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Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review

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Mycotoxins are fungal secondary metabolites that have been associated with severe toxic effects to vertebrates produced by many important phytopathogenic and food spoilage fungi including Aspergillus*,* Penicillium*,* Fusarium*, and* Alternaria *species. The contamination of foods and animal feeds with mycotoxins is a worldwide problem. We reviewed various control strategies to prevent the growth of mycotoxigenic fungi as well as to inhibit mycotoxin biosynthesis including pre-harvest (resistance varieties, field management and the use of biological and chemical agents), harvest management, and post-harvest (improving of drying and storage conditions, the use of natural and chemical agents, and irradiation) applications. While much work in this area has been performed on the most economically important mycotoxins, aflatoxin B_1 and ochratoxin A much less information is available on other mycotoxins such as trichothecenes, fumonisin B_1, zearalenone, citrinin, and patulin. In addition, physical, chemical, and biological detoxification methods used to prevent exposure to the toxic and carcinogenic effect of mycotoxins are discussed. Finally, dietary strategies, which are one of the most recent approaches to counteract the mycotoxin problem with special emphasis on* in vivo *and* in vitro *efficacy of several of binding agents (activated carbons, hydrated sodium calcium aluminosilicate, bentonite, zeolites, and lactic acid bacteria) have also been reviewed.*

Keywords Mycotoxin, detoxification, prevention

INTRODUCTION

Mycotoxins are secondary metabolites which are produced by several fungi mainly belonging to the genera: *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*.[1,2,3,4] While *Aspergillus* and *Penicillium* species are generally found as contaminants in food during drying and storage, *Fusarium* and *Alternaria* spp. can produce mycotoxins before or immediately after harvesting.[5] Up until now, approximately 400 secondary metabolites with toxigenic potential produced by more than 100 moulds, have been reported, with the Food and Agriculture Organization (FAO) estimating that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins,[6–11] leading to significant economic losses.[12]

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Mycotoxins primarily occur in the mycelium of the toxigenic moulds and may also be found in the spores of these organisms[13] and cause a toxic response, termed a mycotoxicosis, when ingested by higher vertebrates and other animals.[14] These secondary metabolites are synthesized during the end of the exponential phase of growth and appear to have no biological significance with respect to mould growth/development or competitiveness.[13,15–17] All moulds are not toxigenic and while some mycotoxins are produced by only a limited number of species, others may be produced by a relatively large range from several genera.[13,16]

The toxic effect of mycotoxin ingestion in both humans and animals depends on a number of factors including intake levels, duration of exposure, toxin species, mechanisms of action, metabolism, and defense mechanisms.[10,17] Consumption of mycotoxin-contaminated food or feed does however lead to the induction of teratogenic, cancerogenic, oestrogenic,

neurotoxic, and immunosuppresive effect in humans and/or animals.[11,18] The mycotoxins of most significance from both a public health and agronomic perspective include the aflatoxins, trichothecenes, fumonisins, ochratoxin A (OTA), patulin, tremorgenic toxins, and ergot alkoloids.[10,11,17,19]

Because of the detrimental effects of these mycotoxins, a number of strategies have been developed to help prevent the growth of mycotoxigenic fungi as well as to decontaminate and/or detoxify mycotoxin contaminated foods and animal feeds. [20,21] These strategies include:

- The prevention of mycotoxin contamination,
- Detoxification of mycotoxins present in food and feed,
- Inhibition of mycotoxin absorption in the gastrointestinal tract.

PREVENTION OF MYCOTOXIN CONTAMINATION

Mycotoxin contamination may occur in the field before harvest, during harvesting, or during storage and processing,[1,22,23] thus methods for the prevention of mycotoxin contamination can conveniently be divided into pre-harvest, harvesting and post-harvest strategies.[24] Whereas certain treatments have been found to reduce specific mycotoxin formation in different commodities, the complete elimination of mycotoxin contaminated commodities is currently not realistically achievable.

Several codes of practice have been developed by Codex Alimentarius for the prevention and reduction of mycotoxins in cereals, peanuts, apple products, and raw materials. The elaboration and acceptance of a General Code of Practice by Codex will provide uniform guidance for all countries to consider in attempting to control and manage contamination by various mycotoxins. In order for this practice to be effective, it will be necessary for the producers in each country to consider the general principles given in the Code, taking into account their local crops, climate, and agronomic practices, before attempting to implement provisions in the Code. The recommendations for the reduction of various mycotoxins in cereals are divided into two parts: recommended practices based on Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP); a complementary management system to consider in the future is the use of Hazard Analysis Critical Control Point (HACCP).[25]

Pre-Harvest Control Strategies

It is well established that mycotoxin contamination of agricultural product can occur in the field as well as during storage.[23,26,27,28] Since phytopathogenic fungi such as *Fusarium* and *Alternaria* spp can produce mycotoxins before or immediately post harvesting several strategies have been developed including biological and cultural control practices to help mycotoxin contamination occurring in this way. The pre-harvest control strategies can be divided with respect to their use for different products, such as cereal grain, nuts, fruits etc. for the prevention and reduction of various mycotoxins.

Prevention Strategies of Mycotoxins in Cereals

The main mycotoxin hazards associated with wheat pre-harvest in Europe are the toxins that are produced by fungi belonging to the genus *Fusarium* in the growing crop. Mycotoxins produced by these fungi include zearalenone (ZEN) as well as trichothecenes and include nivalenol (NIV), deoxynivalenol (DON) and T-2 toxin. *Fusarium* species are also responsible for a serious disease called *Fusarium* Head Blight (FHB), which can result in significant losses in both crop yield and quality. It is important to note that although *Fusarium* infection is generally considered to be a pre-harvest problem, it is possible for poor drying practices to lead to an increased susceptibility for in storage mycotoxin contamination.[29]

Resistance Varieties. There are inherent differences in the susceptibility of various cereal species to FHB, which are reflected in differences in the degree of mycotoxin contamination to which each species is susceptible. The differences between crop species appear to differ between countries. This is probably due to differences in the genetic pool within each country's breeding program and the different environmental and agronomic conditions in which crops are cultivated.[30] Tekauz[31] has shown that DON levels in wheat, barley, and oats were similar when grown under the same field conditions in Western Canada in 2001. However, Langesth and Rudberget[32] have shown that oats had higher levels of DON contamination than barley and wheat in Norway from 1996 to 1999. It has also been reported that in wheat; tall plants without awns, and plants with lax ears tend to have lower rates of natural infection than plants that are short, awned, or which have a high spikelet density.[33]

Only seed varieties recommended for use in a particular area of a country should be planted in that particular area.[25] In recent work by Mesterhazy,[34] wheat varieties most resistant to FHB were shown to reduce DON production to near zero. In fact, resistance seemed to depend to a great extent on inhibition of toxin production directly, since the most aggressive disease-causing fungal strains were also those producing the highest levels of DON. This author suggested that an increased availability of resistant varieties, coupled with the use of appropriate fungicides, was the key in an integrated approach to mycotoxin control associated with *Fusarium.* Similarly, in another field test, resistance to both FHB and DON contamination in different wheat varieties has also been reported.[35]

Obtaining high levels of native genetic resistance in various crop types to toxigenic fungi has proven difficult to achieve. Problems in this regard have centered primarily on the lack of resistant control genotypes and on the lack of involvement of single major genes.[36] Some resistance to *Gibberella* ear rot has however been reported in the past.[37,38] A resistant gene has been identified in *Giberella*[39] and maize hybrids produced which display increased resistance to *Giberella* ear rot.[40]

With respect to genetic resistance to *Aspergillus* infection and subsequent aflatoxin production, since the early 1970s, much work has been done to identify genetically resistant crop genotypes in both laboratory and field based experiments to help control aflatoxigenic mould growth and aflatoxin

biosynthesis.[24,28,41,42] This has led to the identification of a number of well-characterized sources of both resistance to *Aspergillus flavus* infection and to aflatoxin production. These include kernel proteins such as a 14-kDa trypsin-inhibiting protein and others including globulin 1and 2 and a 22-kDa zeamatin protein.[43,44] In addition kernel proteins of maize genotypes GT-MAS and Mp420 have also been identified which are involved in the resistance to *A. flavus* infection and aflatoxin contamination due to greater waxing on kernel surfaces in GT-MAS and Mp420 which can inhibit and/or restrict entry of the aflatoxigenic mould.[45] Recently, analysis of kernel proteins from several genotypes revealed that ribosome inactivating protein (RIP) and zeamatin are resistant to *A. flavus* infection.[28]

In addition the bacterial chloroperoxidese gene have been reported to enhance fungal disease resistance in transgenic tobacco plants, with cotton being tansformed with both CPO and D4E genes in an attempt to reduce aflatoxin contamination of cottonseed. Similarly, three genes that increase tobacco resistance to trichothecenes have recently been identified.[28] In maize, hybrids genetically engineered for insect resistance (by insertion of *Bacillus thuringiensis* genes encoding the endotoxin CryA expressed in the kernel), had kernels that consistently had less fumonisins than kernels from normal plants. Other maize hybrids genetically engineered for fumonisin degradation (by insertion of a gene encoding a fumonisin esterase enzyme, from a gram-positive bacterium) have been produced. These transgenic maize plants can degrade fumonisin B_1 (FB_1) to its hydrolysis product (aminopentol), a compound that however retains most of the FB_1 toxicity.[46]

Field Management. Appropriate field management practices including crop rotation, soil cultivation, irrigation, and fertilization approaches are known to influence mycotoxin formation in the field. Crop rotation is important and focuses on breaking the chain of production of infectious material, for example by using wheat/legume rotations. The use of maize in a rotation is to be avoided however, as maize is also susceptible to *Fusarium* infection and can lead to carry-over onto wheat via stubble/crop residues.[47] It is generally accepted that wheat that follows an alternative host for *Fusarium* pathogens is at greater risk of FHB and subsequent DON contamination of grain. However, there is conflicting evidence that wheat following wheat is more at risk than wheat following a non-cereal crop.[30] Dill-Macky and Jones[48] observed that FHB disease severity and DON contamination of grain was significantly different when the previous crop was maize, wheat, or soya bean; with the highest levels following maize and the lowest levels following soya bean. Codex recommends that crops such as potato, other vegetables, clover, and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculum in the field.[25]

Soil cultivation can be divided into ploughing, where the top 10-30 cm of soil is inverted; minimum tillage, where the crop debris is mixed with the top 10–20 cm of soil; and no till, where seed is directly drilled into the previous crop stubble with minimum disturbance to the soil structure. Any crop husbandry that results in the removal, destruction, or burial of infected crop residues is likely to reduce the *Fusarium* inoculum for the following crop. The benefits of such crop practice are likely to be limited to when wheat follows an alternative host crop for *Fusarium* species.[30] Dill-Mackey and Jones[48] reported that no till (direct drilling) after wheat or maize significantly increase DON contamination of the following wheat crop compared to ploughing, but no till had no effect when the previous crop was soya bean. In Mediterranean climates it is good practice to leave ploughed land exposed to autumn sunshine as a means of destroying fungal material that could otherwise infect the following crop.[47]

Irrigation is also a valuable method of reducing plant stress in some growing situations. It is firstly necessary that all plants in the field have an adequate supply of water if irrigation is used. It is known that excess precipitation during anthesis (flowering) makes conditions favorable for dissemination and infection by *Fusarium* spp., so irrigation during anthesis and during the ripening of the crops, specifically wheat, barley, and rye, should be avoided.[25]

The soil must be tested to determine if there is need to apply fertilizer and/or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development.[25] Fertilizer regimes may affect FHB incidence and severity either by altering the rate of residue decomposition, by creating a physiological stress on the host plant or by altering the crop canopy structure. Martin et al.[49] observed that increasing N from 70 to 170 kg/ha significantly increased the incidence of *Fusarium* infection grain in wheat, barley, and triticale. Recent work by Lemmens et al.[50] in Austria has shown that a significant increase in FHB intensity and DON contamination in the grain was observed with increasing a mineral N fertilizer from 0 to 80 kg/ha. This group concluded that in practical crop husbandry, FHB cannot be sufficiently controlled by only manipulating of the N input.

