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## Chapter

# Fungal Growth and Mycotoxins Production: Types, Toxicities, Control Strategies, and Detoxification

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## Abstract

Fungal growth and the production of mycotoxins are influenced by several factors. Environmental conditions such as temperature, water activity, and humidity affect mycotoxin production and fungal growth. Other factors such as pH, fungal strain, and substrate also play roles. Common mycotoxins include aflatoxins, fumonisins, trichothecenes, sterigmatocystin (STC), citrinin, ergot alkaloids, ochratoxins, zearalenones (ZEAs), patulin, deoxynivalenol (DON), Alternaria toxins, tremorgenic mycotoxins, fusarins, cyclochlorotine, sporidesmin, 3-nitropropionic acid, etc. These toxins cause many health conditions in animals and humans, including death. A comprehensive approach starting from the field before planting, continuing throughout the entire food chain is required to control mycotoxin contamination. Good practices, such as proper field practices before and after planting, good harvest practices and postharvest handling, and proper drying and storage measures, help reduce mycotoxin contamination. Several physical, biological, and chemical techniques have been applied to help reduce/eliminate mycotoxin contamination. Food processing also play slight role in mycotoxins removal.

**Keywords:** Fungal growth, Mycotoxin production, Mycotoxin toxicities, Mycotoxin control and detoxification measures, Factors affecting mycotoxins production

## 1. Introduction

Fungi are members of the group of eukaryotic organisms that mainly include molds and yeasts. Where conditions are favorable, fungi produce mycotoxins, which are naturally occurring toxic secondary metabolites produced by some fungi, mostly molds, which grow on several crops and foods such as nuts, apples, grains, coffee, fruits, spices, etc., before and after harvest. These filamentous fungi are among the microorganisms that metabolize many organic substances such as sugars, lipids, proteins, etc. Fungi are naturally abundant and ubiquitous and have the capability to attack crops in field, after harvest, and during storage, and can survive under several environmental conditions including humidity, temperature,

pH, water activity ( $a_w$ ), etc. [1, 2]. Fungi commonly invade the commodities consumed by animals and humans, and due to their growth on the commodities, they produce low molecular weight secondary metabolites called mycotoxins [3]. Mycotoxins have been recognized as emerging toxins of concern worldwide [4]. Although more than 100,000 fungal species are known, only few, such as species of *Aspergillus*, *Fusarium*, *Penicillium*, etc., are known to produce most of the mycotoxins that significantly affect agriculture, humans, and animals [5].

At present, at least 300 mycotoxins are known, with the widely varied fungal origin, function, structure, toxic potency, and biological effects, although only a few have established significant effects on agriculture, animals, and humans [6, 7]. Many of them have not been sufficiently studied. All mycotoxins identified have between four carbon and complex carbons, which is mainly a result of the different biosynthetic pathways involved in their production [3]. As the production of mycotoxin has not been reported to have any significant biological effect on the growth of fungi, they might play a role in defense mechanisms against several intruders such as insects, animals, nematodes, microorganisms, and even humans [8, 9]. Production of mycotoxins may play role in the maintenance of cell oxidative status at a level essential for the safety of fungi [10]. Several mycotoxins have numerous toxic effects on animals and humans; they pose a real health concern to the public, as they are widely spread in foods worldwide [11].

Mycotoxigenic and pathogenic fungi are common in almost all regions worldwide. They invade, colonize, and grow on many crops, producing mycotoxins under various conditions such as environmental conditions [1]. Several factors have effects on the growth of fungi and production of mycotoxin, and in general, contamination with mycotoxins occurs at various points in the food chain [1]. Fungi presence, however, does not automatically signifies subsequent mycotoxin production, as the conditions required for mycotoxins production are definitive and independent from the conditions that promote the growth of fungi [12, 13]. This chapter throws insight into fungal growth and consequent production of mycotoxins, including their types and toxicities. The chapter also provides methods and strategies for mycotoxins control and detoxification.

