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CHAPTER : 05

Mycotoxin Menace in Stored Agricultural Commodities

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Abstract

Mycotoxins are the diverse groups of toxic secondary metabolites of filamentous fungi and the prime contaminant of agricultural commodities worldwide. These low molecular weight compounds, suspected to be the virulence factor for fungi, often possess properties like hepatotoxicity, carcinogenicity, neurotoxicity, teratogenicity *etc.* Since the ages, many instances of devastating health hazards (mycotoxicosis) induced by mycotoxin contaminated food and feed in humans and animals have been recorded. On the brink of population inflation and resultant food shortage, mycotoxigenic fungal genera like *Aspergillus*, *Fusarium* and *Penicillium* have posed a serious threat to mankind. Mycotoxins like aflatoxin, ochratoxin, deoxynivalenol, zearalenone, fumonisin have gained global concern due to their potential to impair food safety and security. Anywhere in the food chain, mycotoxin contamination of various stored commodities can occur, rendering it as an accumulative process starting from the field, aggravated during the later stages like harvesting, threshing, drying and storage upon receiving conducive environmental conditions. So, a holistic approach comprised of both pre and post-harvest strategies to get rid of the mycotoxin contamination of agricultural commodities is much needed in this era.

Keywords Aflatoxin, Clinical manifestation, Detection, Environmental conditions, Mycotoxins, Prevention strategies

1. Introduction

Among the significant impacts of the post-harvest decay of various agricultural produces, induction of human and animal health hazards attributed to consumption of contaminated food, feed and processed products is the prime one. These unavoidable repercussions of health hazards have brought the term 'mycotoxin' under the light of discussion over the past three decades. The word mycotoxin was derived from the amalgamation of the Greek word '*mykes*' and Latin '*toxicum*', which means 'fungus' and 'poison' respectively. The term was coined on the brink of a mysterious veterinary crisis in 1962 when more than 100000 young turkeys died in England after being fed with peanuts. This death was connected to the aflatoxin poisoning of peanuts and initially recognized as turkey X disease. After this event, drastic and aggravated searches were continued up to 1975 to get the details on these

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toxic secondary metabolites of fungal origin which led to the discovery of more than 200 mycotoxins - the era is now remembered as 'The mycotoxin gold rush'. Since then, mycotoxicology has become a subject of international importance. According to estimates of the Food and Agriculture Organisation (FAO) in 1985, contamination of different food crops with mycotoxin was as high as 25% globally. This estimate was further revalidated and confirmed with exhaustive trials (Eskola *et al.*, 2019) and found to expand under the detailed scrutiny of more sensitive analytical methods. Despite of the difficulties in estimating the exact losses caused by mycotoxin by summing up all the direct and indirect losses in monetary terms, FAO has roughly mentioned the $5 billion impact in the US and Canada alone.

Though most of the moldy or organoleptically objectionable food is often rejected in modern societies, sometimes the presence of mycotoxin in food or beverages does not exhibit any warning signs, thus, leaving no clues behind for visual detection. As with animals, hunger can be expected to favour the ingestion of contaminated food, even by man also where no alternative is available. Hence, the factors like shortage of food due to inflation of population, poverty and lack of scientific knowledge regarding the ideal cultivation, harvesting, storage and processing conditions have augmented the intake of undesirable foods mostly in developing countries. Owing to properties like hepatotoxicity, carcinogenicity, neurotoxicity, mutagenicity, teratogenicity *etc.*, most of the mycotoxins are capable to cause health impairment in humans and animals. These microbial compounds can pave the path into the human food chain either directly through the ingestion of contaminated food grains or via the consumption of animal products from livestock fed by contaminated feeds in an indirect manner.

