



## REVIEW ARTICLE

# MANAGEMENT OF MYCOTOXIN CONTAMINATION IN PREHARVEST AND POST HARVEST CROPS: PRESENT STATUS AND FUTURE PROSPECTS

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## SUMMARY

Fungal secondary metabolites (mycotoxins) produced as contaminants on food and feed commodities are considered to be economically and toxicologically important world wide. Aflatoxins, ochratoxins, citrinin, fumonisins, zearalenone and trichothecenes are important mycotoxins which have been analysed as natural contaminants in various agricultural commodities. Many developed countries have laid down specific regulations for import and export of those items in terms of economic implications. In the light of present status different measures (prevention, elimination, and decontamination/inactivation) have been discussed in this review to minimize the risk of mycotoxin contamination in preharvest, harvest and post harvest conditions.

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## 1. Introduction

Mycotoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause sickness or death in humans and animals. Diseases caused by mycotoxins are called mycotoxicoses. Mycotoxins can be acutely or chronically toxic or both depending on the kind of toxins and dose. In animals, acute diseases include liver and kidney damage, attack on central nervous system (CNS), skin diseases and hormonal effects. Among the mycotoxins, aflatoxins produced by *Aspergillus flavus* Link ex. Fries, *A. parasiticus* Speare, *A. nomius* Kuwartzman et al, are most potent natural carcinogenic compound causing mutation (transversion) of 249<sup>th</sup> codon of P<sup>53</sup> gene (Deng and Ma, 1998). Scientists estimate that there are 300 to 400 mycotoxins presently identified with more being isolated as new techniques and processes evolve. A list of mycotoxins significantly impacting agricultural commodities would include aflatoxin produced by *A. flavus*, and *A. parasiticus*, zearalenone and trichothecenes (particularly deoxynivalenol) produced by *Fusarium* spp., ochratoxin produced by

*A. ochraceus* and fumonisins produced by *F. moniliforme*.

Approximately 25 per cent of world's food crops are affected each year by mycotoxins. The economic effects of mycotoxins are many fold affecting all sections of production and consumption of grain production viz. grain producers, handlers, processors, consumers and society as a whole. Grain producers are affected by limited yields, restricted end markets due to contamination and price discounts. Grain handlers are affected by restricted storage facilities, costs of testing grain lots and loss of end markets. Grain processors incur higher cost due to higher product losses, monitoring costs and restricted end markets. Consumers end up paying higher end product prices due to increased monitoring at all levels of handling and in extreme cases death problems due to consumption of contaminated products. On the other hand societies as a whole end up paying higher costs due to increased regulations, needed research, lower export costs and higher import costs. These costs are found at every level of grain production system, however, it

is almost impossible to estimate the amount of losses. In North Carolina alone the losses to the animal production industry for one year (1992) were \$ 20 millions for poultry, \$ 10 millions for swine, \$ 5 millions for dairy and \$ 1 million for beef and sheep, and \$ 1 million for horses.

Risk of mycotoxin contamination in India is increased due to environmental, agronomic and socio-economic factors. Environmental conditions especially high humidity and temperature favour fungal proliferation. Farming condition in India also sustain fungal and toxin contamination in food and feed. The socio-economic and food security status of the majority of inhabitants leave them little option in choosing good quality products.

## 2. Integrated mycotoxin management programmes.

Strategies to address the food safety and economic issues employ both preharvest and post harvest measures to reduce the risk of mycotoxin contamination in food and feed. Post harvest measures, such as adequate storage, detection and decontamination or disposal as well as continuous monitoring of potential contamination during processing and marketing of agricultural commodities, have proved to be critical and indispensable in ensuring food and feed safety. However, the post harvest contamination is usually the result of preharvest presence of fungal contamination. Preharvest control includes good cultural practices, biocontrol and development of resistant varieties of crops through new biotechnologies. Processed food cannot be safe if prevention, control, good manufacturing practices and quality control are not used at all stages of production. The Hazard Analysis and Critical Control Point (HACCP) approach to processing mycotoxin contaminated commodities should be considered.

