriculture Organization (FAO) estimates that up to 25% of agricultural and food products worldwide are contaminated with mycotoxins(13). Mycotoxin contamination is often linked to drying spices on the ground, creating ideal conditions for fungal growth. According to the Rapid Alert System for Food and Feed (RASFF), the most reported border rejection notifications in the European Union in 2016 were the products contaminated with mycotoxins (14). Mycotoxin contamination has emerged as a critical threat to food security over the past twenty years, with fumonisins being particularly widespread in essential crops. This prevalent contamination by fumonisins necessitates further research into the toxin-producing capabilities of *Fusarium* species (15).

Analytical techniques like enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) can measure mycotoxins. HPLC is mainly useful for simultaneous mycotoxin analysis owing to its specificity and sensitivity. Nevertheless, ELISA may exhibit cross-reactivity with similar compounds and lower sensitivity compared to chromatographic methods (16). Acceptable levels of mycotoxin contamination differ among various countries, including the USA, India, Spain and Iran (2, 17, 18). Additionally, recent advances in rapid detection methods, including biosensors and portable analytical devices, have enhanced the capability for early detection and monitoring of mycotoxin contamination in spices (19). This review aims to provide an in-depth analysis of current challenges and recent developments in mycotoxin detection, examine existing regulatory frameworks and evaluate preventive measures for ensuring the safety and quality of spices in the global food supply chain.

Economically important spices

Spices are unique botanical substances that enhance food scent, flavour and appearance. Since ancient Vedic times, various herbs and plants have been recognized for their therapeutic benefits. The medicinal properties of spices and their ability to combat microbes and inflammation have been documented since the Rigveda era (4500-1600 BCE). Not only do spices enhance the taste of food, but they also promote human health due to their antimicrobial, anti-inflammatory and medicinal qualities. Additionally, they prolong the shelf life of food and serve as natural preservatives (20).

Sold in whole, ground, or blended forms, spices like turmeric, paprika, chili, cloves, star anise, coriander seed, cardamom, cumin and fennel are commonly featured in Asian cuisines. However, geographical location, soil quality and postharvest techniques can influence the attributes of spices. Despite many countries cultivating spices, India stands as the largest producer globally. FAOSTAT data from 2019 reveals that spice production reached 2.8 million tonnes from 1.4 million hectares of harvested land, with Asia accounting for 86% of the total output. Specifically, India produced 1.4 million tonnes of spices in 2019, indicating its dominant position in the market. The top chilli producers in 2016 were China, Mexico and Turkey, underscoring the significance of this crop, especially for emerging economies.

Chilli

Chilli is one of the most commonly used spices worldwide (*Capsicum annum* L.). Chillies are the fruits of *Capsicum* plants from the *Solanaceae* family, originating in America. In Sindh, the most cultivated chilli types are Longi and Sanam. These cultivars are widely used as seasonings in various Sindhi dishes and are profitable in domestic and foreign markets. Chilli products come in multiple forms, such as dried, fresh, powdered, pastes and flakes. Although fresh chillies can be consumed immediately after harvest, most chilli production focuses on processing and dehydration and it is often used in creating spice mixes. Studies suggest that most fungi found in chilli are likely contaminants or pathogens rather than native to the plant (21-23). Among the various spices, *A. flavus* and *A. niger* are the primary sources of fungal contamination in chillies (24). Analysis of OTA contamination in chilli samples from multiple regions revealed predominant fungal species, including *Aspergillus niger*, *A. carbonarius* and *A. ochraceus* (12).

Additionally, recent surveillance studies have demonstrated the co-occurrence of these mycotoxins in dried chilli samples, with AFB1 showing a higher prevalence (82%) compared to OTA (65%) in market samples (25). The Guntur Sannam chilli is renowned worldwide for its distinctive characteristics. Known as an S4 type chilli in trade, it is primarily valued for its strong spice and as a source of *capsaicin*, the active component in chillies responsible for their heat (26). The combination of leaf blight and fruit rot represents a severe threat to chilli production, possibly destroying entire crops (27). Anthracnose (*Colletotrichum capsici*), the most devastating disease in Longi chillies, manifests as dark sunken fruit lesions, leading to significant crop losses (28).

Cinnamon

There are four main types of cinnamon popularly used in various products: Indonesian cinnamon (*C. burmannii*), Chinese cinnamon (*C. cassia*), Vietnamese cinnamon (*C. loureirii*) and Sri Lankan cinnamon (*C. zeylanicum*), although over 250 cinnamon varieties have been identified (29). Throughout history, people have incorporated cinnamon into their culinary practices as a flavour enhancer and aromatic seasoning across diverse cultures. Besides its culinary uses, cinnamon plays a significant role in medicine due to its antibacterial and antioxidant characteristics (30, 31). In a related study conducted in Saudi Arabia, *Aspergillus flavus* and *Aspergillus niger* were identified in cinnamon samples at frequencies of 33% and 75% respectively (32).

Coriander seeds

Coriander seeds, from the plant *Coriandrum sativum* of the Apiace family, are commonly used as a spice. Among fungal pathogens isolated from coriander samples in Bihar, India, *P. verrucosum* showed the highest prevalence (63%), followed by *A. niger* (10.6%) and *A. flavus* (8%) (31). Southeastern Asian countries, which are famous. Fungal pathogens constantly infect them for their reputation in coriander production. Coriander samples from Medan and Indonesia had minimal toxic *Aspergillus* (33).

Pepper

Peppers are widely used as a popular table spice globally. Black pepper is known as the "king of spices" and "black gold. In various regions, including Malaysia and Southeast Asia, peppers are commonly used in curries, soups and meat and poultry marinades. Numerous studies have highlighted the prevalence of fungi in black pepper samples. Black and white peppers are predominately infected with *Aspergillus* species, among other fungal pathogens (33). Black pepper samples from Brazil are highly contaminated with *Aspergillus* spp, such as *A. ochraceus*, *A. parssiticus*, *A. carbonarius*, *A. flavus*, *A. nomius* and *A. niger* (32). Black pepper from Tanzania was found to include AF-producing fungi, including *A. parasiticus, A. flavus* and *A. nomius* (34).

Similarly, white pepper exhibited lower-level contamination with only *Rhizopus* spp *and Aspergillus*. Black pepper samples from Bahrain showed contamination by *A. flavus* species, with 1.12×10^3 CFU/g of fungi at 78% (24).

