

Review

Strategies for controlling and decontaminating mycotoxins in foods and feeds: A review

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Abstract

Mycotoxins are naturally occurring chemical compounds produced by certain genera, such as *Aspergillus*, *Fusarium*, and *Penicillium*, as by-products of their metabolism (secondary metabolites). They are plant pathogens able to cause infection pre-, during, and post-harvest. The most important and economically-relevant mycotoxins of great concern to humans, plants, and animals are aflatoxins, ochratoxin A, fumonisins, and trichothecenes. The present review aimed to compile updated management strategies of mycotoxins in foods and feeds, including control and detoxification techniques. Generally, the strategies are divided into physical, chemical, and biological, and can be implemented during pre-, harvest, and post-harvest. Physical controls pre-harvest includes the development and planting of resistant varieties; during harvest include control of field infections, timely harvest, sufficient drainage, and physical barriers; and post-harvest include storage of harvested commodities under conditions that would prevent and exclude the growth and mycotoxin production by mycotoxigenic fungi. Chemical controls generally involve the use of chemically synthesised fungicides which are often associated with long-term effects on the environment. When compared with chemical and physical controls, biological controls are generally more unique, productive, and environmentally friendly, and when implemented appropriately in the Integrated Pest Management (IPM) strategy, can collectively control the growth and proliferation of mycotoxigenic, and reduce the incidence of mycotoxin production and contamination in foods and feeds.

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Mycotoxins and mycotoxigenic fungi in foods and feeds

Mycotoxins are toxic secondary metabolites produced naturally by certain filamentous fungi also known as moulds (Berthiller *et al.*, 2013). According to Eskola *et al.* (2020), between 60 to 80% of food crops may be contaminated with mycotoxins globally. The co-occurrence of mycotoxins in foods and feeds is of great concern due to the toxic effects they pose to both animals and humans (Wang *et al.*, 2021). The major fungal genera capable of producing mycotoxins are *Aspergillus*, *Fusarium*, and *Penicillium*. Aflatoxins, ochratoxin A, fumonisins, trichothecenes, and zearalenone are the major mycotoxins which are of significant economic importance, and could cause deleterious health effects

(Zheng *et al.*, 2006). Although thousands of fungal metabolites have been discovered, thus far, only a fraction is of great importance in terms of food safety. Of more than 500 mycotoxins that have been identified, about 30% are said to occur in human foods, animal feeds, and occasionally in the environment (Haque *et al.*, 2020). These mycotoxins occur naturally in foods and feeds when these are contaminated by mycotoxigenic fungi under suitable climatic conditions. Mycotoxin production is also affected by several factors like humidity, temperature, drought, improper storage, and insect damage. Mycotoxins get into the human system through direct consumption of contaminated foods, or indirectly as a result of carryover from food products such as milk, eggs, and processed foods (Becker-

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Algeri *et al.*, 2016). Foods can become contaminated by mycotoxins due to infection by mycotoxigenic fungi during preharvest, storage, processing, and distribution of harvested agricultural products (Haque *et al.*, 2020).

Additionally, exposure to mycotoxins can also be due to inhalation or absorption through the skin (Schlosser *et al.*, 2020). Nevertheless, regardless of the contamination route (ingestion, inhalation, or absorption), mycotoxin exposure may result in sickness, lowered performance, and even death in humans and animals. The toxic effects depend on how much has been taken up (dosage), period of exposure (duration), types of mycotoxins, mode of defence, metabolism, and mode of action (Buszewska-Forajta, 2020). An outbreak of mycotoxicoses is the consequence of ingesting food/feed contaminated with mycotoxins. The issue of mycotoxicoses has been reported by many researchers. Also, other adverse effects such as immune system suppression resulting in the inhibition of protein synthesis and cell proliferation, which may reduce immunity, can also be caused by several mycotoxins such as deoxynivalenol and T-2 toxin (Bhat *et al.*, 2010). The effects of mycotoxins in animals may result in lower productivity and immune suppression which leads to a higher incidence of diseases, inflammation of vital organs and reproductive system, and also death (Bhat *et al.*, 2010).

Contamination of foods and feeds by mycotoxins has become a major priority due to their adverse effect. However, the presence of moulds in foods and feeds does not necessarily indicate the presence of mycotoxins. Similarly, the absence of moulds does not guarantee the absence of mycotoxins. Various reviews from different parts of the world have shown that foods and feeds have been contaminated by mycotoxins (Rahman *et al.*, 2020; Yazid *et al.*, 2020). These reviews are constantly updated to assess the efficiency of their mycotoxin management strategies. As earlier mentioned, the mycotoxins that receive the greatest attention as a result of their high frequency and high severity to humans and animals are aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone (Bhat *et al.*, 2010). In feeds, contamination by mycotoxins mainly occurs due to the infected raw materials used in producing the compound feeds (Gruber-Dorninger *et al.*, 2019; Azman *et al.*, 2021). Most contamination of agricultural products is caused by both field and storage fungi (Adebo *et al.*, 2021). Generally, fungal

growth and mycotoxin production occur naturally in the field due to biological factors. However, prolonged storage of agricultural products under extreme conditions such as temperature, humidity, pH value, moisture content, and poor farming practices can also serve as compounding factors, and increase the rate and incidence of mycotoxin production (Marin *et al.*, 2013).

In minimising the risk of mycotoxin exposure towards humans and animals, various international agencies are trying to achieve universal standardisation of regulatory limits for mycotoxins. Regulations were first established in many countries since the 1960s after the discovery of aflatoxins (Anukul *et al.*, 2013). Presently, most countries have regulations and other monitoring programs regarding mycotoxins in their foods and feeds (Eskola *et al.*, 2020). The regulatory limits and standards for mycotoxins vary in different parts of the world (Anukul *et al.*, 2013). However, at the global level, the Codex Alimentarius Commission of the Food, FAO, and WHO, are the major bodies governing the standards for mycotoxins (Eskola *et al.*, 2020). Over the years, more than 100 countries globally have regulatory limits or guidelines for the control of mycotoxins in foods and feeds. The most important factors to be considered for setting a meaningful regulatory limit are risk assessment which involves hazard identification, hazard characterisation, exposure assessment, risk characteristics, and methods of sampling and analysis (Van, 2013).