Selected aspects of an integrated mycotoxin control programme should involve different phases such as ones outlined below:-

### Preharvest procedures

Significant levels of mycotoxins can occur in the food crops in the fields. Some of the strategies for prevention of mycotoxins in the field are

#### Preharvest procedure

- Reduction in plant stress through irrigation, mineral nutrition, protection from insect damage.
- Avoidance of environmental conditions that favour infection in the field eg. Drought, insect infestation, primary inoculum, delayed harvesting etc.

#### Potentially effective

- Good cultural practices viz. crop rotation, cropping pattern, irrigation, timely planting and harvesting and use of biopesticides have protective actions that reduce mycotoxin contamination of field crops.
- Breeding of cultivars resistant to fungal infection.
- Use of crop protection chemicals that are antifungal agent.
- Identification of plant constituents that disrupt aflatoxin biosynthesis or fungal growth and their use in new biochemical marker-based breeding strategies to enhance resistance in crops.

#### Developmental

- Development of transgenic plants resistant to fungal infection.
- Development of transgenic cultivars capable of catabolism/interference with toxin production.
- Development of crops genetically engineered to resist insect damage.
- Development of crop seeds containing endophytic bacteria that exclude toxigenic fungi.
- Exclusion of toxigenic fungi by pre infection of plants with bio-competitive non toxigenic fungal strains.
- The fungal genome of *A.flavus* has been sequenced to understand the regulation of aflatoxin formation by

environmental factors. This information can be used in development of host resistance against aflatoxin contamination by studying the effects of various physiological parameters eg - drought stress on gene expression in toxigenic fungi.

### **Harvesting Procedure**

Mechanical damage of seeds may incur during harvesting. When damage is kept to a minimum during this phase, subsequent contamination is significantly reduced. Field crops should be harvested in timely manner to reduce moisture or water activity ( $A_w$ ) level to a point where mycotoxin formation will not occur.

### **Post harvest procedures**

Even if the contamination occurs or persists after this phase, the hazards associated with toxin must be managed through post harvest procedures, if the product is to be used for food and feed purposes. Storage and processing are the major areas where contamination can be prevented.

### **Post - harvest strategy**

1. Removal of damaged grain and drying of grain to the minimal moisture level.
2. Control of insect and rodent activity and maintenance of appropriate moisture levels and temperature.
3. Appropriate packaging is often successful way of excluding insects and moulds.
4. Frequent cleaning of food/feed delivery systems and short term storage.
  - Use of antifungal agents such as propionic acid and acetic acid.
  - Thermal inactivation is also one of the alternatives for products that are usually heat processed. Fumonisin and ochratoxin levels have been shown to be lower in thermally processed maize and wheat products.

### **3. Regulations**

Regulatory limits are law, violation of which has legal consequences. Regulatory limits or standard provide a benchmark against which effectiveness of food safety programme can be tested. Adverse health effects from the ingestion of these toxins have caused regulatory agencies throughout the world to limit the amount of aflatoxins that are permitted in food or feed that is available for sale. Currently, 77 countries have imposed regulatory limits for mycotoxins. The establishment of regulatory limits may vary in each country depending on level of exposure and sociological, political and economic factors. This results in undue economic burden on growers.

According to FAO estimates, world losses of food stuffs due to mycotoxins are in the range of 1000 million tones a year. Since aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin and fumonisins are the mycotoxins of most concern thereby, stringent mandatory standard, voluntary regulations or guidelines, tolerance level have been laid down for these mycotoxins by many countries. At the international level, CAC (Codex Alimentarius Commission) through its committee on Food Additives and Contaminants (CCFAC) and other relevant commodity Committee is considering the establishment of international guidelines for levels of mycotoxins in food.

An updated status of existing regulations and also the role of CAC in providing a platform to reach a consensus on the maximum permitted levels in food have been reviewed recently (Vishwanath, 2004). Since mycotoxins can occur both in raw products and finished byproducts, this has necessitated the testing of mycotoxins at various stages in food-chain. Important commodities under regulation include - food and feed products: cereal grains and cereal based products, peanuts and their products, nuts and other oil-seeds, milk and milk products, infant and children food, fruit and fruit juices, miscellaneous food products.