Fennel

Multiple fennel varieties exist, sweet fennel (*F. vulgare* var.) and bitter fennel (*F. vulgare* Mill) are the most commonly used. The distinction between these lies in the volatile oil content of their seeds, ranging from 1% to 6%. In bitter fennel, about 10% to 6% of the volatile oil consists predominantly of anethole, comprising half of the oil contents of *A. ochraceus* (5.0%), *A. niger* (5.3%) and *A. flavus* (12.3%), were identified in moderately contaminated fennel samples from India (31). Fungal contaminations such as *A. niger* in fennel were reported with the potential to produce OTA in significant amounts (32). Fennel is widely used to flavour soups, sauces, pastries, confectioneries, bread rolls, liquors, meat dishes and pickled seasonings. Its fruit is often chewed as a masticatory, while its seeds and oil possess various medicinal properties. Fennel leaves are known for their digestive, appetizing and stimulating effects, helping to increase urine secretion and discharge. Additionally, aqueous ethanol extracts from the roots of *F. vulgare* var. dulce have been tested for diuretic activity in rats (35). In fennel ochratoxin A, contamination is less than the permissible limit (31).

Cumin

Cumin is widely used in dishes such as Indian pickles and spice blends like sambar powder. *A.niger* is the major fungal pathogen identified to be contaminated at 4.6 * 10^2 CFU /g of cumin samples in Bahrain(24). *A.niger* was the predominant fungus at 60%, followed by *A. flavus* at 40% and *A. fumigatus* at 20%, all known to produce OTA and AFT.AFB1 and total AFTs were uncommon, with mean amounts of 0.03 µg/kg and 0.05 µg/kg in 57% of Moroccan cumin samples from public markets (36). Studies showed that there was no contamination of cumin samples with *A. niger* with a 0.2 µg/kg detection limit (37).

Mycotoxins in Fungi

Mycotoxins, which are secondary compounds produced by fungi such as *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium*, are known to be present in spices (38). The primary mycotoxins found in spices were OTA and Afs, along with other mycotoxins detected in food products that include fumonisins (FBs), deoxynivalenol (DON), trichothecenes, zearalenone (ZEN) (8, 20). Spices are particularly susceptible to mycotoxin contamination due to cultivation, storage and processing conditions in tropical/ subtropical regions (14). Ochratoxin A's dual threat as a potent kidney toxin and potential carcinogen is particularly concerning in spices. Their frequent consumption as food ingredients enables chronic low-dose exposure that can accumulate harmful effects over time, even at concentrations below regulatory limits (39). It is described below in (Table 1-2).

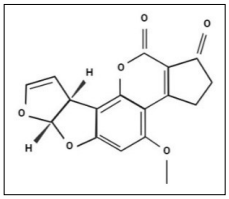
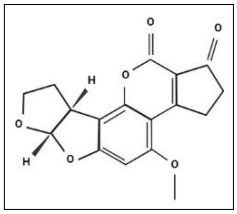
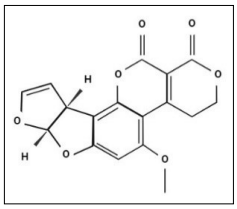
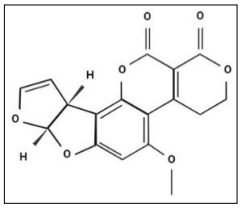
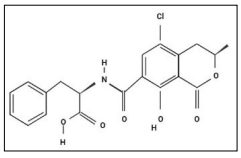
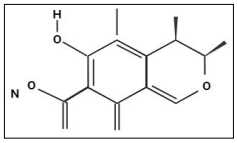
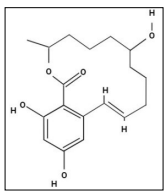
Aflatoxins (AFTs)

AFs exhibit mutagenic solid, carcinogenic and immunosuppressive characteristics, notably more harmful than other mycotoxins (46). AFT represents a significant mycotoxin produced by fungi within the *Aspergillus* genus. *A. flavus*, *A. parasiticus* and *A. nomius* are the primary AFT producers among various *Aspergillus* species (8). AFTs are commonly found in peanuts contaminated with *A. flavus* or *A. parasiticus* in other substances such as dried fruits, nuts, rice, spices, figs and corn (56). There are four main types of aflatoxin: B1, B2, G1 and G2. Research indicates that *A. flavus* primarily releases B toxins, with B1 being the most commonly occurring toxin known for its cancer-causing and genetic-mutating characteristics (57, 58). Cotton and maize stand first in identifying AFTs produced by *A. flavus* (59). Aflatoxins are particularly dangerous in spices because these ingredients are often stored in warm, humid conditions that promote mould growth, are resistant to typical household cooking temperatures and can concentrate in spice

Table 1. List of important mycotoxin and toxin-producing fungal organisms

Mycotoxin	Fungal organisms	Mode of action	Reference
Aflatoxins B1, B2, G1, G2	*A. parasiticus, Aspergillus flavus, A. nomius*	DNA adduct formation, Inhibition of protein synthesis, Lipid peroxidation, Immunosuppression, Carcinogenic (primarily targets liver), P53 mutation induction	(8, 40)
Ochratoxin A	*Penicillium verrucosum, Aspergillus ochraceus, A. carbonarius*	Inhibition of protein synthesis, Disruption of calcium homeostasis, Mitochondrial dysfunction, Competitive inhibition of Phe-tRNA synthetase, Nephrotoxic effects, DNA damage through oxidative stress	(41-44).
Fumonisins B1, B2, B3	*Fusarium verticillioides, F. proliferatum*	Disruption of sphingolipid metabolism, inhibition of ceramide synthase, Cell membrane damage, Alteration of sphinganine/ sphingosine ratio, Neural tube defect induction	(45-47)
Zearalenone	*F.culmorum, F.verticillioides Fusarium graminearum F. semitectum, F.cerealis, F. crookwellense, F.pseudograminearum* and *F.equiseti*	Estrogenic activity, Binding to estrogen receptors, Disruption of hormonal balance, Reproductive system effects, Competitive binding with 17β-estradiol	(20, 45, 46, 48, 49)
Citrinin	*P. viridicatum, P. expansum, Penicillium citrinum, Monascuspurpureus, M. ruber, A. niveus, A.terreus*	Nephrotoxicity, Mitochondrial dysfunction, Oxidative stress induction, Disruption of ion transport, Inhibition of RNA/ protein synthesis	(20, 50-52)

Table 2. Chemical structure of mycotoxins **Source:** Biorender

Mycotoxins	Chemical structure	Reference
Aflatoxin B1		(55)
Aflatoxin B_2		
Aflatoxin G_1		
Aflatoxin G_2		
Ochratoxin A (OTA)		(43)
Citrinin		(20)
Zearalenone (ZEA)		(45)

matrices - a key food safety concern since even small amounts of contaminated spices can introduce significant AFT exposure through regular consumption patterns (45, 60).