2. Mycotoxin and Its Characteristics

Mycotoxins are low molecular weight, chemically diverse groups of toxic secondary metabolites often produced by filamentous fungi and can contaminate various agricultural commodities resulting in sickness and even death in humans as well as in animals. The diseases induced by the ingestion or contact of mycotoxin are known as mycotoxicosis. While all the mycotoxins are of fungal origin, no such rigidity is there for all metabolite compounds produced by the fungi for necessarily being the mycotoxins. They may vary widely in terms of their degree of toxicity, conditions under which they are produced, the products they contaminate, types of health impairment in human and animals. In general, toxins produced by the fungi serve as their virulence factor, probably maintain the oxidative status of cells and proliferate their growth by suppressing other competing microbes through niche modification. Typically, mycotoxins are produced only after the attainment of maximum growth by the fungi but it can be as fast as just 24 hrs of post-infestation. These compounds are capable of remaining toxic in food or feed for a long time even after the death of the fungi. Some evidences showed the traces of aflatoxin in corn stored for 12 years.

3. Historical Records

Entry of the toxicogenic fungi along with the various mycotoxins into the human food supplies can be traced back to time immemorial when mankind first learned to cultivate crops and felt the need for grain storage from one season to another, perhaps 10000 years ago. The science of storing agricultural commodities, mostly cereals, leads the transition of the human race to cultivator from the hunter-gatherer. At that particular time, these practices also provided enormous ecological niches favouring the nourishment of saprophytic fungi on stored grain crops, many of which were potential producers of mycotoxin. The problem of mold damage and the health hazards associated with it were well known to ancient peoples from different parts of the world. Instances of the traditional farmers from the Indo-Gangetic plains are of the greatest shreds of evidence. Their concept of "dry the grains well and keep dry grains dry" for post-harvest storage has been streamed from one after another generation, implicating the need for protection of the grains from various biotic stresses. Several historical events have been documented with more or less scientific evidence regarding mycotoxin ingestion. In the classic literature 'The road to Eleusis' by Wasson *et al.* (1978) an exquisite attempt has been made to link the ancient (15th Century BC to 4th Century AD) Greek ritual 'Eleusinian mysteries' and the mycotoxins. A special beverage '*kykeon*' used on this occasion used to be brewed from barley seeds and additional herbs, which are now suspected to have contained some psychoactive alkaloid compounds produced from the fungi *Claviceps purpurea* or *C. paspali* growing on the barley seeds, leading to the generation of strong mental states immersed in spirituality and intellectuality. Similarly, events like decadence of Etruscans, failure of the expedition of Peter the Great against the Turk in 1772 or 'The Great Fear' of France in 1789, are some of the examples estimated as the influence of mycotoxin poisoning. Even some of the devasting plague epidemics like the plague of Athens (430 BC), the bubonic plague of Europe (14th to 17th Century) are thought to be linked with the consumption of contaminated grains (Ramos *et al.*, 2011). The medieval barbaric genocide of the people accused of witchcraft trials in Salem in 1692 is undoubtedly the most pitiful result of misconception regarding food poisoning with mycotoxin. Ergotism (from the old French word *argot*, representing cock's spur) is the oldest known mycotoxicosis in historical context. The disease was engendered due to the consumption of bread prepared from the contaminated grains collected from the Field infected with *Claviceps purpuea.* Since the ages, several epidemics of ergotism on man and animals have been documented from various parts of the world, like China (in 1100 BC), France (in 857 BC and 994 BC; killed about 20000 and 50000 people), Germany (in 857 AD, 20000 people died), Russia (in 1722, 20000 Russian soldiers died and in 1926-27, 10000 people died). With the increased use of rye grains, the most susceptible host for the fungi *Claviceps*, ergotism devastated vast regions in Central Europe between 1085 and 1095. It was then called St. Anthony's fire (also 'Holy fire' and 'Hell's fire'), in the honour of St. Anthony the Abbot