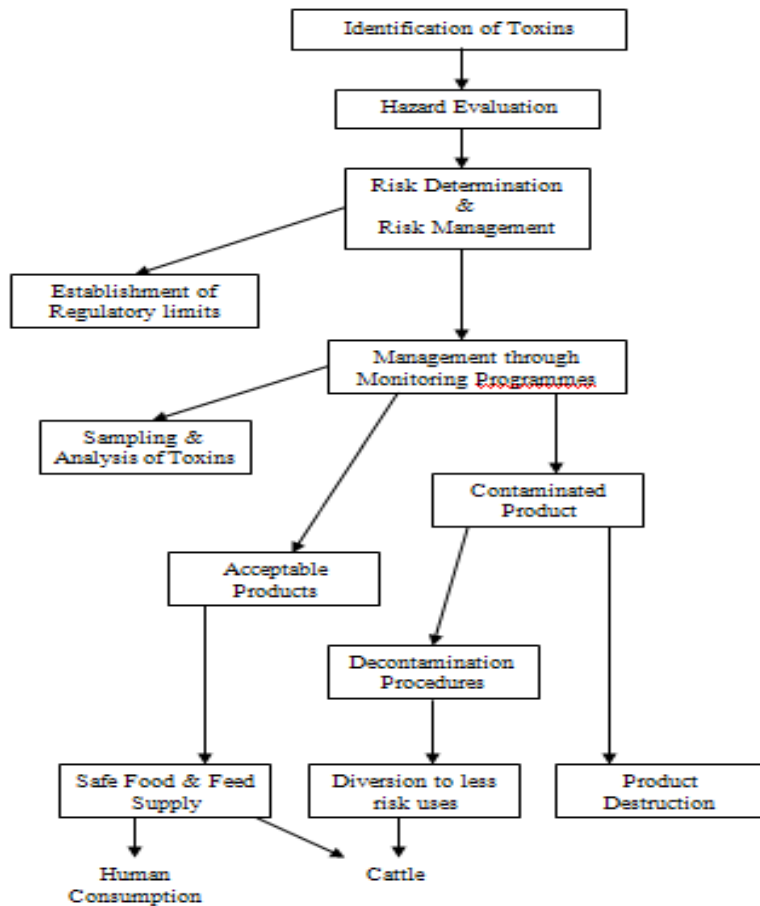
The establishment of regulatory limits may vary in each country depending on level

of exposure and sociological, political and economic factors. A good example of management through a monitoring programme is the aflatoxin control programme used by the state of Arizona (Park, 1993). During the year 1978, almost 910,000 pounds of milk were dumped with contamination levels as high as 10 ppb aflatoxin M<sub>1</sub>. As a result of this huge commercial loss and the need to establish an effective aflatoxin control programme, the state instituted a programme to monitor aflatoxin levels in whole cottonseed and cottonseed products, the major source of aflatoxin contamination in feed.

Once the regulatory limit has been set, sampling plays an important role in

determining the fate of particular product. Adequate random sampling techniques should be used at each point of analysis. It is important to consider the existence of 'hot spot' (or highly contaminated portions of the product). If these highly contaminated portions are not detected, the toxin would then be distributed throughout the lot, rendering a highly contaminated product. Once the level of contamination has been determined through the monitoring programme, the final use of the commodity can be determined. The product may be deemed either safe for human consumption, safe for feed used through treatment or totally unsuitable for use at any point in food-chain(fig-1).

Fig: Management of mycotoxin contamination and food safety



#### 4. Control of mycotoxins

As mycotoxins are potential risk to human and animal health and thereby much time and effort has been expended on seeking methods for removal of or destruction of mycotoxins in contaminated

products. The best approach to the control of mycotoxin is prevention. However, contamination of mycotoxin is sometimes unavoidable, and if prevention fails, other alternative must be considered. The post harvest contamination is the result of

preharvest presence of fungal contamination. Therefore, research focus in the past decades has shifted from post harvest control to a more preventive approach employing various preharvest control measures. In this review, for the sake of convenience we shall discuss control strategies under two broad sub-headings-

Pre harvest control of mycotoxins.