Environmental Conditions. Environmental conditions such as relative humidity and temperature are known to have an important effect on the onset of FHB. For example, it has been shown that moisture conditions at anthesis are critical in *Fusarium* infection of the ears;[29] while Lacey et al.[51] have shown that *Fusarium* infection in the UK is exacerbated by wet periods at a critical time in early flowering in the summer, which is the optimum window for susceptibility. Equally, there is evidence that droughed-damaged plants are more susceptible to infection, so crop planting should be timed to avoid both high temperature and drought stress during the period of seed development and maturation. On the other hand, the planning of harvesting grain at low moisture content and full maturity may be an important control point in the preventing of mycotoxin contamination, unless allowing the crop to continue to full maturity would subject it to extreme heat, rainfall or drought conditions. Delayed harvest of grain already infected by *Fusarium* species is known to cause a significant increase in the mycotoxin content of the crop.[25]

Using of Biological and Chemical Agents. Another factor which is known to increase the susceptibility of agricultural

commodities to toxigenic mould invasion is injury due to insect, bird, or rodent damage.[52] Insect damage and fungal infection must be controlled in the vicinity of the crop by proper use of registered insecticides, fungicides, and other appropriate practices within an integrated pest management control.[25] In France, the Ministry of Agriculture has allowed numerous fungicides including tebuconazole and metconazole to be used for the control of FHB, at or about anthesis, when the plants are more susceptible. In the European Union, fungicides must firstly be shown to be safe to both the environment and to humans before being authorized for use. Nevertheless, the benefits of such fungicide applications is clear given their efficacy in preventing or reducing toxin synthesis in naturally-infected fields.[53]

Part of the integrated control of FHB in wheat production involves the use of fungicides, but this introduces a complication as far as trichothecenes are concerned as there is evidence that under certain conditions, fungicide use may actually stimulate toxin production. This raises particular concerns, since circumstances may arise where the obvious manifestations of FHB are reduced or even eliminated and yet high levels of mycotoxins may be present. Clearly grain affected in this way cannot be identified by visual inspection for signs of FHB (e.g., pink grains) and, in fact, cannot be identified until a specific mycotoxin analysis is carried out.[54]

Research carried out on fungicide use in terms of FHB and mycotoxin development has produced very interesting results. In particular, fungicides in common use have been shown to have differential effects against toxin-forming *Fusarium* species and related non-toxing-forming pathogens such as *Microdochium nivale* on ears.[55] The outcome of the use of fungicides seems to depend on the fungal spices present, and the effect that the particular fungicide has on these species. For example, in recent work commissioned by the Home Grown Cereal Authority, in an experimental situation where *Fusarium culmorum* and *M. nivale* were both present, the use of azoxystrobin showed a significant reduction in disease levels while increasing the levels of DON present in grain.[47] This was believed to be the result of selective inhibition of *M. nivale* by azoxystrobin. *M. nivale* is a natural competitor of toxin-forming *Fusarium* species, particularly *F. culmorum*. Removal of *M. nivale* by the fungicide probably allowed development of the toxigenic species in its place with concomitant increase in toxin formation. This is an important finding as it indicates that the impact of the fungicide is not directly related to mycotoxin production. It follows from these findings that where FHB is caused by *Fusarium* species in the absence of *Microdochium*, disease development is associated with higher levels of toxin. In four field trails, the application of tebuconazole was shown to selectively allow the control of *F. culmorum* and *F. avenaceum* while also reducing DON levels, but showed little control of *M. nivale*. In contrast the application of the other fungicide, azoxystrobin selectively controlled *M. nivale* and allowed greater colonization by toxigenic *Fusarium* species and its use resulted in a increasing in DON levels.[55] Recent in vitro studies looking at a range of *F. culmorum* strains from across Europe showed stimulation in DON production in the presence of epoxiconazole and propiconazole.[56] Ioos et al.[53] also carried out a screen on the efficacy of fungicides, azoxystrobin, metconazole, and tebuconazole at anthesis against *Fusarium* spp., *M. nivale* and on years on naturally infected fields of soft wheat, durum wheat, and barley. The infection levels of *F. graminearum*, *F. culmorum,* and *M. nivale* were significantly reduced by the application *Fusarium* mycotoxin concentration over three of fungicides, with tebuconazole and metconazole effectively controlling the *Fusarium* spp., but they had little effect on *M. nivale*. Although this conclusion concurs with Simpson et al.[55] for tebuconazole, their benefits were apparently seasonal-with tebuconazole controlling these fungi in 2001, while having little effect in 2000 and 2002; while metconazole significantly reduced the levels in 2000 and 2001, but not so in 2002. They also concluded that fungicide treatments in general did not reduce trichothecene contamination in wheat grain in 2000 and 2001, while DON concentration in wheat treated with tebuconazole and metconazole was reduced by 46% and 48%, respectively in 2002.

Alternatively, a limited number of biocompetitive microorganisms have been shown for the management of *Fusarium* infections. Antagonistic bacteria and yeasts may also lead to reductions in pre-harvest mycotoxin contamination. For instance, *Bacillus subtilis* has been shown to reduce mycotoxin contamination by *F. verticilloides* during the endophytic growth phase. Similarly antagonistic yeasts such as *Cryptococcus nodaensis* have also been shown to inhibit various *Fusarium* species.[28] Recent glasshouse studies by Diamond and Coke,[57] involving the pre-inoculation of wheat ears at anthesis, with the two non-host pathogens, *Phoma betae* and *Pythium ultimum* showed a reduction in disease development and severity caused by *F. culmorum*, *F. avenaceum*, *F. poae,* and *M. nivale*.

Prevention Strategies of Mycotoxins in Nuts

The pre-harvest control of aflatoxin contamination of peanuts must take into consideration all the varied environmental and agronomic factors that influence pod and seed infection by the aflatoxin-producing fungi, and aflatoxin production. These factors can vary considerably from one location to another, and between seasons in the same location. Some environments may be particularly favorable to fungal infection and subsequent aflatoxin contamination of peanuts, and in these circumstances it would be necessary to consider whether or not the crop should be grown in such areas. However, for most situations it should be possible to devise agricultural practices that should reduce aflatoxin contamination in peanuts.[58]

The pistachio is a semidry stone fruit consisting of a single kernel enclosed in a thin, bonny shell, which is surrounded by the hull. The main problem with pistachios is the "early split" formed preharvest. The shell partially splits to varying extents at least a month before maturity and harvest.[59] The hull covering the shell usually remains intact, protecting the kernels from invasion by moulds and insects.[60] Normally, the hull does not rupture when the shell splits in the immature pistachio fruit. However,

in a small percentage of the pistachios, the shell and the still adhering hull splits together. This hull rupture, often referred to as "early splitting," is a very important event for infection with the aflatoxin producing fungi *A. flavus*/*A. parasiticus*. An "early split" nut is characterized by a distinct, dark, and smooth-edged split on the hull. The oldest "early splits" have rough and shrivelled hulls and contain the highest levels of aflatoxin; containing up to 99% of all aflatoxin detected. The aflatoxin content in single nuts have revealed that many of the "early splits" contain more than 20 μg kg^{-1} and some above 1000 μg kg^{-1}.[59] Recent work by Bonjar[61] involving the isolation of *A. flavus* from kernels of early splits in pistachio nuts from three orchards in vicinity of Kerman, Iran in 2002 and 2003. The results showed that percent of infection by *A. flavus* and of contamination with aflatoxin in early splits were 2.5% and 12.5%, respectively.

Resistance Varieties. The choice of peanut variety can also be important and therefore before planting, farmers should consult with appropriate plant breeding authorities or agricultural extension services to ascertain the peanut cultivars that have been adapted to their region, and the availability of varieties that are resistant to various factors such as insect attack and microbial and fungal attack that can have an impact on the safety and quality of the peanuts produced.[58]

Field Management. The continued cultivation of peanuts on the same land may lead to a built-up of high populations of *A. flavus/A. parasiticus* in the soil, which will increase the probability of infection and subsequent aflatoxin contamination. Some studies have been carried out on the effect of crop rotation on aflatoxin contamination. In semi-arid environments, populations of *Aspergillus* may be very high, and crop rotations may have little influence on the fungal activity.

There is evidence that peanuts grown in different soil types may have significantly different levels of mould infection. Light sandy soil, for example, favors the rapid proliferation of fungi, particularly under dry conditions. Heavier soils have a higher water-holding capacity and, therefore, there is less likelihood of drought stress occurring, which may be partly responsible for the lower than average levels of aflatoxin contamination in peanuts grown on such soils. It is important to note that soil tests to determine if there is a need to apply fertilizer and/or soil conditioners to avoid plant stress, especially during seed development, which makes peanuts more susceptible to fungal infestation; should ideally be conducted, prior to application. It is also recommended that irrigation be employed to help combat heat and drought stress.[58]

Environmental Conditions. While many factors are known to influence the production of mycotoxins in the field, of these, drought stress during plant growth is among the most important;[1,62,63] with reports indicating that drought stress during peanuts maturation increases the susceptibility of aflatoxigenic mould invasion with and aflatoxins production.[1,63,64]

Using of Biological and Chemical Agents. A number of biological and chemical control agents have been reported to inhibit aflatoxigenic mould growth and subsequent aflatoxin biosynthesis.[1,24] The application of competitive, nonaflatoxigenic strains of *A. flavus*/*A. parasiticus* has clearly been shown to reduce the aflatoxin contamination of agricultural commodities such as peanuts, rice, maize, and cottonseed mainly through competition for substrate and through the production of inhibitory metabolites.[28,62–68]

It is well established that aflatoxin contamination of crops can be suppressed by atoxigenic biocontrol strains. For example the introduction of nonaflatoxigenic strains of *A. parasiticus* in field trials has been reported to result in lower aflatoxin levels in peanut crops, with levels decreasing from 531, 96 and 241 μg kg^{-1} in untreated soils to levels of 11, 1 and 40 μg kg^{-1} for treated soils in three consecutive years (1987, 1988, and 1989), respectively.[66] Similarly, other reports have highlighted that the reductions in aflatoxin levels can be achieved by applying nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* to soils containing developing plants.[64,67,69]

Several phenolics which are secondary plant metabolites synthesized via the phenylpropanoid biosynthetic pathway may exert metabolic effects on the aflatoxin biosynthetic pathway. Acetosyringone, syringaldehyde, and sinapinic acid at concentrations of 4 m mol^{-1} have been reported to reduce aflatoxin biosynthesis by 96%, 74%, and 32%, respectively. It was also demonstrated that phenolics reduce norsolorinic acid accumulation in a *nor* mutant.[70] Similarly, Lee et al.[71] indicated that naturally occuring compounds from plant such as antharaquinones, coumarins, and flavonoids can act as potent inhibitors in the biotransformation of aflatoxin B_1 (AFB_1) to the AFB_1-8,9-epoxide.

It has been also suggested that pesticides and fungicides may be useful in controlling mycotoxin production under field conditions,[41,72] although other investigators have found that pesticides were ineffective in controlling mycotoxin production by *Fusarium* and *Aspergillus* species. Indeed, it might be expected that the primary effect of the use of pesticide during plant growth in the field primarily is in the control of insect damage, thereby reducing the risk of mycotoxigenic mould invasion.[23] Several field trails have demonstrated that fungicides including organophoshorus fungicide, thiabendazole, triadimefon, propiconazole, tolclofos-methyl can be used to control mycotoxin formation,[41] with the timing of pesticide treatment being a particularly important factor in controlling mycotoxin production.[23]

Prevention Strategies of Mycotoxins in Apple Products

Patulin has been found as a contaminant in many mouldy fruits, vegetables, cereals, and other foods, however, the major sources of contamination are apples and apple products. Patulin occurs mainly in mould-damaged fruits although the presence of mould does not necessarily mean that patulin will be present in a fruit but indicates that it may be present. In some instances, internal growth of moulds may result from insect or other invasions of otherwise healthy tissue, resulting in occurrence of patulin in fruit which externally appears undamaged. For this reason, the removal and cutting off of all diseased wood and mummified fruits during the dormant season may be effective in the reduction of patulin contamination. Another equally important factor

is the controlling of pests and diseases which directly cause fruits rots or allow entry sites for patulin-producing moulds. These include canker, eye rot (*Botrytis* spp and *Nectria* spp), codling moth, fruitlet mining tortix moth, winter moth, fruit tree tortrix, blastobasis, sawfly, and dock sawfly.

Apples of poor mineral composition are more likely to suffer physiological disorders in store and hence are more susceptible to particular types of rot especially by *Gloesporium* spp and secondary rots such as *Penicillium*. Where levels of minerals in the fruit for the fresh fruit market are outside optimum ranges, improving calcium and phosphorus levels in the fruit, particularly increasing the calcium/potassium ratio by controlled fertilizer usage, will improve cell structure, which will then reduce susceptibility to rotting.[73]

Harvest Management

Cereals

Harvest is the first stage in the production chain where moisture management becomes the dominant control measure in the prevention of mycotoxin development.[74] Since the mositure content may vary considerably within the same field, the control of moisture in several spots of each load of the harvested grain during the harvesting operation is very important.[25] Another equally important control measure is an effective assessment of the crop for the presence of disease such as FHB. This should be accompanied by an efficient strategy for separation of diseased material from healthy grain.[74] There is evidence that fungal infection can be minimized by avoiding the mechanical damage to the grain and by avoiding contact with soil at this stage.[25]

Nuts

Harvesting plants at optimum maturity and operation of harvesting to avoid damage to agricultural commodities are important factors to bear in mind in order to prevent mycotoxin contamination.[75] For example higher levels of aflatoxin contamination have been reported in over-mature or unmature peanuts.[24] During the harvesting process it is important that every effort is made to avoid physical damage to the agricultural commodities,[22] with crops which have been physically damaged being more susceptible to fungal growth.[72] It has been reported that undamaged seeds of peanuts grown during post-rainy season even under drought-stress conditions, possess a lower risk of aflatoxin contamination.[76] Where harvesting occurs in dry weather, mycotoxin contamination does not usually prove problematic. It does however become a problem where harvesting is done in very humid weather. In many developing countries, the combination of insufficient drying equipment coupled with humid atmospheric conditions results in unacceptable levels of aflatoxin in harvested peanuts, tree nuts, and other foods.[60] The timing of the harvest may also be important, with respect to the level of shell splitting which may occur in pistachios, so that contamination by aflatoxin producing fungi can be minimized at this stage.[77] Harvesting practices such as the using of poles in harvesting nuts in Turkey can lead to crops damage, leaving pistachio nuts which are harvested in this much more susceptible to aflatoxigenic mould invasion during the harvest.[78] Similarly, several investigators have shown that mechanically harvested peanuts are more susceptible to mould invasion during subsequent curing and storage than hand-picked peanuts.[22]

Apple Products

The quality of the fruit resulting from the harvesting is the first step in controlling patulin levels.[79] It has been suggested that, all fruit must be handled as gently as possible to avoid physical damage.[73] With the highest quality hand-picked fruit being used for direct-for-retail sale, processed apple products are usually produced from mechanical harvest, windfalls, insect damage, or culled fruit. Bruises, skin breaks, and other physical damage within these apples provide a perfect entry point for *Penicillium expansum* and other patulin-producing species into the fruit. There are reports in the literature on the effect of fruit quality and harvest method on the patulin content of the resultant juices. In a recent study, patulin was reported to be undetactable in 7 cultivars of tree-picked cider, whereas it was detected between 40.2 and 374 μg l^{-1} in 4 cultivars of ground-harvest cider.[80] It is widely accepted that the picking of fruit in dry conditions, placing in clean bins or other suitable containers, transporting directly to the store, placing in cold storage within 18 h of harvest and cooling to the recommended temperatures (1.5–4.0°C), are all very important steps that must be followed if the overall levels of mould infection is to reduced.[73]

Post-Harvest Control Strategies

Post harverst strategies are important in the prevention of mycotoxin contamination and include improved drying and storage conditions, together with the use of natural and chemical agents, as well as irradiation.