(also known as St. Anthony the Hermit). As the people used to go for a cure in the monasteries dedicated to this Saint. The recovery encountered by many of pilgrims may be due to the far distances of the monasteries from the areas of the outbreak, leaving the source of contaminated rye. Recently in 1978, again an outbreak of gangrenous ergotism devasted the Ethiopian highlands. During the 17th Century, Japan was used to be frequently threatened by another severe mycotoxicosis disease named acute cardiac beriberi connected with the regular eating of moldy rice. The disease was caused by citreoviridin secreted from *Penicillium citreonigrum*. This toxin was earlier believed to be only of historic importance until its reemergence in Brazil a few years ago. Not only on humans, several records of animal epidemics caused by mycotoxin have come under discussion. Among them incidents of Turkey X disease in England, equine leucoencephalomalasia during the Second World War are notable.

4. Factors Influencing Mycotoxin Production

Mycotoxigenic fungi can be traced very frequently in all agricultural zones of the world. The existence of the fungi does not always necessarily mean the mycotoxin contamination at the end. It may be due to the factors promoting fungal growth and subsequent mycotoxin production, which may either be independent or can vary. So, the eradication of causal fungi at least in the visual level does not always ensure the absence of mycotoxins. A number of factors may influence such types of fungal growth and subsequent mycotoxin production. Various stored commodities are prone to be tainted with these toxins anywhere in the food chain, which renders the accumulative mode of the process of contamination, starting from the field, aggravated during the later stages like harvesting, threshing, drying and storage upon receiving conducive environmental conditions.Factors like temperature, water activity, relative humidity, pH, fungal strain and substrate possess inevitable role in mycotoxin production (Daou *et al.*, 2021). Temperature and humidity can influence the competitiveness of mycotoxigenic fungi. Each fungal genera even the different species has an ideal temperature and water activity range specifically required for their germination, growth and mycotoxin production. Due to higher discrepancies in optimal environmental conditions and growth requirements, fungal development and mycotoxin production differ across the geographical regions. Similarly, at field, hygrophilic species of *Fusarium* can be predominant during pre-harvest, since they require a high relative humidity of >90%. After the harvest, hygrophilic fungal species disappear on the prevalence of mesophilic and xerophylic fungal species like *Aspergillus* spp. and *Penicillium* spp., which produce mycotoxins at <80% humidity. If, at the storage, a_w or water activity of food/feed increases with the greater humidity of surroundings, grains become susceptible to fungal growth and mycotoxin production. pH value or the saturation of hydrogen atoms at the medium surrounding the fungi highly influences its growth either by direct action on cell surfaces or through indirect effect on nutrient availability. Some fungi like *Penicilium* sp. and *Aspergillus* sp. are capable of modulating their

surrounding pH, by the production of several secondary metabolites, which provides a better survival chance. Strain or species specificity may generate variation in toxin production. Within the same species of fungus strains may vary in terms of physiological needs, thus producing different mycotoxins. For example, *A. flavus* produces AFB_1 whereas *A. carbonarius* produces ochratoxin. Similarly, separate types of trichothecenes are produced from different strains of the species owing to the allelic variation of a particular gene. On the other hand, filamentous fungi hydrolyze multiple carbon sources to generate energy and promote growth. Simple sugars are readily available for fungal breakdown, but fungal growth will be slowed down with complex ones as they require more digestion.

