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Review

Mycotoxins: Factors influencing production and control strategies

Rouaa Daou[1,2,*], Karine Joubrane[2], Richard G. Maroun[1], Lydia Rabbaa Khabbaz[3], Ali Ismail[2], and André El Khoury[1]

[1] Centre d'Analyses et de Recherche (CAR), Unité de Recherche Technologies et Valorisation agro-Alimentaire (UR-TVA), Faculty of Sciences, Saint-Joseph University of Beirut, Campus of sciences and technologies, Mar Roukos, Lebanon
[2] Department of Food Science and Technology, Faculty of Agricultural Sciences, Lebanese University, Dekweneh, Beirut, Lebanon
[3] Laboratoire de pharmacologie, Pharmacie clinique et contrôle de qualité des medicaments, Faculty of Pharmacy, Saint-Joseph University of Beirut, Beirut, Lebanon

* **Correspondence:** Email: rouaa.daou@net.usj.edu.lb; Tel: +0096170952589.

Abstract: Mycotoxins are secondary metabolites produced by filamentous fungi in food and feed due to several conditions that affect fungal growth and mycotoxin production in different ways. This review aims to explore the different factors that affect mycotoxin production and their control methods. Environmental conditions such as high temperature and humidity increase the risk of fungal growth and mycotoxin production. Other factors that affect contamination include pH, fungal strain, and substrate. To control mycotoxin contamination an integrated approach that starts in the field prior to planting and continues throughout the whole food chain is required, so good practices help minimize contamination at every step to deliver safe products. Good practices include proper practices on the field before and after planting, good harvest practices, appropriate drying measures, and good storage practices. Mycotoxin contamination is inevitable in food and once present they tend to remain as they are very stable compounds, although several physical, chemical and biological techniques could be applied to help minimize contamination. Food processing may also play a minimal role in controlling mycotoxins. Finally, regulations serve to keep food markets free from highly contaminated products, while proper sampling procedures and analytical methods ensure regulations endorsement.

Keywords: filamentous fungi; mycotoxins; environmental conditions; control strategies; good storage practices

Abbreviations: AFM1: aflatoxin M_1; AFB1: aflatoxin B_1; DON: Deoxynivalenol; a_w: water activity; OTA: ochratoxin A; ZEN: Zearalenone

1. Introduction

Filamentous fungi are microorganisms that can metabolize a variety of organic substances including sugars, proteins, and lipids… Fungi are ubiquitous in nature and are capable of attacking crops on the field and/ or during storage and surviving within a wide range of environmental factors including temperature, humidity, and pH. Fungi invade commodities extensively consumed by humans and animals and as a result of their growth on foodstuff, they produce as secondary metabolites, low molecular weight compounds known as mycotoxins [1]. Although there are over 100,000 species of known fungi, only a few of them, namely *Aspergillus spp.*, *Penicillium spp.*, and *Fusarium spp.* are known to be responsible for the production of the majority of mycotoxins that affect agriculture significantly [2,3].

The emergence of modern mycotoxicology started with the aftermath of a veterinary crisis in London, England in 1962. During that time, 100,000 turkey poults died in what was recognized as a mysterious turkey X disease. This disease that was later attributed to the consumption of contaminated peanut meal with secondary metabolites (aflatoxins) alerted scientists to the possibility of the presence of other risky fungal metabolites [4].

Currently, more than 300 mycotoxins are known and possess wide variations in fungal origin, structure, function, and biological effect but only few of them appear to have a significant effect on health and agriculture (Table 1) [5,6]. All identified mycotoxins range from simple four carbon compounds to complex ones [1], and this is due to the difference of biosynthetic pathways that result in their synthesis. As mycotoxin production does not appear to have a significant biochemical effect on fungal growth, they may have developed to play a defensive role against multiple intruders including insects, microorganisms, nematodes, animals, and humans. Their production can also play a role in maintaining the oxidative status of the cell at a level that is essential for fungal safety [7]. Some mycotoxins pose several health effects as they are toxic to humans and animals and they present a real concern to public health because of their widespread in the world food supply [8].

This review aims to explore the factors that affect mycotoxin contamination and control strategies to minimize their frequency in food.

2. Conditions of fungal growth and mycotoxin production

Mycotoxigenic fungi are very common pathogens that are frequent in all agricultural regions in the world. They can invade and grow on a wide range of crops, and are very diverse in a way that allows them to produce mycotoxins under a different set of conditions including environmental ones [13]. Several factors affect fungal growth and mycotoxin production, and generally, contamination with these toxins can occur at different points along the food chain since it is an accumulative process that may start in the field and increase during later stages including harvesting, drying, and storage [13]. However, the presence of fungi does not necessarily mean subsequent mycotoxin contamination since the conditions required to produce mycotoxins are specific and independent from those that promote fungal growth [14,15]. Similarly, the removal of fungi from food does not guarantee the absence of mycotoxins because of their resistant chemical nature.

Table 1. Major mycotoxins, their producing fungi, and affected food types [4,9–12].

Mycotoxin	Producing Fungi	Affected Foodstuff
Aflatoxin B_1, B_2, G_1, and G_2	*Aspergillus flavus* *Aspergillus parasiticus* *Aspergillus nomius*	Wheat, maize, rice, peanuts, nuts, spices, oilseeds, and cottonseed
Aflatoxin M_1	Metabolite of aflatoxin B_1	Milk and dairy products
Ochratoxin A	*Aspergillus carbonarius* *Aspergillus niger* *Aspergillus ochraceus* *Penicillium verrucosum* *Penicillium nordicum* *Penicillium cyclopium*	Wheat, barley, oats, cocoa beans, coffee beans, fruits and fruit juice, dried fruits, and wine
Patulin	*Penicillium expansum* *Byssochlamys nivea* *Aspergillus clavatus*	Fruit and fruit juices, cheese, and wheat
Trichothecenes	*Fusarium sporotrichiodes* *Fusarium langsethiae* *Fusarium graminearum* *Fusarium culmorum* *Fusarium cerealis*	Maize, wheat, barley, oats, grains, and animal feed
Zearalenone	*Fusarium graminearum* *Fusarium culmorum* *Fusarium cerealis* *Fusarium equiseti* *Fusarium verticilliodes* *Fusarium incarnatum*	Maize, wheat, barley, rye and animal feed
Fumonisin B_1, B_2, B_3	*Fusarium verticillioides* *Fusarium proliferatum*	Maize, rice, wheat, sorghum, barley, and oats

Fungal growth is divided into two parts: primary and secondary. While primary growth requires organic compounds for synthesis of biomass and energy production necessary to drive chemical reactions and produce primary metabolites essential to growth; secondary growth happens after a period of maintained growth and may lead, but not in all cases, to sporulation and production of secondary metabolites. The secondary metabolites, such as mycotoxis, don't have significant effects on growth, however, they seem to be produced upon the accumulation of excess primary metabolites precursors serving as a way to reduce their concentration in fungi [15].

Since mycotoxin producing fungi and their target hosts are very diverse, a single set of conditions that contribute to mycotoxin contamination cannot be simply defined. Generally, the major factors that affect mycotoxin production are temperature, a_w, relative humidity, pH, fungal strain and substrate.

2.1. Temperature, water activity, and relative humidity

Climate factors play a key role in determining fungal occurrence [16] so the activity of the fungi and their level of colonization are much determined by predominant environmental conditions most importantly humidity and temperature specially on the field [9]. Those factors, according to Doohan

et al. influence the development, survival, distribution, and frequency of mycotoxigenic fungi and their subsequent toxin accumulation [17]. Temperature and humidity also affect plant growth, strength, and health and influence the competitiveness of mycotoxigenic fungi [13]. Each fungi has an optimal temperature and water activity range for growth, germination, and mycotoxin production. Therefore, no single range of temperature and water activity can be defined as inducing to fungal activity. And due to disparities in environmental conditions and growth requirements, fungal development and mycotoxin production differences among geographical regions are obvious, for example, mycotoxins such as aflatoxins occur more frequently in regions where the climate is tropical and subtropical [18,19].

When humidity and temperature conditions are favorable, fungal invasion can take place and occur at different stages either on the field or later during drying or storage and in some cases a sharp distinction of the stage in which fungal growth was initiated is not possible [15,20]. On the field, during pre-harvest, fungi such as *Fusarium* dominate mostly since they are hygrophilic fungal species that require a relative humidity of 90% and above to germinate and grow. While after harvest, hygrophilic fungi disappear as mesophilic and xerophylic fungal species such as *Aspergillus spp.* and *Penicillium spp.*, germinate, grow and produce mycotoxins at relative humidities of 80 to 90%, and 80% and less, respectively [19]. During storage if the humidity in the surrounding environment exceeds the equilibrium relative humidity of the food, the food will gain moisture and a_w of the food will increase. Increasing a_w during storage is associated with susceptibility to fungal growth and mycotoxin production (Table 2).