Post harvest control of mycotoxins.

### **Preharvest control of mycotoxins**

Ecophysiological conditions are substantially different during preharvest and post harvest stages of agricultural crops. The mould invasion, infestation and mycotoxin elaboration to a great extent depend upon various environmental factors prevailing in the vicinity. Preharvest period begins with the emergence of seedling and continues upto maturity, finally ending with the harvesting of the crops. It is now established that some of the storage fungi (responsible for post harvest mycotoxin production) get associated with food grains from the field (preharvest stage). However, fungi that are associated with standing crops may or may not be able to grow in fields because conditions required for successful mould growth are less favourable in fields than in warehouses and godowns. Moreover, only such moulds are able to grow under fields that exhibit certain degree of parasitism for invading receptive parts of the susceptible varieties of plants. Some preconditions are absolutely essential for preharvest production of mycotoxins. The foremost are availability of toxigenic fungal strain, susceptible host and favourable agro climatic niche (Bilgrami and Choudhary, 1998). In standing crops, host - fungus - environment interaction is critical in predisposition of mycotoxin contamination.

Preharvest control includes good cultural practices such as insect control, irrigation during drought conditions, planting and harvesting dates, cropping patterns etc. Pre harvest prevention especially through host resistance is probably the best and widely explored strategy for control of mycotoxins. Currently new biotechnological approaches

are employed for preharvest control of mycotoxins.

### **Enhancement of host resistance**

#### **Breeding**

Host resistance enhancement can be achieved through identification of germplasm resistance to aflatoxin and also identification of natural resistance mechanisms and traits (Brown et al, 2003; Cleveland et al, 2003). Aflatoxin contamination involves multiple chromosome regions and several genes (Davis and William, 1999). Therefore, attempt to select resistant trait, while maintaining desirable agronomic characteristics, have slowed down due to failure to identify expressed genes and proteins involved in resistance. Therefore, present approach is to elucidate the biochemical mechanisms that confer resistance in corn kernels that are vulnerable to aflatoxin contamination. These mechanisms could be used to enhance germplasm through marker assisted breeding and/or genetic engineering (Brown et al, 1998).

Genetically engineered *A.flavus* with gene construct consisting of GUS ( $\alpha$ -glucuronidase) or Green Fluorescent Protein (GFP) reporter gene linked to an *A. flavus*  $\alpha$ -tubulin gene promoter for monitoring fungal growth (Huang et al, 1997; Brown et al, 2003) or with the reporter gene linked to an aflatoxin biosynthetic pathway gene, could provide a quick and economical way to indirectly measure aflatoxin levels. It is now possible to accurately assess fungal infection levels and to predict the corresponding aflatoxin levels in the same kernels as a result of fungal infection. In this way, it is now possible to determine whether kernel resistance mechanisms are affecting fungal growth or aflatoxin biosynthesis. Chromosomal regions with resistance to *A. flavus* and inhibition of aflatoxin production in corn have been identified through Restriction Fragment Length Polymorphism (RFLP) (White et al, 1998). However, limiting the growth of aflatoxigenic fungi might at times not be enough to maintain aflatoxins "at acceptable" levels in corn crops.

Therefore, identification of compounds (or plant constituents) that disrupt aflatoxin biosynthesis or fungal growth and their use in new biochemical marker - based breeding strategies to enhance resistance in crops to aflatoxins, could potentially save the agricultural industries thousands of millions of dollars. Studies of the genetics of aflatoxin biosynthetic pathway for understanding how and why this fungus makes aflatoxin have enabled scientists to examine strategies to interrupt aflatoxin synthesis, thereby, preventing aflatoxin contamination of crops. The fungal genome of *A.flavus* has been sequenced to understand the regulation of aflatoxin formation by environmental factors (Bhatnagar, 2010). This information is being used to assist in the development of host resistance against aflatoxin contamination by studying the effect of various physiological parameters eg- drought stress on gene expression in toxigenic fungi.