5. Major Mycotoxin-Producing Fungal Genera and the Toxins Produced by Them

The fungi responsible for the origin of mycotoxins and resultant mycotoxicosis can broadly be classified under three different groups according to the stage of infection of the substrate: (i) field infecting fungi, (ii) storage fungi and (iii) advanced deterioration fungi. Numerous species from the fungal genera like *Aspergillus* and *Penicillium* can cause infection of cereals, fruits *etc.* in field as well as in storage. So, they can fit well in both field infecting and storage fungal groups. Whereas, mycotoxigenic as well as plant pathogenic genus like *Fusarium* is mostly restricted to the field infecting group. The growth of "advanced deterioration fungi" normally not meant for infesting whole grains, rather, they easily colonize on damaged grains, requiring higher moisture content for proliferation. Fungi like *A. clavatus*, *A. fumigatus*, *Scopulariopsis*, *Chaetomium*, *Rhizopus*, *Mucor* and *Absidia* are the instances. Other than these major fungal genera, *Trichothecium*, *Myrothecium*, *Trichoderma*, *Stachybotrys*, *Tilletia*, *Sclerotinia*, *etc.* are also capable of producing mycotoxin. In any filamentous mycotoxigenic fungi the genes involved in the biosynthesis of secondary metabolites (like: mycotoxin) are accommodated together in clusters on chromosomes. Such types of gene clusters are commonly referred as secondary metabolite gene clusters or SM clusters. The genes of the cluster modulate various chemical reactions progressing to the biosynthesis of different toxins from the initial condensation of acetyl CoA and malonyl CoA in every case (Kolawole *et al.*, 2021). Most of these clusters exhibit almost similar characteristics to all toxicogenic species of a genus. Some of the major mycotoxigenic fungal genera, the toxins produced by them and clinical manifestation of these toxins are discussed here.

5.1. Aspergillus *and* Penicillium

These two fungal genera from the class Ascomycetes are the opportunistic plant pathogens. Species from these genera are capable of inducing disease symptoms on numerous plants and plant products, at various stages of the food supply chain. The yellow mold of peanut, ear rot of maize, black mould of different fruits are some of the devastating diseases caused by

various species of *Aspergillus*. Whereas, most of the *Penicillium* species are opportunistic storage mold, often being the cause of economically important post-harvest diseases like green and blue mold. Along with food spoilage, they are capable of producing mycotoxins. Some of them *i.e.* aflatoxin, ochratoxin, patulin are the major global concern of the date due to their vast detrimental effects on food and feed.

5.1.1. Aflatoxins

Aflatoxins are the difuranocoumarin derivatives, resultant of the polyketide pathway driven by many species of this genus, but mostly by the two species *A. flavus* and *A. parasiticus*. Though encountered less frequently, a few other species are also known to produce aflatoxin. A few of them are *A. bombycis*, *A. nomius, A. ochraceus*,and *A. pseudotamari*. Remarkable qualitative and quantitative differences are noted among the various toxicogenic species of this genus. This group of mycotoxins is the major contaminant of food and feed worldwide specially in hot and humid regions. Grains that are inadequately dried and hold a moisture level >14% are highly prone to aflatoxin contamination at temp of >20 °C. Various crops like rice, maize, peanuts (groundnuts), cotton seed, Brazil nuts, spices and figs are highly prone to aflatoxin contamination in storage. About 19 Afs analogues have been discovered till date, but those belonging to B series (AFB_1, AFB_2) and G series (AFG_1, AFG_2) are of utmost importance regarding their toxicity and occurrence. The B and G nomenclature was posed due to the formation of two fluorescent colours *i.e.* blue and green respectively, by them when tested on thin layer chromatography plates under the exposure of UV light; whereas the subscript numbers indicated the major and minor compounds respectively.

Clinical Manifestation of Aflatoxin Contamination

Among the various aflatoxins, AFB_1 is known to be one of the most formidable carcinogen compounds for livers in humans and other mammals. Aflatoxin acute toxicity can also be promoted by AFB_2. This particular compound can either bind or inhibit the key enzymes of intermediary metabolism, developing necrosis of liver cells. A descending potency series expressing the degree of aflatoxin toxicity and carcinogenicity, namely, $AFB_1 > AFB_2 > AFG_1 > AFG_2$, has been established for the aflatoxin-induced mutagenic activity along with DNA damage. Two hydroxylated compounds, AFM_1 and AMF_2, which are derivates of AFB_1 and AFB_2, can be formed and excreted in the milk of lactating animals including humans upon consuming contaminated food. Similarly, sterigmatocystin (ST) is also reported to be acutely toxic and hepatic carcinogenic. Acute poisoning incidents on humans and animals by aflatoxin like in Taiwan (1967), Kenya (1982), Uganda (1970) expressed a wide range of symptoms in the victims. This included edema of the legs, abdominal pain, vomiting, palpable livers, Reye's syndrome, jaundice, *etc.*