Table 2. Fungi types, their growth and mycotoxin production optimal conditions reported in different studies [10,24–27].

Fungi	Growth		Optimal growth		Optimal for toxin production	
	Temperature	a_w	Temperature	a_w	Temperature	a_w
Aspergillus flavus/ parasiticus	15–44 ℃	0.91–0.99	35 ℃	0.95	33 ℃	0.99
Aspergillus ochraceous	10–40 ℃	0.80-0.98	24–31 ℃	0.96–0.98	25–30 ℃	0.98
Aspergillus carbonarius	8–40 ℃	0.90–0.93	32–35 ℃	0.94–0.99	30–35℃	0.98

As for temperature requirements, most fungal species are mesophiles that grow within a temperature range of 5 to 35 ℃ with optimum growth taking place at a range between 25 to 30 ℃ [21]. There are also fungal species that can tolerate low temperatures known as psychrophiles, and others that can bare high temperatures referred to as thermophiles [9]. Temperature conditions that promote growth are related to chemical reactions necessary for development that happens inside the fungi most efficiently at the optimal temperature ranges allowing for an accelerated growth pace. However, when the temperature shifts from the optimal range the reaction rate declines or may even stop leading to a growth halt [22].

Conditions that promote fungal growth may not always lead to mycotoxin production. However, generally, a temperature range between 25 and 30 ℃, a water activity higher than 0.78, and relative humidity between 88% and 95% are considered as favorable for fungal growth and subsequent mycotoxin production [23]. For example, it has been observed for *Aspergillus spp.* that the conditions

that promote germination are within a wider range than ones that support fungal growth, which in turn, can take place over a broader range of conditions than mycotoxin production [19] (Table 2).

2.2. pH

The medium surrounding the fungi and its pH value play a role in fungal development and mycotoxin production. pH value or the saturation of hydrogen atoms in the medium surrounding the fungi affects its growth either through direct action on cell surfaces or through indirect effect on nutrient availability. Fungi possess the ability to modulate the surrounding pH through secreting acids or alkali, for example, Pennicilium sp. and Aspergillus sp. can acidify the surrounding by secreting gluconic and citric acids [28]. The capacity of controlling the pH gives the fungi a better chance to survive within the host. pH, on the other hand, can influence aw and temperature interactions since it affects metabolic processes specially ones related to sporulation and morphogenesis [29].

pH value has also been shown to affect the biosynthesis gene expression, as for example and according to Brzonkalik, "the genes responsible for OTA production in Penicillium verrucosum are expressed at pH 8" [30]. The effect of pH on the production of certain types of mycotoxins is yet to be determined separately for every kind, but it is well established that acidic conditions promote germination and mycotoxin production in most cases. For example, aflatoxin production needs a pH value of 4.0 and in its case, the lower the pH the higher the synthesis [7,15]. Similarly, OTA is observed at much higher levels when *Aspergillus ochraceus* are at lower pH ranges [30]. Fumonisin B_1, in turn, is not stable in an alkaline medium and needs a pH of 4.0 to 5.0 to be synthesized [7] and trichothecene production, as well, is induced under acidic conditions [15].

2.3. Fungal strain

Fungal species vary in toxicity and the production of mycotoxins is sometimes restricted to some types of fungi and at many times it is even limited to specific strains within a species [31]. And according to Laubscher et al. mycotoxin production is affected by "strain specificity, variation, and instability" [32]. This is evident since within the same species strains can display differences in the optimum conditions needed to promote growth and toxin production and different strains of same species may produce different types of mycotoxins. For example, *Aspergillus flavus* can grow at a temperature range of 15–44 ℃ and produce AFB1 unlike *Aspergillus carbonarius* that thrive at a wider temperature range of 8–40 ℃ and produce OTA [19].

2.4. Substrate

Mycotoxigenic fungi can grow on various types of substrates but the exact reason why fungi predominate on a specific food item is still unclear. However, since the nutrients required for their growth, mainly carbon and nitrogen, are widely found in food items especially ones rich with carbohydrates [33], molds can be found on almost all kinds of foodstuff. Nevertheless, substrates that promote fungal growth may not automatically be considered as supporters to mycotoxin production knowing that the conditions which promote toxin production are usually more constrained than those required for growth. In general, the production of mycotoxins is influenced mainly by the interaction of several factors in a substrate including pH, temperature, and composition especially the presence of

simple sugars [34]. The interaction of multiple factors within a substrate imposes limitations on fungal growth, germination, and mycotoxin production since in the presence of all promoting factors, the lack of one single factor may affect fungal growth and halt its development.

Osmotic pressure in a substrate affects fungal growth and mycotoxin production and many studies showed that it participates in determining the physiological responses of the fungi and it affects the biosynthesis of secondary metabolites including mycotoxins [35]. In addition to that, studies have shown that upon osmotic stress fungi are able to adjust their physiology in a way that enhances their adaptation and survival [35].

On the other hand, sugars are composed of carbon molecules and naturally, filamentous fungi possess the ability to hydrolyze multiple carbon sources to produce energy and support growth [36]. Therefore, in the presence of sugars, especially simple ones that are readily available for breakdown, fungal growth will be more frequent. While upon the domination of complex sugars, fungal growth will be slower since those require more digestion to attain readily absorbable units of carbon. Simple sugars might also induce the production of mycotoxins, for example, Liu et al [37] showed that an increase of soluble sugars concentration to 3.0% and 6.0%, especially sucrose, maltose, and glucose promoted AFB1 production in cell culture. Similarly, Uppala et al. showed that more AFB1 production by *A. flavus* was caused by increasing the sugar content of the medium [38].

2.5. Climate change effects on mycotoxins

Climate change is becoming more obvious and environmental changes are expected accordingly, this include an increase in global temperature that is anticipated to increase by 1.5 to 4.5 ℃ at the end of the 21st century [39]. An increase in precipitation, extreme weather conditions such as heat waves and prolonged cold winter, flooding, and droughts are also expected. This change will be coupled by an accumulation of gases in the atmosphere including carbon dioxide that is expected to double or triple in concentration within the next 25 to 50 years [40]. Global warming and climate change can greatly affect food security including the decrease of yields, decrease of crop quality, and the increase of food safety issues rendering some products unsuitable for human consumption. Global change is expected to affect many aspects of the food chain (Table 3) in what relates to mycotoxins especially that they are mainly affected by environmental factors [41].

Climate change is expected to affect regions in varied ways, and according to the European Food Safety Authority, some geographical regions will be affected in advantageous ways while others will experience detrimental effects [42]. For example, according to Medina et al. Southern Europe and the Mediterranean basin will experience significant changes that lead to mycotoxin prevalence increase and yield production decrease, while in northern Europe the climate change effects are expected to be positive [43].

Table 3. Climate change effects on atmosphere, agriculture, mycotoxin production, and crop storage.

Climate change impact on different elements	Specific effect
Effect on weather	Increase in global temperature; Increase in precipitation; Extreme weather conditions (prolonged warm or cold episodes); Flooding; Droughts; Accumulation of gases in the atmosphere (CO_2)
Effect on agriculture	Decrease of yields; Decrease of crop quality; Increase of pest and insect population, spread, and attacks; Early maturing and ripening of crops; Decreased plant resilience; Changes in crop pathology
Effect on mycotoxins	Increase or decrease in mycotoxin production according to regions and prevalence of climatic conditions optimal for mycotoxin production such as temperature and humidity
Effects on storage	In uncontrolled storage: Increase in the risk of fungal invasion and mycotoxin production; Formation of hotspots in storage; Increase of intragranular CO_2; Increase grain respiration; Increase in insect and pest attacks

Since fungal growth, germination, and mycotoxin production are governed by environmental factors and are prevalent under a set of optimal conditions, climate change and the shift in temperature and humidity might have varied effects on mycotoxin production. For example, some mycotoxins that are normally produced at low temperatures might not be produced as the later shift to higher levels, while others that are dominant in sub-tropical and tropical areas such as aflatoxins might start to be produced in usually temperate regions due to the expected increase in temperature in those areas and this was previously evident in Italy where a set of hot and dry episodes in 2003 and 2004 induced the colonization of Aspergillus flavus and the production of aflatoxins [44,45]. Therefore, each mycotoxin will be affected in different ways according to the prevalence of their optimal production conditions.