Regulatory limits for mycotoxins in foods and feeds often involve aflatoxins, fumonisins, ochratoxin A, trichothecenes, and zearalenone (Siri-Anusornsak *et al.*, 2022). EU guidance levels and recommended limits vary in different food and feed materials such as feeds, cereals, cereal products, maize, and maize products. The limit of aflatoxin B₁ is 20 µg/kg in all feed materials; the sum of fumonisin B₁ and B₂ is 60,000 µg/kg in maize and maize products; zearalenone is 2,000 µg/kg in cereals and cereal products, and 3,000 µg/kg in maize by-products; ochratoxin A is 250 µg/kg in cereals and cereal products; and deoxynivalenol is 8,000 µg/kg in cereals and cereal products, and 12,000 µg/kg in maize by-products.

Factors affecting mycotoxin contamination in foods and feeds

Mycotoxigenic fungi can invade, colonise, and produce mycotoxins in the field, during storage,

processing, and transportation under suitable environmental conditions which can be affected by intrinsic and extrinsic factors. The most important factors that may influence the growth and mycotoxin production of mycotoxigenic fungi are temperature, pH, substrate, light, and water activity (a_w) which vary among different fungal species (Mannaa and Kim, 2017b). Generally, the temperature at which mycotoxigenic mould grows ranges from 10 to 40.5°C, relative humidity > 70%, and pH 4 - 8 (Neme and Mohammed, 2017). However, during storage, the major environmental conditions that affect the growth and mycotoxin production are water activity and temperature (Norlia *et al.*, 2020). For instance, in tropical regions with high rainfall, humidity, and temperature, *A. flavus* may dominate other fungal species that have lower optimum growth temperatures (Wagacha and Muthomi, 2008). However, these mycotoxigenic fungi will continue to produce mycotoxins during storage under suitable conditions. Therefore, the growth and mycotoxin production of each species depend on optimum conditions. What is worse, with the current climate condition, a shift in growth and mycotoxin production is expected to occur as a result of climate change factors (Medina *et al.*, 2015b).

Climate factors

Climate change is described as the changes that occur in the atmosphere as a result of fluctuations in temperature, wind pattern, atmospheric gases, and rainfall (Senghor *et al.*, 2017). Other changing trends in the environment could also lead to climate change such as ocean temperature, increase in global air, melting ice, and rising global average sea levels. Studies have shown that the changing trends in temperature, drought occurrence, precipitation, and subsequent increase in CO₂ will also have a significant effect on food security, food safety, food supply, plant diseases, and pathogens. The European Food Safety Authority (EFSA) reported that in Europe, the effect could be regional. It has also been suggested that the effect in the Mediterranean basin may be negative with extremely high temperatures, elevated CO₂, change in rainfall, and drought, and will have an impact on food production and storage (Medina *et al.*, 2015b). Based on the current data, atmospheric CO₂ is expected to double or triple due to the climate change impact in the next 25 to 50 years (Medina *et al.*, 2017). Consequently, the influence of climate change will also increase/decrease mycotoxin

contamination depending on the fungal species and crop commodities involved.

In previous years, several predictions about the climate have been made describing what may happen to mycotoxins due to the impact of climate change (IPCC, 2007). One of the well-known predictions and hypotheses about the effect of climate change on mycotoxins is the interaction between water activity and temperature on the growth and mycotoxin production of fungi. This scenario has been studied on *Aspergillus*, where the impact of temperature, CO₂, and water activity was determined on growth and mycotoxin production. At 34°C, 0.97 - 0.92 a_w and various levels of CO₂, the growth rate of *A. flavus* was unaffected; however, aflatoxin B₁ production was optimum at the same temperature at 0.95 a_w (Medina *et al.*, 2015a). The optimum concentration of aflatoxin was obtained at 0.9 - 0.92 a_w at 21°C after 21 days of incubation by *A. flavus* (Mousa *et al.*, 2013). According to Klich (2007), the optimum temperature for the production of aflatoxins may vary, ranging from 24 - 30°C depending on the strain. Similarly, Lahouar *et al.* (2017) also reported the effect of temperature, water activity, and incubation time on toxigenic *A. tubingensis* and *F. incarnatum* isolates obtained from sorghum seeds. The optimum growth rate of *A. tubingensis* was at 37°C and 0.99 a_w , while for *F. incarnatum*, the growth rate was optimum at 25°C and 0.99 a_w . For *Fusarium*, growth is more favourable in temperate weather at a temperature range from 26 - 28°C, and water activity greater than 0.88 a_w , while for *A. flavus*, growth is better at warm temperatures (Marroquín-Cardona *et al.*, 2014). Additionally, it has also been suggested that other factors such as CO₂ levels, temperature, and water activity (3-way interaction) will play a vital role in the relationship between climate change and mycotoxin production (Mshelia *et al.*, 2020).