5.1.2. Ochratoxin

Currently, a number of the species from the genus *Aspergillus* and one

species of the genus *Penicillium* are known to produce ochratoxin. Species of *Aspergillus* responsible for ochratoxin secretion are *A. ochraceus*, *A. alliaceus*, *A. carbonarius*, *A. auricomus*, *A. melleus*, *A. glaucus* and *A. niger*, whereas, *Penicillium verrucosum*, a frequent contaminant of barley grain, is the only species verified to produce ochratoxin from this genus. Among the 20 different ochratoxin analogue compounds identified, ochratoxin A (OTA) is regarded to express the highest toxicity and it is a potential nephrotoxin. Presence of ochratoxin A has been reported from the grains of barley, oats, wheat, rye, coffee beans *etc.* But, the toxin has been vigorously studied since 1965 due to its high frequency of contamination with barley worldwide. In a few cases, ochratoxin have also been suspected to be present in certain wines, mostly due to the contamination of grapes with *A. carbonarius*.

Clinical Manifestation of Ochratoxin Contamination

Ochratoxin A, the potential nephrotoxin to a number of animal species, have the most prolonged half-life required for its elimination from the body of any species examined. Though multiple ways can be portrayed by the compound to disrupt cellular physiology, but inhibition of the enzymes involved in phenylalanine metabolism, mitochondrial ATP production and stimulation of lipid peroxidation are the prime ones. Ochratoxin has been traced in blood, animal tissues and milk (including human milk). The toxin contamination is believed to be associated with pork consumption, resulting in porcine nephropathy. A progressive chronic nephritis and urinary tract tumors reported from the populations residing on the bank of the Danube river of Romania, Bulgeria, commonly known as endemic Balkan nephropathy, is estimated to be a fatal ochratoxicosis disease.

5.1.3. Patulin

During the 1940s, Patulin was first isolated from the fungus *Penicillium patulum* (later known as *P. urticae*, now *P. griseofulvum*) as the principle antimicrobial active component of the species. Depending on the origin, the compound was also regarded as clavacin, claviformin, expansin, mycoin c and penicidin. Several fungi species belonging to the genera *Penicillium*, *Aspergillus*, *Byssochlamys* and *Paecilomyces* have been reported to produce patulin. Among the *Aspergillus* species, patulin production is restricted to three of the Clavati group: *Aspergillus clavatus*, *A. giganteus* and *A. longivesica*. From the genus *Penicillium* at least 13 patulin producing species are reported: *P. carneum*, *P. clavigerum*, *P. coprobium*, *P. dipodomyicola*, *P. expansum etc.* In between 1950 and 1960s patulin was thought to aggravate antibiotic activity, thus being used for treatments of common cold and fungal skin infections. But soon after its toxicity to both plants and animals was evaluated, it was reclassified as mycotoxin. Currently, the blue mold fungus *P. expansum*, which is also the cause of the soft rot of apples, cherries, pears, crab apples, strawberries, black mulberries, peaches *etc.* has been regarded as the prime source of patulin contamination.

Clinical Manifestation of Patulin Contamination

Patulin is not carcinogenic compound, rather data on its genotoxicity against mammalian cells were evident. In animals, some of the acute symptoms initiated by patulin contamination may include the damage of liver, spleen, kidney and toxicity reaction to the immune system. Cases of nausea, gastrointestinal disturbances and vomiting have been recorded in humans.