Climate change can also affect global mycotoxin contamination indirectly through (1) the increase of pest and insect populations, global spread, and attacks, (2) early maturing and ripening of crops, (3) decreased plant resilience, and (4) change in host pathology upon the presence of CO_2 in the atmosphere [40,43,46].

But since fungi tend to adapt to change it is complex to accurately determine the effect of climate change on mycotoxins and further studies need to be done in this domain [40].

Climate change can also affect harvest and drying since unexpected heavy rains due to extreme weather conditions cause farmers to harvest immature kernels and store them without drying, therefore increasing the risk of mycotoxin contamiantion. On the other hand, in regions where warm conditions are predicted to dominate, faster crop growth cycles and earlier harvest are expected leading to reduced drying needs before storage [47].

As climate change progresses storage of food becomes an important measure to mitigate probable shortages due to decreased agricultural yields. However, environmental changes accompanied by warm and humid weathers can heavily affect storage and cause unfavorable interactions between different factors leading to accelerated grain deterioration. Storage problems are mostly expected in developing countries especially on-farm storage sites where storage conditions are not well controlled. In warm climates fungi such as *Aspergillus flavus* are expected to become the most threatening since they survive in high temperatures and cause mycotoxin production such as aflatoxins, and in regions with high humidity and damp atmospheres, the risk of fungal invasion in stored grains also increase [47].

Amid changed environmental conditions and with reduced grains quality that are expected to be less resistant to fungal attacks, factors that induce fungal growth and mycotoxin production will prevail in storage including the formation of hotspots in storage, an increase of intra-granular CO_2, increased grain respiration leading to an elevated a_w in stored grains. In addition to that, insect attacks might further worsen the case of contamination by damaging the crops rendering them more prone to fungal attack [47].

Therefore, under any climatic conditions, it is highly crucial to store crops and grains in controlled storage facilities that maintain a safe temperature and humidity levels and provide proper aeration systems to maintain grain quality and protect it from fungal attacks and mycotoxin contamination. (Further details on proper storage conditions are more explicated in the article).

3. Mycotoxins control and prevention strategies

Mycotoxins production in nature is unavoidable and most foods consequently are at a risk of being contaminated. The destruction of contaminated crops results in huge economic losses, so mycotoxin control through the food chain is essential. Mycotoxins are very diverse and can be produced at several stages, by several fungi, and on several crops, so a single control strategy for all mycotoxins cannot be adopted and applied universally. However, some practices can be implemented to avoid their entry and minimize their frequency in food products. Currently, no method is available for total control of mycotoxins, and the development of a food safety program concerning contamination control is complex. A successful approach might be to adopt an integrated food safety system that involves proper quality practices at each stage of production to minimize mycotoxins frequency in the end product (Figure 1). Such practices would include applying proper measures during pre-harvest, harvest, drying, storage, and processing [48].

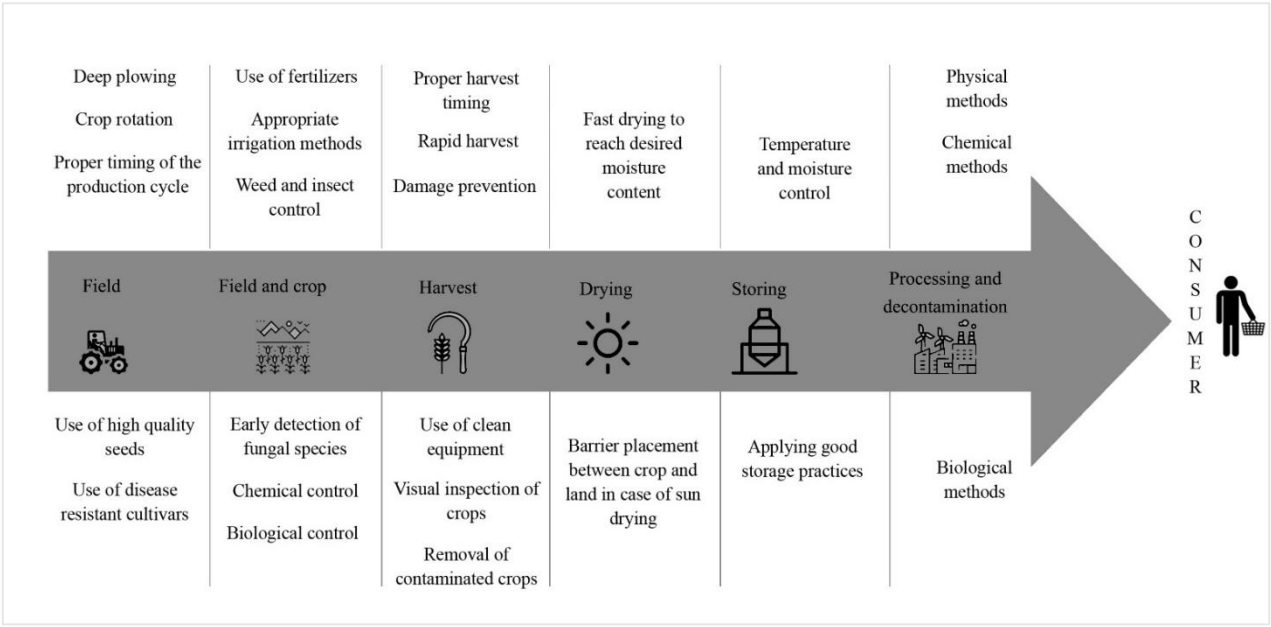

Figure 1. Proper practices to minimize mycotoxin contamination along the food chain.

3.1. Proper field practices

The majority of fungi are considered as phytophathogens as they infect the plants on the field [9], so management of contamination during pre-harvest is extremely important since it presents the first route of mycotoxin introduction into food. Generally, fungi that dominate on the field are species of *Fusarium spp.*, *Cladosporium spp.*, and *Alternaria spp.* On the other hand, *Aspergillus spp.* and *Penicillium spp.* can be found on field but at low rates and in general, the extent of contamination is expected to be higher wherever climatic conditions are in principle favorable to mycotoxin contamination [49]. While it is impossible to completely prevent mycotoxin development at pre-harvest, it is still extremely important to develop strategies that aim at reducing contamination during this phase. Those strategies should be of a high priority since decreasing the inoculum concentration at pre-harvest is considered crucial to the quality of the subsequent product. Therefore, in order to adopt proper strategies, a sufficient understanding of the toxigenic fungi, type of crops, field management, and harvesting practices should be applied [50]. In the field, many factors can contribute to mycotoxins presence such as drought stress, insect infestation, heat, poor soil fertility, and delayed harvesting…[50,51] Proper field practices include field preparation and management before planting, and field and crop management after planting.

3.1.1. Field preparation and management prior to planting

Field preparation before planting is crucial to control fungal attack and mycotoxin contamination and it includes; tilling and deep plowing, crop rotation, timing the production cycle, and the use of high-quality seeds or disease-resistant cultivars.

Tilling and deep plowing are essential to remove any remaining plant material. Previous crop residues that persist on the ground eventually deteriorate and harbor soil-borne fungi increasing their readiness to invade any new crops. So plowing buries the debris underground making them inaccessible to fungal inhabitation [52]. Tilling may also increase water availability to crops by minimizing the compressed layers of soil [53]. On the other hand, crop rotation prevents fungal species build-up [50,51] and it has been shown that mycotoxin contamination is higher in plots where the same crops are grown over consecutive years [52] since molds that might be well-established on a plant can prevail from a year to another if the same kind was planted continuously [54]. Planning the dates of planting and timing the production cycle are crucial as well to achieving vigorous crops at harvest. It is specifically critical to plan this cycle ahead of time to prevent early or late maturing of the plant and avoid the harvest at a time of rainfall or high relative humidity [52,54]. Seeds used for planting are of extreme importance too, since they are the foundation of any new crop. Therefore, good seed quality contributes to the growth of healthy plants that can withstand fungal attacks. Seeds must be inspected to ensure the absence of any disease or pest attack, otherwise, they will not germinate or they will be prone to fungal invasions that will successively increase the risk of mycotoxin contamination. Using resistant cultivars, on the other hand, may present a successful approach to prevent disease and control toxin contamination. At present, there are no totally resistant varieties, but partially resistant ones exist that can be used, but those do not provide protection against all genera of fungi. Partially resistant seeds are also mostly effective in cooler temperature climates, while, the resistance is needed to a bigger extent in tropical and sub-tropical regions where fungal infections are more frequent [52]. Resistant seeds may not be available in markets and they are more expensive than regular ones, so farmers in developing countries may not have access to those seeds which affects the quality of their

product. Alternatively, farmers resort to farm-saved seeds, and this practice would be safe in case the seeds were stored at appropriate temperature and humidity conditions that protect them from infection.