Improving of Drying and Storage Conditions

The practices that reduce mycotoxin contamination may differ depending on the climate of the region and the type of the crop. However, general practices to avoid fugnal infection of different crops are discussed below.

Cereals. Mycotoxigenic fungal growth can arise in storage as a result of moisture variability within the grain itself or as a result of moisture migration results from the cooling of grains located near the interface with the wall of the storage container/silo.[18] Thus control of adequate aeration and periodical monitoring of the moisture content of silos plays an important role in the restriction of mycotoxin contamination during the storage period.[22]

The moisture levels in stored crops is one of the most critical factors in the growth of mycotoxigenic moulds and in mycotoxin

production,[81] and is one of the main reasons for mycotoxin problems in grain produced in developing countries.[82] Cereal grains are particularly susceptible to grow by Aspergilli in storage environments. The main toxigenic species are *A. flavus* and *A. parasiticus* for aflatoxins,[2] and *Penicillium verrucosum* is the main producer in cereals for OTA[83] while *A. ochraceus* is typically associated with coffee, grapes, and species,[25] aflatoxins can be produced at a_w values ranging from 0.95 to 0.99 with a minimum a_w value of 0.82 for *A. flavus*, while the minimum a_w for OTA production is 0.80.[2] It has been reported that *A. flavus* will not invade grain and oilseeds when their moisture contents are in equilibrium with a relative humidity of 70% or less. The moisture content of wheat at this relative humidity is about 15%, and around 14% for maize,[25] but it is lower for seeds containing more oil, approximately 7% and 10% for peanuts and cottonseeds, respectively,[22] while *A. parasiticus* has been reported to produce aflatoxins at 14% moisture content in wheat grains after 3 months of storage.[84] The minimum moisture content for growth of *P. verrucosum* is 16–17%, while for OTA production the moisture content needs to be approximately 1% higher.[25] With respect to OTA it has been reported that this mycotoxin was produced by *P. verrucosum* in maize stored at 21% relative humidity after 4 week, reaching a maximum of 3.6 ppm after 8 weeks.[85]

The second critical factor influencing post harvest mould growth and mycotoxin production is temperature.[22,75] Both the main aflatoxin producing *Aspergillus* strains *A. flavus* and *A. parasiticus* can grow in the temperature range from 10–12°C to 42–43°C, with an optimum in the 32 to 33°C range.[86] Aflatoxins are produced at temperatures ranging from 12 to 40°C,[2] while OTA production by *P. verrucosum* occurs between 10 to 25°C,[87] with several studies highlighting the relatively high incidence of mycotoxins such as aflatoxins and ochratoxins in foods and feeds in tropical and subtropical regions.[23,88,89] The control of temperature of the stored grain at several fixed time intervals during storage may be important in determining mould growth. A temperature rise of 2–3°C may indicate mould growth or insect infestation.[25]

Until recently, little if any work has been carried out on monitoring how spoilage fungi interact with each other in the stored grain ecosystem, and the effect that this has on mycotoxin production. Recent studies by Magan et al.[90] have shown that the system is in a state of dynamic flux with niche overlap altering in direct response to temperature and a_w levels. It appears that the fungi present tended to occupy separate niches, based on resources utilization, and this tendency increased with drier conditions. In another study this group has looked at the influence of species interaction and changing environmental conditions on mycotoxin production. They have shown that the production of DON and NIV by *F. culmorum* is strongly influenced by competition with other fungi.[91]

With respect to storage facilities Codex recommend they be dry, well-constructed structures that provide protection from rain, drainage of ground water, and protection from the entry of rodents and birds, ideally with minimum temperature fluctuations.[25]

Nuts. The primary goal for aflatoxin prevention in storage is to prevent mould development on the peanuts due to condensation or leaks in the warehouse. The a_w of peanuts varies with moisture content and temperature must be carefully controlled during storage.[58] For example, Abdulkadar et al.[92] have reported that climate conditions of high humidity and temperature increase the incidence of aflatoxin contamination. It is known that stock piling of peanuts can cause heat built-up and moisture accumulation with resultant mould growth and aflatoxin contamination. To prevent an increase in aflatoxin contamination occurring during storage and transportation, it is important to control the moisture content, the temperature in the environment, and the hygienic conditions.[58] In peanuts which are particularly susceptible to Aspergilli infection the minimum moisture content for *A. flavus* growth is 8–10% at around 82% relative humidity, and aflatoxin production on peanuts is optimum at between 15–35% moisture content.[93] However, to improve stability and avoid Aspergilli contamination, the pistachios should be dehulled and dried to a moisture content of 5–7%, corresponding to an a_w of less than 0.70 at 25°C, as soon as possible after harvest, usually within 48 hours.[59]

It is well established that rapid crop drying may be useful in controlling aflatoxin contamination in storage and that in addition that crops containing different moisture values are not stored together.[24] It is also well established that mould invasion is facilitated as a result of increased moisture levels of stored commodities.[20,81] Moisture abuse can even occur in crops with very low moisture content such as those harvested in many Asian, and African countries as well as in European countries such as Turkey, as a result of intermittent rainfall and dewing.[24] An example of this is the pistachio nuts in Turkey which although sun-dried post harvest can occasionally become contaminated with aflatoxigenic as a result of storage in silos under high moisture conditions.[78] Another factor to bear in mind is the fact that if fungal growth does occur in storage, moisture will be released during metabolism, which will lead to the growth of other fungal species and to the production of mycotoxins such as OTA.[94]

Since mycotoxin-producing moulds are obligate aerobes, it seems likely that mycotoxin production could be prevented or at least reduced by modification of atmospheric gases in storage silos; such as by using carbon dioxide, nitrogen, carbon monoxide, and sulphur dioxide. Previous work on peanuts reported that increases in the concentration of CO_2 in storage silo resulted in significant reductions in aflatoxin production within stored peanuts.[22]

Apple Products. Conditions of apple storage are important with respect to mould growth. Rapid cooling and maintenance of store atmosphere conditions is known to improve the condition of the fruit. Ideally fruit should be loaded and cooled to less than 5°C in 3–4 days and to optimum temperatures within a further period of 2 days. In addition, controlled atmosphere conditions have been reported to reduce mould growth in apples. Controlled atmosphere conditions may be achieved within 7–10 days from the start of loading, and ultra-low O_2 regimes (i.e. less than 1.8% O_2).[73] For example, *Botrytis cinerea* growth on stored

apples has been shown to be retarded by increasing CO_2 levels from 0 to 15%,[95] while growth of *Rhizopus stolonifer*, *B. cinerea* and *P. discolor* has been shown to be inhibited by a combined treatment with 80% O_2 and 20% CO_2.[96] Alternatives, to room storage are the use of packaging materials such as polyethylene, which through atmospheric control, has been shown to reduce patulin production in apples.[97] Recent work by Jackson et al.,[80] has shown that patulin was not detected in cider from culled, tree-picked apples stored for 4 to 6 weeks at 0 to 2°C, but was detectable at levels between 0.97 and 64 $\mu g\ l^{-1}$ in stored, tree-picked, unculled fruit.

The use of controlled atmospheric storage has also been recommended in the manufacturing of various products. A precedent for this can be seen in mould spoilage of cheese where Taniwaki et al.[98] showed that *Mucor plumbeus*, *F. oxysporum*, *Byssochlamys fulva*, *B. nivea*, *P. commune*, *P. roqueforti*, *A. flavus*, and *Eurotium chevalieri* growth could be reduced in atmospheres containing 20–40% CO_2 with 1–5% O_2 and less AFB_1, AFB_2, roquefortine C, and cyclopiazonic acid (CPA) occurring in cheese, compared with those stored in air.

The Use of Natural and Chemical Agents

Various natural and chemical agents are known to prevent both mycotoxigenic mould growth and mycotoxin formation. For example phosphine, a highly toxic gas used for the protection of cereals against insects and mould invasion, is effective at concentrations ranging from 1000 to 2000 ppm in inhibiting *A. flavus* and *A. parasiticus* growth and in preventing mycotoxin biosynthesis.[99,100] The effect of fungicides on mould growth and mycotoxin biosynthesis is affected by several factors such as chemical type, rate of application, crop type, fungal species, and storage conditions.[23] Other fungicides such as dichlorvos, landrin, malathion, and diazinon are also effective in inhibiting AFB_1 formation.[41,101] In addition the fungicide, iprodione (3-(3,5-dichlorophenyl)-N-(1-methyletthyl)-2,4-dioxo-1-imidazolidine carboxamide) can be used as an effective agent in agricultural commodities to prevent the growth of various fungal species such as *Alternaria*, *Aspergillus*, *Penicillium*, *Monilia*, *Botyrtis*, *Didymella*, *Rhizochtonia*, and *Sclerotinia*. In addition it is known that iproidine at a concentration of 5 $\mu g\ g^{-1}$ of medium can suppress the growth of *A. parasiticus*,[102] while a similar concentration of 5 μg/g of medium, decreases the production of AFB_1 and OTA by *A. flavus* NRRL 1290 and *A. ochraceus* NRRL 3174 by more than 50%, respectively.[103]

Herbicides treatments have also been shown to affect both the growth of mycotoxigenic fungi and subsequent mycotoxin production. Glufosinate-ammonium [butanoic acid, 2-amino-4-(hydroxymethylphosphiyl)-ammonium salt] (GA), has been shown to inhibit *A. flavus* growth and AFB_1 formation, with treatments of 2,000 μg GA per ml leading to reductions of 90% in AFB_1 being observed.[104] In addition synthetic pesticides have recently been reported to reduce levels of OTA contamination in experimental viticultures with 96.5% reductions being observed in samples treated with azoxystrobin and 88% reductions in samples treated with dinocap and penconazole.[105]

The ability of sorbic acid and its sodium and potassium salts, as well as sodium benzoate to inhibit aflatoxigenic mould growth and aflatoxin production, is well established,[106–109] while SO_2 has been reported to inhibit the growth of *Byssochlamys* species associated with patulin production.[110] Potassium sorbate (0.05 – 0.15%) has been reported to inhibit the growth of *P. patulum* and *P. roqueforti*, as well as patulin production,[111] while sodium benzoate at 0.4% results in more than a 25% reduction in AFB_1 produced by *A. parasiticus*.[107] Chourasia[112] has shown that the yield of mycelia and sclerotia, and aflatoxin production by *A. parasiticus* is inhibited by propionic and citric acid, while combined treatments of propionic acid (0.05–0.5%) and nisin (500–1000 ppm) have been shown to display fungistatic properties by significantly inhibiting the growth of *A. ochraceus* and *F. moniliforme* and in controlling the production of AFB_1 and AFG_1 by *A. parasiticus*.[113] In addition it has also been reported that ethyl or methyl benzoate (at 0.02%) completely inhibits both aflatoxin biosynthesis and growth of *A. flavus* by 78% and 61%, respectively,[108] while other compounds such as trifluoperazine, an anti-calmodulin agent (at 1mM) completely inhibits aflatoxin production in *A. parasiticus* NRRL 2999.[114]

Surfactant molecules which have both lipophilic and hydrophilic effects have been shown to inhibit aflatoxigenic mould growth and aflatoxin biosynthesis, with Triton X-100, Tergitol NP-7, Tergitol NP-10, polyoxyethylene 10 lauryl ether, and Latron AG-98, (at 1% w/v) reducing aflatoxin production by between 96 and 99%.[115] Heavy metal ions in the form of sodium selenite and potassium tellurite (0.05–4%) have also been shown to inhibit both growth and mycotoxin production in *A. parasiticus*, but this approach is unlikely to be used in foods or feeds due to the potential of heavy metal contamination.[116]