Agricultural practices

In the field, management practices may have a significant effect on fungal contamination and mycotoxin production by mycotoxigenic fungi (Marroquín-Cardona *et al.*, 2014). However, these management practices alone might not be effective once mycotoxin contamination has occurred. Harvesting crop products at a premature stage may increase the risk of fungal contamination (Marroquín-Cardona *et al.*, 2014). Aside from this, delayed harvesting, intercropping, and continuous cultivation may also increase the risk of mycotoxin

contamination. Inadequate harvesting techniques can also promote fungal contamination and mycotoxin production (Luo *et al.*, 2018). This is very common in developing countries where people are more exposed to mycotoxins due to a lack of modern agricultural technologies (Bhat *et al.*, 2010). Furthermore, fungal pathogens may continue to produce mycotoxins during the harvesting and storage of crops. For instance, *Fusarium* spp. are the most common plant pathogens which occur on the field, and their life cycle is very similar. The pathogens may survive on debris, soil, and other residues, and may produce macroconidia which can be dispersed to susceptible plants during growing seasons, thus leading to infection under favourable conditions (Cobo-Díaz *et al.*, 2022). Therefore, Codex has pre- and post-harvest recommendations which include adequate inspection of storage facilities, and harvested products should be kept clean (free from debris and any plant residues that may harbour fungi). Fungal spores (conidia) can survive in the ground and other residues for several months, and may lead to infection year after year without proper management practices (Marroquín-Cardona *et al.*, 2014). Subsequently, the availability of moisture content in harvested grains and high relative humidity of the surrounding air are the most important factors for fungal proliferation and mycotoxin contamination in stored grains. Due to

this, adequate drying immediately after harvest will be of great measure (Matumba *et al.*, 2021).

Strategies for mycotoxin control

According to Peng *et al.* (2018), strategies developed for the control of mycotoxins are based on Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), and also Hazard Analysis and Critical Control Points (HACCP). Recently, Matumba *et al.* (2021) proposed five major keys for the prevention and control of mycotoxins in grains which include: 1) sustaining plant's vigour and health; 2) reducing mycotoxigenic fungal population in growing plants and storage; 3) rapidly reducing the moisture content of grains and avoiding rehydration; 4) safeguarding husks/hulls or pericarp/testa; and 5) cleaning and removing mycotoxin high-risk components. These measures can be undertaken pre-, during, and post-harvest (Figure 1).

These strategies may help prevent the development of mycotoxigenic fungi and mycotoxin formation, but once mycotoxin contamination has occurred in foods or feeds, management must be done through a post-harvest detoxification procedure. Several researchers have also reported the use of methods for control of mycotoxins at pre- and post-harvest phases as shown in Table 1.

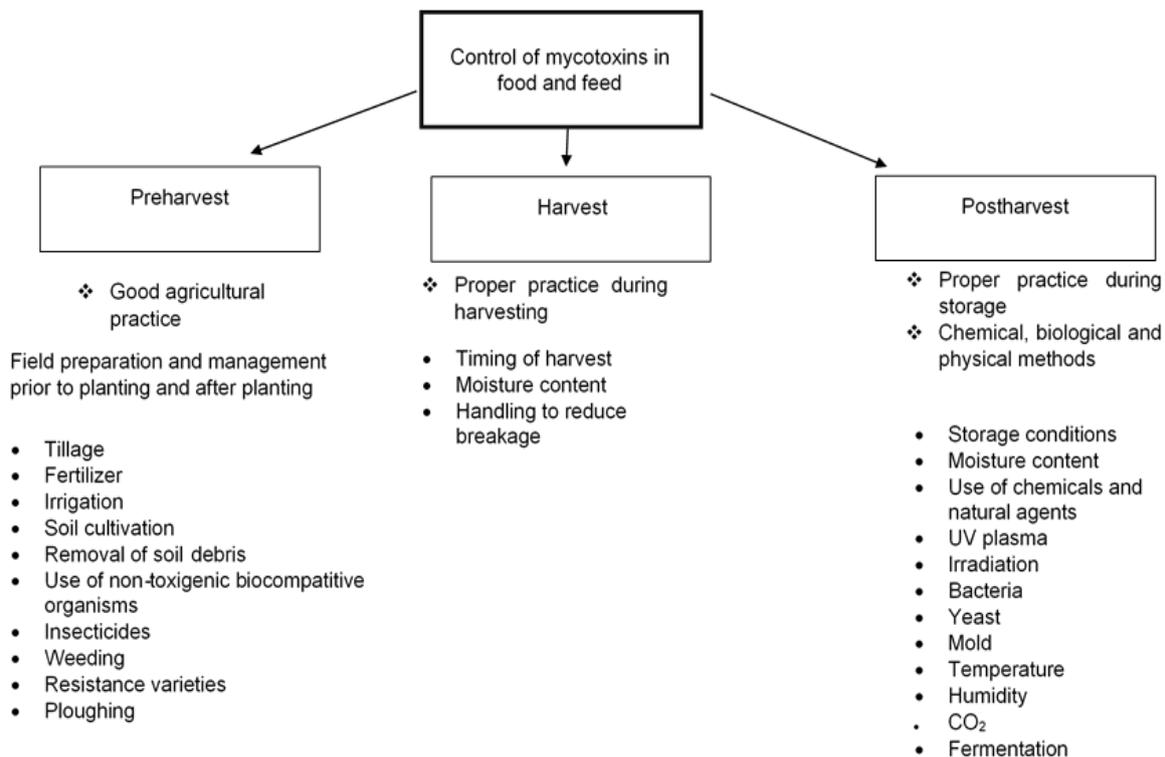


Figure 1. Strategies for controlling mycotoxins in foods and feeds.

Table 1. Control of mycotoxins in foods and feeds at pre- and post-harvest phases.