3.1.2. Field and crop management after planting

After planting, facilitating the growth of healthy plants by implementing proper field practices and reducing stress on the crops minimizes fungal growth and mycotoxins production. This stage includes the use of fertilizers, appropriate irrigation methods, weed and insect control, chemical control, and biological control [50,51].

The use of fertilizers improves plant health and maintains its resistance towards disease and fungi. Nutrient availability is very important for plant vigor and lack of proper plant nutrition leads to breaking in the stem of the plant making it more exposed to fungal invasion. So, in case nutrients were deficient in the soil, fertilizers can be used to increase soil fertility. However, fertilizer application must be accurate in timing and quantity since over-application may expose the plant into further stress making it more prone to pest and mold attacks. Heavy application may also be hazardous on human health since fertilizers contain, in addition to essential nutrients, heavy metals such as lead, cadmium, chromium, and arsenic that might accumulate in the plant or contaminate underground water. Exposure to fertilizers by farmers can also lead to many respiratory and dermal health problems such as cough, chest tightness, difficulty breathing, skin rashes, and dermatitis [55].

Appropriate irrigation can also prevent mycotoxins accumulation and it includes two main aspects; irrigation timing and method. Proper timing can prevent drought stress that results in plant cracking and facilitates fungal spores' entrance. The irrigation method, in which splashing is controlled is also essential to prevent fungal spreading [52]. Weed and insect control is also crucial to prevent disease in crops and further fungal invasion. Weeds contribute to contamination by acting as reservoirs of fungal inoculum and by competing for water and nutrients with the crops hence rendering them weak [56]. Therefore, weed removal should be continuously practiced. Insects, on the other hand, can cause fungal dissemination and make the grains more vulnerable to infection by causing physical damage. Hence, it is important to keep the area clean from plant debris, since removing any residual plants or vegetable matters makes food unavailable for rodents and reduces pest attack possibilities. The application of insecticides at appropriate doses, as well, can help control the frequency of attack.

3.1.2.1. Early detection of fungal species

Although the presence of fungi is not a definite indicator of mycotoxin contamination, their presence may imply an increased risk of contamination in case suitable conditions for mycotoxin production were found. Therefore, early detection of filamentous fungi in crops that allows for corrective measures is crucial. Methods to detect filamentous fungi need to be accurate, rapid, and reliable. Some types of methods are available mainly the mycological methods and the proteomic and genomic techniques [57]. Mycological methods involve common culturing techniques performed through multiple steps including culture, isolation, and identification. This process requires different media and incubation settings of time and temperature and may require subsequent methods to isolate mycotoxins produced and assess the toxicity of the filamentous fungi. Mycological methods are generally time-consuming and labor-intensive. On the other hand, proteomic and genomic techniques identify filamentous fungi at the molecular level and according to El Khoury et al. "the development

of molecular biology techniques for the genetic differentiation of species has resulted in substantial advances in taxonomy due to their sensitivity and specificity" [58]. Genomic methods include techniques such as Polymerase Chain Reaction (PCR) in which the DNA sequence of filamentous fungi is compared to sequences attained and deposited in the Gene bank to identify the fungal species tested. The success of the PCR method depends highly on the reliability of the reference gene sequence. And due to the high sensitivity of the PCR method, the detection of specific target DNA-molecules is allowed even in a complex mixture that allows the exact identification of the filamentous fungi present [59].

Hence, when filamentous fungi are identified at early stages decontamination methods could be applied on the field to prevent germination and growth of fungal species and prevent subsequent ycotoxin production.

3.1.2.2. Chemical control

Chemical control through using fungicides is at present the most effective way to control fungal invasion and subsequent mycotoxin contamination [9]. However, studies regarding fungicides are controversial; while many studies showed their effectiveness, others considered that at some instances fungicides can stimulate mycotoxin production and present a threat due to the healthy visual appearance it implies on mycotoxin contaminated crops [60]. Additionally, fungicide is a type of pesticide, and their use has been correlated with health and environmental risks. Uncontrolled intensive application of pesticides has been particularly reported in developing countries resulting in high exposure either through skin contact, inhalation, or ingestion of contaminated food and/ or water. Accordingly, pesticides can be metabolized, excreted, deposited, and/ or accumulated in human bodies causing several health effects [61]. In fact, several studies and experimental data linked pesticide exposure to cancer at different sites and to toxic effects on the nervous, reproductive, respiratory, endocrine, and immune systems [62]. Pesticide health outcomes depend on several factors including its type, exposure route and duration, and the health status of the exposed individual. For example, pesticide residues may be specifically dangerous on susceptible groups such as infants, pregnant women, elderly, and immunosuppressed people. Exposure to pesticides, as well, might compromise possible additive and/ or synergistic effects due to the application of several types on crops and plants rather than a single one [61].

3.1.2.3. Biological control

Biological control includes the application of harmless fungal species that serve to compete with toxic fungi and inhibit their pathogenic activity. Although this measure is not very practical, but it presents a safer alternative for chemical control methods. The idea behind this control is to introduce a strain of non-harmful biological agents such as bacteria or yeasts to compete with the pathogenic fungi for resources and reduce its growth and its mycotoxin producing ability. For example, non-aflatoxigenic strains of *Aspergillus spp.* are applied as biocontrol agents to compete with aflatoxigenic strains and prevent their domination and the subsequent production of aflatoxins [63]. According to Dorner, upon the application of non-aflatoxin producing strain of *Aspergillus parasiticus* to the field soil, significant decrease in aflatoxin contamination levels was achieved [64]. Also, in a previous study, aflatoxin contamination decreased significantly upon the application of atoxigenic strains of *Aspergillus flavus* to cotton rows in Arizona [65].

But despite its evident benefits, this method has several limitations that might discourage its use. First, non-toxic fungal strains, even though help in reducing mycotoxin contamination, may produce other toxic metabolites that may prove harmful to humans. Second, those strains could lead to an underestimation of mycotoxin contamination since they can affect metabolic pathways of fungi and lead to modified mycotoxin derivatives production. Third, the biocontrol agent used might impact other microorganisms found in nature. Lastly, mycotoxin production ability can be transferred from a parent fungi to a descendant one through the crossing of non-toxigenic strains with toxigenic ones leading to the possible reproduction of successive toxin-producing fungi [63].

3.1.3. Proper practices during harvest

Harvest is a critical stage for mycotoxin control in which moisture content becomes the most important parameter for crop protection. To decide harvest time, it is very important to take into consideration three factors including the predominant climatic conditions, the possibility of insect, pest, rodents, and bird's infestation, and the availability of drying amenities and storage warehouses. In ideal cases, harvest must start after a period of dry weather, but in many circumstances around some regions in the world, this option is not practical. So many crops might be harvested in wet weather conditions making them more susceptible to fungal growth and mycotoxin contamination, or, in a different scenario, some crops may be kept for longer times on the field which increases the risk of fungal attack and of insects, pests, rodents, and birds' attacks. Harvest must be performed in a rapid way but it is also crucial to avoid mechanical damage during the process, especially, when using heavy equipment like tractors. Similarly, it is essential to visually examine the crops and check for any symptoms of fungal disease and accordingly separate the contaminated from the healthy ones. It is also very essential to check for the cleanliness and hygiene of the equipment used for harvesting so no fungal cross-contamination takes place from one batch to another.

3.1.4. Proper practices during drying

Grains and crops are sometimes harvested with high moisture contents requiring therefore appropriate drying to be stored safely. Drying is an old practice that is used to protect agricultural produce from fungal infection and avoid thereby economic losses. This process may begin prior to harvest and continue until storage, and it is very important to reach the desired moisture content during drying to avoid the risk of fungal invasion at subsequent stages. Preferably, to prevent fungal attack, drying must be performed at the fastest rate possible, but this might be influenced by many factors including harvest practices and crop nature; as for example, the maize kernels covered by leaves dry at a slower rate than exposed sorghum heads [52]. Upon completion of drying, the crops must be transferred as soon as possible to storage.