Ochratoxin A (OTA)

Out of these, OTA is the most prevalent mycotoxin in food, originating from *P. verrucosum* and various Aspergillus species, namely *A. ochraceus* and *A. carbonarius* (8). *A. ochraceus* and *A. carbonarius* dominate OTA production in tropical regions, commonly contaminating spices and crops during both the cultivation phase and postharvest storage under warm, humid conditions. The ochratoxin A (OTA) is primarily produced by four key fungal species: *A. ochraceus, A. niger, A. carbonarius* and *P. verrucosum* (41-33). Fungi-producing OTAs' are well favoured in regions with tropical climates, promoting the synthesis of OTA (61).

Citrinin

Citrinin(CIT) is a mycotoxin produced by several *Penicillium* spp (50). Although CIT was discovered for the first time in 1931 from *Penicillium citrinum*, several other fungi were also found to produce the mycotoxin, such as *A. terreus, A. niveus, Monascusruber, M. purpureus, P.viridicatum* and *P.expansum* (20). Studies have confirmed that citrinin is toxic to kidneys, as shown in a prior investigation with chickens contaminated by *P. citrinum* (62). However, it is classified under group 3 carcinogen, indicating their low lethality by the International Agency for Research on Cancer (IARC).

Fumonisins

The primary producers of fumonisins are *Fusarium* species, particularly *F. verticillioides* (63-65). Twenty-eight fumonisins, which have been identified, were sorted into four different groups (46), among which fumonisin B (FB) was found to be the most common group, comprising FB1, FB2 and FB3. Necrotic lesions identify the syndrome with a liquid consistency in the white matter region of horses' brains (8). There has been an increasing interest among researchers in exploring the presence of FBs in spices. Spices and herb samples collected in China were identified with the presence of FB1 and FB2 (66).

The research demonstrated that mouldy samples, accounting for 42.5%, were tainted with FB2 and FB1 and averaged concentrations of 165.9 and 129.0 µg/kg respectively. In contrast, usual samples, comprising 8.6%, exhibited contamination levels of 165.9 and 256.8 µg/kg for FB1 and FB2 respectively. Additionally, assessed herbs and spices were obtained from Polish markets, where FBs were identified in the samples at levels ranging from 5.29 to 62.78 µg/kg for total FBs (67,68).

Trichothecene (TC)

F. graminearum and *F. culmorum* are among the common species in agricultural goods, besides other species capable of producing TCs, including *Cephalosporium, Cylindrocarpon, Dendrodochium, Myrothecium, Trichoderma, Trichothecium* and various *Stachybotrys* species (51, 53, 54). Field crops such as Paddy, Barley, oats, corn, rye and wheat are frequently tainted with TCs (69).

Zearalenone (ZEA)

Zearalenone (ZEA), previously referred to as F-2 toxin, is categorized as a non-steroidal estrogenic mycotoxin (37). ZEA is synthesized by certain *Fusarium* species, primarily *F. graminearum* and *F. semitectum* (45). Additional producers of ZEA include *F. culmorum, F. verticillioides, F. cerealis, F. crookwellense, F. pseudograminearum* and *F. equiseti* (48, 69).

Impact of Environmental and Agricultural Factors on Mycotoxin Production

The aflatoxin biosynthesis pathway is modelled by non-biological factors such as temperature, water activity, pH and carbon/nitrogen availability (70). These environmental factors serve a dual role: enhancing *A. flavus* colonization while simultaneously inducing the transcription of genes involved in aflatoxin production (71,72). Fungal growth and toxin production are favoured by higher water activity, specifically with aflatoxin synthesis reaching optimal levels at 0.99 aw when combined with temperatures of 29-30 °C (73, 74).

Aflatoxin biosynthesis and fungal growth are inhibited at temperatures outside the 25-37 °C range, while water activity below 0.85 aw reduces toxin production, with complete cessation occurring at 0.70-0.75 aw (74). Mycotoxin contamination in spices is significantly influenced by poor soil management practices, particularly inadequate crop rotation, improper field preparation and contaminated soil conditions (75). Improper irrigation practices, such as over-irrigation, water stress and the use of contaminated water, can adversely affect crop health and soil quality (76). Delayed harvesting, such as leaving crops in the field too long or harvesting under wet conditions, can increase the risk of spoilage and contamination (14). Inadequate drying, including slow or improper methods and incomplete drying, can lead to the growth of spoilage organisms and reduce crop quality (77). Poor storage conditions like high humidity, inadequate ventilation, temperature abuse and pest infestation can lead to contamination, spoilage and product degradation (78). In the agricultural sector, proper sanitation and hygiene practices are absent throughout the process, from harvesting to storage and transportation (79, 80). The most suitable temperature range for the survival of *A. flavus* is around 30 °C with the most effective production occurring between 25 °C and 30 °C (81, 82). Even with their low water activity levels, spices are at risk of fungal contamination, influenced by the cultivation location, harvesting procedures and processing techniques (61). Fungal contaminations and mycotoxin production are significantly promoted in warm and humid tropical regions, which are also ideal conditions for spice cultivation (83).

The mycotoxins contaminating the spices are viable from the point of infection through the storage unit and can show their impact when consuming the food products containing the spices (84).

Among storage fungi, *Aspergillus* species, especially *A. flavus and A. niger*, are commonly isolated from spices due to their tolerance to low moisture conditions and ability to produce mycotoxins (85, 86). *Penicillium* is another common storage fungi that can produce and secrete mycotoxins, especially in tropical regions (87). Southeast Asia's climate is predominantly tropical, making it one of the world's most climate-vulnerable regions. Predicted changes in temperature, CO_2 levels and rainfall patterns are expected to intensify in this region due to climate change (88). The hot and humid conditions present for much of the year promote the growth of mycotoxin-producing fungi, which release toxic metabolites, leading to contamination in food and feed (89). The tropical zone, between 23.5° North and 23.5° South, has high temperatures year-round because the

Sun's rays fall directly here. This direct sunlight is concentrated over a smaller area, causing more heat per unit and making it the hottest climate zone (90). Spices, valued for their unique flavours and aromatic compounds, are inherently vulnerable to fungal contamination due to their hygroscopic nature, which allows moisture absorption from the environment, creating ideal conditions for fungal proliferation (91). Container transportation and temperature abuse during maritime shipping significantly increase spice contamination levels during transit (92) (Fig. 1.).

Impact of mycotoxin

Ochratoxin A (OTA) exhibits nephrotoxic and reproductive toxicity, with demonstrated transplacental transmission and documented hepatotoxic, neurotoxic and immunosuppressive effects. At the same time, OTA and aflatoxins contaminate diverse food matrices, including cereals, nuts, dried fruits, spices, legumes, alcoholic beverages and herbs at varying concentrations (37) (Fig. 2.).