## 2. Fungal growth conditions and mycotoxin formation

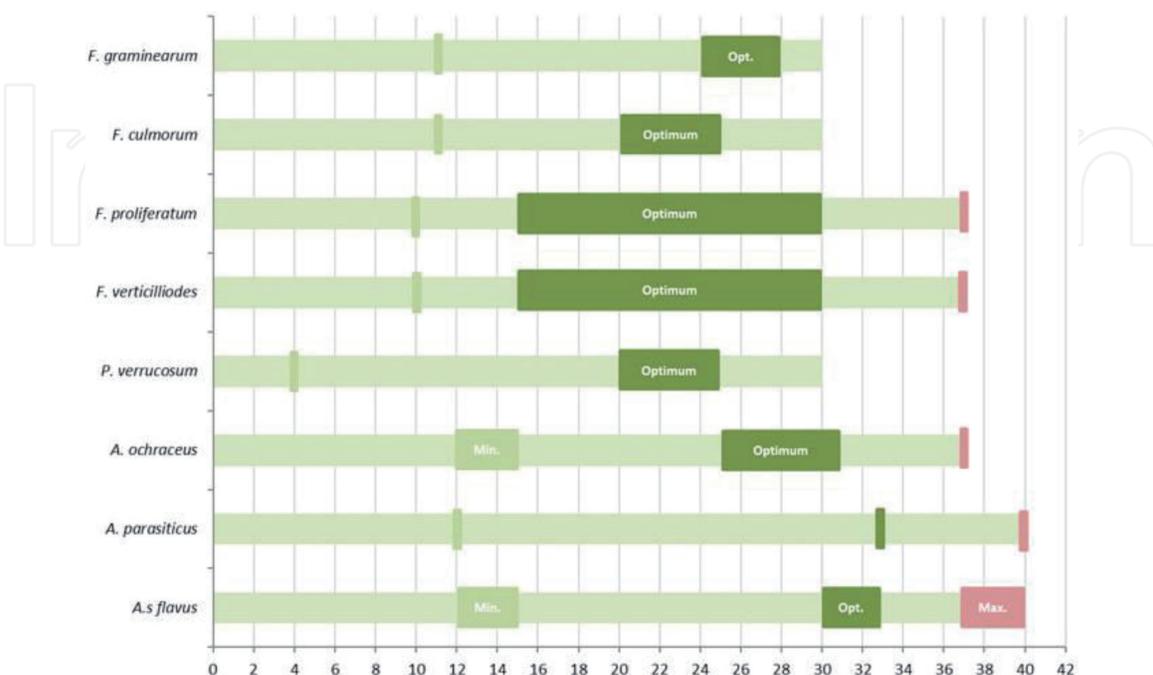
Several factors influence fungal invasion, colonization, growth, and consequent production of mycotoxins [14]. The most significant conditions favorable for growth of fungi and production of mycotoxin include temperature and  $a_w$ . The optimum temperature for production of mycotoxin by several molds range from 20 to 30°C. Generally, in the tropics and subtropics which are characterized by warm climate, aflatoxins B1, B2, G1, G2, M1, and M2 (AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2 respectively) are of main concern, whereas fusariotoxins, e.g. trichothecenes occur mostly in regions with moderate climate [15, 16]. Additionally, stress factors, including mechanical damage, insect ingression, weed competition, high crop densities, poor fertilization, and drought, can weaken the natural defense mechanism of plants and, as a result, promote fungal colonization, mycotoxin production, and formation of toxins. The optimum conditions for production vary along with temperature, substrate, humidity, and type of mycotoxin [17–19]. Interactions between temperatures and  $a_w$  with respect to *F. Verticillioides* growth and mycotoxins production have been studied [17]. The study reported that at 0.995  $a_w$ , the optimal *F. verticillioides* growth rate ranged from 20 to 25°C, however, when the  $a_w$  reduced to 0.98, the optimal temperature for growth shifted to 30–35°C. The conclusion was opposite for the production of mycotoxins. For example, the optimum  $a_w$  and temperature for

the production of fumonisin B1 (FB1) were 0.98–0.995 and 20°C respectively [17]. This shows that the optimum conditions required for mycotoxins production differ from those required for their growth. **Figure 1** shows the minimum, optimum, and maximum required by some fungi for the production of mycotoxins.

The growth of Fungi is categorized into primary and secondary growths. In primary growth, organic compounds are required for the biomass synthesis and production of energy needed to drive chemical reactions to produce the primary metabolites that are essential for growth; secondary growth takes place after the phase of maintained growth and may, sometimes, lead to sporulation and secondary metabolites production [1]. The secondary metabolites, including mycotoxins, have no significant impacts on the fungal growth, but appear to be produced as a result of the excess accumulation of the precursors of primary metabolites, as a means to reduce their concentrations in the fungi [12]. As the fungi that produce mycotoxin and their targeted hosts are diverse in nature, it is difficult to define a single set of conditions which ultimately leads to mycotoxin production. However, in general, the main factors which affect the production of mycotoxins, include temperature, relative humidity, aw, substrate, fungal strain, and pH.

## 2.1 Temperature, relative humidity, and water activity

Environmental factors play significant roles in determining the occurrence of fungi; the fungal activities and colonization are predominantly determined by conditions such as temperature and humidity [21]. These factors influence the mycotoxigenic fungal prevalence, development, frequency, distribution, and survival, and their subsequent accumulation of toxin. Also, humidity and temperature affect plant growth, health, strength, and influence the mycotoxigenic fungal competitiveness. Due to differences in growth requirements and the environmental factors, fungal development and production of mycotoxin differ from one geographical region to another [22–24]. Before harvest, on the field, fungi *Fusarium* species mostly dominate since they are hygrophilic and require at least 90% relative



**Figure 1.** Minimum, optimum, and maximum range of temperatures (°C) for the production of mycotoxins (adapted from [20]).

humidity (RH) to germinate/grow. Whereas after harvest, the hygrophilic fungi are not seen, as xerophilic and mesophilic fungi, such as *Penicillium spp.* and *Aspergillus spp.*, germinate and grow, leading to mycotoxins production at 80% or less RH and 80 to 90% RH, respectively [23]. In storage, if the surrounding environment's RH is more than the food's equilibrium RH, the food gains moisture and its  $a_w$  increases [25]. Increased  $a_w$  during storage increases the food vulnerability to fungal invasion, germination, growth, and production of mycotoxin. The major optimal factors for fungal growth and mycotoxin production are shown in **Table 1**.

For temperature requirement, most fungi are mesophilic and grow in range of temperature between 5 and 35°C, with optimal growth occurring around 25–30°C [1]. In addition, there are species of fungi called psychrophiles (tolerant to low temperatures) and thermophiles (can bare high temperatures). When there is shift in optimal temperature range (see **Table 1**), it may lead to a halt in growth [28]. The conditions that encourage the growth of fungi may not necessarily result in the production of mycotoxins. However, temperatures of 25–30°C,  $a_w$  above 0.78, and RH of 88–95% favor fungal growth and production of mycotoxin [23, 25]. The most common mycotoxins, their toxicities, and fungi that produce them are shown in **Table 2**. Most of these mycotoxins are carcinogenic, genotoxic, mutagenic, immunotoxic, teratogenic, etc.