Sun-drying was the most popular way of drying crops for a long time. However, in many cases it might not be practical and safe enough since ambient weather conditions may allow extending drying times, increasing, therefore, the risk of fungal attack specifically in large batches. This way, as well, may not be suitable in areas dominated by fluctuating weather conditions since there is always a risk of mold inhabitation due to sudden rainfalls. Some farmers, during sun-drying, tend to place the crops on the soil making them more prone to fungal infection, but this can be solved by placing a barrier or a platform between the soil and the harvested crops. Another technique used is solar drying that also

depends on the sun to generate heat that warms the air used for drying in the system. This procedure can be affected by many factors including geographical location, crop size, and the size of production. Alternatively, mechanical drying could be used in which warm dry air is forced into the product hence achieving the desired result at faster rates. For large mass production drying can be executed using superheated steam drying or infrared radiation [66].

3.2. Proper storage practices

As agriculture is advancing and the human population is growing, the yield of production is increasing, creating the need to store cereals, seeds, and grains in silos or storage facilities for long periods of time. As mentioned previously, it is extremely important to take serious measures that limit fungal invasion and mycotoxin contamination before any product reaches storage since the key to a safe and efficient storing process is the introduction of uncontaminated foods. Otherwise, if the product to be stored is highly contaminated little can be done to prevent further accumulation of fungi or mycotoxins [51]. In storage, several techniques can be applied to prevent fungal infection and germination and to keep the product in a sound condition, while on the other hand any mishandling of the product can lead to rapid deterioration of quality. Several numbers of fungi can inhabit stored grains, but mainly fungal species of low frequency in the field become of significant importance during storage such as *Aspergillus spp.* and *Penicillium spp.*, while field fungi that require high a_w become of less importance [67,68]. Major damage can be created by storage fungi including; mycotoxin production, quality reduction, nutritional losses, and heating initiation [9]. The conditions found in storage that led to fungal growth may not always be inductive to mycotoxin production, and similarly, the absence of fungi may not be a guarantee of the absence of mycotoxins from the commodity since toxins tend to persist even after the fungi disappears. Many factors affect fungal growth and their ability to germinate in storage, most importantly a_w and temperature. Mainly storage fungi are capable of growing at a relative humidity of 70 to 90% [9], and they can thrive over a temperature range of 10–40 ℃ with the optimum temperature at a range of 25–35 ℃ [69]. In addition to a_w and temperature, other factors also affect storage safety including; grain physical condition, grain nutrient composition, inter-granular air level, microbial interactions between different species in a bulk system such as bacteria and yeasts, usage of inhibiting materials such as chemical preservatives, storage time and the hygienic conditions [9]. Those factors interact during storage, exert significant effects on each other, and influence the extent of colonization. Fortunately, the conditions in the stored grain could be more controlled than those in the field and the stability of the product can be preserved using several methods. And since a_w and temperature are the most critical measures of storage safety, ideally both must be monitored and controlled. Accordingly, relative humidity must be maintained below 70% over the whole period of storage and products must be preserved at low temperatures since this can reduce fungal activity and keep the product safe for the desired storage period. The product temperature is a good indicator of storage quality and any fluctuations could lead to the respiration of the grain and condensation resulting in internal pockets of increased a_w in the stored product. Subsequently, upon the increase of a_w, xerophytic fungi retain their capability to grow, and their metabolic processes, afterward, lead to the production of water resulting in wetter substrates that can support a broader range of fungal growth [13]. Fungal activity leads as well to metabolic heat that raises the temperature hence supporting the growth of more fungi.

Therefore, storage is a very critical stage along the food chain that could affect subsequent product safety and quality, and it is very crucial to keep the conditions of storage strictly monitored and controlled over the whole storing span. Effective temperature and humidity monitoring and aeration systems in storage facilities are recommended so any problem is storage can be detected at an early stage when interventions could still be effective. Quality checks must be performed regularly, and this may include visual assessments for any signs of damage, pest attack, or mold growth, in addition to microbiological and chemical assessments through periodically collecting representative samples from the bulk and assessing it for quality parameters including, temperature, moisture content, and microorganism contamination. Pest invasion must also be prevented since their attack can increase moisture levels, lead to physical damage of the crop, and cause fungal dissemination. In general, many measures can be taken to assure the safety of the product through using good storage practices summarized in Table 4.

Table 4. Good storage practices to reduce fungal and mycotoxin contamination [9,13].

Element	Measures
Grain	Check for appropriate physical conditions prior to storage
	Check that appropriate moisture content is achieved before storage
	Check for the presence of disease or fungal infections
Storage facilities	Check soundness and suitability
	Perform weatherproofing
	Ensure sanitation of building and equipment
	Remove previous crop residues
	Install impermeable moisture barriers on floors
Insect, rodents, and birds prevention	Apply insecticide to building before use
	Use insect and rodent trapping
	Seal any holes in the building to prevent the entry of rodents and birds
Maintenance and monitoring	Install devices to measure temperature and moisture
	Calibrate devices regularly
	Monitor the presence of pest infestation
	Monitor any physical damage or signs of disease in crops
Quality assessment	Assess visually the presence of any pest infestation
	Assess visually the presence of any physical damage or fungal invasion
	Perform periodic microbiological and chemical assessments
Record keeping	Record data
	Retain tested samples

4. Methods for detoxification and decontamination of mycotoxins

Controlling mycotoxins in the early stages along the food chain and its primary prevention at critical points in the field and during harvest and storage are very crucial [70,71]. As this approach remains the best one to preserve the quality of food and protect it from mycotoxin contamination, it may not always be certainly effective and mycotoxins may appear in the subsequent food commodities that are destined for use in food processing or in animal feed. Therefore, several methods for decontamination and detoxification of mycotoxins have been developed, but despite that, there is no single technique that has proved effective against the wide array of mycotoxins that might occur

simultaneously in a food commodity [9]. For a single method to be effective and successful, it must be a comprehensive one comprising many properties [9]. First, this method should be able to completely destroy, inactivate, or remove the toxin along with any residual fungal spores. At the same time, it must preserve the nutritional value and the technological properties of the commodity. This method shall be preferably integrated into the food processing system in a cost-effective way that is easily operated without damaging the equipment and without posing any danger on the workers or the environment. Finally, this method must be approved by regulatory organizations for food production purposes. So far, the methods developed comprise techniques that may be able to remove or decontaminate mycotoxins in food products through physical, chemical, biological means, or through an integrated approach combining more than one technique. Those methods have not proven to be totally effective and they might possess many advantages and disadvantages (Table 5).

Table 5. Different decontamination means of mycotoxins in food, their advantages, and disadvantages [70–74].

	Physical decontamination	Chemical decontamination	Biological decontamination
Examples	Sorting Sieve cleaning Density segregation Washing De-hulling Steeping Extrusion cooking Steam heating Infrared heating Microwave heating Radio frequency heating Irradiation Cold plasma Photocatalytic detoxification	Organic acids Hydrochloric acid Ammonium hydroxide Hydrogen peroxide Sodium bisulphite Chlorinating agents Ozone Formaldehyde Natural substances such as herbs, spices, and their extracts	Bacteria Yeasts Mold Algae
Advantages	Effective against some mycotoxins Low change in food properties Does not involve usage of chemicals	Effective against some mycotoxins Affordable	Effective against some mycotoxins Inexpensive Environment friendly Does not involve usage of chemicals
Disadvantages	Impractical Might be limited to large-scale industries with sophisticated equipment Time-consuming Expensive In case of thermal treatment possible changes in color and food quality	Possible health effects Formation of toxic byproducts Enhancing bioavailability of masked mycotoxins Time consuming Environmentally toxic	Time consuming Impractical More effective in controlled laboratory settings

4.1. Physical decontamination

Physical control techniques comprise the separation of damaged or contaminated crops from healthy ones and they include methods like sorting, sieve cleaning, density segregation, washing, de-hulling, and steeping that help reduce the concentration of mycotoxins. In this process water soluble mycotoxins can be partially removed from the outer surface of the grain using water or water solutions, however, before using this method the solubility of the mycotoxin should be considered [72].

Physical decontamination also comprises destroying of mycotoxins through heat treatment and irradiation [73]. Thermal processes such as steam, infrared, microwave, radiofrequency, and extrusion heating developed as innovative decontamination approaches [74]. Heat treatments that combine the ultimate time/temperature conditions may present the most important intervention for mycotoxin control, however, many mycotoxins are very heat stable [70] and require, to be destroyed, very high temperatures and long processing durations that may not be achievable in conventional food production processes. Thermal methods and due to the application of high temperatures might change the physical qualities of food including color, it may impose burn like characteristics to certain spots on the food items especially the surfaces and edges, and it may degrade heat-sensitive nutrients [74].