5.2. *Fusarium*

The well-known phytopathogenic genus *Fusarium* and the numerous species belonging to it are recognized to cause several economically important diseases around the different climatic zones of the world. Apart from resulting lower crop yield and inadequate quality, *Fusarium* species are also the potential producers of several mycotoxins in the grains. Mycotoxins produced by *Fusarium* spp. do not always originate at the store, most of cases the infection starts directly from the field. The most relevant mycotoxins from this genus are trichothecenes, zearalenone and fumonisins.

5.2.1. Trichothecenes

More than 200 trichothecene analogues have been identified worldwide and they are all widely categorized into four different types, *i.e.* A, B, C and D. Among these groups, type A (includes T2 toxin) and type B (includes deoxynivalenol or DON) are recognized well regarding the vast contamination potential of them to various agricultural commodities around the world. Various trichothecene groups of toxins have been reported from corn, rye, oats, rice, wheat, barley and other crops. Infection by these fungal pathogens visibly appears as whitish or pinkish mold growth at the tip of the corn ear. Prevalence of trichothecene contaminations has frequently been observed in Asia, Europe, North and South America.

Clinical Manifestation of Trichothecene Contamination

In general, trichothecene groups of toxins inhibit protein synthesis in animal and human by disrupting the synthesis of DNA and RNA. It causes harm to the active cells setting on the lining the gastrointestinal tract, skin, lymphoid, erythroid cell; suppresses the antibody level, immunoglobins; disrupts the hormonal balance by inhibiting cytokinin. The manifestation of the disease mostly expressed as weight loss, bloody diarrhoea, lesions on beak or mouth, dermal necrosis, hemorrhage and reduction in milk or egg production. Poultry species are more susceptible to type A trichothecenes than type B. Prominent oral symptom like yellow caseous plaques on mucosa of the hard palate, at angle of mouth and tongue can be observed on poultry.

5.2.2. Deoxynivalenol (DON)

Deoxynivalenol, commonly known as vomitotoxin, is a potent type B class of trichothecene toxic to agricultural commodities. DON is commonly secreted by *F. graminearum* and ocassionaly by *F. culmorum* in some geographical areas. This fluorescent mycotoxin may co-exist with zearalenone, fumonisin, *etc*. The causal fungus *F. graminearum* typically invades the corn cobs and other small grains like wheat, oats and barley in later harvesting stage. Unlike the species of *Aspergillus* and *Penicillium*, this organism thrives better in

cool, moist conditions. In corn, contamination occurs with the windblown conidia landing on the silks, whereas for small grains, conidia of *Fusarium* penetrate during anthesis through the emerging anthers. The resultant disease is either ear rot of corn or head blight of small grains. This particular mycotoxin is mandatory for the fungus to develop disease. Upon infection wheat head may turn into pink and premature, uneven ripening can lead to the formation of blanched or tombstone kernels. Pink scab, a generalized term has been imposed on the commodities showing contamination. As the toxin is stable during storage, milling, processing and also resistant to thermal processing, it has become a major concern.

Clinical Manifestation of DON Contamination

Potential health implications for humans are administrated under fairly low exposure to DON. Several gastrointestinal issues like vomiting, diarrhoea and inflammation are often observed. Most commonly affected animals are the swine and they experience reduced intake of contaminated grain. On eating they may vomit . Similarly, vomiting syndrome can be induced on consuming contaminated grains in humans also. DON is a potential immunosuppressive and leads to kidney problems in animals. Adverse effects are seen with baking and malting using the contaminated grain.

5.2.3. Zearalenone

This mycotoxin chemically is a phenolic resorcyclic acid, produced by the *F. graminareum* and *F. culmorum*. It often co-exists with deoxynivalenon. Though zearalenone is a prime contaminant of maize, its occurrence in wheat, barley, sorghum and rye have also been recorded worldwide. As with wheat, maize and sorghum, zearalenone rises in grains before harvest but in case of other grains, insufficient evidences failed to determine whether the chemical produced on pre-or post-harvest stage.