#### Resistance Associated Protein (RAP)

Two kernel proteins, one 28 KDa and inhibitory to *A.flavus* growth, the other over 100 KDa in size and primarily inhibitory to toxin formation were identified from resistant corn inbred (Huang et al, 1997). Chen et al. (1998) observed kernel protein profiles of 13 corn genotype which revealed a constitutively expressed 14 KDa Trypsin Inhibitor Protein (TI), present in relatively high concentrations in seven resistant corn lines, but at low concentration or absent in six susceptible lines. The mode of action of TI against fungal growth may be partially due to inhibition of fungal amylase, limiting *A.flavus* growth but also for toxin production (Woloshuk et al, 1997). TI also demonstrated antifungal activity against other mycotoxigenic species. The identification of these proteins and their corresponding genes may provide markers for plant breeders and facilitate the introduction of antifungal genes through genetic engineering into other aflatoxin susceptible crops.

Using 2-D gel electrophoresis, several resistant and susceptible genotypes were compared and over a dozen such protein spots, either unique or 5 fold upregulated in resistant lines, were identified and isolated.

These proteins were grouped into three categories based on their peptide sequence homology viz. storage, proteins (eg- Globulins - GLB<sub>1</sub>, GLB<sub>2</sub>; and late embryogenesis proteins) stress responsible proteins (Aldose reductase, Glyoxylase 1 and heat shock protein), antifungal protein (eg- TI). Further studies are necessitated in relation to physiological and biochemical characterization. In addition, researches in genetic mapping, plant transformation using resistance associated protein gene (RAP gene), gene silencing experiments and marker associated breeding elucidating the role of stress related RAP in kernel are under progress in order to develop preharvest preventive control.

#### Biocompetitive agent

Microbes have been suggested as an agent of control of mycotoxin contamination. *Aspergillus niger* when cultured with *A.flavus* on maize substrates suppressed aflatoxin production by lowering the pH of the substrates. Choudhary (1992) has reported that *Fusarium*, *Trichoderma*, and *Rhizopus* sp. co-inhabiting maize kernels were responsible for inhibiting aflatoxin production by *A.flavus*. Reduction in aflatoxin contamination of cotton seeds has been achieved by application of a non-aflatoxigenic strain of *A. flavus* to soil around developing cotton plants (Cotty, 1992) and patent was sanctioned (US Patent Number 5, 171, 686) for the use of non-aflatoxigenic *A. flavus*.

Mycoparasites of plant pathogens, *Trichoderma* spp., has been accepted as most potent biological control agent for certain plant diseases. Its mycoparasitism involves a complimentary action of antibiosis, nutrient competition and cell-wall degradation enzyme such as  $\alpha$ -1-3-Glucanase, proteases and chitinases (Sinha and Choudhary, 2008). Since chitin is a major component of most fungal cell-walls, has been attributed to its chitinase biocontrol activity of *Trichoderma* (El-Kantatny et al, 2001). Several hydrolases of *Trichoderma* were recently identified and purified. Some of the genes coding them were cloned and sequenced and transformant were obtained, which

confirmed that over production of single protease or chitinase resulted in better biocontrol agent. *T. viride* was found to inhibit the production of aflatoxin B<sub>1</sub> (73.5%) and aflatoxin G<sub>1</sub> (100%) when cultured with *A.flavus* (Bilgrami and Choudhary, 1998).

Microbes, *Bacillus pumilus* is also reported to inhibit the growth and aflatoxin production by *A. flavus* to the extent of 99.2%. An active compound being produced by *B. pumilus* was identified (Sinha and Choudhary, 2008).

*Exophiala spinifera*, *Rhizocardiella atrouirens* (Black yeast fungi) and Gram Negative bacterium (*Caulobacter* spp.) isolated from mouldy maize kernels have been found to extensively metabolize fumonisins to CO<sub>2</sub>. These microorganisms produce fumonisins catabolizing enzymes, such as esterase, which lead to the formation of hydrolysed fumonisin B<sub>1</sub> (AP<sub>1</sub>) plus tricarboxylic acid. Fumonisin esterase enzymes were expressed in transgenic maize plants. Lower levels of fumonisin B<sub>1</sub> accompanied by an accumulation of AP<sub>1</sub> were observed in kernels of transgenic maize plants as compared to conventional plants. Levels of fumonisins in transgenic maize hybrids with kernel expression of insecticidal *Bacillus thuringiensis* protein, cry A(b) were lower as compared to conventional maize hybrids.