Mycotoxins can enter the body through various pathways, including contact, inhalation and ingestion. A primary concern among these toxic components is AFTs; consistent consumption of mycotoxin at a level ranging from 10 to 50 µg/kg or above can reflect negatively on human health due to their teratogenic, immunosuppressive, carcinogenic and mutagenic effects (93). Approximately five billion people worldwide are exposed to mycotoxins daily through conventional food sources and unidentified pathways (94). Excessive consumption of mycotoxin results in intoxication, a condition referred to as mycotoxicosis (95). Mycotoxin accumulation causes acute or chronic toxicity and temporary or long-term adverse effects such as neurotoxicity, teratogenicity, cytotoxicity, mutation, hepatotoxicity and carcinogenicity. OTA is also nephrotoxic and its target organ is the kidney. Overdose of ochratoxin can cause kidney failure and death also (31). Mycotoxins (particularly aflatoxins, ochratoxin A and trichothecenes) disrupt cellular bioenergetics by inhibiting mitochondrial electron transport chain complexes I-IV, resulting in decreased ATP production and increased reactive oxygen species (ROS) generation, which collectively leads to cellular energy depletion and oxidative damage (96, 97). Aflatoxin B1 and ochratoxin A disrupt glucose homeostasis by inhibiting GLUT4 transporter expression and IRS-1 phosphorylation in the insulin signalling cascade, resulting in impaired glucose uptake, insulin resistance and reduced glycogen synthesis in muscle and liver cells (98).

Mycotoxins prevent the replication of RNA and DNA by interacting with nucleic acids at the cellular level (99). Acute toxicity is reflected in rapid allergic changes and symptomatic effects, which can be diagnosed using drugs. Chronic toxicity is the result of prolonged consumption of toxins, leading to irreversible conditions such as cancer and severe fatality (8). In Gambia, children consuming AFT-contaminated food had a lower secretory immunoglobulin A(IgA) rate (43). Moreover, Contamination with AFT has also led to changes in lymphocyte subsets and distribution among Ghanaian adults, indicating that AFTs may weaken cellular immunity and reduce infection resistance (100).

Techniques involving mycotoxin detection

Early detection of mycotoxins is paramount for ensuring food safety and minimizing public health risks, with recent research demonstrating its vital role in preventing acute and chronic toxicity exposures, protecting vulnerable populations like children and the immunocompromised and identifying contamination sources before widespread distribution (19). Early detection strengthens food security by enabling screening of raw materials before processing, preventing contaminated products from entering the food chain and supporting sustainable

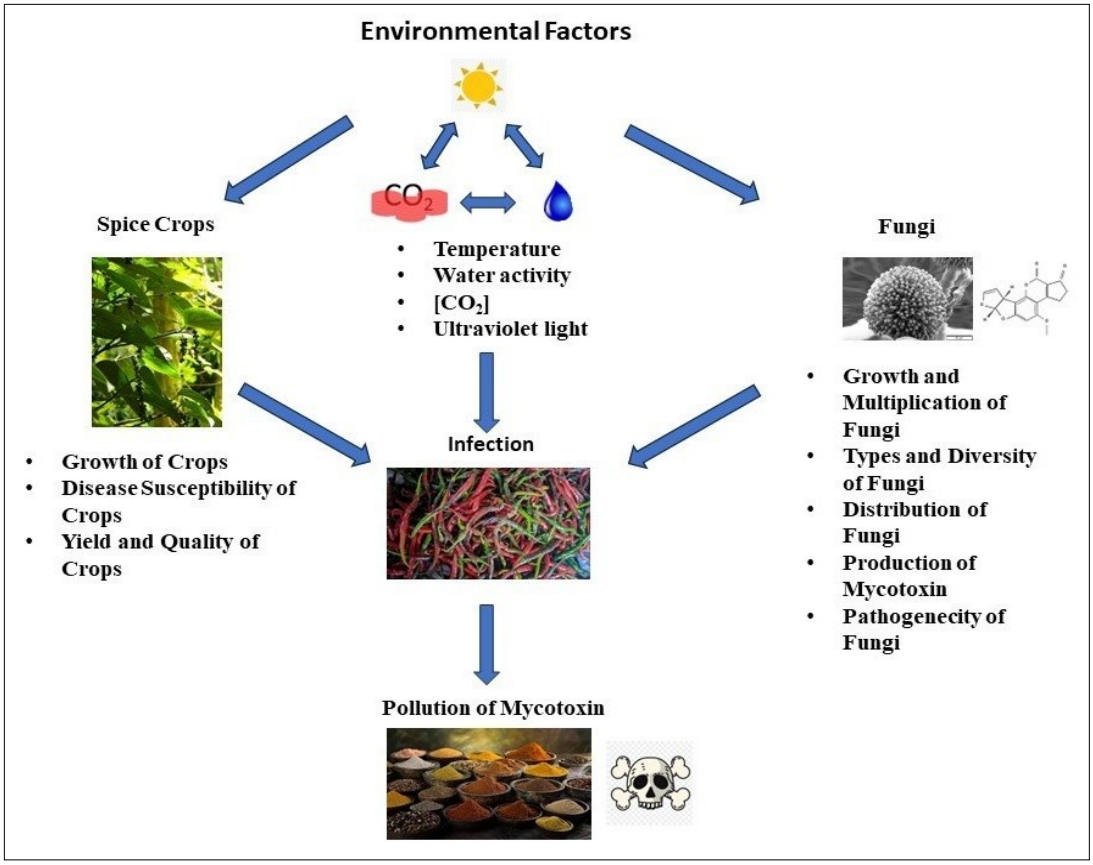


Fig. 1. Impact of environmental factors on toxin contamination.