A more promising approach, and a possible alternative to fungicide treatment in the prevention of mycotoxin formation, particularly post-harvest; is the potential use of antagonistic bacteria, fungi, and yeast (Table 1). It is well established that antagonistic yeast can reduce the growth of spoilage moulds both *in vitro* and under simulated full-scale storage conditions.[117] *Pichia anomala* and *Saccharomyces cerevisiae* have been shown to reduce OTA accumulation *in vitro* in two isolates of *P. verrucosum*, while *Pichia anomala* can reduce OTA levels synthesized by *P. verrucosum* from 100 000 ng g^{-1} to <10 ng g^{-1} level in wheat at 25°C after 21 d.[118] In another study, Shantha[119] reported that several fungal cultures including a *Phoma* sp., *Mucor* sp., *Trichoderma harzianum*, *Trichoderma* sp. 639, *Rhizopus* sp. 663, *Rhizopus* sp. 710, *Rhizopus* sp. 668, *Alternaria* sp. and some strains belonging to the *Sporotrichum* group could inhibit AFB_1 biosynthesis by $\geq$90%. Interestingly Picco and co-workers have reported that *F. proliferatum* can inhibit AFB_1 production in *A. flavus*, when both fungi are co-cultured, under optimal growth conditions.[120] In addition, it has been reported that two antifungal compounds namely fusapyrone and deoxyfusapyrone isolated from rice cultures of *F. semitectum* exhibited toxic activity against some pathogenic and mycotoxigenic fungi

Table 1 Effect of antogonistic microorganisms on the prododuction of mycotoxins

Antagonistic micr.	Activity against to:	Effects observed	Reference
Lb. casei	*A. parasiticus* NRRL 2999	AFB_1 and AFG_1 production diminished by 36% and 23%, respectively	El-Gendy and Marth (1981)[124]
Streptococcus lactis ATCC 11454	*A. flavus* (V3734/10)	Significant reduction of the aflatoxin production	Coallier-Ascah and Idziak (1985)[125]
Lb. acidophilus	*A. parasiticus* NRRL 2999	Prevention of aflatoxin production	Karunaratne et al. (1990)[126]
Lb. delb. subsp. *bulgaricus*	*A. parasiticus* NRRL 2999	Prevention of aflatoxin production	Karunaratne et al. (1990)[126]
Lb. plantarum	*A. parasiticus* NRRL 2999	Prevention of aflatoxin production	Karunaratne et al. (1990)[126]
Pediococcus pentosaceus	*A. parasiticus* CBS 92170	Aflatoxin production completely prevented	Luchese et al. (1992)[127]
Lb. plantarum CH	*A. parasiticus* CBS 92170	Aflatoxin production completely prevented	Luchese et al. (1992)[127]
Lb. alimentarus CH33	*A. parasiticus* CBS 92170	Aflatoxin production completely prevented	Luchese et al. (1992)[127]
Lb. sake CH10	*A. parasiticus* CBS 92170	Aflatoxin production completely prevented	Luchese et al. (1992)[127]
Lactobacillus spp.	*A. flavus*	Prevention of aflatoxin production	Gourama and Bullerman (1995)[134]
Lactococcus spp.	*A. parasiticus*	Stimulation of aflatoxin production	Gourama and Bullerman (1995)[134]
Lb. casei CCM 1825	*P. citrinum*	73.2% reduction in citrinin production	Gourama (1997)[130]
Lb. casei CCM 1825	*P. expansum*	74.3% reduction in patulin production	Gourama (1997)[130]
Lb. casei pseudoplantarum	*A. parasiticus* NRRL 2999	Production of AFB_1 and AFG_1 decreased by 80 and 92%, respectively	Gourma and Bullerman (1997)[135]
Brevibacterium linens	*Aspergillus flavus*	Prevention of fungal growth and aflatoxin formation	Osman (2004)[145]
Pichia anomala	*P. verrucosum*	Decreased OTA production to non-detectable levels	Petersson et al. (1998)[118]
Saccharomyces cerevisiae	*P. verrucosum*	Decreased OTA production to non-detectable levels	Petersson et al. (1998)[118]
Bacillus pumilus	*A. parasiticus* NRRL 2999	98.4–99.9% reduction in aflatoxin formation	Munimbazi and Bullerman (1998)[123]
Fusarium proliferatum	*A. flavus*	Significantly reduction in aflatoxin formation	Picco et al. (1999)[120]
Pseudomonas fluorescens	*P. expansum*	Patulin production diminished by 44–70%	Florianowicz (2001)[143]

such as *Alternaria alternata*, *A. fumigatus*, *Penicillium* spp., *Phoma tracheiphila*, *Ascochyta rabiei*, *Cladosporium* spp., and *B. cinerea*.[121] Similarly, aflastatin isolated from *Streptomyces* sp. MRI 142 has been reported to completely inhibit aflatoxin production by *A. parasiticus* at the concentration of 0.5 μg ml^{-1}.[122]

Munimbazi and Bullerman[123] reported the inhibition of aflatoxin production by *A. parasiticus* by up to 98.4–99.9%, by extracellular metabolites of six *Bacillus pumilus* isolates, while in another study, Dock et al.[95] reported an effective antagonistic effect on the growth of *B. cinerea* growth by an *Erwinia* sp. in apples stored under modified atmospheres.

Alternatively, lactic acid bacteria or their antifungal metabolites have been studied as natural preservatives to inhibit mycotoxigenic mould growth and mycotoxin production in recent years.[124–133] Lactic acid bacteria are of special interest as preservation organisms since they have a long history of use in food and are GRAS "generally regarded as safe" organisms and as such any compounds isolated from lactococcal species, may be particularly useful in the preservation of foods from mould spoilage and mycotoxin contamination.

Even though in many cases the antifungal and antimycotoxigenic potential of lactic acid bacteria are still unknown, it is widely believed that inhibition of mycotoxin synthesis is due to microbial competition, the depletion of nutrients, low pH, and also due to the production of heat-stable low-molecular weight of metabolites which are produced by lactic acid bacteria.[129,134–137]

Earlier work reported antifungal activity from a *Lactobacillus* casei strain that inhibited both growth and aflatoxin production in *A. parasiticus*.[124] Later, Coallier-Ascah and Idziak[125] reported that aflatoxin production by *A. flavus* were significantly reduced in the presence of *Streptococcus lactis* culture, while an antifungal activity from a *Leuconostoc mesenteroides* strain was reported but no specific substance was identified.[138] *Lb. casei pseudoplantarum* 371, an isolate from a silage inoculant, has been reported to inhibit production of AFB_1 and AFG_1 by 80% and 92%, respectively.[135] In another study, Corsetti et al.[139] reported an antifungal activity from *Lb. sanfrancisco* CBI which was caused by the formation of short-chained fatty acids, including caproic acid which inhibited spoilage moulds from the genera *Monilia*, *Aspergillus*, *Penicillium*, and *Fusarium*. Other

antifungal compounds including benzoic acid, mevalonolactone, methylhydantoin, and cyclo-(glycyl-L-leucyl) which act synergistically with lactic acid to inhibit *F. avenacum*, have also been reported to be produced from *Lb. plantarum*;[140] while a *Lb. plantarum* silage isolate has been shown to produce a range of antifungal cyclic dipeptides.[141] In addition Rees[136] has reported the inhibition of aflatoxins by metabolites such as benzoic acid, cyclo (glycl,l-leucyl) methylhycantoin, and nevalonactone produced by specific lactic acid bacteria. Croci et al.[142] have also reported the ability of *Lb. casei* and *Lactococcus lactis* subsp. *lactis* to inhibit the production of aflatoxin by *A. parasiticus* NRRL 2999 in a synthetic medium. With respect to the inhibition of *Penicillium* species, cultures of *Lc. lactis* and *Lc. casei* as well as cultures of *Lc. cremoris* and *Lb. delbrueckii* subsp. *bulgaricus* have been reported to both decrease hyphae biomass, by between 25–97%, and to completely inhibit patulin synthesis in *P. expansum*.[143]

More recently Lb. coryniformis subsp. coryniformis Si3 has been reported to display a broad spectrum antifungal activity against A. fumigatus, *A. nidulans, P. roqueforti, Talaromyces flavus, Mucor hiemalis, F. graminearum, F. culmorum, F. sporotrichoides*, and *F. poae*.[144] This activity is associated with the production of a low molecular weight proteinaceous heat stable compound, which is similar to the antifungal/antiaflatoxigenic heat stable activity reported from Lb. casei supernatants.[130]

Natural plant extracts and spices are also known to prevent mould growth and mycotoxin production.[146,147,148,149,150,151] Specifically extracts from Egyptian plants namely, *Lupinus albus* (Leguminosae), *Ammi visnaga* (Umbelliferae), and *Xanthium pungens* (Compositae), have been shown to inhibit the growth of an aflatoxin of *A. flavus* in a dose-dependent manner.[152] Essential plant oils and their components have also been demonstrated to protect maize kernels from infection by *A. flavus*. Oils of *Ocimum basilicum* (basil), *Syzygium aromaticum* (clove), *Thymus vulgaris* (thyme), and *Cinnamomum zeylanicum* (cinnamon) among others are known to inhibit the development of *A. flavus* on maize kernels,[153] while in a similar study carried out by Juglal et al.[150] spice oils of eugenol, cinnamon, oregano, mace, nutmeg, tumeric, and aniseed displayed antifungal activity against *A. parasiticus* and *F. moniliforme*. The production of AFB_1, AFB_2, and AFG_2 by *A. parasiticus* was completely inhibited at 0.1 μl ml^{-1} of clove, with AFG_1 production reduced by 66%; while 78% reductions in fumonisin B_1 (FB_1) formation by *F. moniliforme*, when treated with 2 μl ml^{-1} clove oil, were also reported. In addition essential oils from 12 different medicinal plants, and in particular thyme and cinnamon have been shown to inhibit growth and mycotoxin production in *A. flavus*, *A. parasiticus*, *A. ochraceus*, and *F.moniliforme*.[154] Juglal and coworkers have suggested that the inhibitory effects exerted by spices and herbs may rely at least in part on phenolic compounds such as coumarins and flavonoids.[150]

There have been a number of reports citing the inhibitory effects of onion extracts on *A. flavus* growth, with an ether extract of onions, thio-propanol-S-oxide, being demonstrated to inhibit growth. In addition Fan and Chen[155] reported that welsh onion ethanol extracts depressed the mycelial growth and aflatoxin production of some strains of aflatoxin-producing fungi. Black and red pepper extracts have been shown to reduce aflatoxin production in *A. parasiticus* IFO 30179 and *A. flavus* var *columnaris* S46.[156] In addition patulin formation in *P. expansum* can be inhibited by lemon and orange oils if used in sufficient concentration, while at concentrations of (0.05–2.0%) more than a 90% reduction in aflatoxin formation by *A. flavus* has been demonstrated.[157] The essential oil of lemongrass at concentrations >0.2 mg ml^{-1}, has a similar effect on aflatoxin formation in the fungus.[151] In another extensively study, Selvi et al.[158] demonstrated that *A. flavus* growth and AFB_1 production were both inhibited by an extract, containing mainly garcinol; from the tropical shrub/tree *Garcinia indica* at 3000 ppm. With respect to OTA production, spice essential oils of oregano (*Origanum vulgare*), mint (*Menta arvensis*), basil (*Ocimum basilicum*), sage (*Salvia officinalis*), and coriander (*Coriandrum sativum*) have been shown to be effective against ochratoxin-producing fungi, with oregano and mint oils completely inhibiting the growth of *A. ochraceus* NRRL 3174 and OTA production after 21 days at the concentrations of 1000 ppm.[148]

Irradiation

Radiation is typically categorized as either ionizing (IR) or non-ionizing (NIR), with IR involving X-rays, and gamma (γ) rays and NIR involving UV rays, microwaves, infrared rays, and radio waves. Despite much public debate on the safety of irradiated foods it is becoming more frequently used in the sterilization of a wide variety of foods.[159] In 1980 the FAO/IAEA/WHO Expert Committee on food irradiation (JECFI) indicated that irradiation of any food commodities up to an average dose of 10 kGy causes no toxicological hazards and no special microbiological or nutritional problems.[160] Thus in relation to mycotoxin prevention irradiation has been used to inhibit mycotoxin biosynthesis during storage period, and many studies have been conducted to access the use of γ-irradiation in particular to prevent mould growth and mycotoxin formation.[161,162]

Refai et al.[163] have reported both the complete inhibition of growth by *A. ochraceus* and subsequent ochratoxin production in poultry feed concentrates at a radiation dose of 4 kGy and confirmed an earlier report on the inhibition pof OTA production at dose levels from 2 to 5 kGy.[164] While in another study, Aziz et al.[162] reported that a dose level of 3.0 kGy irradiation was sufficient to inhibit considered as a sufficient dose for inhibition of AFB_1 production in *A. flavus*, following 45 days of storage. However at lower dose levels such as 2 kGy up to 52.2 μg kg^{-1} AFB_1 was produced during the same storage period. This compared favorably however with AFB_1 levels of 1380.3 μg kg^{-1} present in non-irradiated controls.

Not all fungi however respond to irradiation in the same way as is evidenced by the report of Aziz and Smyk[165] where they

showed that exposure to near UV radiation induced the synthesis of both AFB_1 (200 ppm) and OTA (210 ppm), in non-toxigenic fungal strains of *A. flavus* EP-63 and *A. ochraceus* P-153 respectively and it is clear that the effectiveness of irradiation in the inhibition of mould growth and mycotoxin biosynthesis is both strain and dose dependant, as well as being influenced by humidity and storage conditions.[12,161,162]

DETOXIFICATION OF MYCOTOXINS

The prevention of mycotoxin contamination prior to harvest or during post-harvest and storage is not always possible necessitating decontamination before the use of such materials for food and feed purposes.[166] Therefore various detoxification processes play an important role in helping prevent exposure to the toxic and carcinogenic effect of mycotoxins.[167] Detoxification of mycotoxins is typically achieved by removal or elimination of the contaminated commodities or by inactivation of the toxins present in these commodities by physical, chemical, or biological methods.[168] On the other hand, detoxification of products by chemical treatments is not allowed in the European Union. The mixing of contaminated products with good quality products is also prohibited.[169]

According to the FAO any decontamination process to reduce the toxic and economic impact of mycotoxins needs the following requisites:[10,20,166,170,171]

- It must destroy, inactivate, or remove mycotoxins;
- It must not produce or leave toxic and/or carcinogenic/mutagenic residues in the final products or in food products obtained from animals fed decontaminated feed;
- It should not adversely affect desirable physical and sensory properties of the product;
- It must be capable of destroying fungal spores and mycelium in order to avoiding mycotoxin formation under favorable conditions;
- It has to be technically and economically feasible.