In case of deoxynivalenol of (DON) 12, 13 epoxide ring is essential for the toxicity and removal of this ring results in a significant loss of toxicity. Intestinal microflora are capable to detoxifying DON by enzymatic reduction of epoxide ring resulting in the metabolite DOM-1, that is known to be non-toxic. In an another investigation *Agrobacterium* - *Rhizobium* group bacteria, isolated from soil was found to transform 70% of DON to 3 - keto - DON after a day incubation.

Different micro-organisms (bacteria, fungi and yeast) are reported to convert Zearalenone (ZEA) to  $\alpha$  and  $\beta$  - zearalenol. However, Oestrogenicity of these metabolites are similar to ZEA and thereby this transformation cannot be regarded as detoxification.

Micro-organisms like *Acinetobacter calcoaceticus*, *Phenylobacterium immobile* and a

non toxigenic strain of *Aspergillus niger* are reported to convert ochratoxin A (OTA) to less toxic  $\alpha$ -OTA.

### Other methods

Green Fluorescent Protein (GFP) is being utilized to monitor gene expression and food colonization by *Aspergillus flavus* (Brown et al, 2003). These studies demonstrated that the modified GFP encoded by pNucEm<sup>2</sup> and pGAP<sup>33</sup> was highly expressed in *A.flavus*. The intensity of fluorescence is sufficient to allow the visualization of a GFP- containing strain under a standard laboratory UV-light. The use of these constructs in strains of *A.flavus* could facilitate the detection of the fungus in substrates such as soils or foods and can be useful in screening corn genotype for resistance to aflatoxin accumulation and making screening faster and more commercial.

Aqueous plant extracts of cinnamon, peppermint, basil, origanum, epizote, clove and thyme caused total inhibition of fungal development on maize kernels and optimal dosage varies from 3 to 8 per cent. *O.methoxycynamaldehyde* from cinnamon has been reported to be highly effective against *A.flavus* and *A.parasiticus*. Bilgrami et al (1992) have reported inhibition of mycelial growth and aflatoxin production on maize grains when treated with onion, garlic and eugenol. Caffeine appears to inhibit aflatoxin synthesis by restricting the uptake of carbohydrate, which are ultimately used by the mould to synthesize aflatoxins. Capsanthin of *Capsicum annum* (Red chilli) was also reported to check growth and toxin production at all tested concentrations. Phenolics like tannic acid, caffeic acid and phloroglucinol at 0.01 M concentration prevented aflatoxin production by more than 55%. Bankole and Joda (2004) observed efficacy of lemongrass (*Cymbopogon citrates*) powder and essential oil on *A.flavus* growth and aflatoxin contamination. Recently, components of *Eucalyptus* oil limonene and geranial have confirmed the antifungal activity (Adegoke et al, 2000; Lee et al, 2008; Mengai et al, 2010).

### Post harvest control of mycotoxins

Once crop becomes infected under field conditions, the fungal growth continues usually with increasing vigour at post harvest stage and in storage. Most fungi cannot grow below a critical moisture level. *A.flavus* cannot grow in corn with moisture content less than 12 to 13 per cent. If corn is dried below this level no additional growth of fungus or production of aflatoxin will occur if proper storage practices are followed. In addition, fungus can survive in residues left in storage and feeding facilities and thereby, produce mycotoxins under such conditions. Food and feed residues should be discarded soon and storage and feeding facilities should be decontaminated. Periodical evaluation of storage suitability be monitored with the help of CO<sub>2</sub> - sensor.

However, in most of the cases preharvest and post harvest preventive strategies have to be supplemented with control strategies viz Elimination and/or Detoxification (inactivation) of mycotoxins.