Method	Target	Substrate	Phase	Reference
Biological				
Fungicides with the <i>Bacillus subtilis</i> strain. BIOUFLA2	<i>F. verticillioides</i>	Maize	Post-harvest	Guimarães <i>et al.</i> (2020)
<i>Trichoderma viride</i>	<i>F. moniliforme</i>	Maize	Pre- and post-harvest	Yates <i>et al.</i> (1999)
<i>Bacillus subtilis</i> CE1	<i>F. verticillioides</i>	Maize root	Pre-harvest	Cavaglieri <i>et al.</i> (2005)
<i>Aspergillus flavus</i> strains NRRL 21882, 18543, and 30797 are non-aflatoxigenic strains	<i>A. flavus</i>	Soil	Pre-harvest	Weaver and Abbas (2019)
Aflasafe GH01 and Aflasafe GH02	<i>A. flavus</i>	Maize, groundnut	Post-harvest	Agbetiameh <i>et al.</i> (2020)
Atoxigenic <i>A. flavus</i> L. morphotype isolates (Ka16127, La3304, La3279, and Og0222)	<i>A. flavus</i>	Maize, groundnut	Pre-harvest	Bandyopadhyay <i>et al.</i> (2019)
<i>Aspergillus flavus</i> AF36	<i>A. flavus</i>	Almond, fig, pistachio	Post-harvest	Ortega-Beltran <i>et al.</i> (2019)
Rhizobacteria (<i>Bacillus safensis</i> RF69, <i>Bacillus sp.</i> RP103, and <i>Bacillus sp.</i> RP242)	<i>F. verticillioides</i>	Maize	Pre-harvest	Einloft <i>et al.</i> (2021)
<i>T. harzianum</i> , <i>T. asperellum</i> , and <i>T. virens</i>	<i>F. solani</i> and <i>F. oxysporum</i>			
Mytoolbox Af01	<i>A. flavus</i>	Maize	Pre- and post-harvest	Savić <i>et al.</i> (2020)
<i>T. harzianum</i> strain T22	<i>F. verticillioides</i>	Maize	Pre-harvest	Ferrigo <i>et al.</i> (2014)
<i>Bacillus cereus sensu lato</i> strain B25	<i>F. verticillioides</i>	Maize	Pre-harvest	Lizárraga-Sánchez <i>et al.</i> (2015)
Chemical				
Metconazole, azoxystrobin, and tebuconazole	<i>F. culmorum</i> , <i>F. graminearum</i> , and <i>Microdochium nivale</i>	Wheat	Pre-harvest	Edwards <i>et al.</i> (2001)
Chitosan	<i>Fusarium</i> spp.	Maize, wheat	Post-harvest	Zachetti <i>et al.</i> (2019)
Physical				
Sorting	<i>A. flavus</i> and <i>F. verticillioides</i>	Maize	Post-harvest	Aoun <i>et al.</i> (2020)
Sorting, winnowing, washing, crushing combined with dehulling	Aflatoxins and fumonisins	Maize	Post-harvest	Fandohan <i>et al.</i> (2005)
Sorting and resistance	<i>F. verticillioides</i>	Maize	Pre- and post-harvest	Morales <i>et al.</i> (2019)
UV-C irradiation	<i>Aspergillus</i> spp., <i>Penicillium</i> spp., and <i>Fusarium</i> spp.	Brown rice, black rice	Post-harvest	Ferreira <i>et al.</i> (2021)

Pre-harvest strategies

Contamination of agricultural products by mycotoxigenic fungi may occur at pre-harvest. Most of the common fungi that occur on the field are *Fusarium*, *Cladosporium*, and *Alternaria*. Other species also occur at a lower rate such as *Aspergillus* and *Penicillium* which are regarded as phytopathogenic fungi producing mycotoxins under favourable conditions (Daou *et al.*, 2021). Several strategies have been developed to help reduce and prevent the occurrence of these fungi, such as GAP, which include crop rotation, planting of resistant varieties of crops, tilling, on-time planting, control of insect damage, and weed eradication (Adebiyi *et al.*, 2019).

Resistance varieties are achieved by intentional manipulation of plant species to create the desired genotype and phenotype for a specific purpose which involves genetic engineering, gene editing, mutation breeding, hybrid breeding, and in-breeding. Planting of resistance varieties seems to help reduce fungal attacks, for instance *Fusarium* spp., which are responsible for *Fusarium* head blight which in turn results in significant losses of crop yield and quality (Varga and Tóth, 2005). However, the difference in susceptibility of cereals to *Fusarium* head blight has been observed which depends on plant species and locations. In maize and groundnut, a positive effect has also been observed in the prevention and reduction of aflatoxins. The mechanism of resistance includes good husk coverage, the presence of protein inhibiting fungal growth, wax, and cutin layers which help in the prevention of fungal infection, growth of fungi after infection, and inhibition of aflatoxin biosynthesis after infection (Soni *et al.*, 2020).

Crop rotation is an important strategy that prevents the accumulation of fungal species on the field when crops are grown consecutively for years. Crop rotation helps in breaking the continuous chain of fungal attacks due to carryover from the previous season. Tilling and ploughing are essential, where the topsoil that contains previous debris which may harbour soil-borne fungal species is inverted and mixed (Golob, 2007).

Other field management strategies are very crucial such as planting date, timely weeding, use of chemicals, biological control of fungal infection, control of insects and weeds, and planting of sound seeds (Matumba *et al.*, 2021). Soil fertiliser improves nutrient availability that provides good health, and

maintains plant resistance to diseases and fungal attacks. However, deficiency in soil nutrients may lead to the breaking of stems, thereby exposing the plants to fungal invasion. Therefore, care must be taken to avoid over-application of fertiliser which also exposes the plants to stress, thus making them more prone to pest and fungal attacks (Daou *et al.*, 2021). Crop density, proper irrigation, and modelling of mycotoxin risk at the field could be used to prevent plant infestation by mycotoxigenic fungi and mycotoxin contamination pre-harvest (Ariño *et al.*, 2009). Furthermore, other measures such as plant quarantine, phytosanitary measures, and control of insect pests may be essential for preharvest management of mycotoxins (Gahatraj *et al.*, 2020).