## 2.2 Fungal strains

There is variation in the toxicity of Fungal species and their mycotoxins production may usually depends on species, strains, and/or genera. The production of mycotoxin is influenced by strain stability, variation, and specificity [29]. Strains of the same species can have different optimum conditions required for growth and production of mycotoxins; also, strains of the same species can produce one or more different mycotoxins. For instance, while *Aspergillus flavus* can thrive within 15 to 44°C and produce aflatoxin B1 (AFB1), *Aspergillus carbonarius* thrives at 8 to 40°C and produce ochratoxin A (OTA) [23].

## 2.3 pH

The surrounding medium and its pH influence the development of fungi and production of mycotoxins. pH of the surrounding medium affects fungal growth either by direct or indirect actions on cell surfaces or on nutrient availability

Fungi	Water activity	Temperature for growth	Optimal water activity	Optimal temperature	Optimal water activity for mycotoxin production	Optimal temperature for mycotoxin production
<i>Aspergillus carbonarius</i>	0.90–0.93	8–40°C	0.94–0.99	32–35°C	0.98	30–35°C
<i>Aspergillus flavus</i> ,	0.91–0.99	15–44°C	0.95	35°C	0.99	33°C
<i>Aspergillus ochraceous</i>	0.80–0.98	10–40°C	0.96–0.98	24–31°C	0.98	25–30°C
<i>Aspergillus parasiticus</i>	0.91–0.99	15–44°C	0.95	35°C	0.99	33°C

**Table 1.** Optimal conditions for fungal growth and mycotoxin production [26, 27].

Mycotoxins	Common fungal species	Foods commonly found	Toxicity
Aflatoxins (Aflatoxins B1, B2, G1, G2, M1, M2)	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i> , <i>Aspergillus bombycis</i> , <i>A. pseudotamarii</i> , <i>A. nomius</i> , etc.	Cereals, seeds, vegetables, nuts, legumes, fruits, etc.	Liver cancer; target DNA; hepatocellular carcinoma; mutagenic and teratogenic effects. Aflatoxin M1 is a metabolite of aflatoxin B1 and is commonly found in milk and dairy products
Alternaria toxins (altertoxin, alternariol methyl ether, alternariol, altenuene, tentoxin, tenuazonic acid)	<i>Alternaria</i> species such as <i>Alternaria solani</i> , <i>Alternaria japonica</i> , <i>Alternaria dauci</i> , <i>Alternaria triticina</i> , <i>Alternaria tenuissima</i> , <i>Alternaria brassicae</i> , <i>Alternaria alternata</i>	Fruit, grains, beer, fruit juices, vegetables, seeds, vegetable juices, wine, peppers, tomatoes, dried fruit, flour, bran, wheat, cereal products (e.g. rice and oat flake), sunflower seeds, sunflower oil, etc.	Although most <i>Alternaria</i> toxins show low acute toxicities, alternariol methyl ether and alternariol are genotoxic, cytotoxic, carcinogenic, and mutagenic effects, with scientific-based findings from toxicological studies, in vitro, involving mammalian and bacterial cells. Tenuazonic acid has phytotoxic and antibacterial properties and acute toxicities for dogs, chicken, and mice; it also causes hematological disorders in humans.
Emerging fusarium mycotoxins (enniatiins, NX-2 toxin, beauvericin, moniliformin, fusaproliferin, etc.)	<i>Fusarium</i> species, such as <i>F. verticillioides</i> , <i>F. subglutinans</i> , <i>F. proliferatum</i> , <i>F. arthrosporioides</i> , <i>F. chlamydosporum</i> , <i>F. redolens</i> , <i>F. acuminatum</i> , <i>F. avenaceum</i> , <i>F. oxysporum</i> , <i>F. beomiforme</i> , etc.; <i>Beauveria bassiana</i>	Corn, rice, corn products, seeds, nuts, coffee, tree nuts, dried fruits, beans, vegetable oil, etc.	They are potentially toxic to humans and animals. Beauvericin has antifungal, insecticidal, and antibacterial properties, and may have toxic effects leading to apoptosis induction, increased cytoplasmic calcium concentration, and fragmentation of DNA in cell lines of mammals.
Ergot alkaloids	Clavicipitaceae (e.g. <i>Neotyphodium</i> and <i>Claviceps</i> ) and Trichocomaceae (e.g. <i>Penicillium</i> and <i>Aspergillus</i> ) families. <i>Claviceps purpurea</i> is the dominant producer	Rye, barley, wheat, triticale, oats, etc.	Ergot alkaloids are both harmful and beneficial to humans. Causes ergotism. Gangrenous and convulsive forms of toxicities. Can cause delirious seizures, St. Anthony's Fire, and fits. Can cause
Fumonisin (fumonisins B1, B2, B3, etc.)	species of <i>Fusarium</i> (such as <i>F. verticillioides</i> , <i>F. nygamai</i> , <i>F. fujikuroi</i> , <i>F. Proliferatum</i> , <i>F. oxysporum</i> , <i>F. Globosum</i> , etc.), <i>Aspergillus awamori</i> , <i>A. niger</i> etc.	Corn, corn products, asparagus, rice, beer, soybeans, beans, sorghum, etc.	Inhibits sphingolipids synthesis. Linked to esophageal and liver cancer in human, atherosclerosis in monkeys, equine leukoencephalomalacia in horse, porcine pulmonary edema and pulmonary artery hypertrophy in swine, and kidney and liver cancer in rodents.
Ochratoxins (Ochratoxins A, B, C)	<i>Aspergillus</i> and <i>Penicillium</i> genera, such as <i>Aspergillus ochraceus</i> , <i>Aspergillus carbonarius</i> , <i>Aspergillus niger</i> , <i>Penicillium verrucosum</i> , etc.	Cereals, seeds, fruits, legumes, vegetables, nuts, etc.	Ochratoxins have immunotoxic, neurotoxic, hepatotoxic, teratogenic, and nephrotoxic activities; cause nephropathy in pigs; In human, ochratoxin A was linked to urothelial tumor, renal failures, chronic interstitial nephropathy, and Balkan endemic nephropathy.