On the other hand, non-thermal treatments such as irradiation may be effective through partially lowering mycotoxin contamination since they absorb the radiation energy [72] and it may be practical in large scale industries. However, there is public distrust of irradiated food products and this method is still not widely applied since radiation can penetrate cells and cause DNA damage leading to mutations. Despite that, the European Commission approved the dose of 10 kGy as the maximum allowable dose applied on food after FAO/IAEA/WHO Expert Committee demonstrated that this level does not pose a danger to human health [72].

A novel physical method that is of recent interest to researchers is the use of non-thermal techniques such as cold plasma for fungal and mycotoxin removal. Cold plasma is an ionized gas that contains partially ionized atoms and molecules with approximately zero net charge [75]. Treatment by cold plasma was shown in many studies to cause fungal cell wall and DNA destruction allowing intracellular component leakage [75–79]. Other studies on the cold plasma effect on mycotoxins showed that they were either partially or completely destroyed at a rapid rate [75,80–82]. The mechanism of mycotoxin destruction efficiency is related to the molecular structure of mycotoxins, the plasma chemical nature, and their subsequent interaction and according to Misra et al. "destruction could be related to the free radicals produced during the treatment, or the presence of UV photons, ozone, or reactive ions and electrons" [75]. Cold plasma treatment is distinctive from traditional methods since it allows for rapid decontamination at ambient temperature and pressure conditions without altering the food quality [23,75]. Nevertheless, some studies showed that cold plasma treatment might affect lipids in food, and since it is a surface treatment with low penetration ability it might not be practical in the large-scale industry especially when treating irregularly shaped food or bulk material [83]. Further studies are also needed to discover if this treatment has any cytotoxic effects [83].

Another non-thermal emerging technique that can be used for the removal of mycotoxins is the photocatalytic detoxification of mycotoxins in foods. This process comprises a chemical reaction induced by the absorption of photons by a solid photocatalyst which results in oxidation/ reduction reactions on the surface of the photocatalytic material causing the formation of free radicals that interact with contaminants such as mycotoxins and aids in degrading or converting them to less toxic compounds through oxidation reactions [84]. Many studies have already established the effect of

photocatalytic detoxification on mycotoxins [84–89]. This process is efficient, economically feasible, and environmentally convenient, however, analysis of application must be carried out to check for changes in food quality including nutritional and sensorial ones, and to check for any possible chemical or toxin residues following the treatment [84].

Although physical techniques seem acceptable since there would be limited change afterwards in the properties of the commodity, there usage is still considered unpractical and limited only to large scale industries since they might be time-consuming and expensive.

4.2. Chemical decontamination

Chemical methods employ chemical compounds treatments with acids, alkalis, reducing and oxidizing agents, that are either of organic or synthetic nature. Those chemicals are used to detoxify mycotoxins upon their addition to the food commodity. According to Karlovsky et al. "chemicals can be introduced to food through mixing, packing, fumigation, or immersion" [73]. Chemical agents involved in this technique include; organic acids, ammonium hydroxide, hydrochloric acid, hydrogen peroxide, sodium bisulphite, chlorinating agents, ozone, formaldehyde, and natural substances such as herbs, spices, and their extracts [71,73].

Chemical treatment has shown to be effective in the removal of some mycotoxins, however, the chemicals used are mainly weak ones and the majority of mycotoxins might be resistant to them. As for strong chemicals, particularly acids, they cannot be used in food treatment since their usage leads to various toxic effects such as the formation of other chemical compounds [72]. Chemical transformation might also lead to the production of other mycotoxin metabolites and the release of mycotoxins from masked and entrapped forms hence increasing their bioavailability [90].

Treatment with ozone was shown to be promising since it can degrade mycotoxins through reacting with bonds in the mycotoxin chemical structure especially double bonds in mycotoxins such as AFB1. Ozone can be applied in gaseous form avoiding the increase in moisture; however, ozone treatment might require a prolonged time to be effective and it may cause oxidation of fat components hence affecting food quality. By-products might also be formed during the process so safety of such treatment must be taken into consideration [91].

As many of the chemical treatments have proved affordable and effective against mycotoxins, their usage is still banned by the European Union in foods since they can pose some health risks due to possible toxic byproducts generated in the process, and according to European Commission regulation, "foodstuff containing mycotoxins shall not be deliberately detoxified by chemical treatments" [13,92]. While in other regions around the world chemical treatments are only approved for certain mycotoxins, mainly AFB1 in feedstuffs [72]. Chemical treatment usage is also time-consuming and may disturb the environment.

4.3. Biological decontamination

Biological means of decontamination apply microorganisms such as; bacteria, yeasts, molds, and algae. Biological decontamination became specifically demanded after the trend to avoid physical and chemical methods and use natural substance treatments instead. The microorganisms used in biological means may be able to bind, degrade, or modify mycotoxins into less toxic substances in certain foods animal feed through acetylation, glucosylation, deamination, hydrolysis, or decarboxylation [71]. For

example, OTA can be transformed into phenylalanine by the action of some plants, bacteria, yeasts, or mold [93]. Some microorganisms and enzyme systems can be added as well to animal feed that allows the detoxification or degradation of the mycotoxin in the gastrointestinal tracts of ruminants.

Lactic acid bacteria and yeasts are most used in decontamination as they are able to reduce mycotoxins by binding them onto the cell surface or by transforming them into less toxic products [72]. Enzymatic catalysis is also used and they have promising potential in mycotoxin contamination, however, more studies are needed to prove their efficacy and safety.

Biological methods are inexpensive and present no risk to the environment since no chemical substances are used. However, their use can be time-consuming and impractical in some cases. Many biological means, as well, proved to be effective only in laboratory settings so further studies are needed to test their efficacy in food. Another major drawback is the limited ability to decontaminate multiple mycotoxins simultaneously [90].

4.4. Effect of processing on mycotoxins

Food processing is a procedure that comprises the application of any physical, chemical, or biological technique to alter the food quality or shelf-life and make it more suitable for human consumption. Some processes are used to remove or decrease organisms in food commodities such as bacteria, microbes, and molds. Mycotoxins, on the other hand, are affected differently through food processing since their presence depends on multiple factors including their very high stability [94] and resistance to temperature, the presence of other constituents including enzymes, the pH and moisture content of the food product, in addition to the temperature. Processing of a food product may involve complex actions, and at each stage mycotoxins concentration can be affected in different ways; it either decreases, increases, or remains stable. For many commodities, such as cereals, the complex processing procedure may lead to a final product that has lower mycotoxin concentration than the raw crop used. While, in other processes such as cheese making, mycotoxins could become more concentrated in the final cheese product than it was in the raw milk. Since one of the aims of processing is to reduce mycotoxins in food, a thorough understanding of the mycotoxins' reaction to processing, the processing procedure itself, and the conditions of the food commodity is extremely crucial to achieve the desired results and maximize mycotoxin removal. Several effects on different mycotoxins by food processing techniques used are summarized in Table 6.

5. Role of regulations, sampling, and analytical methods in mycotoxin control regulations

Mycotoxins occur as natural contaminants, and they are present worldwide in crops, food products, and animal feed. Mycotoxins total exclusion is unattainable, and control and decontamination methods do not guarantee mycotoxin-free commodities. Their presence and dispersal along the markets due to trade, as well, made it impossible to ban mycotoxins completely from food. Instead, many countries have resorted to set levels that ensure consumer protection and food safety and keep the exposure to mycotoxins as low as possible. In this context, tolerances, guidelines, and residue levels have been set in several countries [9] and maximum admissible levels have been established for mycotoxins that occur in several food commodities especially ones traded and consumed extensively. The number of countries adopting mycotoxin regulations worldwide increased over time, and more recent detailed regulations have been enforced globally with a special emphasis

on official sampling procedures and analytical methods [9]. According to FAO, "In 2002, at least 100 countries had mycotoxin regulations for food and/or feed world-wide, and the total population in these countries, by the time, represented approximately 90% of the world's inhabitants" [9].

In general, food legislations must serve to protect the economic interests of both food producers and traders. So ideally, it is preferable to harmonize regulations, especially in countries with trade contracts such as the European Union or MERCUSOR (trade agreement between Argentina, Brazil, Uruguay, Paraguay, and Venezuela), and adapt an international food safety standard regarding mycotoxins. While in fact, mycotoxin regulations around the world vary significantly and the absence of a unified and transparent approach led to a wide range of disparities in guidelines with several differences in maximum admissible levels set between different countries. Additionally, in many developing countries especially ones facing food availability issues, regulations may not be present or present but not enforced.