During harvest and post-harvest strategies

During harvesting, drying, and threshing, transfer of mycotoxins from contaminated to healthy crops is highly likely. Therefore, care must be taken during the handling of crops through contact with the equipment, and avoid breakage since injured grains are prone to fungal attacks (Bruns, 2003). Post-harvest management strategies are aimed to create a favourable environment that can prevent the growth of mycotoxigenic fungi or control mycotoxin production below the dangerous level (Gahatraj *et al.*, 2020). However, post-harvest processing and storage are the major important areas to be considered where contamination can be prevented (Bruns, 2003). Storage conditions have a significant effect on fungal growth and mycotoxin production. During storage, the most common fungal species that can inhabit the stored grains are *Aspergillus* and *Penicillium* (Wambacq *et al.*, 2016). These fungal species are regarded as storage fungi able to damage the stored grains such as the production of mycotoxins, reduction of crop quality, losses of nutrients, and heating initiation (Suleiman and Kurt, 2015). Some other factors such as temperature, moisture content, and relative humidity may affect fungal growth and mycotoxin production during storage (Mannaa and Kim, 2017b). Most storage fungi are capable of growing at a temperature range of 10 - 40°C. However, the optimum temperature for mycotoxin production is between 25 - 35°C, with a relative humidity of at least 65%, and water activity of about 0.65 a_w (which is about 13% moisture content in cereals) (Atanda *et al.*, 2011). According to Neme and Mohammed (2017), to obtain safe grains during the

entire storage period, relative humidity must be maintained at about 70%, and temperature lowered to minimise the growth and metabolism of the fungi. Maintaining temperature is good storage practices that prevent the increase in water activity due to grain respiration (Daou *et al.*, 2021). Furthermore, the use of modified atmospheric storage has been proven to have an effect on fungal growth such as a decrease in oxygen tension and an increase in CO₂ (Atanda *et al.*, 2011). Generally, storage practices such as controlled temperature, humidity, and moisture levels in either stored grains or storage houses may prevent fungal growth and mycotoxin contamination (Luo *et al.*, 2018).

In addition to these, some other factors such as grain physical condition, grain nutrient composition, intergranular air level, storage time, and hygienic conditions may also affect storage safety. During storage, several other techniques of decontamination may also be applied which include physical, chemical, and biological methods (Luo *et al.*, 2018; Daou *et al.*, 2021). Several methods have been developed to prevent, control, and decontaminate mycotoxigenic pathogens and mycotoxin at both pre- and post-harvest stages in food and feed products. Methods used for the decontamination of mycotoxins are shown in Figure 2.

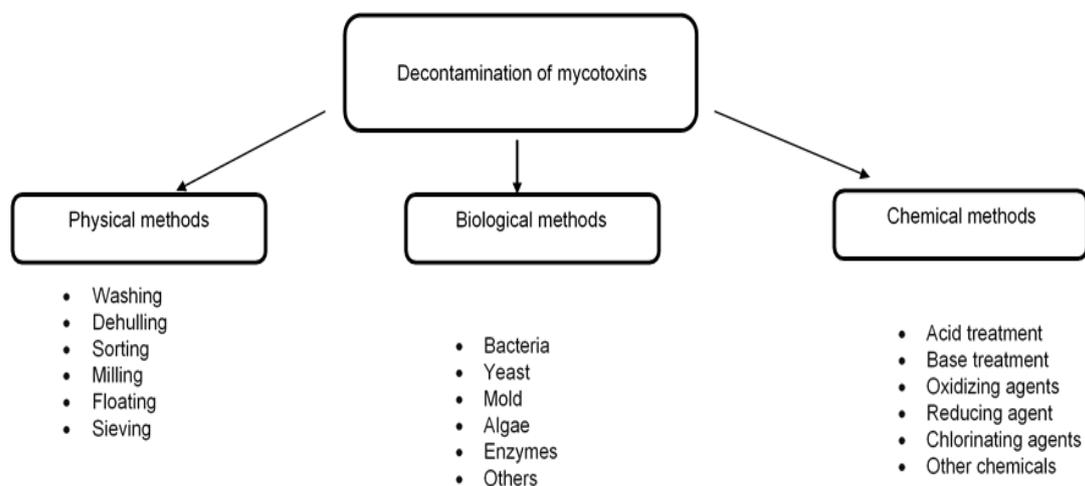


Figure 2. Various mycotoxin decontamination methods.

Physical methods

Post-harvest management strategies involve some physical approaches such as sorting, segregation, floating, washing, boiling, roasting, dehulling, steeping, and sieving to reduce the concentration of mycotoxins present (Agriopoulou *et al.*, 2020). These processes involve the removal of dust, inferior or infected kernels, and foreign materials, which are expected to reduce the level of mycotoxins as required by the EU (Peng *et al.*, 2018). The simple technique of washing using water or sodium carbonate solutions has significant effects in reducing the concentrations of deoxynivalenol, zearalenone, and fumonisins in grains (Varga and Tóth, 2005). According to Matumba *et al.* (2021), over 90% of target mycotoxins have been eliminated by hand sorting which increases the safety of products.

Physical methods such as heat treatment (steam, infrared, microwave, and extrusion cooking) have also been used to destroy mycotoxins in foods

(Peng *et al.*, 2018). Several thermal processes such as extrusion cooking were found to reduce the level of ochratoxin A in various food matrices (Castells *et al.*, 2006; Lee, 2020). Non-thermal processes such as gamma irradiation have also been reported to successfully reduce the level of ochratoxin A in feeds (Refai *et al.*, 1996). However, the efficiency of irradiation methods depends on several factors such as appropriate dose, environmental condition of the irradiated product, and microbial loads (Jeong and Jeong, 2018). The major advantages of this method are its high efficacy and environmental friendliness (Liu *et al.*, 2020). Nevertheless, the control of mycotoxins through physical methods is often laborious, inefficient, and impractical on a large scale (Udomkun *et al.*, 2017). Therefore, farmers often turn to chemical controls instead.

Chemical methods

Various chemicals are effective in controlling the level of mycotoxins in foods and feeds, and they

are classified as acids, bases, oxidising agents, reducing reagents, chlorinating reagents, salts, and others (Varga and Tóth, 2005). Ammonia (base) has been used for the treatment of seeds to reduce the concentration of mycotoxins such as aflatoxins, fumonisins, and ochratoxin A to an undetectable level, and also to inhibit the growth of mycotoxigenic fungi (Agriopoulou *et al.*, 2020). Also, the mixture of glycerol and calcium hydroxide has been used for the detoxification of mycotoxins (Luo *et al.*, 2018). However, the use of bases on food intended for human consumption has been forbidden by the EU.