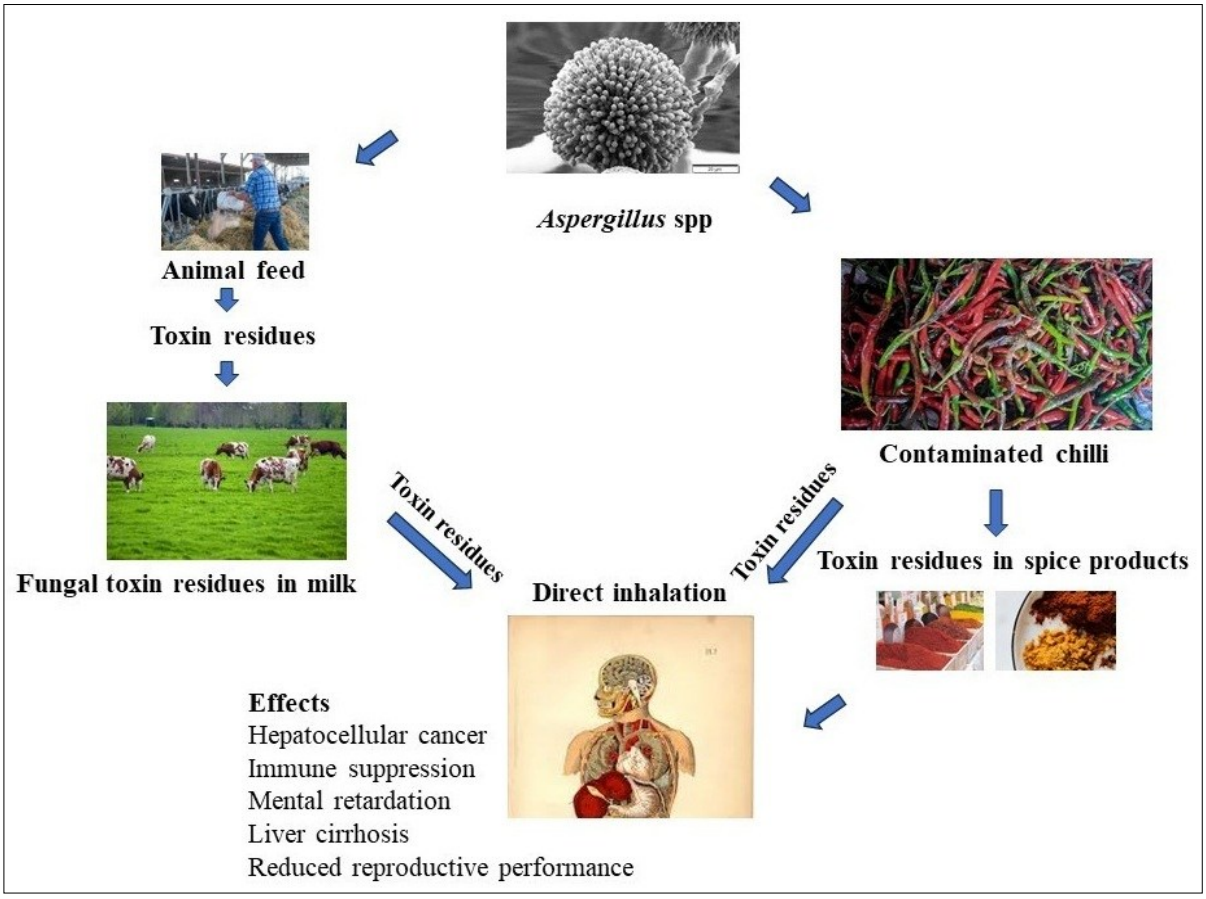


Fig. 2. Impacts of mycotoxin on human health from a toxicological perspective.

agriculture practices (101). Since the discovery of mycotoxins, a variety of approaches have been utilized for their analysis, with chromatographic methods, immunoassays and rapid strip screening tests being the predominant methods for mycotoxin detection and analysis (102, 103) (Table 3).

Chromatographic methods

In chromatographic analysis, separation occurs through physical interactions when the liquid mobile phase permeates through or moves along the solid stationary phase, causing a differential distribution of components between these phases (108, 109). Commonly utilized chromatography methods include high-performance liquid chromatography (HPLC) integrated with diode array, fluorescence and UV detectors, thin-layer chromatography (TLC), gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), (102, 103). Methods belonging to the chromatographic classification have been developed explicitly for the precise quantification of mycotoxins because they can effectively ascertain, recognize and measure various toxic substances (110).

Thin - layer chromatography

Thin-layer chromatography (TLC) has demonstrated its cost-effectiveness and simplicity as a method suitable for the quantitative and qualitative detection and analysis of multiple mycotoxins. This is mainly due to its cost efficiency, simplicity and the presence of UV light fluorescent spots. However, it is essential to note that TLC exhibits lower sensitivity and accuracy levels, which present challenges in achieving precise quantification (102, 111). Conversely, liquid chromatography (LC) is known for its reliable separation capacity, particularly when combined with the sensitivity of tandem mass spectrometry. This combination has solidified LC as a powerful technique capable of detecting and quantifying mycotoxins in various circumstances (112, 113). The detection limit for aflatoxins using fluorescence detection after derivatization was reported to be 0.1-0.5 ng per spot (114). The detection limit for fumonisins using fluorescence detection after derivatization was reported to be between 100-500 ng per spot (115).

Table 3. Comparison of analytical techniques: TLC, HPLC and LC-MS/MS in terms of cost, sensitivity, accuracy and applications

Techniques	Cost	Sensitivity	Accuracy	Application	References
TLC	Low (inexpensive setup and materials)	Low to moderate sensitivity	Moderate accuracy; qualitative/semi-quantitative	Qualitative analysis, preliminary compound identification, purity checks	(102)
HPLC	Moderate to High (equipment and solvents are costly)	Higher sensitivity than TLC in the ng/mL range	High accuracy for quantitative analysis, but can be impacted by matrix complexity.	Quantitative analysis, pharmaceutical testing, complex mixture separation	(104)
LC-MS/MS	Very High (expensive setup and operation costs)	Very high sensitivity, capable of detecting low ng/mL or even pg/mL concentrations	Extremely high accuracy, especially for complex mixtures due to MS specificity	Used in biopharmaceuticals, proteomics, metabolomics, clinical diagnostics and drug development	(105–107)

High-performance liquid chromatography (HPLC)

The concurrent analysis of multiple mycotoxins is achieved using HPLC due to its high sensitivity and specificity as a chromatographic technique (19, 44). HPLC uses diverse discoverers and adsorbents, including fluorescent (FLD) and UV-visible (UV), which are contingent upon the presence of chromophores employed for mycotoxin examination. Occasionally, mycotoxins are detectable directly in HPLC-FLD owing to inherent fluorescence (37). Detection limits are approximately 50 ng g^{-1} for FB1 and 100 ng/g for FB2 (115). Fluorescence detection (FLD) with post-column derivatization is widely used for detecting aflatoxins in food and feed due to its high sensitivity. For aflatoxins B1 (AFTB1) and B2 (AFTB2), the method can detect levels as low as 0.1-0.5 ng/g, while for G1 (AFTG1) and G2 (AFTG2), detection limits range from 0.2-0.6 ng/g. (116). The limits of detection (LOD) for aflatoxins (AFTs), ochratoxin A (OTA) and zearalenone (ZEA) were 0.004-0.012 ng/g, 0.05 ng/g and 0.5 ng/g respectively, while the limits of quantification (LOQ) were 0.015-0.05 ng/g, 0.2 ng/g and 2 ng/g, respectively (117). HPLC provides excellent resolution for complex mycotoxin mixtures, mainly using advanced columns with sub-2 µm particle sizes (118). Modern UHPLC systems achieve faster analysis times while maintaining high separation efficiency (44). The development of comprehensive HPLC methods capable of simultaneously detecting multiple mycotoxin classes, integrating novel stationary phases for improved separation and implementing gradient elution programs optimized for complex matrices has been demonstrated (119). Enhanced sample cleanup protocols using immunoaffinity columns and QuEChERS (101). Matrix-matched calibration strategies for accurate quantification (120).