Mycotoxins	Common fungal species	Foods commonly found	Toxicity
Patulin	<i>Penicillium expansum</i> , <i>A. clavatus</i> , <i>Penicillium patulum</i> , <i>Penicillium griseofulvum</i> , <i>Penicillium urticae</i> , <i>Penicillium crustosum</i> , etc.	Apples, apple products, fruits, cereals, legumes, vegetables, nuts, seeds, etc.	Mutagenicity, carcinogenesis, immunotoxicity, teratogenicity, and neurotoxicity are chronic and acute effects of patulin showed on cell cultures. Patulin has neurotoxic and immunotoxic effects in animals, but no reliable evidence has shown its carcinogenicity to human, although studies have shown human toxicities, such as hemorrhage, ulceration, vomiting, and nausea
Sterigmatocystin	Species of <i>Aspergillus</i> , such as <i>A. versicolor</i> (major producer), <i>A. aureolatus</i> , <i>A. amstelodami</i> , <i>A. ruber</i> , <i>A. Chevalieri</i> , <i>A. sydowi</i> , <i>A. quadrilineatus</i> , etc. Also produced by species of <i>Emiricella</i> , <i>Chaetomium</i> , <i>Penicillium</i> , and <i>Bipolaris</i>	Peanuts, corn, wheat, grain products, barley, rice, etc.	Sterigmatocystin has teratogenic, mutagenic, and carcinogenic effects. Can cause hepatic toxicity in most animals; bloody diarrhea and death in cattle; hepatocellular carcinoma and squamous cell carcinomas in rats; LD50 in mice is $\geq 800$ mg/kg
Trichothecenes, e.g. deoxynivalenol (vomitoxin), anguidine, T-2 toxin, 3- and 15- acetyldeoxyvalenol, nivalenol, HT-2 toxin, crotoxin, diacetoxyscirpenol, macrocyclics, etc.	Species of <i>Fusarium</i> (such as <i>Fusarium crookwellense</i> , <i>F. graminearum</i> , <i>F. poae</i> , <i>F. culmorum</i> ), <i>Myrothecium</i> , <i>Trichoderma</i> , <i>Cephalosporium</i> , <i>Stachybotrys</i> , <i>Spicellum</i> , <i>Verticimonosporium</i> , <i>Trichothecium</i>	Rice, oats, wheat, vegetables, rye, barley, maize, etc., and animal foods, such as liver, eggs, milk, and kidney	Trichothecenes can diffuse into cells, and block translation by interacting with eukaryotic ribosomes. Trichothecenes exposure affect nearly all key systems in vertebrates, cause alimentary toxic aleukia (ATA) in humans, etc. They inhibit DNA, RNA, and protein synthesis, and also cause lipid peroxidation, apoptosis, inhibit mitochondrial functions, cause changes in neurotransmitters, and cytokine activation.
Zearalenone (formerly referred to as F-2 toxin)	<i>Fusarium</i> species, such as <i>F. crookwellense</i> , <i>F. cerealis</i> , <i>F. semitectum</i> , <i>F. equiseti</i> , <i>F. graminearum</i> , <i>F. culmorum</i> , etc.	Maize, soybean, oats, barley, wheat, rice, rye, sorghum, grain products, etc.	Zearalenone chronic administration can cause uterine fibroids, pituitary adenomas, hepatocellular carcinoma, and liver damage in mice, and chronic progressive hematotoxicity, testicular atrophy, cataracts, retinopathy, and nephropathy in rats; Among other animals, pig is more prone its toxicities. Zearalenone or its metabolic compounds are known to bind transcription factors, including pregnane X receptor involved in expressing enzymes in pathways of biosynthesis

Mycotoxins	Common fungal species	Foods commonly found	Toxicity
Other common mycotoxins (Fusarins [fusarins A– F], Tremorgenic mycotoxins, Cyclochlorotine, Sporidesmin, 3-nitropropionic acid,)	Fusarins are produced by the species of <i>Fusarium</i> , such as <i>Fusarium verticillioides</i> (formerly <i>Fusarium moniliforme</i> ), <i>Fusarium graminearum</i> ( <i>Fusarium venenatum</i> ), <i>Fusarium poae</i> , <i>Fusarium sporotrichioides</i> , <i>Fusarium oxysporum</i> . Tremorgenic mycotoxins are produced by <i>Aspergillus terreus</i> , species of <i>Penicillium</i> genus, etc.; <i>Pithomyces chartarum</i> produces Sporidesmin; Cyclochlorotine is produced by <i>Penicillium islandicum</i> ; 3-nitropropionic acid (3-NPA) is produced by the species of <i>Arthrinium</i> ;	Many foods and feeds	Fusarins are mutagenic; 3-nitropropionic acid interjects mitochondrial electron transport; Cyclochlorotine interrupts myofibrils and is hepatotoxic in animals; Due to the hydrophobicity of sporidesmin, it can be integrated easily into the membranes of cells, in which it changes the organization of the bilayer Tremorgenic mycotoxins cause “staggers syndromes” in livestock, and are linked to neurological conditions, such as seizures, tremors, mental confusion, and even death in humans.