Synthetic chemicals have been used for controlling mycotoxigenic fungi and infestation by insects for decades due to their antifungal activities. According to Nesci *et al.* (2008), butylated hydroxyanisole and propylparaben have significantly reduced the population of *Aspergillus* spp. in maize after six months of storage. Ozone (O₃) is a very famous chemical used due to its oxidising ability which could be in liquid or gas form. Ozone has shown a significant effect on the degradation of aflatoxin B₁ and aflatoxin G₁, with the latter has been proven to be the most sensitive (Agriopoulou *et al.*, 2016). Generally, chemical control through the use of fungicides is regarded as the most effective method of controlling fungal infestation and mycotoxin contamination even though the use of fungicides is considered controversial. The use of fungicides has been correlated with risks to humans, animals, and the environment (Daou *et al.*, 2021). It has been reported that pesticides metabolise, deposit, or accumulate in the human system, thus resulting in severe health issues (Nicolopoulou-Stamati *et al.*, 2016). Nevertheless, the toxicity of pesticides to human health depends on the types of pesticides, route of exposure, duration, and health status of the exposed individual (Daou *et al.*, 2021).

Biological methods

Biological control has been proven to be one of the efficient alternatives to chemical control of fungal occurrence on the field, and to reduce the levels of mycotoxins in foods and feeds (Yazid *et al.*, 2020). The application of biological means in the detoxification of mycotoxins involves the use of microorganisms, enzymes, and metabolites to bind and degrade the mycotoxins. Studies have shown that the use of microorganisms such as bacteria, moulds, and yeasts has a significant effect on mycotoxin

degradation in foods and feeds (Mannaa and Kim, 2017a; Luo *et al.*, 2018). This method involves the application of harmless fungal species to inhibit pathogenic activities through nutrient or habitat competition on the field. For example, a non-toxicogenic strain of *A. flavus* and *A. parasiticus* has successfully decreased aflatoxin contamination in maize, cotton, pistachio, and peanuts (Agriopoulou *et al.*, 2020). Furthermore, fungi such as *Rhizopus*, *Trichoderma*, *Clonostachys*, and *Penicillium* spp. have significant effects on the detoxification of mycotoxins (Taheur *et al.*, 2019).

Some bacteria can also bind and detoxify mycotoxins in various food products (Taheur *et al.*, 2019). For example, *Bacillus velezensis* RC 218 and *Streptomyces albidoflavus* RC 87B showed 30% reduction of *Fusarium* head blight in wheat on the field (Palazzini *et al.*, 2018). Biocontrol is also applicable during storage. However, despite the benefit of this method, several limitations may discourage its uses such as the possibility of producing toxic metabolites that might be harmful, and the possibility of conversion to modified forms (Kagot *et al.*, 2019). The use of competing yeast has also been proven to be important for inhibiting the growth of some mycotoxigenic fungi and mycotoxin contamination (Luo *et al.*, 2018). This is of greater interest since yeast can also produce a beneficial antibacterial compound which is important to both humans and animals, and because yeast does not produce secondary metabolites and allergens (Tilocca *et al.*, 2019). Table 2 shows a summary of methods of biological decontamination of mycotoxins in foods and feeds.

Mycotoxin binders (adsorbents)

Adsorbents are mycotoxin binders which are considered the common methods of mitigating mycotoxins associated with health risks upon exposure. Adsorbents are large molecular compounds that bind mycotoxins in contaminated feeds. The major mechanism involved includes the reduction of the bioavailability of mycotoxins. However, they are only effective if their stability is retained in the gastrointestinal tract of animals, and are excreted through urine and stool (Haque *et al.*, 2020). The principle of this technique helps reduce the uptake of mycotoxin and distribution in the blood and target organs if stable in the digestive tract channel, and excreted in urine and faeces (Jard *et al.*, 2011). In

Table 2. Biological methods of mycotoxin decontamination.

Mycotoxin	Product	Process	Reference
Type-A trichothecene (diacetoxyscirpenol, T-2 toxin, HT-2 toxin, neosolaniol, T2-triol) and type-B trichothecene (3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, fusarenonX, nivalenol, verrucarol)	Food, feed	Microorganisms	Ahad <i>et al.</i> (2017)
Aflatoxins (B ₁ , B ₂ , G ₁ , G ₂), ochratoxin A	Food, feed	Bacteria	Biernasiak <i>et al.</i> (2006)
Fumonisin B ₁	<i>In vitro</i>	Enzymes	Li <i>et al.</i> (2021)
Aflatoxin B ₁	<i>In vitro</i>	Enzymes	Branà <i>et al.</i> (2020)
Fumonisin (FB ₁ , FB ₂ , FB ₃ , HFB ₁)	Maize	Enzymes	Alberts <i>et al.</i> (2021)
Ochratoxin A	<i>In vitro</i>	Bacteria	Guimarães <i>et al.</i> (2018)
Aflatoxins (B ₁ , B ₂ , G ₁ , G ₂)	-	Bacteria	Guimarães <i>et al.</i> (2018)
Aflatoxin B ₁	Milk	Bacteria	Marrez <i>et al.</i> (2018)
Deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin	Wheat	Bacteria	Juodeikiene <i>et al.</i> (2018)
Aflatoxin M ₁	Milk	Yeasts	Hassan <i>et al.</i> (2021)
Patulin	<i>In vitro</i>	Yeasts	Li <i>et al.</i> (2019)
Deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, deoxynivalenol-3- β -D-glucoside	Wheat	Bacteria	Trakselyte-Rupsiene <i>et al.</i> (2022)
Aflatoxin B ₁	Beverage	Bacteria	Taheur <i>et al.</i> (2020)
FB ₁ , T-2 toxin	Sorghum	Bacteria	Adebo <i>et al.</i> (2019)
Aflatoxin B ₁ , deoxynivalenol, fumonisins, T-2 toxin, zearalenone	<i>In vitro</i>	Bacteria, yeasts	Chlebicz and Śliżewska (2020)
Aflatoxin B ₁ , B ₂ , ochratoxin A	Almond, peanut	Bacteria	Taheur <i>et al.</i> (2019)
Aflatoxin B ₁ , zearalenone, ochratoxin A	Milk	Bacteria	Taheur <i>et al.</i> (2017)
Aflatoxin M ₁	Milk	Bacteria	Foroughi <i>et al.</i> (2018)
Deoxynivalenol, 15-acetyldeoxynivalenol, alternariol, deoxynivalenol-3-glucoside, T-2 toxin, HT-2 toxin, enniatin B ₁	Wheat	Bacteria	Zadeike <i>et al.</i> (2021)
Aflatoxins (B ₁ , B ₂ , G ₁ , G ₂)	Nut	Organic acid	Jubeen <i>et al.</i> (2020)