Regulations serve as a safeguard for food markets from contaminated imported commodities. In many cases, commodities are rejected due to food safety threats, for example, according to the European Commission Rapid Alert System for Food and Feed "RASFF" annual report, mycotoxins were the primary hazard in border rejection from non-EU countries in 2018 with a total of 569 notifications [111]. The results of strict regulations, while retaining the benefits of the importing country, may create an economic imbalance and affect exporting countries that can face difficulties finding new markets or maintaining their usual ones. This may also lead to the abundance of foods contaminated with mycotoxins in local markets especially in developing countries that are the main contributors to mycotoxin contamination world-wide, specifically countries where hot climate is dominant. In developing countries, there is also a lack in regulations, monitoring, and supervision leading to more mycotoxin contamination. In this part of the world food shortage may be already a problem so drastic measures can cause scarcity of food and extreme prices, so according to Egmond and Jonker, "regulatory philosophy should not jeopardize the availability of some basic commodities at reasonable prices" [9,112]. And therefore, to reach realistic protection, regulations must be derived from the cooperation between all stakeholders based on experiences from science, consumers, industry, and policymakers [9].

5.1. Sampling

Quantifying mycotoxin concentration is one of the most important steps of controlling it in food and feed, and it is used in regulatory activities, quality assurance, decision making, and research. Sampling presents the first part of mycotoxin quantification that is based on drawing a representative sample of the whole lot and assuming that the mycotoxin concentration it contains is equal to that in the bulk. To attain a proper sample a specific procedure is usually followed: first, a bulk sample is collected from a lot, then this bulk sample is reconstituted through blending and mixing, and finally, a test sample is drawn out of it to be used in the analysis (Figure 2).

Table 6. Sample of some processing methods and their effect on different mycotoxins.

Commodity	Processing method	Processing details	Mycotoxin	Result
Barley	Cleaning	Physical removal of infested kernels	OTA	Decreased by 2–3% [95,96]
Kernels, seeds, and nuts	Cleaning	Physical removal of infested kernels	Aflatoxins	Decreased by 40–80% [95,97]
Barley and corn	Washing	3 washings with distilled water	DON	Decreased by 65–9%
			ZEN	Decreased by 2–61% [73]
Cocoa beans	Mechanical shelling	Removal of cocoa bean shells	OTA	Decreased by 48%
	Hand shelling			Decreased between 50–100% [73]
Whole wheat	Dry-milling	Milling into component fractions	OTA	Reduced OTA in white flour Increased OTA in bran and offal [96]
Small grain cereals	Milling	Milling into component fractions	DON	Increased in bran Decreased in flour [73,98]
Dough	Fermentation	Artificially contaminated dough covered with damp cloth and fermented at 30 ℃ for 15 mins	OTA	No change [73,99]
Bread	Baking	Artificially contaminated dough baked at 244 ℃ for 25 mins	OTA	No significant reduction [96]
Corn-meal muffins	Baking	Baked at 218 ℃ for 20–25 mins	Aflatoxins	Decreased by 13% [9,100]
Rice	Ordinary cooking	Cooked at 160 ℃ for 20 mins in a commercial electric cooker with 200 mL of distilled water	AFB1	Decreased by 34%
	Pressure cooking	Cooked at 160 ℃ for 20 mins in a commercial electric pressure cooker fixed at 15 lb/in^2 (0.10 MPa)		Decreased by 70% [73,101]
Maize grits	Extrusion cooking	Cooked in three extrusion barrels at temperatures of 120, 140, & 160˚C and three moisture contents of the corn grits 18, 22, & 26%, mixing and non-mixing mode were also applied for different barrels	ZEN	Decreased by 65–83% [73,102]

Continued on next page

Commodity	Processing method	Processing details	Mycotoxin	Result
Maize grits	Extrusion cooking or roasting	Corn grits spiked with FB1 at a level of 5 μg/g were extruded in a co-rotating twin-screw extruder at different temperatures (140, 160, 180 and 200 ℃) and screw speeds (40, 80, 120 and 160 rpm)	Fumonisins	Decreased by 34–95% [95] Decreased with increased temperature and decreased screw speed
Beer	Brewing	A single-temperature infusion mash was used (65 ℃ for 60 min), with no addition of exogenous brewing enzymes. Then, the worts were boiled for 1 h. Hopping was achieved using liquid CO_2 hop extracts. Fermentation was done using an ale yeast at 18 ℃ for 4 days. After a three-day warm maturation at 13 ℃, the beer was cooled to 3 ℃, and the yeast was harvested. The beer was conditioned for 6–8 d at 0 ℃, then bottled and stored at 0 ℃.	OTA	Decreased by 68–87% [103]
Cocoa beans	Roasting	Cocoa beans artificially contaminated with aflatoxins at a concentration of 220.7 ng/g were roasted at 250 ℃ for 15 minutes	Aflatoxins	Decreased by 71% [104]
Coffee	Roasting and grounding	Cooked in a commercial pressure cooker, using a home stove for 45 minutes at 115 ℃ and 13 psi.	OTA	Decreased by 84% [73,105]
Pistachio nuts	Roasting with lemon juice and/or citric acid	Roasting at 90, 120, or 150 ℃ for 30 min and 60 min in an electrical oven	AFB1	Decreased by 76.6%–93.1% [73,106]
Milk	Pasteurization	Pasteurized at 95 ℃ for 3 minutes	AFM1	No change [107]
Cheese curd	Cheese processing	Milk inoculated with a starter culture and incubated at a temperature of 35 ℃ for 20 mins. Rennet was then added and the curd was ready after 45 mins. The curd was the cut and left for 6h for drainage and then they were kept at 16 ℃ for about 7 days until their pH dropped to 4.6.	AFM1	AFM1 concentration was 4 times higher in curd than milk [73,108]
Cheese	Cheese processing	Milk samples and manufactured cheese samples were collected directly from manufacturers.	AFM1	AFM1 concentration was higher in cheese than milk [73]

Continued on next page

Commodity	Processing method	Processing details	Mycotoxin	Result
Yogurt	Milk fermentation	Starter culture was added to 10 L of milk and fermented at 42 ℃ until pH 4.0 and 4.6 were achieved in different batches.	AFM1	Decreased by 13% at pH 4.6 Decreased by 22% at pH 4.0 [73,109]
Maize	Microwave treatment	Treated at 2450 Hz applied on a 2 g moist corn samples until temperatures of 150–175 ℃ were achieved.	DON	Decreased by 40% [73]
Apple juice and cider	UV treatment	Using a commercial UV machine that is composed of a stainless steel outer unit containing three inner chambers of quartz tubes connected in sequence. Apple juice was pumped through a thin layer between the outer steel unit and inner quartz tubes. The juice's UV exposure was calculated by the following equation: UV dose (mJ/ cm^2) = irradiance x exposure time (Test operated at 0 to 99.4 mJ/ cm^2)	Patulin	Decreased by 5–73% Increase in reduction value was shown with increased UV dose [110]
Vegetable oil	UV exposure	Naturally and a fortified contaminated corn oil were exposed to sunlight for 30 weeks	ZEN	Decreased up to 90% by isomerization of natural trans-ZEN into cis-ZEN [73]

Improper sampling methods could lead to wrong estimations of mycotoxin concentrations and subsequent misclassifications of the lots. Eventually, this would lead to undesirable economic and health consequences. Fortunately, mycotoxin-sampling plans can be designed in a way that decreases error of estimation although no mycotoxin concentration can be determined with a 100% certainty. A mycotoxin-sampling plan is defined by two pillars: analytical procedure to be used for quantification and a determined accept/reject limit [113]. The sampling plan must be characterized with both accuracy and precision, where accuracy is defined as the closeness of measured mycotoxin concentration in a sample to the true one in the bulk, and precision as the closeness of concentrations in measured replicate samples to each other [113]. Generally, most mycotoxins are heterogeneously distributed, and sampling should be done randomly, hence, giving every individual item in bulk an equal chance to be chosen [13]. Two errors can be introduced in case sampling was done in the wrong ways: bias and variability that are associated with accuracy and precision, respectively.