**Table 2.** Common mycotoxins, their producing fungi, and known toxicities [1, 2, 25].

respectively. Fungi can modulate the pH of the surrounding medium through secreting alkali or acids; species of *Aspergillus* and *Penicillium* can acidify the surrounding through citric and gluconic acids secretion [30]. The ability to control the pH provides fungi a better possibility of surviving in their host. In addition, the pH can have influence on the interactions of temperature and  $a_w$ , as it affects metabolic processes, including morphogenesis and sporulation [31].

The pH can also affect the gene expression for biosynthesis, e.g. at pH 8, the genes responsible for production of ochratoxin A by *Penicillium verrucosum* are expressed [32]. Although the pH effect on the production of some mycotoxins has not been fully established for every type of mycotoxins, however acidic conditions are known to promote germination and production of mycotoxin. Production of aflatoxins requires pH 4.0 and the pH has inverse relationship with the level of synthesis [12]. In the same way, OTA levels are higher when *Aspergillus ochraceus* are at low pH [32]. Fumonisin B1 (FB1) is unstable in alkaline medium and requires pH 4.0–5.0 for synthesis; production of trichothecenes is initiated in acidic conditions [12].

## 2.4 Substrate

Mycotoxigenic fungi grow on several substrates, however, the major reason for their predominate on some foods has not been sufficiently established. The nutrients needed for the fungal growth, mostly nitrogen and carbon, are commonly found in foods, especially those rich in carbohydrates, molds are found in many foods [33]. Substrates that encourage the growth of fungi may not necessarily be support mycotoxin production, as conditions that promote the production of mycotoxins are usually different from those needed for fungal growth (see **Table 1** and **Figure 1**). Generally, mycotoxins production is greatly influenced by the interactions between many factors in substrate, such as temperature,  $a_w$ , pH, and composition (e.g. simple sugars). The substrate's osmotic pressure affects the growth of fungi and the production of mycotoxin, and several studies reported that it can aid in evaluating fungal physiological responses, as well as can affect the secondary metabolites biosynthesis such as mycotoxins biosynthesis [34]. On the other hand, upon osmotic stress fungal species adjust their physiological responses to enhance their survival and adaptation [34].

Sugars have carbon and filamentous fungi have the natural ability to hydrolyse several sources of carbon to support growth and produce energy [35]. Consequently, in sugars presence, such as the presence of simple sugars, which readily breakdown, there is higher frequency of fungal growth. When complex sugars dominate, fungal growth is slower as the complex sugars need further digestion to yield simpler units of carbon that are readily absorbable. Simple sugars may contribute to the mycotoxins production. [36] reported that increase in the concentration of soluble sugars to 3 and 6 percent, especially maltose, glucose, and sucrose, promoted production of AFB1 in cell cultures. [37] also reported that more production of AFB1 by *Aspergillus flavus* resulted from an increase in the medium sugar levels.

## 2.5 Effects of climate change on mycotoxins production

Climate change has resulted in changes in most environmental conditions, including rise in global temperature which is expected to increase by 1.5–4.5°C by 2100 [38]. A rise in droughts, precipitation, flooding, and extreme weather conditions are expected [39]. Climate change and global warming affect food security greatly, including reduction in crop quality, reduction in yields, and increased food

safety challenges making some crops unsafe for human and animal consumption. Global change affects mycotoxin production, mainly by affecting the environmental conditions that influence their production [40].

Climate change affects different regions in various ways, with some regions having advantage whereas the opposite is the case for other regions [41]. The Mediterranean basin and Southern Europe most likely experience significant changes resulting in increase in mycotoxin prevalence, while the effects of climate change are anticipated to be positive in northern Europe [42]. As fungal growth, their germination, and production of mycotoxins are largely influenced by environmental conditions, especially the optimal conditions, temperature changes and change in humidity induced by climate change may several effects on production of mycotoxins. The mycotoxins usually produced at low temperature may not be produced at higher levels, whereas others predominant in tropical and sub-tropical regions, including aflatoxins, may be produced in temperate regions as a result of the expected rise in temperatures in these regions; for example, in Italy, in 2003 and 2004, hot and dry conditions resulted in the *Aspergillus flavus* colonization and aflatoxins production [43]. Different mycotoxins can be affected differently, usually based on the optimum conditions required for their production. Climate change also affects mycotoxins production indirectly via the increase of pest and insect populations, global spread, and attacks, early maturing and ripening of crops, decreased plant resilience, and change in host pathology upon the presence of CO<sub>2</sub> in the atmosphere [42, 44].

### 3. Mycotoxins prevention and control

The production of mycotoxins has shown unavailability and, as a result, many foods are being contaminated regularly. Mycotoxins greatly and widely vary and are produced by many fungi at various stages, on many crops, and consequently, a specific strategy for controlling all the mycotoxins has proven difficult with little or no success in decontaminating the affected foods or reducing all the mycotoxins to safe levels; most specific control strategies may only be effective in reducing the levels of specific types of mycotoxins. However, certain control measures can be employed to prevent or minimize their entrance, production, and occurrence in foods. Currently, no method has proven sufficient to totally control all mycotoxins. A successful strategy may adopt a combination of food safety system involving suitable quality measures at every production stage to reduce the frequency of mycotoxins occurrence in the final food products, which would include taking appropriate measures before, during, and after harvest. **Table 3** shows the overview of the action mechanisms of mycotoxins.