addition, the connection of mycotoxins to binding agents occurs through various methods such as hydrogen bonding, hydrophobic bonding, and electrostatic adsorption. The most important criterion for assessing the efficiency of mycotoxin adsorbents is their performance at acidic or neutral levels (pH levels). Most importantly, the major factors that play a major role in the efficiency of adsorbents are their physical properties such as pore size, dissociation constant, surface accessibility, total charge, and distribution and physicochemical features of mycotoxins (Peivasteh-Roudsari *et al.*, 2021).

Several adsorbents such as minerals [aluminosilicates, hydrated sodium calcium aluminosilicate (HSCAS), montmorillonite KSF, bentonite, zeolite, sepiolite, and synthetic polymers]

are being used as effective strategies for preventing mycotoxins passage from the gastrointestinal tract into the blood and organs of animals. Among all, aluminosilicate minerals are the largest class of adsorbents, widely used for the decontamination of mycotoxins. Although many aluminosilicate adsorbents can adsorb aflatoxins and fumonisin B₁, they appear to be ineffective at absorbing other mycotoxins including deoxynivalenol and zearalenone (Mussaddeq *et al.*, 2000). The binding efficiency of mineral adsorbents depends on both the structures of the binders (surface area, charge distribution, and pore size of adsorption) and mycotoxins (charge distribution, polarity, and shape) (Kabak *et al.*, 2006). Hydrated sodium calcium aluminosilicate (HSCAS) are commonly used as anti-

caking agents in animal feed. It has been shown to act as an enterosorbent that tightly and selectively binds aflatoxins in the gastrointestinal tract of animals, thus decreasing their bioavailability and toxicity (Harper *et al.*, 2010). HSCAS is highly efficient in adsorbing aflatoxins but fails to prevent the toxic effects of *Fusarium* mycotoxins, such as fumonisins or trichothecenes (Harper *et al.*, 2010). Furthermore, the addition of HSCAS to aflatoxin-contaminated diet contributed to a significant improvement in the biochemical, haematological, and immunological parameters, as well as in the retention of minerals (Abbès *et al.*, 2010).

The safety and efficacy of bentonite as a feed additive have also been evaluated by the EFSA. It has been observed that bentonites are not genotoxic, and are not absorbed when used as a feed additive, hence providing no direct toxicological risk for the animal. They are considered to be eco-friendly, cheap, and have high efficiency in the removal of mycotoxins from animal feed (Phillips *et al.*, 2019). According to Carraro *et al.* (2014), milk contaminated with aflatoxin M₁ (0.080 mg/L) was decontaminated with bentonites to an acceptable limit, although slight changes in the nutritional profile of milk were observed. Also, raw and lyophilised bentonite decreased aflatoxin B₁ from 92.8 - 98.6% at different conditions (temperature, pH, time, and dose) (Bettiol *et al.*, 2022). However, due to the limited binding effects of bentonites, they are not effective for the adsorption of other mycotoxins (Čolović *et al.*, 2019).

Zeolites are aluminosilicate compounds with effective capabilities in reducing mycotoxins and other toxic compounds. According to Papaioannou *et al.* (2005), zeolites have been used as feed additives in the prevention of certain farm animal diseases. It has been reported that natural zeolite-clinoptilolite is effective in the adsorption of aflatoxins and other mycotoxins such as fumonisins (Daković *et al.*, 2010). Also, the adsorption of zearalenone was observed to increase with the use of modified zeolite at pH levels of 3, 7, and 9 (Daković *et al.*, 2005). However, the addition of 0.5 - 2% of zeolite diet decreased serum levels of zinc, copper, and manganese, while increasing the level of aluminium (Toprak *et al.*, 2016).

Montmorillonite showed effectiveness in binding and reducing the toxicity of polar mycotoxins such as aflatoxins (Nones *et al.*, 2015). Zhang *et al.* (2020) reported that the adsorption of deoxynivalenol by pillared montmorillonite modified with

aluminium, iron, and titanium was effective at pH 2.0 and 6.8, ranging from 14.7 - 23.4% and 21.8 - 27.4%, respectively. Furthermore, montmorillonite (20 - 1000 µg/mL) could inhibit cell proliferation, and induce oxidative stress and membrane damage between 24 and 72 h, with more remarkable cytotoxicity after long-term exposure of about ten days (Baek *et al.*, 2012). However, they were less effective in binding low polar and hydrophobic mycotoxins such as zearalenone and ochratoxin A due to their hydrophilic negatively-charged surfaces (Marković *et al.*, 2017). Besides, the major shortcomings of modified montmorillonite are relatively low organic carbon content in organic montmorillonite, and low adsorption effect on weak polar zearalenone (Sun *et al.*, 2020).

Sepiolite is a natural clay mineral (hydrated magnesium-rich silicate) that has a high adsorption ability, with fibre-shaped crystalline morphologies, a large specific surface area, and unique pore structure (Tian *et al.*, 2019). The synthesis of flower-like mesoporous magnesium silicate composites from sepiolite has been used for the adsorption of aflatoxin B₁ with 100% efficiency (Li *et al.*, 2022). It has been observed that sepiolite has a selective ability in the adsorption of aflatoxins, with limited adsorption efficiency of other mycotoxins (Vila-Donat *et al.*, 2018).