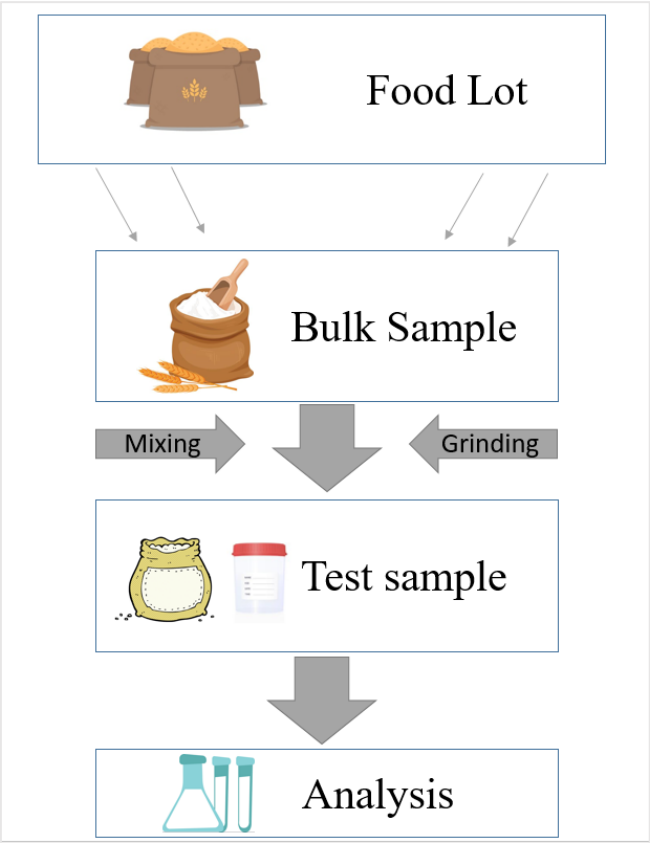

Figure 2. Test sample collection procedure.

Bias is introduced when a sample is drawn using equipment and procedures that decrease the chance of any item in the bulk of being selected. For example, if the sample was collected from a single location, too many contaminated particles may be collected or may be missed otherwise. Therefore, to avoid bias appropriate equipment that are regularly checked for their quality must be used in sampling, and small sample portions should be taken from several locations in the lot and mixed together in order to reconstitute the sample. Variability, on the other hand, may appear due to differences in mycotoxin distribution among the particles in a lot, therefore increasing a sample size may improve precision.

Samples can be either drawn from a static lot that is contained in storage containers, bins, or bags or from a dynamic lot that is defined as any commodity transferred through a moving stream. Generally, it is easier to obtain a more representative sample from dynamic lots [113]. Different methods to collect proper samples from the two types of lots are presented in Table 7.

Table 7. Sample collection methods from static vs. dynamic lots [9,13].

Static lots		Dynamic lots
In a single container	A probing device should be used to collect samples from different locations in the lot according to a probing pattern. The probe must be able to reach the bottom of a container, should not decrease the chance of any item from being selected, and should not alter any item contained in the lot.	Small parts of a sample should be collected at pre-determined and frequent, time intervals throughout the whole flow period.
In separate containers or bags	The bulk sample should consist of many sub-samples taken from many containers dispersed along the storage.	Sampling can be executed manually or automatically using programmed equipment such as cross-cut samplers.
In a container with limited access	The sample should be collected when the food product is being placed into the container, or when being discharged.	

Upon establishing sampling criteria much care must be taken to ensure that the interests of the buyer and the seller are maintained fairly. Two types of mistakes may occur in case mycotoxin concentrations were not assessed properly due to errors in the sampling plan (Figure 3). First is the false-positive error, where safe lots that contain mycotoxin concentrations within the acceptance limits may test bad and get rejected by the buyer. This is referred to as the seller's risk since an economic loss will result to the supplier due to this error. Second is the false-negative error, where contaminated lots may test negative and get accepted. And this is known as the buyer's risk since accepting a bad lot may cause health and economic consequences knowing that this product could be incorporated into further food processes.

Figure 3. Mycotoxin analysis decision errors due to sampling mistakes.

5.2. Analytical methods

Following proper sampling, analysis presents an essential and primary step in controlling mycotoxins. Suitable analysis that provides accurate and precise results are required for many reasons;

first to help control mycotoxin contamination and decrease admission of contaminated food commodities into a country, second to establish proper food safety control strategies regarding mycotoxins such as HACCP, and third to confirm the results of the control strategy followed. Proper analysis procedures are also required in research projects that provide reliable information on mycotoxin contamination allowing, therefore, for the right decision making.

Analytical procedures are characterized by different criteria, namely; speed and cost of analysis, technical skills required to perform the method, and the type of data they provide i.e. qualitative or quantitative. According to Richard et al. "the most desirable method incorporates all three: they are rapid, easy to use, and quantitative" [13]. But in fact, this kind of perfect method is not available and the choice of the procedure to be used requires an assessment of the analysis's purpose to determine the relative importance of every aspect and make compromises accordingly. Until now, mycotoxin analysis methods established are complex and time-consuming since they require major sample extraction and cleanup prior to quantification. In the first phase of extraction, shaking or blending are used to pull out mycotoxins from the solid phase into an organic solvent (liquid phase). The choice of the organic solvent to be used for each commodity depends on its ability to extract the desired mycotoxin and its suitability with the test procedure; safety measures and the cost of wasted solvent are also taken into consideration. After that, the liquid phase, in which the mycotoxin is evenly distributed, is separated from the solid matrix through filtration or centrifugation. Next, cleanup is executed and it implicates further isolation of the toxin from the extract. Clean-up is usually performed using solid-phase extraction (SPE) that might be designed to trap either the mycotoxin or the impurities. Immunoaffinity column (IAC) is an example of SPE; in which attached to a solid phase are antibodies specific to mycotoxins that work on binding them while allowing the rest of the sample to pass through. Then mycotoxins are eluted after that using an organic solvent that denatures the antibodies resulting in a pure mycotoxin-solvent solution. This solution is then incorporated into the last step of analysis. In this phase, many chromatographic methods can be used such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) to quantify a mycotoxin concentration. HPLC is the most used method because of its high precision, selectivity, and sensitivity [114] although it is expensive, time-consuming, and requires for operation highly skilled technicians.

Other rapid methods that need minimal or no clean-up are available such as enzyme-linked immunosorbent assay (ELISA). This method relies on antigen-antibody specific and selective reactions. In principle, anti-mycotoxin antibodies are added to be captured by immobilized antibodies bound to the microtiter plate of ELISA, after that samples are added in order for targeted mycotoxins to get bound by anti-mycotoxin antibodies. Then a substrate/ chromogen is added that binds to available antibody sites, followed by a stop solution that causes a color change in the wells of the plate. Lastly, a photometric absorbance measurement is done and the mycotoxin concentration is computed from the attained absorbance.

ELISA compromises many benefits since it is rapid, simple, cost-effective, and efficient. But despite that, such methods remain less reliable and accurate due to the high risk of false-positive or false-negative results, and due to the instability of the reagents supplied and the need for their proper refrigerated storage at all times to maintain quality [115]. Accordingly, ELISA has been established as a qualitative screening method [116], and any sample result that proves to be contaminated using the ELISA test must be further confirmed by more vigorous chromatographic techniques such as HPLC [117].

6. Future trends in mycotoxin research

Mycotoxin contamination is prevalent so future strategies must concentrate on the need to control fungal contamination and mycotoxin production along all the food chain starting from production till the food reaches the customers. The synergistic toxic effects of mycotoxins occurring simultaneously in food should be considered, as well as the probable presence of masked mycotoxins. Conventional screening methods that are reliable, convenient, rapid, and cheap are needed, and the development of methods that quantify masked mycotoxins is of extreme importance. More research, exposure assessments, and safety evaluations are needed to evaluate the potential toxicity of masked mycotoxins and mycotoxin byproducts. Further research on the safety of physical, chemical, and biological decontamination methods are needed, and strategies that combine an integrated decontamination approach must be developed to maximize mycotoxin removal from food to the most possible extent.

Regarding climate change, it is very crucial to conduct researches that quantitatively estimate the impacts on fungi and mycotoxins. According to Medina et al. future research should focus on discovering how mycotoxin production patterns would change and how some secondary mycotoxins could become primary ones with the emergence of climate change [40]. Research must also focus on the shift of mycotoxin contamination according to regions and aid in discovering the current agricultural control practices' effectiveness on mycotoxin control in changed climate conditions.

7. Conclusions

Mycotoxin contamination in food might not be inevitable and its presence could threaten the food security of many countries especially developing ones. However, the implementation of proper methods from the beginning of the food chain until the end including all stages of production like planting, harvest, drying, storage, processing, packaging, transport… helps to decrease the level of contamination and maintain it below the tolerable levels assigned by different countries. Strict recommendations that preserve the benefit of the consumer in the first place need to be enforced and rapid and reliable analysis methods to determine fungal and mycotoxin contamination must be applied to ensure food safety.

Conflict of interest

The authors declare that there is no conflict of interest.

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