Liquid chromatography (LC)

Using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) is currently the predominant method for detecting mycotoxin. This method is renowned for its exceptional selectivity and sensitivity, eliminating the necessity for derivatization or purification steps (121). The integration of LC with MS/MS (LC-MS/MS) presents a robust diagnostic technique known for its high sensitivity, selectivity and dependability (106-107). LC-MS/MS-based multi-methods have become increasingly important as they facilitate the quick detection and sometimes quantification of various mycotoxins in diverse food categories and animal feeds (99). LC-MS/MS has extended its application in detecting several mycotoxins such as DON, T-2 toxin, AFTB1, B2, G1, G2, HT-2 toxin and OTA in grains of legume (122). LC-MS/MS can detect multiple toxins at deficient concentrations (ppb or lower). The tandem mass spectrometry (MS/MS) process allows for high specificity, reducing interference from other substances in complex matrices (106, 107). This instrumentation makes it suitable for automation, allowing labs to handle large sample volumes efficiently and with consistent results. This is particularly useful in regulatory and quality control settings (123). It can detect toxins, including those with different chemical structures (e.g., polar and non-polar compounds), providing flexibility in multi-toxin analysis (124). It is a faster analysis compared to traditional methods,allowing for detecting multiple toxins in one analytical run, saving time and resources (125). The aflatoxin detection limits were reported as 0.005-0.01 µg/kg for AFTB1 and AFTB2 and 0.008-0.015 µg/kg for AFTG1 and AFTG2 (126). The detection limits for ochratoxins were reported as 0.01-0.05 µg/kg for OTA and 0.02-0.06 µg/kg for OTB (127). LC-MS/MS enables simultaneous detection of multiple mycotoxin classes with high specificity and sensitivity, achieving detection limits of 0.01-0.5 µg/kg across diverse mycotoxins, including masked and emerging forms (4). Tandem MS/MS analysis offers enhanced specificity and sensitivity (5). The simultaneous detection of regulated, emerging and masked mycotoxins, which can analyze over 50 mycotoxins in a single run and reduce sample preparation requirements compared to conventional methods, has been achieved (4).

Immunochemical Methods

Immunochemical methodology obviates the need for proficient and extensively trained personnel to address potential issues during the separation process, thereby reducing labour intensity and time consumption and establishing superiority over chromatographic and spectrophotometric methodologies. The most prevalent immunochemical techniques utilized in the examination of aflatoxins include enzyme-linked immunosorbent assay (ELISA), immunosensors, Radioimmunoassay (RIA) and immunoaffinity column assay (ICA) (8, 128).

The detection process depends on specific polyclonal and monoclonal antibodies designed for these toxins (75). Recently, biosensors, including piezoelectric, electrochemical, optical and variants, have been integrated to quantify mycotoxins in food items (129). Nanoparticle-based Biosensor and Surface Plasmon Resonance (SPR) techniques function by detecting refractive index changes, earning their classification as 'label-free' detection systems (4, 5). The SPR method allows for the real-time optical recognition of multiple analytes involving fluorescence polarization and near-infrared fluorescence sensors, displaying promising fluorescence detection and quantification capabilities. By utilizing this technique, a fluorescence-labelled PT derivative bound to antibodies exhibits increased emission of fluorescence polarization. This technique's detection range for PT in food products extends from 6 to 102 µg/L (130).

Biosensors, particularly PT ones, could prove valuable in real-time mycotoxin monitoring in the food sector. However, the short stability of the bio-recognition elements in effect with the long-term self-life of biosensors, insufficient selectivity, in particular with enzyme inhibition-based biosensors and the relatively high cost of antibodies in comparison with artificial recognition elements are various challenges faced in executing detection methods using biosensors. The realm of nanotechnology is evolving in mycotoxin detection. Nanoprobes that include nano-silver, graphene and magnetic nanoparticles are utilized to detect different mycotoxins (36). Another rapid technique, the electronic nose, relies on food aromas and odours.

Fungal contaminations in food leave a trail of volatile byproducts that can be detected using GC-MS, whose properties are correlated with fungal activities during infection (8). The fungal-contaminated samples are differentiated from healthy samples utilizing the integration of NIR hyper-spectral imaging and spectroscopy ranging from 700 to 2500 nm. Various alternative approaches have surfaced alongside conventional methods, including aggregation-induced emission, molecularly imprinted polymers, electronic noses and fluorescent polarization.

The utilization of the electronic nose has been documented in the identification of various mycotoxins, such as FBs and Afs, DON in maize and wheat and wheat bran, respectively (131, 132). Detection of AFTB1 in broad bean sauce peanut oil and OTA in coffee and wine by developing an Aggregation-Induced Emission (AIE) dye-based sensor (133, 134). Chronic exposure to sub-ppb levels of aflatoxins can lead to liver damage and immunosuppression (70). Improved detection methods help enforce strict regulatory limits (134). Enhanced sensitivity was reported using quantum dot-labeled antibodies (4). The strict regulatory limits for AFTM1 in milk, set at 0.05 ppb by the EU and 0.5 ppb by the FDA, preclude the use of sample dilution or reduced injection volumes in analysis, as these techniques can raise detection limits above these critical threshold values (135, 136). A novel immunoassay system utilizing microfluidic and protein microarray technologies was developed for rapid mycotoxin screening, demonstrating high sensitivity with detection limits between 0.03 and 1.24 ng/mL (137).

Regulatory limits

Implementing preventive measures and regulations is crucial to shield consumers from exposure to mycotoxins. The primary goal is establishing upper limits for mycotoxins in food products to promote fair trade and protect public health. Regulatory thresholds have been set by more than a hundred nations across the globe (13). It is described below (Table 4).

Management of mycotoxins

The global spice industry, particularly in tropical regions, faces a prevalent issue of mycotoxin contamination. It is imperative to enforce stringent pre and postharvest measures to mitigate the escalation of mycotoxin levels (139, 140). Furthermore, the adoption of practices such as adherence to good manufacturing practices (GMP), good storage practices (GSP) and good agricultural practices (GAP) can significantly contribute to the reduction of mycotoxin production (141) (Fig. 3).