Removal from Contaminated Commodities

Physical Separation

Since detoxification of mycotoxins by chemical applications is not an acceptable practise in some regions, physical segregation of contaminated crops is a very important option for the producer.

Sorting. Since the majority of mycotoxin contamination usually occurs in a relatively small number of seeds or kernels then the segregation and sorting of damaged, discolored, crops containing visible mould growth can lead to the removal of significant quantities of mycotoxins from these crops.[21,22,171–173] The results from the various studies indicate that sorting for quality (discarding scalpers, stained floaters, and hand pick outs) removes a large part of the aflatoxin present at harvest time.[174–178]

Manual, mechanical, and electronic methods can all be used for the segregation of crops.[22,173] Manual selection is based on the fact that damaged kernels will vary in size, shape, and color and that obvious visible mould growth will be present on the affected kernels. For example, sorting which removes visibly mouldy apples reduces the level of mould to an acceptable level, and makes a major contribution towards achieving an acceptable level of patulin in the final product.[179] While manual selection is the simplest way for the physical removal of contaminated grains it is a time-consuming procedure and this in many cases limit its applicability.[166] However, a partial removal of aflatoxin can be achieved by fluorescence sorting of the maize, cotton seed, and dried figs, where contamination can be observed by fluorescence following illumination with UV light,[22,171,180] with a positive correlation being reported between the observation—bright greenish–yellow fluorescence under longwave (365 nm) UV light and the presence of aflatoxin in these commodities.[24,181]

Electronic sorting devices while useful in the elimination of contaminated seeds are unlikely to be used on a large scale industrially, due to economic factors. In any case problems have been encountered with this approach in the seperation of contaminated pecans, due to the inherent intense fluorescence of the kernels.[168] The recognition of dark stain present on the lips of the shells of "early splits" as a basis for machine sorting has been reported.[59] However, a combined electronic and hand-sorting regimen has been developed by the peanut industry in the United States to facilitate a reduction in aflatoxin levels in peanut products for human consumption.

Density Segregation. Another effective method for reducing mycotoxin levels is the use of flotation and density segregation which has been reported to decrease significantly the mycotoxin content of crops, particularly the aflatoxin levels in toxic maize kernels.[3,166,171,182] Mould-damaged, mycotoxin-contaminated kernels exhibit differing physical properties from non-damaged kernels and so may be separated by density segregation in certain liquids, or fractionation according to specific gravity tables. Density segregation and removal of kernels buoyant in water and saturated sodium chloride solution are known to reduce DON, ZEN, and aflatoxins levels in cereals. It has been also reported that gravity seperation can be very useful in reducing DON levels in grains. It has also been shown that the use of specific gravity tables which allow the removal of the least dense fractions containing the tombstone kernels can reduce DON contamination by 68 to 85% in wheat containing 4–7 mg kg^{-1} toxin.[183]

Washing. A simple method to reduce *Fusarium* mycotoxins such as DON, ZEN and fumonisins in grains or maize cultures has been proposed by Scott,[171] which involves washing procedures using water and sodium carbonate solution. Washing barley and maize three times with distilled water has been reported to reduce the DON level by 65–69%. Thus washing might be a useful treatment to use prior to wet milling or ethanol fermantation, otherwise the cost of drying grains remains prohibitive.[183]

Such approaches are known to be successful in the reduction of patulin levels in apples with reports indicating that patulin levels in apples can be reduced from 920 ng g^{-1} to 190 ng g^{-1} following an initial water treatment, with appreciable levels of patulin being transferred from the solid to the aqueous phase.[184] These researchers also reported that the removal of rotten and damaged fruit prior to processing by hand resulted in marked reductions in patulin levels from 2335 to 55 ng g^{-1}. These finding were subsequently supported by Acar et al.[185] who reported that washing and handling of apples to remove external surface dirt and topical chemical residues before processing was a critical step in the removal of patulin from apples, with average reductions in patulin levels of 54% being observed during the washing and handling stages.

Milling. It is possible that the removal of certain grain components during milling can result in a reduction in toxin levels in contaminated grain. In commercial dry milling, fumonisins are found in flaking grits, flour, germ, and bran. The pattern of distribution after experimental dry milling varies slightly for different types of maize, but in general the levels are lower in grits and higher in germ, bran, and fines. The distribution of DON in the various milling fractions of wheat largely depends on the degree of fungal penetration of the endosperm, therefore milling grain having surface contamination results in flour with low mycotoxins levels.

In standard milling practice 66% reduction of OTA can be obtained in the production of white flour from hard wheat contaminated 618 μg kg^{-1} OTA after inoculation with *P. verrucosum*. On the other hand, a 40% reduction can be obtained for soft wheat.[183]

Extraction with Solvents

A number of solvents are capable of extracting mycotoxins from contaminated food materials such as oilseed peanut and cottonseed.[20,21] The most commonly used solvents include 95% ethanol, 90% aqueous acetone, aqueous isopropanol, 80% isopropanol, hexane-methanol, methanol-water, acetonitrile-water, hexane-ethanol-water, and acetone-hexane-water.[20,171] While solvent extraction can effectively remove aflatoxin from oilseed meals without either the formation of toxic byproducts or any reduction in nutritional properties or quality, the large-scale application of this technique, however, is limited by high cost and problems related to disposal of the toxic extracts.[20] Even though some polar solvents like methanol and ethanol are effective in the reduction of aflatoxins from contaminated commodities, there are problems with the additional co-extraction of solids from these materials. For example it has been reported that extraction with 80% isopropanol completely removed aflatoxins in cottonseed and peanut meal, but it also removed between 8.7% and 9.5% in meal solids.[186]

Adsorption

Some adsorbents can bind and remove mycotoxins from aqueous solutions.[20,171,187] Two extensively suggested mycotoxin binding agents are active charcoal and bentonite. The particle size of adsorbent and heat treatment have been reported to affect the degradation level of mycotoxins.[188]

Activated charcoal, at levels of 3–5 g l^{-1} applied for 5 minutes have been shown to considerably decrease patulin levels in apple juice.[189] Similarly Huebner et al.[190] have reported that a composite carbon adsorbent in a fixed-bed adsorption system effectively reduced patulin levels in apple juice, although the appearance and task of the juice was affected, while Karadeniz and Ekşi[173] have reported the complete removal of 30 μg ml^{-1} of patulin from apple wine by active charcoal. In a similar study the patulin content of apple juice was decreased from 62.2 μg l^{-1} to 30.8 μg l^{-1} by the treatment with 3% activated charcoal for 5 min.[187]

In addition, bentonite has been shown to remove 65-79% of AFM_1 from milk,[188] as well as up to nearly 100% of aflatoxins from liquid solution.[24] While in another study by Kane et al.[191] 99% of AFB_1 was removed from peanut oil by adding clays such as attapulgite, kaolin, and novasil at 0.5 g l^{-1}.

Inactivation in Contaminated Commodities

Physical Methods

Numerous physical strategies have been applied for mycotoxin decontamination. These include thermal inactivation and inactivation through irradiation.[168,171]

Heat-Treatment. Most mycotoxins are relatively heat-stable within the range of conventional food processing temperatures (80–121°C), so little or no destruction occurs under normal cooking conditions such as boiling and frying, or even following pasteurization.[52,192,193] The sensitivity of mycotoxins to heat treatment is affected by many factors including the moisture content, pH, and ionic strength of food.[20,167,188,194] Roasting or frying for periods of up to 30 minutes at temperatures of between 150–200°C can result in residual levels of between 20–60%.[52] Degradation by heat treatment depends on the type of mycotoxin and its concentration, the extent of binding between the mycotoxin and the food constituents, the degree of heat penetration, as well as the heating temperature and the processing time.[20] Destruction of mycotoxins in foods by different heat treatments is summarized in Table 2.

Aflatoxins have decomposition temperatures ranging from 237°C to 306°C. Solid AFB_1 is quite stable to dry heat up to its thermal decomposition temperature of 267°C.[20,167] It has been observed that increasing the moisture content of the food results in an enhanced degradation of aflatoxins.[22] For example, 74.8% of aflatoxins (B_1+B_2) were degraded by heating at 100°C for 1 h at a moisture content of 30% in contaminated cottonseed meal, while 32.7% of the degradation occured in a similar meal containing 6.6% moisture, under similar conditions. It has been suggested that the presence of moisture in foods helps in opening the lactone ring in AFB_1 to form a terminal carboxylic acid; which then undergoes a heat-induced decarboxylation.[20]

Table 2 Destruction of mycotoxins in foods by different heat treatment

Treatment	Food	Mycotoxin	Initial level ($\mu g g^{-1}$)	Destruction(%)	Reference
Heating: 90°C, 20 min	Apple juice	Patulin	0.22	18.8	Kadakal and Nas (2003)[198]
Heating: 100°C, 20 min	Apple juice	Patulin	0.22	26.0	Kadakal and Nas (2003)[198]
Heating: 100°C, 30 min	Buffer solution	DON	2	Negligible	Wolf and Bullerman (1998)[200]
Heating: 60°C, 60 min	Milk	CPA	1	9	Prasongsidh et al. (1998)[202]
Heating: 80°C, 60 min	Milk	CPA	1	18	Prasongsidh et al. (1998)[202]
Heating: 100°C, 60 min	Milk	CPA	1	30	Prasongsidh et al. (1998)[202]
Roasting: 150°C, 30 min	Peanuts	AFB_1	1	38	Ozkarslı (2003)[196]
Roasting: 150°C, 30 min, 2% NaCl	Peanuts	AFB_1	1	41.5	Ozkarslı (2003)[196]
Roasting: 150°C, 30 min, 5% NaCl	Peanuts	AFB_1	1	47.6	Ozkarslı (2003)[196]
Microwave roasting: 0.7 kw, 8.5 min	Peanuts	AFB_1	1	48–61	Pluyer et al. (1987)[195]
Microwave roasting: 0.9 kw, 1.5 min	Peanuts	AFB_1	1	50.3	Ozkarslı (2003)[196]
Microwave roasting: 0.9 kw, 1.5 min, 2% NaCl	Peanuts	AFB_1	1	30.5	Ozkarslı (2003)[196]
Microwave roasting: 0.9 kw, 1.5 min, 5% NaCl	Peanuts	AFB_1	1	38.6	Ozkarslı (2003)[196]
Evoparation: 70°C, 20 min	Apple Juice	Patulin	0.22	9.5	Kadakal and Nas (2003)[198]
Evoparation: 80°C, 20 min	Apple Juice	Patulin	0.22	14.1	Kadakal and Nas (2003)[198]
Extrusion: 120°C, 18% moisture	Corn Grits	ZEN	4.4	83	Ryu et al. (1999)[204]
Extrusion: 140°C, 18% moisture	Corn Grits	ZEN	4.4	83	Ryu et al. (1999)[204]
Extrusion: 160°C, 18% moisture	Corn Grits	ZEN	4.4	77	Ryu et al. (1999)[204]
Extrusion: 180°C, 15% moisture	Corn Flour	AFB_1	0.05	25	Cazzaniga et al. (2001)[9]
Extrusion: 180°C, 15% moisture	Corn Flour	DON	5	98	Cazzaniga et al. (2001)[9]

Pluyer et al.[195] showed that roasting of peanuts at 150°C for 30 min caused a 30% reduction in aflatoxin levels, while a 60% reduction in aflatoxin levels have been reported up roasting at 150°C for 90 min.[193] In another study, Ozkarslı[196] achieved 38%, 41.5%, and 47.6% degradation of AFB_1 in unsalted, and 2% and 5% salted peanuts respectively, by traditional roasting at 150°C for 30 min.

Patulin has a melting point of 111°C[197] and it has been reported that approximately 90% of patulin can be degraded by heat treatment at 105°C for 29 h.[188] In addition, Kadakal and Nas[198] have demonstrated that heat treatments of 90 and 100°C result in patulin degradation levels of 18.8% and 26%, respectively. It should be noted however that patulin is more heat stable under acidic conditions.[184,185]

ZEN is quite heat resistant surviving temperatures of 120°C for 4 hours,[52] although reductions in ZEN levels have been reported in aqueous buffered solutions at different pH values, with complete removal of ZEN being observed in less than 30 min at 225°C.[199] Wolf and Bullerman[200] have also reported differences in the effects of heat treatments at differing pH values on mycotoxin degradation. In this case DON levels were unaffected by heat treatments of 100–120°C at pH 4.0 and 7.0, but at pH 10.0 heat treatments of 120°C for 30 min or 170°C for 15 min resulted in the complete degradation of DON. A similar phenomenon has also been observed for moniliformin (MON) where the percentage of MON reduction was correlated positively with increasing temperature and pH, with heating at pH 10 causing major reduction i.e. 99% degradation at 175°C after 60 min.[201]

In another study, Prasongsidh et al.[202] reported that heating of milk contaminated with 1 μg cyclopiazonic acid (CPA) ml^{-1} for 24 h at 60, 80 and 100°C resulted in only 9–17%, 20–34% and 49–50% reductions in the CPA content of milk, respectively. In similar work, 87–100% degradation of fumonisin in maize and maize products was achieved by heat treatment at 150–200°C,[171] while roasting corn meal samples at 218°C for 15 min resulted in the complete removal of FB_1 in contaminated maize meal samples.[203]

Extrusion cooking has been reported as being a useful technique in the destruction of some naturally occuring food toxins. For example extrusion cooking of the corn grits resulted in reductions in ZEN ranging from 77 to 83%, 74 to 83% and 66 to 77% at 120°C, 140°C and 160°C, respectively.[204] Similarly, Cazzaniga et al.[9] reported a greater than 95% reduction in DON in maize flour following extrusion cooking (140–200°C).