#### 3.1 Mycotoxins control using appropriate field practices

Most fungi are phytopathogens that infect the crops in field, and their pre-harvest management is very important. In general, fungi that mostly predominate in field include *Alternaria spp.*, *Cladosporium spp.*, and *Fusarium spp.* However, *Penicillium spp.* and *Aspergillus spp.* also occur in field at low levels and the contamination levels in general are usually higher anywhere climate conditions are favorable to the production of mycotoxin [45]. Whereas it is unlikely to totally prevent the production of mycotoxins in the field before harvest, it is of extreme importance to adhere to strategies which aim to reduce contamination to the barest possible minimum in preharvest. To choose and implement suitable strategies, a sufficient knowledge about the mycotoxigenic fungi, crops mostly affected, harvesting

Action	Mechanism
DNA effects	There are two major types of interactions between nucleic acids and mycotoxins; reversible and noncovalent or irreversible and covalent. The covalent and irreversible interaction between DNA and AFB1 results in the formation of N7-guanine adduct.
Effects on hormones	ZEA has structural similarity to 17 $\beta$ -estradiol; the effects of ZEA on receptors of estrogen explain its fertility problems on humans and animals. Ergovaline, an ergot alkaloid, reduces levels of prolactin in animals by acting as an agonist of dopamine.
Epigenetic properties	Few mycotoxins change the levels of DNA methylation
Important metabolic enzymes inhibition	OTA, citroviridin, and AFB1 affect the metabolism of carbohydrates, while rubratoxin B and trichothecenes interfere with metabolism of lipid. Fumonisin B1 inhibits argininosuccinate synthetase. Fumonisin B1 chemical structure has high similarity to those of sphinganine and sphingosine, the sphingolipids backbones. Consequently, fumonisins inhibit ceramide synthase competitively.
Ionophore	Beauvericin and enniatins that are produced by the species of <i>Fusarium</i> have ionophoric activities specific to potassium and cause influx of potassium into the matrix of mitochondria, followed by swelling of the mitochondria.
Mitochondrial interactions	By binding covalently to the enzyme active site, 33-NPA permanently inactivates succinate dehydrogenase. Acrebol, from <i>Acremonium exuviarum</i> , inhibits mitochondrial complex III, consequently causing ATP depletion by inhibiting the chain of respiration. Fumonisin B1 was found to obstruct the mitochondrial complex I in human neuroblastoma cells and rat primary astrocytes, resulting in reduced cellular and mitochondrial respiration and an increase in reactive oxygen species (ROS) generation, with calcium signaling deregulation.
Necrosis and apoptosis	AFB1 cytotoxic effects in lymphocytes of humans involve necrosis, caspase activation, and apoptosis.
Protein interaction	The plasma albumin binds to aflatoxins. After oxidation of AFB1 by cytochrome P450s, two epoxides are formed and they react with the lysine $\epsilon$ -amino group, forming AFB1-albumin adducts. Aflatoxins are immunosuppressive, and in several studies, they suppress immune response mediated by the cell and impairs phagocytosis and chemotaxis. Most immunotoxic properties of fumonisin B1 may be as a result of its capability of altering the levels of mRNA and/or expression of IL-1 $\beta$ , IFN- $\gamma$ , and TNF- $\alpha$ in several scientific experiments. Penitrem obstructs uptake of glutamate and GABA ( $\gamma$ -aminobutyric acid) into cerebellar synaptosomes, modulating the function of GABA receptor. One of the ways patulin exerts its toxicities is by causing a dose- and time-dependent phosphorylation increase of c-Jun N-terminal kinase, protein kinases 1 and 2 regulated by extracellular signal, and p38 kinase, contributing to downstream effects including cell death and DNA damage. A mycotoxin known as Secalonic acid D, which causes "cleft palate", phosphorylates the binding protein of cAMP response element.
Ribosomal binding	Ochratoxin A competes with phenylalanine-tRNA ligase and inhibits synthesis of protein; both aspartame and phenylalanine reduce toxicity of OTA by competing with it. Trichothecenes toxicities are due to their capability to bind the eukaryotic ribosomes' 60S subunit and inhibit the reaction of peptidyl transferase.
RNA polymerase effects	AFB1 has inhibitory effects on chromatin-bound RNA polymerase which is DNA-dependent and, consequently interferes with synthesis of RNA. Luteoskyrin and patulin also inhibit RNA polymerase.

**Table 3.**  
*Mycotoxins action mechanisms [2].*

practices, and proper field management play important role [46]. Many factors such as delayed harvesting, poor soil fertility, heat, insect infestation, and drought contribute to the production of mycotoxins in the field [46, 47]. Appropriate practices in the field include the management and preparation of field before planting, and proper management of crop and field after planting.

### *3.1.1 Preparation and management of the field before planting*

Preparation of the field prior to planting is critical in controlling fungal invasion and the consequent production of mycotoxins. Deep plowing, tilling, production cycle, use of disease-resistant cultivars, crop rotation, use of high-quality seeds, etc. play important role. Deep plowing and tilling can be essentially used for the removal of remaining plant materials. Previous residues of crops which persist on the soil end up deteriorating and harboring soil-borne fungi, which increase their possibility of invading new crops. Plowing puts debris under the ground, making them not accessible to inhabitation by fungi. Tilling may also increase water availability to crops by minimizing the compressed layers of soil [48]. Additionally, rotation of crops prevents the build-up of fungi; it was reported that the production of mycotoxins is higher in lands where same crops are consecutively grown for years, as molds that may colonize a plant can occur from year to year if same crop was continuously planted [46–48]. Seeds for planting are also very important. Seeds with good quality contribute to the health of plants' growth to withstand fungal invasion.