Table 4. Maximum levels of aflatoxins in spices under EU legislation reported (138)

Toxin	Spices	Maximum level (µg/kg)	
		B	Sum of B_1, B_2, G_1 and G_2
Aflatoxin	Dried spices include fruits from *Capsicum* spp. (whether whole or ground, such as chillies, cayenne, paprika, chilli powder), *Piper* spp. (comprising white and black pepper), *Myristicafragrans* (commonly known as nutmeg) and *Curcuma longa* (known as turmeric). Additionally, mixed dried spices that contain any combination of the spices as mentioned above are also considered.	5,0	10,0
	Ginger (*Zingiber officinale*) (dried)	5,0 10,0	5,0 10,0
Ochratoxin	Dried spices (including whole or ground chillies, chilli powder, cayenne, or paprika) other than capsicum species		15
	Capsicum spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne or paprika)		20

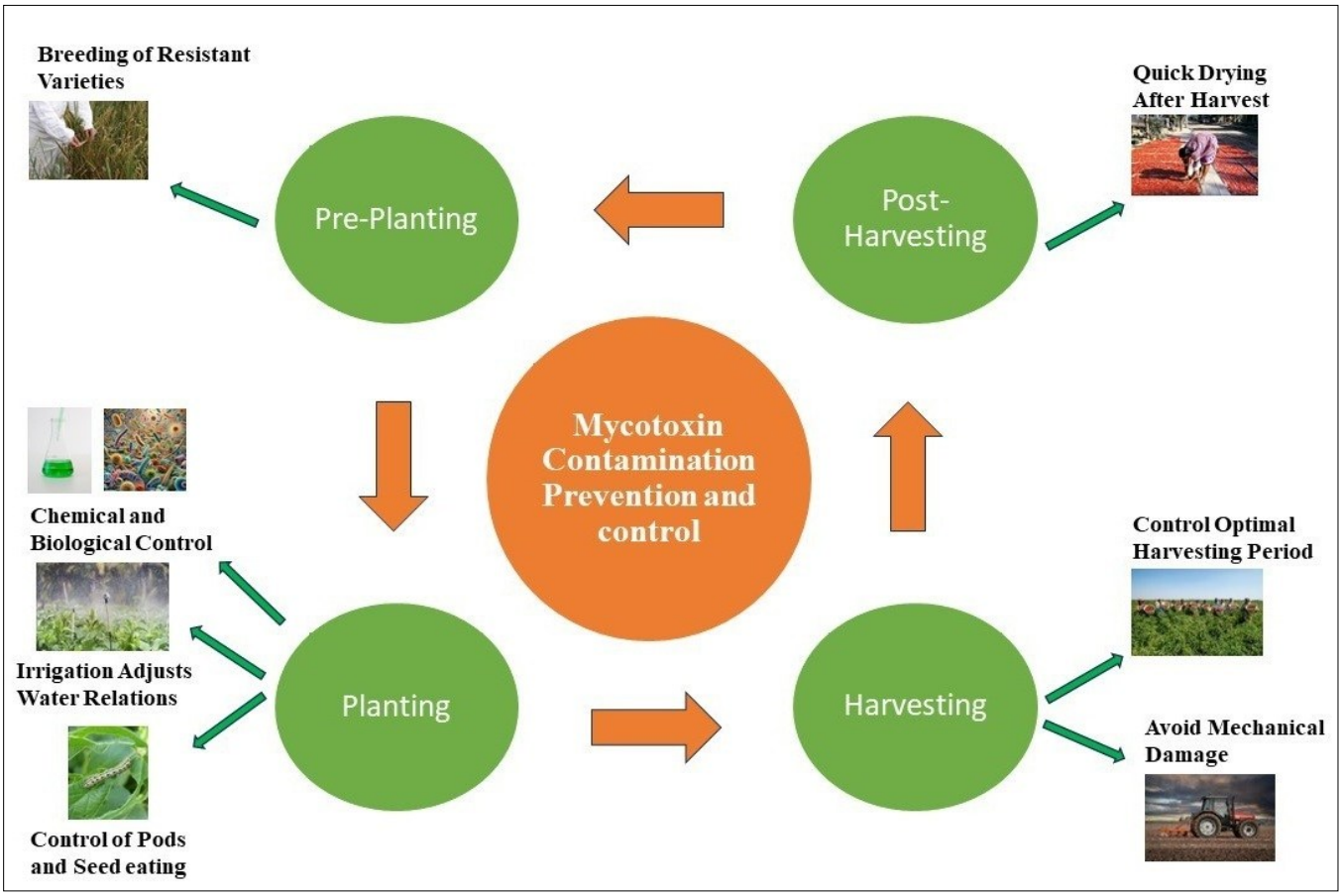


Fig. 3. Methods for preventing mycotoxin contamination in spices.

Preharvest

Crop rotation strategies in spices, such as rotating pepper with ginger and turmeric with pulses, help manage pests, improve soil health and reduce disease incidence (142, 143) demonstrate the effectiveness of these practices in spice cultivation. When applied appropriately, organic soil amendments such as compost, green manure, biochar and animal manure enhance soil fertility and structure (4). Microorganisms like lactic acid bacteria, *Bacillus licheniformis, B. subtilis and Saccharomyces cerevisiae* have effectively detoxified mycotoxin in pre-and postharvest stages (144). The extract from neem leaves has been reported to reduce AFT contamination in cereals during storage (145). Amid growing concerns over synthetic preservatives, there is increasing interest in natural food protection methods to enhance food quality, extend shelf life and safeguard against biodegradation by mycotoxigenic microbes (146). Integrated management combines cultural practices, biological control and soil management to enhance agricultural sustainability (147).

The choice of genetically modified seeds or fungal-resistant crop varieties with antifungal attributes presents favourable strategies. Moreover, pesticides, fertilizers and appropriate irrigation practices can be advantageous.

Utilizing native, suitable toxigenic *A. flavus*, the biological management of aflatoxins has recently become a viable method for reducing crop contamination (148). Application of pre-harvest fungicide can reduce the production of mycotoxins by specific A. flavus strain in Capsicum powder, growth of fungal strains that produce aflatoxin, identified and isolated from chilli, paprika and smoked paprika significantly inhibited *in vitro* when grown in 3% extract agar supplemented with 80% mancozeb and 25% tebuconazole, administrated at a concentration of 3.5 and 0.75g/L respectively, regardless of strain or environmental conditions (149).