Heat supplied in the form of high energy microwaves can also destroy mycotoxins.[167,171] For example, Pluyer et al.[195] have reported reductions in the levels of contamination in peanuts, in the range of 50–60% and 32–40% for AFB_1 and AFG_1 respectively; after microwave roasting at 0.7 kw for 8.5 min. In another report, Das and Mishra[205] reported that an enzyme treatment of peanuts meal followed by microwave treatment at 1 kw for 15 min increased the degradation level of AFB_1 from 53% to 97% without affecting the organoleptic qualities of the meal, while microwave roasting at 900 watt for 1.5 min has been shown to reduce AFB_1 levels by 50.3%, 30.5%, and 38.5% in unsalted, 2% and 5% salted peanuts, respectively.[196]

In summary the use of elevated temperatures to detoxify contaminated commodities is largely hindered by the impairment of both the nutritional and organoleptic properties of the foods, coupled with doubts regarding the potential generation of toxic pyrolysate at elevated temperatures, however treatments providing high temperatures and pressures such as extrusion cooking may prove useful in future decontamination regimes.

Ionizing Radiation. As previously mentioned radiation has proven useful in the post harvest control of mycotoxins in storage, in addition however ionizing involving x-rays and γ rays have been extensively investigated as methods for the

degradation of mycotoxins.[171] Irradiation is a non-thermal treatment and is increasingly being referred to as "cold pasteurization" given that it can eliminate food borne pathogens without increasing in product temperature,[160]and that overall average doses of 10 kGy presents no toxicological hazard.[12,160]

The incativation of mycotoxins by γ-radiation is dependent on the degree of the radiation dose, and by the type of food and mycotoxin.[167] In addition water plays a critical role in the destruction of aflatoxin by γ-radiation, since radiolysis of water initiates free radical reactions leading to aflatoxin degradation.[20,167] 75% and 100% reductions in the toxicity of peanuts meal containing AFB_1 have been reported following γ-radiation at a dose of 1 and 10 kGy, respectively,[20] while Refai et al.[206] have reported that a dose of 5 kGy totally degraded the aflatoxin found in basterma samples.

Aflatoxins are also sensitivity to UV radiation at 222, 265, and 362 nm, with the greatest absorption occurring at 362 nm.[20,167] This has proven useful in the removal of AFM_1-from milk, with UV radiation at 365 nm for 20 min destroying 56.2% of AFM_1-contaminated milk (1ppb)[207] and in dried figs; with UV treatment for 30 min resulting in 45.7% of AFB_1 present being degraded.[208]In addition peanut oil treated with a UV light for 2 hours resulted in 40–45% destruction of the aflatoxins present.[209]

Alternatively, solar energy can also be used for the destruction of mycotoxins, with the UV rays present in the sunlight playing an important role.[20,167,171,208,210,211] For example it has been reported that approximately 70% of AFB_1 in artificially contaminated coconut oil was destroyed by solar energy.[210] Additionally, Kane et al.[191] showed that AFB_1 (at levels of 600 ppb) was fully removed from peanut oil in transparent glass and translucent plastic containers after 18–24 h of effective exposure to sunlight.

Chemical Methods

A wide range of chemicals have been shown to reduce, destroy, or inactivate mycotoxins.[167,168,170,171] These chemicals include (a) acids (e.g. hidrochloric acid), (b) bases (e.g. ammonia, sodium hyroxide), (c) oxidizing agents (e.g. hydrogen peroxide, ozone), (d) reducing agents (e.g. bisulfite), (e) chlorinating agents (e.g. sodium hypochlorite, chlorine dioxide and gaseous chlorine), and (f) miscellaneous reagents such as formaldehyde.[167,171]

Acid-Treatment. Acid treatment of the aflatoxins, AFB_1, and AFG_1 results in their conversion to the hemiacetal forms AFB_{2a} and AFG_{2a}, respectively through incorporation of water,[167,188,212] while treatment with HCl (pH, 2) is reported to reduce AFB_1 levels by 19.3% within 24 h.[188] In another report, Tabata et al.[212] revealed that AFB_1 and AFG_1 were completely degraded after treatment with a 1% solution of HCl or H_2SO_4, with 13% and 18% reductions in AFB_2 and AFG_2 levels being observed respectively when compared to control samples.

Treatment with Bases. Inorganic and organic bases are efficient and relatively inexpensive chemicals with which to detoxify aflatoxins and other important mycotoxins from large quantities of contaminated agricultural products.[22] Among these chemical agents, ammoniation is an approved procedure for the detoxification of aflatoxin-contaminated agricultural commodities and feeds.[188,213–218] It is the method of choice in some North American States (Arizona, California, Texas, Georgia, Alabama); and in France and Senegal for detoxifying aflatoxins in contaminated peanut, cotton, and maize meals.[170,171,216] Ammoniated peanut meal is routinely imported by several European Union member countries. In contrast, the US Food and Drug Administration (FDA) does not as yet permit interstate shipment of ammoniated cottonseed or maize.[171]

The ammoniation process uses ammonium hydroxide or gaseous ammonia, both of which are equally effective in detoxifying aflatoxins in peanut, cotton and maize meals.[167,170] The success of aflatoxin detoxification by ammonia treatment is governed by the quantity used, the reaction time, as well as the temperature and pressure levels employed.[170] High pressure treatments are more effective in aflatoxin detoxification than treatments under atmospheric pressure, with the additional benefit that ammoniation under high pressure requires lower levels of ammonia with less processing time. The level of destruction from high pressure ammoniation can be increased by increasing the temperature with destruction of AFB_1 by 2% ammonia under atmospheric pressure/ambient temperature for 24 h was 88%, being reported; while AFB_1 in contaminated grain was inactivated by more than 99% by a high temperature/high pressure (121°C/2 bar) ammoniation procedure.[216] Indeed a high temperature/high pressure ammoniation procedure (80–120°C/35–50 psi) has been commercially used to detoxify aflatoxin from cottonseed in Arizona, Texas, and California and from cottonseed meal in Arizona.[171]

AFB_1 degradation by ammonia occurs as a result of hydrolysis of the lactone ring followed by decarboxylation to two major non-toxic compounds namely AFD_1 and loss of the cyclopentenone ring to give a compound of molecular weight 206[167,170,171,213] AFD_1 appears much less toxic than the parent AFB_1 molecule in the range of 130–20 000.[170]

Other mycotoxin such as FB_1have also been shown to be degraded following ammoniation with a high pressure (60 psi)/ ambient temperature or atmospheric pressure/high temperature (125°C) ammonia treatment resulting in 79% of FB_1 destruction in contaminated maize.[171] Similarly, Norred et al.[219] reported a 30 to 45% destruction of FB_1 by ammoniation in contaminated maize.

The effect of alkaline agents such as sodium, potassium, or calcium hydroxides on the destruction of mycotoxins is slightly less than ammonia treatment.[170] The destruction order of different alkali on AFB_1 in solution at 110°C is potassium hyroxide > sodium hydroxide > potassium carbonate > sodium carbonate > potassium bicarbonate > ammonium hydroxide > sodium bicarbonate > ammonium carbonate.[167] The treatment of peanut meal (containing 30% moisture) with a 2% sodium hyroxide at 100°C for 120 min reduced AFB_1 to trace quantities.[22]

Oxidizing Agents. Ozone or triatomic oxygen (O_3) is a powerful oxidant and reacts across numerous chemical groups, although it is particularly effective with olefinic double bonds.[220] Several oxidizing agents have successfully been used to detoxify mycotoxins from contaminated agricultural products. Treatment of cottonseed and peanut meals with aqueous O_3 has been shown to destroy aflatoxins.[221] It is also known that, the C=C unsaturated double bond of the terminal furan ring of AFB_1, AFG_1, and AFM_1 is susceptible to O_3 and other oxidizing agents. However, aflatoxins which lack a double bond in the terminal furan ring such as AFB_2, AFG_2, and AFM_2 are resistant to oxidation by ozone.[22,75]

Several studies have indicated that the O_3 treatment reduces AFB_1 levels by 91% in cotton-seed meal containing 22% moisture after heat treatment at 100°C for 2 hours.[22] In another report, McKenzie et al.[220] showed that AFB_1 and AFG_1 were rapidly degraded using 2% O_3, while AFB_2 and AFG_2 were more resistant to oxidation and higher levels of O_3 (20%) were required for rapid degradation, and treatment with 10 weight % O_3 for 15 seconds resulted in the levels of CPA, FB_1, OTA, patulin, and ZEN present being reduced to undetectable levels. In addition, O_3 has been reported to reduce the mutagenicity of AFB_1.[222]

Hydrogen peroxide has been used on a commercial scale to detoxify aflatoxins, ZEN, and DON,[223] and the treatment of dried figs with H_2O_2 and sodium bisulfite, resulted in decreases in AFB_1 of 65.5% following during 72 h storage.[208]

Reducing Agents. Sodium bisulfite, is commonly used as a food and drink additive due to its enzymatic degradation inhibitor, antioxidant, and bacteriostatic properties. In addition it can be used to detoxify mycotoxins, and in particular AFB_1 and AFG_1.[21,167,170,171,224] The main reaction product following exposure to AFB_1 is a sulfonate, also called AFB_1-S, which forms as a result of the insertion of $NaHSO_3$ to the double bond present in the terminal furan ring.[167,170,171] This reduces the mutagenic potential of the AFB_1 molecule. Dried fig fruit spiked with 250 ppb AFB_1 which was treated with 1% sodium bisulfite resulted in the degradation of 28.2% of the AFB_1.[208] Similarly, sodium bisulfite treatments at 0.5% and 2% destroyed 80% and 90% of AFB_1, respectively in maize.[188] It has also been reported that 200 ppm of sodium sorbate treatment resulted in 68% of AF ($B_1 + B_2 + G_1 + G_2$) destruction in dried figs contaminated with 100 ppm AFB_1. In addition, the using of sorbic acid in combination with physical treatments such as heat and UV radiation potentiates its degradative ability. For example it has been reported that the levels of AF ($B_1 + B_2 + G_1 + G_2$) treated with SO_2 (2000 ppm)-heat (65°C), SO_2-UV radiation, and SO_2-heat-H_2O_2 (0.2%) decreased by 79%, 81% and 95%, respectively.[225]

Sodium bisulfite has also been shown to destroy DON in maize and patulin in apple juice,[24,171] with 96.3% degradation of patulin in apple juice being reported.[226]

Chlorinating Agents. Aqueous chlorine is commonly used in the food industry to sanitize food processing equipment and to wash a variety of raw materials such as fruits, nut meats, fish, frog, and meat prior to processing.[167] In addition chlorinating agents such as chlorine and sodium hypochlorite have also been reported to destroy mycotoxins;[167,210,227,228] with chlorine at concentrations of 11 mg gas per gram of copra meal spiked with AFB_1 resulting in more than 75% of destruction of the mycotoxin AFB_1.[229] In addition, it has been reported that the application of gaseous chlorine at 10% degraded more than 90% of aflatoxins in peanut meals.[210] With respect to other mycotoxins, Yazıcı and Velioğlu[228] have shown that applications of thiamine hydrochloride and pyridoxine hydrochloride resulted in the reduction of patulin in apple juice by 100% at the end of a 6 months storage period at 22 ± 2°C.

Other Treatments. A variety of other chemicals such as formaldehyde, potassium permanganate, sodium borate, and 75% methanol have been shown to be effective in the detoxification of several mycotoxins,[167,170] with 0.5% formaldehyde in particular reducing AFM_1 levels from 1.1 μg to 0.05 μg in milk samples.[170] However, the use of many of these agents in food and feed is restricted by safety problems that may induce to form toxic residues.[167]

Thus while many of the chemical treatments outlined above may destroy mycotoxins present in many foods and feeds, in many cases they significantly decrease the nutritional value of the foods or produce toxic products or other products with undesirable effects; thus limiting their widespread use.