In 2009, the European Union (386/2009/EC) approved the use of feed additives as mycotoxin-detoxifying agents to prevent mycotoxicoses in farm animals. According to the European Food Safety Authority (EFSA), the maximum safe level of bentonite and sepiolite is 20 kg/tonne/complete feed, which is regarded safe for all livestock. Also, the safe level of algae-interspaced bentonite is 0.125 kg/tonne/complete feed for pigs (Horky *et al.*, 2022). Presently, di-octahedral bentonite was the only authorised anti-aflatoxin additive by the EU Regulation in 2009 (EC, 2013). However, the major challenges associated with the implementation of these strategies include high costs, unproven performance in large-scale implementation, low removal efficiency, the generation of secondary pollution (toxic chemicals), and large quantities of sludge formation.

Generally, clay adsorbents have been very effective on aflatoxin B₁; however, their efficacy is only limited to aflatoxin B₁, and have negative effects on the bioavailability of minerals, trace elements, and may also adsorb nutrients (Galvano *et al.*, 2001).

Therefore, due to these reasons, the use of organic adsorbents (activated carbon, microfibers and bioactive adsorbent, organic aluminosilicate or modified clay) was proposed. According to Peivasteh-Roudsari *et al.* (2021), magnetic nanoparticles (Fe_2O_3 , Fe_3O_4 particles coated with chitosan, magnetic carbon nanocomposite, and nanocellulosic compound mixed with retinoic acid) are also effective adsorbents for the removal of mycotoxins.

Apart from these methods, biotransforming agents (BA) that involve the degradation of mycotoxins by converting them into non-toxic metabolites are also being used. Bacteria, moulds, yeasts, and enzymes can transform these mycotoxins into less toxic metabolites through glucuronidation, acetylation/deacetylation, ring cleavage, isomerisation, esterification, sulphation, glycosylation, hydroxylation, oxidation, hydrogenation, de-epoxidation, methylation/demethylation, glycosylation, and deamination (Wielogorska *et al.*, 2016).

Other probiotics, antioxidants, and plant extracts are also common as mycotoxin detoxification agents (Holanda and Kim, 2021; Ramli *et al.*, 2021). Several plant extracts such as essential oils and their bioactive compounds have been used for their antimycotoxigenic and antifungal for effective detoxification of mycotoxins (Chaudhari *et al.*, 2019). For instance, clove oil, turmeric oil, and eugenol can inhibit the growth of *Aspergillus* and the production of aflatoxin B₁. A recent study investigated the effect of chemically treated durian peel on the adsorption efficiency of aflatoxins, deoxynivalenol, zearalenone, fumonisins, and ochratoxin A which showed that adsorption was > 80%, except for deoxynivalenol (Adunphatcharaphon *et al.*, 2020). Generally, the use of biological treatment is often preferred over chemical treatment in the detoxification of mycotoxins as it is considered environmentally safe (Awuchi *et al.*, 2021).

Emerging and green technologies such as high-pressure processing (HPP), ohmic heating, pulsed electric field (PEF), cold plasma, as well as ultrasound methods have also been identified as useful strategies used in fungal and mycotoxin decontamination in foodstuffs (Alizadeh *et al.*, 2021). HPP could alter the structure of mycotoxins, thus decreasing their toxicity and ability to thrive in the environment. PEF, however, may result in the death of the cells by demolishing the position of the cell

membrane such that a transmembrane voltage gets formed *via* the assembly of potential diversities between the biofilms of the inside and outside parts, thus reaching a certain threshold after which permanent cell structural changes occur (Suchanek and Olejniczak, 2018).

Cold plasma is a non-thermal process used for the detoxification of mycotoxins. The mechanism involved in the detoxification of mycotoxins using cold plasma is the disruption of mycotoxigenic fungal cellular activity by destroying the fungal DNA and cell wall which may lead to the leakage of intracellular component, and reduction/elimination of mycotoxin production (Adebo *et al.*, 2021). However, studies have shown that cold plasma may be difficult to apply in large-scale industries, especially for the treatment of foods with irregular shapes and bulky foods, and it also has a negative effect on lipids (Gavahian and Cullen, 2020). Another non-thermal process is the application of irradiation (UV, gamma, and electron beam) for mycotoxin decontamination. Studies have shown that several irradiation methods have significant effect on the decontamination of mycotoxins (Luo *et al.*, 2014; 2017; Markov *et al.*, 2015). Although Peng *et al.* (2018) reported that this method has proven to be effective in mycotoxin decontamination, the major concern is its high cost, oxidation of vitamins and lipids, and changes in colour and flavour of foods.

Way forward

The occurrence of mycotoxins in foods and feeds is unavoidable, and has been a serious issue affecting food safety and food security globally since it is entirely impossible to create a condition or surrounding which is free or devoid of fungi or fungal spores which are mostly airborne and air-transmitted (aerial or horizontal contamination). This thus has negative effects on animals, humans, and plants. Dealing with the contamination of agricultural products by mycotoxins remains the key challenge that affects many countries across the globe, especially those that heavily rely on agriculture for food and income. This too has led to the development and implementation of various control and preventive strategies for crops during pre-, harvest, and post-harvest. Mycotoxin decontamination and detoxification approaches deliver a wide range of outcomes. However, more research must be undertaken and encouraged to help supplement existing information, such as experimental studies on

the use of natural products such as essential oils in the prevention of mycotoxins pre- and post-harvest. Furthermore, additional studies on the evaluation of agricultural practices on the overall occurrence of mycotoxigenic fungi and mycotoxins are also warranted, and must be implemented in tandem with other control strategies such as in the Integrated Pest Management (IPM) strategy since no individual method can eliminate mycotoxigenic fungi and mycotoxins in the agricultural settings.

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