An ideal fungicide should effectively concurrently hinder both mould growth and AFT production. Chilli was evaluated with bioagents, plant extracts and fungicides for contamination with aflatoxin in *in-vitro* and *in vivo* conditions. The development of mould was significantly reduced by 0.3% mancozeb (91.1%), followed by captan (85.2%) and carbendazim (73%). Suppression of (100%) A. flavus was recorded with the application of nimbicidin, Pongamia oil and neem seed kernel extract (NSKE) at a concentration of 5%. A. flavus was inhibited by an indigenous isolate of Pseudomonas fluorescens with a suppression rate of 74.9% exceeding *Trichoderma harzianum* with a recorded inhibition rate of 70.4% *in vitro*. The supplements performed exceptionally *in vitro* were selected for real-time challenges against *A. flavus* in the agricultural field. Among these, fruits treated with captan displayed the lowest infection rate by A. flavus (1.6%), followed by those treated with NSKE (2.2%), *P. fluorescens* (2.0%) and nimbicidin (7.8%), in comparison to the control (38.3%). In terms of field assessment, the lowest incidence was observed in the chilli plot treated with NSKE spray (1.6%), which was similar to *T. harzianum* (2.6%), captan (2.2%) and *P. fluorescens* (2.4%) treatments, contrasting with the control (7.4%). It is advisable to utilize mancozeb (0.3%), NSKE (5%) or *P. fluorescens* (1×10^8 CFU/mL) through preharvest spraying on chilli plants ten days before harvest to effectively manage aflatoxins (AFTs) at the agricultural level (150).

Harvest and Postharvest

Efficient management practices during harvesting and postharvest stages can mitigate the escalating levels of mycotoxin in spices. It is essential to prevent physical harm to the bark of cinnamon, seeds of cumin, pods of peppers, roots of ginger and turmeric and leaves of bay plants caused by insect infestations or harvesting tools. Fruits damaged by physical means or fungal contamination must be eliminated. Appropriate hygienic washing techniques to eliminate dirt or soil from the plant surfaces. Extreme hygiene must be employed when separating dirt or soil from plant surfaces. After the cleaning process is completed, promptly initiating the drying phase is imperative. Since moisture provides a favourable environment for mould growth, it is essential to dry spices quickly to prevent the proliferation of mould. The moisture content should be decreased to approximately 10%, with the water activity level below 0.7. The effects of drying red peppers on concrete and soil surfaces were explored in a study. The red peppers were halved and dried outdoors, with one group placed on soil and the other on concrete. After drying, the pepper halves were left to incubate for a week. Interestingly, the peppers dried on concrete surfaces did not exhibit a significant production of aflatoxins; however, 6 samples out of 10 produced high levels of aflatoxin contamination when dried on soil surfaces.

The predominant fungi present in the soil were found to be *A. flavus* and *A. parasiticus,* indicating that the contact of chilli with soil during soil drying of chilli might be the significant factor contributing to the contamination of aflatoxin in ground-dried red peppers. It is crucial to ensure appropriate storage and transportation conditions to uphold the quality of spices. Transport vehicles and storage facilities should be maintained in excellent, dry conditions and protected from insects. Due to their hygroscopic nature, spices can absorb moisture from the surroundings, creating an environment conducive to mould growth and mycotoxin production, especially in warm and humid climates (151).

Similarly, aflatoxin levels and mould growth on hot peppers during a 5-month storage period at 20 °C, 25 °C and 30 ^{0}C. The hot peppers were stored in low-density polyethene bags and jute bags. The findings revealed that storing the peppers at higher temperatures of 25 °C and 30 °C resulted in a 61% increase in AFT concentrations compared to those stored at 20 °C. However, there was no report of AFT contamination during the initial 90-100 days of the storage period in polyethene bags. In contrast, the contamination was reported in hot peppers when stored in jute bags during the same period. The results showed the advantage of storing hot pepper in polyethene bags compared to jute bags. The prolonged storage of hot pepper combined with a gradual increase in temperature and aeration permeability in jute bags over polyethene bags contributed to the rise in fungal growth and aflatoxin contamination (152). Cold plasma is a novel non-thermal technology that utilizes reactive species (e.g., O, O_3, OH, NO, NO_2) to degrade mycotoxins, converting them into less toxic compounds while preserving food quality (153). An alternative to traditional open-air Sun drying is solar driers, which require lower investments compared to advanced fossil fuel drying techniques. This is particularly beneficial for most developing countries in climatic zones with significantly higher insolation than the global average of 3.82

kWh/m^2 per day (154). Preserving food materials like meat, vegetables, fruits, spices and herbs through open-air sun drying is considered one of humankind's earliest systematic technological activities (155). A survey conducted in several countries in the Asia-Pacific region identified the most promising and popular solar driers as (i) natural convection cabinet type, (ii) forced convection indirect type and (iii) greenhouse type (156).

Conclusions and Future Perspectives

Current challenges

Current detection methods face significant constraints, including high costs, time-intensive processes, limited field sensitivity, challenges in multi-mycotoxin detection, complex sample preparation requirements and dependence on specialized expertise and laboratory facilities. The management of mycotoxins faces critical challenges due to climate change-induced proliferation, emerging fungal resistance to treatments and inadequate storage infrastructure in developing nations, creating significant obstacles to effective control and prevention. The substantial financial burden on small-scale farmers and the complexity of meeting diverse regulatory requirements across different geographical regions further compound these challenges, making comprehensive mycotoxin management increasingly complex.

Emerging technologies

Modern mycotoxin detection is evolving through innovative technologies, including highly specific biosensors with aptamers and antibodies, accessible smartphone-based platforms, sensitive, rapid test kits, machine learning-driven automated analysis systems and portable spectroscopic devices, all aimed at improving detection accuracy and accessibility.

Microbial biocontrol represents a sustainable approach to mycotoxin management through the strategic use of non-toxigenic fungal strains, antagonistic *Bacillus* and *Pseudomonas* species, advanced formulations for enhanced efficacy, integrated control methods for synergistic effects and field-validated biocontrol agents, as supported by extensive research.

Gene-editing technologies, particularly CRISPR-Cas9, are transforming mycotoxin resistance in crops through targeted applications in developing resistant varieties, modifying mycotoxin biosynthesis pathways, enhancing plant defence mechanisms, creating fungal-resistant crops and ensuring safety through rigorous assessments, as demonstrated by recent research advances.

Future directions

By combining IoT-based environmental monitoring systems, modified atmosphere packaging, real-time sensor technologies for moisture and temperature control, blockchain-enabled supply chain traceability and automated intelligent warehousing systems, modern storage solutions are revolutionizing mycotoxin management and creating a more efficient and reliable storage ecosystem, bolstered by recent research advances. Producers, processors, regulatory agencies, the scientific community and consumers are responsible for maintaining a safe and high-quality spice supply. Contaminants like mycotoxins are inevitable and impossible to eradicate. However, effective monitoring programs and regulatory limits can significantly reduce the levels of mycotoxins in consumer products.

Acknowledgements

The authors gratefully acknowledge the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, for providing the platform to publish this article.

Authors' contributions

SV and TM conceived the idea and wrote the manuscript. TM gave ideas and SV designed the diagrams and tables. SV and TM revised the manuscript. MJ, PV, KP, JH and KD finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Claude and Gravity Write to improve the language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publications' content.

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