Biological Methods

As outlined above, the use of many of the available physical and chemical methods for the detoxification of agriculture products contaminated with mycotoxins is restricted due to problems concerning safety issues, possible losses in the nutritional quality of treated commodities, coupled with limited efficacy and cost implications.[166] This has led to the search for alternative strategies such as biological agents. Advances in this area have been aided by recent advances in molecular biology, genetic engineering and microbial genomics coupled with the discovery of the very broad catabolic potential present within the microbial world.[131,166,230]

A number of different fungal cultures have been shown to detoxify AFB_1. Fungal strains such as *Trichoderma* sp. 639, *Phoma* sp., *Rhizopus* sp. 668, *Rhizopus* sp. 720, *Sporotrichum* sp. ADA, *Sporotrichum* sp. SF, and *Alternaria* sp. have been shown to degrade AFB_1 to levels between 65% and 99% in 5 days at 28 ± 2°C.[119] Similarly, Varga et al.[231] reported the ability of various *Aspergillus* strains such as *A. fumigatus*, *A. japonicus*, and *A. niger* to degrade OTA in liquid YES media, with *A. niger* degrading OTA to ochratoxin-α and phenylalanine in solid media and liquid cultures. Additionally, *Acinetobacter calcoaceticus* has been shown to degrade 100% of OTA in ethanol minimal salts medium at an initial OTA concentration of 10 μg ml^{-1} at 30°C within 120 h.[232]

Various fermentation processes have also been shown to result in reducing the toxic effects of mycotoxins. For example maize contaminated with F_2 toxin when used as a substrate for a *Candida intermedia*, fermentation resulted in a 10-fold reduction in toxin activity.[233] In traditional beer fermentations 49%

of the destruction of ZEN has been reported while in 69% of ZEN present was converted to the less toxic form β-zearalenol, during fermentation by *S. cerevisieae*.[234] Given the low levels of aflatoxin and α or β-zearalenol were not found in Canadian and European beers, together with quite low levels of OTA, it has been suggested that mycotoxins present in agricutural products may be removed by ethanol fermentation.[166,234] Similarly, patulin in apple juice and OTA in barley have been reported to be destroyed by ethanol fermentation.[230] Alternatively however, it is a well-established fact that OTA has been found in a wide variety of wines, indicating that it does in fact survive the fermentation process.[235,236]

Some strains of *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* have been shown to degrade OTA and AFB_1 in milk by fermentation.[10,230] Similarly, it has been reported that, 90–97% of AFM_1 was destroyed during the fermentation of milk containing 600–1400 μg/kg AFB_1.[210]

There have been many reports on the ability of lactic acid bacteria to inhibit the mutagenic potential of mycotoxins.[237,238] For example *Lactobacillus* sp LA2 has been shown to inhibit mutagenic potential of AFB_1, AFB_2 and AFG_1 in the range of 56.6-77.4%.[238] Similarly, Park and Rhee[239] highligted that *Lb. plantarum* KLAB21 isolated from the Korean traditional fermented vegetable, *kimchi*, possess a strong antimutagenic activity against AFB_1. The antimutagenic potential of *Lb. plantarum* KLAB21 against AFB_1 were found to be 82.5% on *Salmonella typhimurium* TA 100 and 78.2% on *S. typhimurium* TA 98.

Flavobacterium aurantiacum can significantly remove AFB_1 from a liquid medium and a variety of food products including milk, peanuts, and corn without leaving toxic by-products.[240–243] Ozkaya[244] observed that *F. aurantiacum* strain NRRL-B-184 removed 79.9–98.9%, 92.6–99.8%, and 88.7–100% AFB_1 from phosphate-buffered saline, in peanuts and red pepper, respectively within 48 h. In another study by D'Souza and Brackett,[245] the divalent cations Ca^{+2} and Mg^{+2} were shown to stimulate AFB_1 degradation by *F. aurantiacum*. However, the bright orange pigmentation of this microorganism limits its use in food or feed fermentations.[240]

THE INHIBITION OF MYCOTOXIN ABSORPTION IN THE GASTROINTESTINAL TRACT

One of the most recent approaches to the prevention of mycotoxicosis in livestock is the addition of the non-nutritionally adsorbents in the diet that bind mycotoxins in the gastrointestinal tract and capable of reducing their bioavailability.[10,246,247–250] The addition of adsorbents to feeds is the most widely applied way of protecting animals against mycotoxins.[183] Activated carbons (AC), hydrated sodium calcium aluminosilicate (HSCAS), zeolites, bentonites, and certain clays are the most studied adsorbent and they possess a high affinity for mycotoxins.[248,251,252] Aluminosilicates are the preferred adsorbents, followed by AC and special polymers, however, the adsorption efficiency depends on the chemical structure of both the adsorbent and the mycotoxin.[183] Additionally, a yeast cell wall derived esterified glucomannan have been tested for the adsorption of mycotoxins.[253] The cell walls polysaccharides (glucan and mannan), proteins, and lipids have been reported to exhibit numerous different adsorption mechanisms, e.g. hydrogen bonding, ionic, or hydrophobic interaction.[252]

The physical structure of the adsorbent including dose, the total charge and charge distribution, the size of the pores and surface area, as well as physico-chemical properties of the mycotoxins play a significant role in the achievement of mycotoxin-binding by adsorbent materials.[252,254] Mycotoxins that contain polar functional groups such as aflatoxins can be adsorbed by many effective adsorbents including certain clays e.g. montmorillonite and zeolite-clinoptolite. However, fairly non-polar mycotoxins such as ZEN and OTA are not effectively adsorbed on the hydrophilic negatively charged surfaces of these unmodified minerals.[254] Table 3 shows the results of some studies conducted *in vitro* for the detoxification of mycotoxins by different adsorbents.

AC are known as one of the most effective and nontoxic group of sorbents with a high surface to mass ratio (500–3500 m^2/g).[10,246,252] They are formed by pyrolysis of several organic compounds.[10,251,252] It was previously reported that hydrogen bonding playing a significant role in the binding mechanism for activated carbon.[255] They determined HSCAS and activated choarcoal binding was nearly 100% available in both water and simulated gastrointestinal fluid. In another report, Galvano et al.[256] determined the adsorption abilities of the AC ranging from 0.80 to 99.8% and 1.83 to 98.93% for OTA and DON, respectively. Similarly, Avantaggiato et al.[250] investigated the effectiveness of AC and cholorestyramine in reducing the intestinal absorption of ZEN. They observed that, the use of 0.25 to 2% AC or chlostyramine resulted in a reduction of ZEN absorption of 43 to 84% or 19 to 52%, respectively.

HSCAS from natural zeolite has been the most extensively investigated adsorbent because of possessing high affinity for aflatoxins.[10,215,247,251] Several *in vivo* and *in vitro* experiments have shown that HSCAS is the most effective adsorbent for use against aflatoxins. In a very extensive study, Edrington et al.[257] observed a significant reduction in carry-over of AFM_1 in Turkey poults fed 0.75 mg AFB_1 kg^{-1} body weight along with 0.5% AC or 0.5% HSCAS (Table 4). Additionally, Galvano et al.[246] performed to confirm the ability of AC and HSCAS to reduce the carry-over of AFB_1 from feed to milk and whether could be used *in vivo* conditions. They observed 50% and 36% carry-over reduction by AC and HSCAS, respectively. Similarly, it has been reported that the use of 0.5% HSCAS resulted in a significant reduction in carry-over of AFM_1 in urine samples.[258]

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline-hearth cations characterized by infinite three-dimensional structure.[10,251] When compared with zeolite, HSCAS showed much higher adsorption abilities toward aflatoxins. When HSCAS or zeolite were added in a

Table 3 *In vitro* adsorption of mycotoxins by different adsorbents

Adsorbent	Mycotoxin	Adsorption Index (%)	Reference
Activated carbon	AFB_1	>99	Diaz et al. (2002)[253]
Activated carbon	AFB_1	>90	Lemke et al. (2001)[255]
Activated carbon	OTA	0.8-99.8	Galvano et al. (1998)[256]
Activated carbon	DON	1.8-98.9	Galvano et al. (1998)[256]
HSCAS	AFB_1	>90	Lemke et al. (2001)[255]
HSCAS	OTA	13.2	Galvano et al. (1998)[256]
HSCAS	DON	3.9	Galvano et al. (1998)[256]
Zeolite	AFB_1	99	Tomašević-Čanović et al. (2003)[254]
Zeolite	ZEN	5	Tomašević-Čanović et al. (2003)[254]
Zeolite	OTA	40	Tomašević-Čanović et al. (2003)[254]
Organozeolite	OTA	41-52	Daković et al. (2003)[261]
Sepiolite	OTA	10.5	Galvano et al. (1998)[256]
Sepiolite	DON	4.5	Galvano et al. (1998)[256]
Clinoptilolite	AFB_1	6	Lemke et al. (2001)[255]
Na-bentonite	AFB_1	95-98.5	Diaz et al. (2002)[253]
Ca-bentonite	AFB_1	98.5	Diaz et al. (2002)[253]
Esterified glucomannan	AFB_1	96.6	Diaz et al. (2002)[253]

concentration of 0.5% to rat diets containing 2 mg AF kg^{-1} body weight, the excretion of AFM_1 in urine of rats were significantly decreased.[259] In similar work, Oğuz et al.[260] observed that the using of clinoptilolite, a natural zeolite at 1.5% and 2.5% concentration to broiler chickens diet containing 2.5 mg kg^{-1} aflatoxins were effective in the avoiding of aflatoxicosis. Regarding mycotoxins other than aflatoxins, Dakovié et al.[261] indicated that the degree of hydrophobicity plays a role in OTA adsorption on organo-zeolites. They observed that the toxic effects of OTA in the gastrointestinal tract can be prevented by the use of organo-zeolite in animal feed.

Alternatively, bentonites are commonly used in the adsorption of mycotoxins.[10,251] It has been extensively used in the clarification of beverages and decoloration of oils.[251] The adsorption ability of bentonites mainly depends on the interchangeable cations (Na^+, K^+, Ca^{++}, and Mg^{++}) present in the layers.[10]

Ellis et al.[262] demonstrated that the addition of 2% bentonite to the feed of growing fish contaminated with 20 μg AFB_1 kg^{-1}

Table 4 *In vivo* adsorption of mycotoxins by different adsorbents

Adsorbent	Concentration (%)	Mycotoxin	Effects observed	Reference
Activated carbon	0.5	AFB_1	Reduced excretion of AFM_1 in turkey poults, no protective effects against aflatoxicosis	Edrington et al. (1996)[257]
Activated carbon	1	AFB_1	76% reduction in AFM_1 concentration in milk of lactating goats	Nageswaro Rao and Chopra (2001)[248]
Activated carbon	2	AFB_1	Carry-over reduction of AFM_1 in milk of lactating cows diminished by 50%	Galvano et al. (1998)[256]
HSCAS	0.5	AFB_1	Significant reduction of urinary excretion of AFM_1 in rats	Sarr et al. (1995)[258]
HSCAS	0.5	AFB_1	Significant decrease of urinary excretion of AFM_1 in turkey poults	Edrington et al. (1996)[257]
HSCAS	0.5	AFB_1	Reduced excretion of AFM_1 in urine of rats	Abdel-Wahhab and Nada (1998)[259]
HSCAS	2	AFB_1	Carry-over reduction of AFM_1 in milk of lactating cows diminished by 36%	Galvano et al. (1998)[256]
HSCAS	0.5	AFB_1	Significant decrease of urinary excretion of AFM_1 in rats	Mayura et al. (1998)[263]
HSCAS	0.5	AF	Decrease of bioavailability of AF in the gastrointestinal tract of rats; protection against aflatoxicosis in animals	Abdel-Wahhab et al. (2002)[249]
Zeolite	0.5	AFB_1	No significant effect (rats)	Mayura et al. (1998)[263]
Montmorillonite	0.5	AF	Reduction of bioavailability of AF in the gastrointestinal tract of rats; protection against aflatoxicosis in rats	Abdel-Wahhab et al. (2002)[249]
Clinoptilolite	1.5 –2.5	AFB_1	Protection against aflatoxicosis	Oğuz et al. (2000)[260]
Bentonite	2	AFB_1	80% reduction of AFB_1 level in liver and kidney of fish	Ellis et al. (2000)[262]
Bentonite	1	AFB_1	66% reduction in AFM_1 level in milk of lactating goats	Nageswaro Rao and Chopra (2001)[248]

Table 5 Binding of aflatoxins by viable bacteria *in vitro* conditions.