### *3.1.2 Management of crop and field after planting*

Facilitating the healthy plants growth after planting through implementing proper practices in the field and decreasing the stress on crops reduces fungal growth and production of mycotoxins [47, 49]. Fertilizers application improves the health of plants and maintains their disease and fungal resistance. Availability of nutrients is important for plant life and lack of proper nutrition of plant results in a break in the plant stem, exposing it to more invasion by fungi and other microorganisms [50]. Proper irrigation also has the capacity to prevent accumulation of mycotoxins via method and timing of irrigation. Proper timing can prevent drought stress, while the method of irrigation that control splashing can help prevent the spreading of fungi. The control of insect and weed is also important in preventing crop diseases and invasion by fungi [2, 51]. Fungicide application at proper doses can also help in controlling the fungal invasion and consequent production of mycotoxins.

#### *3.1.2.1 Early fungal detection as a control means*

Fungi presence does not necessarily indicate mycotoxin production; however, their presence implies an increased mycotoxin production risk if the conditions are suitable for the production of mycotoxin. Consequently, early detection of fungi which allows corrective measures can be very critical in controlling mycotoxin production [52]. When fungi are detected at early stages, the methods of decontamination (see [2]) in the field can be used to prevent fungal germination and growth, which in turn prevent subsequent production of mycotoxins.

#### *3.1.2.2 Biological control after planting*

After planting of crops, biological control measure largely includes the applying harmless species of fungi that compete with mycotoxigenic fungi, inhibiting their pathogenic activities. While this measure seems practically challenging, it presents safer control methods that are ecofriendly. This measure implies introducing a strain of harmless bio-agents, e.g. yeasts or bacteria, which compete with mycotoxigenic fungi for resources, thereby reducing their growth and ability to produce mycotoxin. For instance, *Aspergillus spp.* strains that do not produce aflatoxins are

applied as biological agents to compete with the strains that produce aflatoxins and prevent their dominance and subsequent aflatoxins production [53, 54]. A study reported that after applying non-aflatoxigenic strain of *Aspergillus parasiticus* on the soil in the field, significant reduction in the levels of aflatoxin was attained [55]. Same was the case after non-toxic strains of *Aspergillus flavus* were applied to cotton row. However, this biological control method has limitations that may discourage its wide application. First of all, the biological agent used may have impact on other natural occurring microorganisms. Secondly, non-toxigenic strains of fungi, even though they can help in reducing the production of mycotoxins, may produce other metabolic compounds that might be toxic to humans and animals. Thirdly, the non-toxigenic strains may result in underestimating the levels of mycotoxins, as they may have effects on the fungal metabolic pathways and cause the production of modified derivatives of mycotoxins [54]. Additionally, the ability to produce mycotoxins may be transferred from one fungi to another (a descendant) via non-toxigenic strains crossing with toxigenic strains, resulting in likely reproduction of successive fungi that produce mycotoxins [54].

### *3.1.2.3 Chemical control after planting*

Use of fungicides as chemical control is one of the current most effective ways to proper control of crop invasion by fungi and consequent production of mycotoxins [53, 56]. Use of chemicals such as captan, mancozeb, cinnamaldehyde, citronella oil, tea tree oil, monocerin, sulfur, etc. has been recommended.

## **3.2 Appropriate measures during harvest**

Another critical stage for controlling mycotoxin production is during harvest, where moisture plays the most crucial role for the protection of crops. Harvest should start after dry weather condition. Harvesting crops in wet weather may make them more vulnerable to the growth of fungi and subsequent mycotoxin production; also, keeping crops on the field for a long period of time can increase the risk of invasion by fungi, birds, rodents, pests, and insects, all of which can contribute to mycotoxin production through one way or the other [1]. It is important to prevent mechanical damage during harvest.

## **3.3 Postharvest control and prevention**

### *3.3.1 Appropriate storage*

Measures should be taken to limit fungal invasion and the production of mycotoxins before food products reach storage facility. If commodities are highly contaminated before reaching storage facility, it is difficult and more complicating to prevent further fungal or mycotoxins accumulation [47]. During storage, proper techniques should be applied to avoid fungal invasion and germination. Several fungi inhabit stored grains, especially fungal species that are infrequent in the field such as *Penicillium spp.* and *Aspergillus spp.*, whereas fungi in the field that require high water activity pose less significance during storage [57]. Several factors and storage conditions affect fungal growth and germination capacity during storage, especially temperature and  $a_w$ . The fungi active in storage can grow at 70–90% relative humidity, and they usually thrive at temperatures of 10 to 40°C with 25–35°C range of optimum temperature [58]. Use of chemical preservatives, hygienic conditions, and storage time can also affect fungal growth and mycotoxin production. Temperature and water activity should be monitored and properly controlled during

storage. The relative humidity should be kept below 70% throughout storage. The foods should be kept at low temperatures to reduce fungal activity during storage. The temperature of the stored food can be used as a good storage quality indicator.