### CO<sub>2</sub> Sensor

It is essential for grain industry to have effective management programmes to protect against economic loss due to contamination from stored-product insects, moulds and mycotoxins. Manual grain inspection (human sensory exposure) and measuring grain temperature are the main tools used by the farmers and grain industry for monitoring proper storage conditions (Bortosik et al, 2008). Human sensory exposure literally means having personnel "walk" to the grain mass, smell the grain aeration discharge stream and look at the grain. Human sensory exposure for mould spoilage and other quality parameters could be biased and it varies from person to person. These cables are routinely placed in modern grain bin. Unfortunately, a temperature cable will not detect the fungal growth several feet away from the cable until the size of the spoiling grain mass is large enough to raise the temperature around the volume of the temperature cable. These limitations are overcome with CO<sub>2</sub> sensors.

CO<sub>2</sub> sensors can be effectively used to monitor early detection of spoilage during storage (Zagrebenyev et al, 2001; Bortosik et al, 2008; Maier et al, 2010). CO<sub>2</sub> sensors can effectively be used to detect stored product insect infestation and grain spoilage due to mould infections well before spoilage detection by traditional methods such as visual inspection, smell and temperature cables. Such an early warning system would provide more timely information to farmers to make correct management decision to avoid the cost of spoilage mitigation measures such as turning, aeration and fumigation. This would help in continuing to store grain or market it early to avoid further quality deterioration.

### Elimination of mycotoxins from contaminated food/feed

Mycotoxins in contaminated commodities usually reside in relatively small number of seeds or kernels. Those seeds or kernels can be removed following one of these methods -

### Physical separation

The principle of this method is based on the identification of damaged kernels in the seed lots because of variations in size, shape, colour and also visible mould growth. Aflatoxin contaminated kernels are usually damaged, shriveled or discoloured (Natrajan et al, 1975). Significant amount of aflatoxins in shelled peanuts can be removed by electronic sorting and hand picking (Dickens & Whitaker, 1975).

However, complete removal of all contaminated particles or aflatoxin cannot be expected with physical methods of separation. Since the toxin can diffuse into the interior of the kernel, residual contamination may be present at very low levels in the final product. If there is a high level of residual contamination, other procedures must be used to manage the residual contamination in final product.

Similar process based on fluorescence has been suggested. There appears to be good correlation between the presence of aflatoxins and the occurrence of a Greenish-yellowish fluorescence (GYF) in the



contaminated seeds under UV-light (Ashworth et al, 1968). However, this type of separation was not successful in pecans because of inherent intense fluorescence in the kernels (Escher, 1974).

Another approach is through floating and density segregation. It was observed that 95% of the aflatoxin in 21 of 29 samples of peanuts was contained in kernels that floated in tap water (Kirskey et al, 1989). Mean aflatoxin level was decreased from 301 ppb to 20 ppb using floating as a separation mechanism (Phillips et al, 1994). There is still aflatoxin residue though separation has shown to significantly reduce aflatoxin contamination and thereby, can be considered as an effective first line defense for certain products.

Corn screening (based on size and broken corn kernels) usually contains about 10 times the fumonisin content compared to intact corn (Murphy et al, 1993). Significant amount (Ca 25%) of Deoxynivalenol (DON) can also be removed by cleaning and polishing. Apples rotten with *Penicillium expansum* contain patulin, is mostly used for commercial juice production. It has been reported that trimming of apples to remove the rot reduces patulin content by 93% to 99% (Lovett et al, 1975). Physical separation or trimming of apples before processing is the best method to reduce patulin contamination.

### Filtration

Aflatoxin was removed up to 90% through single filtration, but in recirculation of oil, they could achieve even up to 100% removal since aflatoxin in crude peanut oil remains in finely suspended form and can easily be separated by filtration. Basappa and Sreenivasamurthy (1979) at CFTRI, India have developed a special filter pad system which can easily be adopted in oil mills to remove aflatoxin from crude oil. This filter pad can be prepared by impregnating fuller's earth salt slurry in between two filter cloth layers and dried completely at 100°C for eight hours. The pads can be stored under desiccated conditions up to 2 months without loss in activity.