Clinical Manifestation of Zearalenone Contamination

Typical estrogenic syndromes are produced in animals on the consumption of zearalenone contaminated grains. Mostly swine are affected. Infertility of swine and dairy cattle due to the degeneration of reproductive structures is often observed. Piglets of swine are produced small and weak. Male swine show feminization, atrophy in the testis and enlargement in mammalian gland.

5.2.4. Fumonisins

Two species, *i.e.*, *F. verticillioides* and *F. proliferatum* are known as the prime producers of fumonisin toxin in wheat and maize. These pathogens of maize are responsible for inducing Fusarium ear and stalk rot and seedling blight. Maize grain grown in temperate regions and maize-based food products are highly susceptible to fumonisins production.

Clinical Manifestation of Fumonisin Contamination

Fumonisins are estimated as probable carcinogens linked to the development of esophageal and liver cancer. It can cause developmental abnormalities

in the embryo, malformations of brain and spinal cord. Acute toxicity of fumonisins can cause diarrhoea and abdominal pain. Fumonisins received particular attention due to induction of leucoencephalomalasia in horses.

Other than these major toxins, hundreds of toxins are also known. Such as fumitremorgin (from *A. fumigatus*), luteoskyrin (*Penicillium citreoviridin*), citrinin (*P. citinum*), verruculotoxin (*P. verruculosum*), fusariogenin (*F. sporotrichoides*) *etc*. Several mycotoxises have gained the importance, like: sick building syndrome (by *Stachybotrus chartatum*), lupinosis in cattles, pink rot dermatitis in humans (by *Sclerotinia sclerotiorum* of celery) *etc*.

6. Detection Methods of Mycotoxins in Food and Feed Material

Evaluation of the presence of mycotoxins in stored food and feed material is the preliminary requirement for enacting any laws to fix the legislation limits for these toxic compounds. Due to their complex structural backbones and the presence in low concentration, difficulties in detection are often faced. Any of the detection methods of secondary metabolites is comprised of sampling, sample preparation and analytical procedure in general. Several immune-assay based methods and chromatographic separation procedures are widely used for this detection purpose. Immune-assay based methods are mostly driven by the principle of recognition of the specific antibodies by the mycotoxins that serve as antigens in the reaction. Detection reaction is typically expedited by the presence of radioactive or chromogenic or fluorescent markers. In the absence of markers, these methods can be accomplished with natural fluorescence of a few mycotoxins or by measuring the conductivity. Procedures like Enzyme Linked Immusorbent assay (ELISA), Lateral Flow Immunoassay (LFIA), Fluorescence Polarization Immunoassay (FPIA) are some of the instances. For quantitative analysis of the mycotoxin contamination, chromatographic separation is the most widely used strategy. These are the exceptionally selective, accurate and reproducible methods frequently demanding expensive instrumentation facilities along with chromatographic expertise. In case of the feed material analysis, Liquid Chromatography is the most common method, although gas chromatography (GC) and thin layer chromatography (TLC) are also recognised. For simple, rapid and *in-situ* assessment non-invasive methods like infrared spectroscopy (IR) techniques and Raman spectroscopy have been developed. These types of methods enable prompt decision-making, thus avoidance of the possible loss of an entire lot can be ensured, but are quite expensive.

7. Prevention and Control of Mycotoxin Contamination

Strategies for the prevention and control of mycotoxin contamination in food and feed have been elaborately recommended by FAO. According to the phase where the measure is taken, the strategies are gradually divided into 3 subsequent levels. The basic or primary prevention strategies should be executed before the fungal infection attains the threshold and mycotoxin contamination starts. These strategies are the most important ones and

prophylactic. Primary prevention strategies are mostly based on keeping the conditions unsuitable for promoting fungal growth. Several practices that have been recommended under the primary prevention strategies include suitable schedule for pre-harvest, harvest and post-harvest.