### *3.3.2 Chemical detoxification and decontamination*

Chemical methods make use of chemical treatments with oxidizing agents, reducing agents, alkalis, and acids which are synthetic or organic. The chemicals are employed in the detoxification of mycotoxins after adding the foods. Chemicals can be introduced through packing, fumigation, mixing, or immersion [59]. Chemicals commonly used include formaldehyde, ozone, chlorinating agents, sodium bisulfite, hydrogen peroxide, hydrochloric acid, ammonium hydroxide, organic acids, and natural substances (e.g., spices, herbs, and their extracts) [59–61]. Treatment with chemical is effective in removing some mycotoxins, although they are mostly weak chemicals and most mycotoxins can be resistant to the chemicals. Ozone treatment is promising as it has the ability to degrade mycotoxins via reacting with bonds in the structure of mycotoxins, such as the double bonds in aflatoxin B1. By-products may form in the process [62, 63].

### *3.3.3 Physical detoxification and decontamination*

Physical measures to control mycotoxins mostly comprise separating contaminated and damaged crops from wholesome crops using methods such as sorting, steeping, dehulling, washing, density segregation, sieve cleaning, etc., to reduce the mycotoxins levels [60, 64]. In physical process of decontamination, mycotoxins that are soluble in water can be removed partially from the grain outer surface using water or solutions of water [62]. Physical methods also include mycotoxins destruction and removal using irradiation and heat treatment [59]. Thermal processes including extrusion heating, radiofrequency, microwave, infrared, steam, boiling, etc. have been applied as innovative methods of mycotoxin decontamination [63]. Heat treatments that make use of a combination of the temperature conditions and time might give the most significant approach to control mycotoxins, however, most mycotoxins have heat stability and require very high temperatures and prolonged durations of processing for destruction, which may not be achieved in conventional food processing [64, 65] or destructive to the food constituents such as nutrients [63]. Non-thermal treatments like irradiation might be effective by partial lowering of mycotoxin levels, as the mycotoxins absorb energy of radiation and can be widely applied at industrial scale [62]. However, Irradiation is not widely applied due largely to public distrust of irradiated foods, since radiation has the ability to penetrate cells, causing DNA damage that results in mutations. In spite of that, the European Commission has approved 10 kGy dose as the maximum dose allowable for food application after it was demonstrated that this level poses no danger to humans [62].

Cold plasma is a recent novel non-thermal physical method used for the removal of fungi and mycotoxins. Cold plasma is ionized gas with partially ionized molecules and atoms with net charge approximately zero [66]. Cold plasma treatment caused the destruction of fungal DNA and cell wall, leading to the leakage of intracellular components [66–69]. Cold plasma partially or completely destroyed mycotoxins quickly [66, 70]. The efficiency of mycotoxins destruction mechanism is associated with their molecular structures, the nature of plasma, and subsequent interactions; destruction may be associated with the production of free radicals in the course of the treatment regimen, or the UV photons and ozone presence, or reactive electrons and ions [66]. Treatment with cold plasma is distinctive from conventional methods, as the mycotoxins can be rapidly decontaminated at ambient

pressure and temperature conditions without affecting the quality of the food [66]. However, some studies indicated that treatment with cold plasma may have effects on lipids, and it may be difficult to apply at large-scale industries, especially for the treatment of foods with irregular shape and bulky foods [71].

Photocatalytic detoxification is another emerging non-thermal technique used for mycotoxins removal from foods. The process involves chemical reaction initiated by photons absorption by solid photocatalyst, resulting in redox reactions on the photocatalytic material surface, leading to free radicals' formation which interact with the contaminants (mycotoxins) and helps degrade or convert them to lesser toxic substances via oxidation [72]. Several studies have reported photocatalytic detoxification effects on mycotoxins [72–75].

#### *3.3.4 Biological detoxification and decontamination*

Biological decontamination strategy makes use of microorganisms (algae, molds, yeasts, and bacteria). The microorganisms may bind, modify, or degrade the mycotoxins to lesser toxic compounds in some feed and foods through decarboxylation, hydrolysis, deamination, glucosylation, and/or acetylation [60]. Ochratoxin A can be converted to phenylalanine by some bacteria, plants, mold, and yeasts [76]. Some enzymes and microorganisms can be added to feed for mycotoxins degradation/detoxification in the ruminants' GI tracts. Yeasts and Lactic acid bacteria are mostly used for decontaminating mycotoxins as they can reduce their levels by binding to their cell surface or by converting them to lesser toxic substances [62]. Additionally, enzymatic catalysis can be used as they have promising applications in decontaminating mycotoxins [77].

## **4. Conclusion**

Fungi commonly invade the commodities consumed by animals and humans, and due to their growth on the commodities, they produce low molecular weight secondary metabolites called mycotoxins. Environmental conditions such as temperature, water activity, and humidity affect mycotoxin production and fungal growth. Other factors such as pH, fungal strain, and substrate also play roles. The conditions that encourage the growth of fungi may not necessarily result in the production of mycotoxins. Common mycotoxins include aflatoxins, zearalenones (ZEAs), patulin, deoxynivalenol (DON), fumonisins, trichothecenes, sterigmatocystin (STC), citrinin, ergot alkaloids, ochratoxins, Alternaria toxins, tremorgenic mycotoxins, fusarins, cyclochlorotrine, sporidesmin, 3-nitropropionic acid, etc. These toxins cause many health conditions in animals and humans, including death. A comprehensive approach starting from the field before planting, continuing throughout the entire food chain is required to control mycotoxin contamination. Good practices, such as proper field practices before and after planting, good harvest practices and postharvest handling, and proper drying and storage measures, help reduce mycotoxin contamination. Several physical, biological, and chemical decontamination methods can be used to reduce/eliminate mycotoxin levels. More studies are required to develop methods and techniques that can effectively reduce all mycotoxins in foods and feeds.

## **Conflict of interest**

The authors declare no conflict of interest.

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