Bacteria	Bacterial conc'n (CFU ml^{-1})	AF conc'n (μg ml^{-1})	% AF bound	Reference
Lb. acidophilus E-94507	1×10^{10}	5 AFB_1	18.2	Peltonen et al. (2001)[273]
Lb. acidophilus CSCC 5361	1×10^{10}	5 AFB_1	20.7	Peltonen et al. (2001)[273]
Lb. acidophilus ATCC 4356	1×10^{10}	5 AFB_1	48.4	El-Nezami et al. (1998)[265]
Lb. acidophilus LA1	10^9	0.15 AFM_1	18.3	Pierides et al. (2000)[272]
Lb. acidophilus NCC 12	10^8	0.1 AFM_1	30.5	Kabak and Var (2004)[275]
Lb. acidophilus NCC 36	10^8	0.1 AFM_1	28.0	Kabak and Var (2004)[275]
Lb. acidophilus NCC 68	10^8	0.1 AFM_1	25.7	Kabak and Var (2004)[275]
Lb. rhamnosus E-97800	1×10^{10}	5 AFB_1	22.7	Peltonen et al. (2001)[273]
Lb. rhamnosus CSCC 2420	1×10^{10}	5 AFB_1	33.1	Peltonen et al. (2001)[273]
Lb. rhamnosus LBGG	10^{10}	5 AFB_1	75.3	El-Nezami et al. (1998)[265]
Lb. rhamnosus LC705	10^{10}	5 AFB_1	76.1	El-Nezami et al. (1998)[265]
Lb. rhamnosus LBGG	2×10^{10}	5 AFB_1	78.5	Kankaanpaa et al. (2000)[264]
Lb. rhamnosus GG	10^8	0.15 AFM_1	50.7	Pierides et al. (2000)[272]
Lb. rhamnosus LC705	10^8	0.15 AFM_1	46.3	Pierides et al. (2000)[272]
Lb. rhamnosus 1/3	10^8	0.15 AFM_1	18.1	Pierides et al. (2000)[272]
Lb. rhamnosus GG	10^{10}	5 AFB_1	76	Haskard et al. (2000)[270]
Lb. rhamnosus LC705	10^{10}	5 AFB_1	77	Haskard et al. (2000)[270]
Lb. plantarum E-79098	1×10^{10}	5 AFB_1	28.4	Peltonen et al. (2001)[273]
Lb. paracasei F19	10^{10}	5 AFB_1	28	Peltonen et al. (2000)[269]
Lb. crispatus M247	10^{10}	5 AFB_1	6	Peltonen et al. (2000)[269]
Lb.crispatus MU5	10^{10}	5 AFB_1	20	Peltonen et al. (2000)[269]
Lb. fermentum CSCC 5362	1×10^{10}	5 AFB_1	22.6	Peltonen et al. (2001)[273]
Lb. johnsonii CSCC 5142	1×10^{10}	5 AFB_1	30.1	Peltonen et al. (2001)[273]
Lb. johnsonii LJ-1	10^{10}	5 AFB_1	31	Peltonen et al. (2000)[269]
B. lactis CSCC 5094	1×10^{10}	5 AFB_1	34.7	Peltonen et al. (2001)[273]
B. lactis Bb-12	10^{10}	5 AFB_1	17	Peltonen et al. (2000)[269]
B. longum CSCC 5304	1×10^{10}	5 AFB_1	37.5	Peltonen et al. (2001)[273]
B. longum Bl 24	10^8	0.1 AFM_1	26.7	Kabak and Var (2004)[275]
B. bifidum Bb13	10^8	0.1 AFM_1	32.5	Kabak and Var (2004)[275]
Propionibacterium freu. ssp. *shermani* JS	10^{10}	5 AFB_1	34.1	El-Nezami et al. (1998)[265]
P. freu. ssp. *shermani* JS	10^{10}	5 AFB_1	22	Haskard et al. (2000)[270]

in a 7 day test period, resulted in an 80% of AFB_1 in liver and kidney tissues, compared to control fish not fed bentonite. Similarly, Abdel-Wahhab et al.[249] observed that the addition of 0.5% montmorillonite which is commonly the main constituent of the clay known as bentonite or 0.5% HSCAS to the rats diets containing 2.5 mg kg^{-1} aflatoxin ($B_1 + B_2 + G_1 + G_2$) resulted in a significant improvement in the hematological and biochemical parameters and histological picture of the both liver and the kidneys. In a very extensive study, Nageswaro Rao and Chopra[248] compared the effect of the supplementation of sodium bentonite and activated charcoal on aflatoxin carry-over in milk of lactating goats fed AFB_1 contaminated ration. They determined a reduction of 65.26% (600 ppb AFB_1 + 1% bentonite) and 76.09% (600 ppb AFB_1 + 1% activated charcoal) in the carry-over of AFM_1 from feed to milk in comparison to control on the 14th day of the experiment. In another study, Diaz et al.[253] tested nine agents consisting of 4 activated charcoals, 3 sodium bentonites, a calcium bentonite and esterified glucomannan for their activity in the adsorption of AFB_1 in a novel *in vitro* assay. All nine agents were able to adsorb more than 95% of the 5 μg AFB_1 in solution.

In recent years, there has been increasing interest in the hypothesis that the absorption in consumed food can be inhibited by microorganisms in the gastrointestinal tract.[264] Numerous investigators showed that some dairy strains of lactic acid bacteria and bifidobacteria were able to bind aflatoxins effectively.[265–269] The aflatoxin-binding capacity of the viable and heat-killed bacteria is presented in Tables 5 and 6, respectively. Although the mechanisms of aflatoxin binding by specific lactic acid bacteria and bifidobacteria are unclear, specific lactic acid bacteria are proposed to decrease the absorption of mutagens/carcinogens by bacterial binding in the small intestine.[270] It is suggested that the binding of AFB_1 to lactic acid bacteria is a physical phenomenon that is associated with the bacterial cell wall structure.[266,268,270–273]

Cell wall peptidoglycans and polysaccharides have been suggested to be the two most important elements responsible for the binding by lactic acid bacteria.[267,269,270,271] Similarly, El-Nezami et al.[265] reported that all the gram-positive strains tested were more efficient than *Escherichia coli*, suggesting that the bacterial ability to remove AFB_1 is dependent on the cell wall structure. Oatley et al.[268] determined that the cell surface hydrophobicity might have been related to aflatoxin binding. They also reported that, the most efficient binder to AFM_1, *Bifidobacterium bifidum* BGN4 (46%) have shown a very high cell surface hydrophobicity than the other tested strains. Additionally, Peltonen et al.[269] reported that the differences in AFB_1 binding by the tested strains are probably due to different bacterial cell wall and cell wall envelope structures. However, it is likely that multiple components are involved in AFB_1 binding.[273]

Table 6 Binding of aflatoxins by heat-killed bacteria *in vitro* conditions

Bacteria	Bacterial conc'n (CFU ml^{-1})	AF conc'n ($\mu g\ ml^{-1}$)	% AF bound	Reference
Lb. rhamnosus GG	10^{10}	5 AFB_1	30.5	El-Nezami et al. (1998)[266]
Lb. rhamnosus GG	10^{10}	5 AFB_1	83	Haskard et al. (2000)[270]
Lb. rhamnosus GG	10^{8}	0.15 AFM_1	57.8	Pierides et al. (2000)[272]
Lb. rhamnosus LC 705	10^{10}	5 AFB_1	28.5	El-Nezami et al. (1998)[266]
Lb. rhamnosus LC 705	10^{8}	0.15 AFM_1	51.6	Pierides et al. (2000)[272]
Lb. rhamnosus 1/3	10^{8}	0.15 AFM_1	39.9	Pierides et al. (2000)[272]
Lb. acidophilus LA1	10^{9}	0.15 AFM_1	25.5	Pierides et al. (2000)[272]
Lb. acidophilus LC1	10^{10}	5 AFB_1	74.7	Haskard et al. (2001)[276]
Lb. acidophilus ATCC 4356	10^{10}	5 AFB_1	69.7	Haskard et al. (2001)[276]
Lb. gasseri ATCC 33233	10^{9}	0.15 AFM_1	61.5	Pierides et al. (2000)[272]
Lc. lactis ssp cremoris ARH74	10^{9}	0.15 AFM_1	38.9	Pierides et al. (2000)[272]
Lc. lactis ssp cremoris	10^{10}	5 AFB_1	40.1	Haskard et al. (2001)[276]
Lc. lactis ssp lactis	10^{10}	5 AFB_1	58.1	Haskard et al. (2001)[276]
Bifidobacterium spp. JO3	10^{10}	10 AFB_1	41	Oatley et al. (2000)[268]
Bifidobacterium spp. JR20	10^{10}	10 AFB_1	37	Oatley et al. (2000)[268]
Bifidobacterium spp. CH4	10^{10}	10 AFB_1	37	Oatley et al. (2000)[268]
Bifidobacterium spp. Bf 6	10^{10}	10 AFB_1	25	Oatley et al. (2000)[268]
B. adolescentis 14	10^{10}	10 AFB_1	31	Oatley et al. (2000)[268]
B. bifidum BGN4	10^{10}	10 AFB_1	46	Oatley et al. (2000)[268]

El-Nezami et al.[265] showed that 24-h old cultures of *Lb. rhamnosus* strain GG and *Lb. rhamnosus* strain LC-705 were able to remove 80% of AFB_1 within 24 h. In similar work, Shah and Wu[274] have demonstrated that the binding abilities of AFB_1 by six strains of probiotic bacteria were found to range between 20 and 50% within 180 min. In another research by Peltonen et al.[269] the binding of AFB_1 from phosphate buffered saline by probiotic bacteria was found to range from 5.8 to 31.3%. In another study by Peltonen et al.[273] the detoxification ability of AFB_1 by *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* strains were found to range between 17.3–59.7%, 5.6–41.1%, and 18.0–48.7%, respectively. In addition, El-Nezami et al.[267] indicated that probiotic lactic acid bacteria are capable of binding AFB_1 under *in vivo* conditions. They observed that, 74% of the reduction in the uptake of AFB_1 by the intestinal tissue, in the presence of *Lb. rhamnosus* GG takes place within 60 min.

It was previously reported that the binding order of aflatoxins ($AFB_1 > AFB_2 > AFG_1 > AFG_2$), correlates with a decreasing polarity of the compound and is also consistent with hydrophobic interactions playing a role in the mechanisms.[270] Similarly Pierides et al.[272] showed that AFM_1 was removed less than AFB_1. They reported that this might have been due to the additional -OH group possessed by AFM_1 resulting in increased polarity of this molecule. The removal of AFM_1 from phosphate-buffered saline by viable *Lactobacillus* strains was found to range from 18.1% to 53.8% in 15–16 h. Kabak and Var[275] determined that the abilities of tested strains to bind AFM_1 were found to range between 25.7% to 32.5% and 21.2% to 29.3% for phosphate-buffered saline and skim milk, respectively. Among the organisms studied, *Bifidobacterium bifidum* Bb13 demonstrated significantly higher (32.5%) AFM_1 binding ability in phosphate-buffered saline than the other strains.

CONCLUSIONS

Strategies to prevent mycotoxin contamination of food and animal feed have been discussed. It is clear that mycotoxins can contaminate agricultural produce both in the field as well as during storage. The use of pre-harvest control strategies for such resistance varieties, field management, the use of biological and chemical agents, harvest management, and post-harvest applications, including improving drying and storage conditions, together with the use of natural and chemical agents, and irradiation have clearly been shown to be important in the prevention of mycotoxigenic mould growth and mycotoxin formation. The importance of drying and moisture control during storage is generally well understood by the industry, in terms of the importance of prevention of fungal contamination. Interesting results have been reported on the potential use of biocompetitive agents in different biological control strategies to prevent the pre-harvest aflatoxin contamination of crops such as peanuts, rice, maize, and cottonseed. It is clear that much more work must be conducted to identify various crop genotypes which are resistant to mycotoxigenic fungus infection and subsequently mycotoxin formation. It is also clear that a combination of the development of crop species with resistance to toxigenic fungi and biocompetitive non-mycotoxigenic strain technologies may yield one of the most effective strategies for prevention of mycotoxin contamination.

Several natural plant extract and spice oils of eugenol, cinnamon, oregano, onions, lemongrass, tumeric, mint, and chemical compounds (fungicide, herbicide, and surfectant) are known to prevent both mycotoxigenic mould growth and mycotoxin formation during post-harvest season. In addition to application of plant extracts and chemical agents, as well as antagonistic microorganisms such as lactic acid bacteria with their antifungal properties, seem to be potentially very effective in the prevention

of mycotoxin formation. The precise antifungal properties of lactic acid bacteria are still largely unresolved but may involve microbial competition, as well as extracellular metabolites which are heat-stable and of low molecular weight. Again, further investigations are clearly needed to gain a better understanding of this antifungal action.

Various physical and chemical strategies have also been developed to help prevent mycotoxin contamination, including physical separation, extraction with sorbents, and adsorption. The fluorescence sorting of maize, cottonseed, and figs by examination under UV light is known to be the cheapest and the simplest acceptable way for the screening of aflatoxins. It is clear that no single currently available physical or chemical detoxification method will be suitable for all foods and animal feeds. The effectiveness of a method in the detoxification of mycotoxins depends on the nature of the food, environmental conditions such as moisture content, temperature, as well as the type of mycotoxin, its concentration and the extent of binding between mycotoxin and constituents. While a range of chemical compounds, including hydrochloric acid, ammonia, hydrogen peroxide, O_3, sodium bisulfite, and chlorine seem to hold great potential in the detoxification of mycotoxins unfortunately their use significantly decreases the nutritional value of the foods or produces toxic derivatives in the treated product with undesirable sensory properties. This will severely limit their widespread use. At the same time it should be noted that chemical treatment is not allowed within the EC for commodities destined for human consumption.

Recently there has been an increasing interest in the use of bacteria, yeast, and fungi to help reduce the toxic effect of mycotoxins. While most studies to date on mycotoxin detoxification by microorganisms have been undertaken under laboratory conditions, there is data on the effective use of *F. aurantiacum* in the detoxifying AFB_1 from various food products, including milk, peanuts, maize, and red pepper without leaving toxic endproducts. One potential drawback here is the production of a bright orange pigment by the organism which restricts its use in the detoxification of food and in feed fermentations.

The most recent approach to the problem has been the use of mycotoxin-binding agents in the diet that sequester the mycotoxin in the gastrointestinal tract thus reducing their bioavailability. Although AC, HSCAS, aluminosilicate, zeolite, and bentonite have shown good potential for use in the animal feed to help overcome aflatoxicosis, the future *in vivo* investigations must focus on other problematic mycotoxins. Interestingly lactic acid bacteria and bifidobacteria have been shown to bind AFB_1, but mechanistic studies need to be conducted on the precise binding mechanism while the conditions favoring the release of bound toxin molecules need to be investigated as well.

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