7.1. Primary prevention strategies

7.1.1. Pre-Harvest

(1) Crop varieties selection: Healthy, pests and disease-free seeds should be used for sowing/transplanting to ensure a vigorous crop stand capable of enduring the biotic stresses.

(2) Sowing date: Alteration of the sowing date can partially escape the infection by various fungal pathogens at later stages.

(3) Avoiding insect attack: Insects serve as the vectors transporting the fungal spores to the stored grains or create critical environmental points within the bulk of commodities which favour fungal growth and promote toxin production successively (like significantly higher infection by *Aspergillus flavus* and *A. parasiticus* in damaged grains).

(4) Biocontrol techniques: Biocontrol techniques have been implied to fight against fusariotoxicosis. Non-aflatoxigenic strains of the same species of *Aspergillus* could be used as agents prompting the bio-competitiveness.

7.1.2. Post-Harvest

(1) Proper storage: Keeping a moisture content to minimum <13% for starch cereal, <11% for soyabean is good for mold prevention, restricts the chances of generation of high moisture pockets. Range of 12-15 °C temp. is ideal for storing. In case of cold drying, at 5-8 °C mold growth ceases. A low oxygen concentration (<1%) along with augmentation of carbon dioxide concentration are efficient in preventing mould development.

(2) Sorting: Sorting of grains should be accomplished based on their appearance or density patterns. For example, apple dissection before making apple juice or washing wheat before spaghetti preparation can contribute to 95% reduction in patulin or a 23% removal of DON.

7.2. Secondary Prevention Strategies

If the invasion of some fungi already begins in commodities at an early phase, this level of prevention will then be required. To prevent further deterioration and mycotoxin contamination, existing toxigenic-fungi should be eliminated or growth should be terminated. Several measures suggested are as follows.

(1) Re-drying of the products should be done to stop the growth of infested fungi.

(2) Elimination of contaminated seeds.

(3) Inactivation or detoxification of mycotoxin contaminated food and feed.

7.3. Tertiary Prevention Strategies

Tertiary prevention strategies are implied only when the products are heavily

infested. For example, peanut oil extracted from low-grade peanut seeds contains extremely high quantities of aflatoxins, which can be eradicated during oil refining process via absorption and alkalinization. Only a few practices are recommended under this stage: (1) Complete destruction of the contaminated products (2) Detoxification or destruction of mycotoxins to the minimal level: The most frequently used techniques for detoxification mycotoxins is based on decreasing their bioavailability by the inclusion of various binding agents or adsorbents. These adsorbents will be considered as efficient only if it is stable in an animal's digestive tract and channelled away along with bounded mycotoxins via urine and faeces. Phyllosilicates like bentonite and montmorillonite could adsorb 40-100% ochratoxin in wine. Cholestyramine resin, a synthetic polymer used to treat humans, has been found to adsorb zearalenone. Among the bio adsorbent *Saccharomyces cerevisiae*, was found to bind with AFB_1 and reduced the detrimental effects of it in broiler diet. Other than the adsorbents, several physical processes like thermal treatment, degradation by extrusion, irradiation, ammoniation, deammoniation can be used efficiently for detoxification of mycotoxins.

8. Conclusion

Mycotoxin contamination is a major challenge to the food production sector. Several instances of mycotoxin menace have brought forth the realization that not only improvement in production technology is sufficient, but protection of the produces from various biotic stresses is also the key factor toward food safety and security. Studies of the mycotoxins, the chemistry behind them, molecular events associated with their synthesis and genes comprising the fungal secondary metabolite cluster are much needed to design preventive strategies. Detection of mycotoxin in food and feed material is the preliminary requirement for the enactment of fixing any legislation limits for such toxic compounds. As the mycotoxin contamination in stored commodities is an accumulative process spanned over pre-harvest, harvest, drying, post-harvest storage phases, a holistic approach comprised of management and control strategies in every stage can be a solution to cope up the problem.

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