### Milling

In the processed products the levels of aflatoxin vary with the nature of processing, food materials and the affinity or solubility of toxin in the products. Laboratory studies have reported that during wet milling of inoculated corn, aflatoxin B<sub>1</sub> was distributed in the milling fractions viz- it went primarily in steep water (39% to 42%), fibre (30% to 38%), with the remainder found in gluten (13% to 17%), germ (6% to 10%) and starch (only 1%). In a good risk management plan, individual fraction should be considered for further utilization. In another investigation (CFTRI, India) it was found that 85% of the aflatoxins present in groundnut seeds goes into the cake after crushing in expeller oil mill or hydraulic press, and only 15% remained in oil (Basappa & Sreenivasamurthy, 1974).

Other mycotoxins (zearalenone, DON, nivalenol) are also distributed during in wet milling of corn kernels. Recent wet milling laboratory scale studies on naturally contaminated corn have shown that the toxin remains mostly in gluten while the germ remains free of the toxin. The starch fractions which account for 65% to 71% of milled products were free of detectable zearalenone.

Additional chemicals can be added to the steeping solution so that the modified process would be used to ensure the safety of the final product.

The concentration of toxin in a particular fraction simplifies the process of risk management.

In dry milling of naturally contaminated corn, the highest levels of aflatoxin B<sub>1</sub> occurred in germ and hull fractions, but distribution varied with the contamination level. In artificially contaminated rice, aflatoxin was greatly reduced by milling, with more than 95% in bran and polish fraction (Achroder et al, 1968). In drum wheat, aflatoxin B<sub>1</sub> was determined to be in peripheral parts of the kernels. Upon milling, the aflatoxin concentration in the flour varied according to the quality of the final product. Dry cleaning of the zearalenone naturally contaminated corn does not significantly reduce toxin levels in corn lot.

On industrial perspective, combination of cleaning, sorting and milling can significantly reduce the risk of mycotoxins.

### **Solvent extraction procedure**

Extraction of aflatoxin with solvent offers several advantages-

- (a) The aflatoxin can completely be removed under suitable conditions.
- (b) There is little likelihood of forming from the aflatoxins other products having adverse physiological activity.
- (c) Extraction can be carried out and the solvent can be recovered without nutritional loss in many cases.

The disadvantages are -

- (a) Special solvent extraction equipment may be required.
- (b) The extraction of some of the soluble components with aflatoxins.
- (c) The added cost of traditional processing.
- (d) The possible introduction of off-flavour.

Solvent able to fit all the criteria, however, is not an easy task. Other factors become involved like cost of solvent, percentage of recovery, toxic affect of solvent and its residues, and from safety point of view the inflammability, explosiveness and boiling point, may also be taken into considerations.

Aflatoxin is soluble in polar solvent such as methanol and is insoluble in water and petroleum hydrocarbons. The extraction methods used in analysis of aflatoxins employ chloroform/water and acetone/water to remove the mycotoxins. Aqueous isopropanol has been found to be an effective solvent for removal of aflatoxins from both contaminated cottonseed and groundnuts. Six extractions with 80% aqueous isopropanol at 60°C resulted in complete removal of aflatoxin in both meals. A binary solvent system of 90% acetone and 10% water (by weight) reduced the aflatoxin content of pre pressed cottonseed and groundnut meal to less than 10µg/kg in small scale batch extractions and less than 40 µg/kg in continuous plant extractions.

Removal of all aflatoxins from peanut meal by an aqueous solution of calcium chloride has also been reported (Sreenivasamurthy et al, 1971).

There are, however, many disadvantages inherent with the use of these solvent systems. Aqueous alcohol and aqueous acetone removes 2.8 to 10 % of soluble carbohydrate from groundnut products making such processes uneconomical. There is also a problem of disposal of the toxic extracts. Acetone has also been reported to produce undesirable off-flavours due to mesityl oxide on the detoxified product. High concentrations of iso-propanol are also unsuitable as they cause formation of protein gels.

### **Inactivation of mycotoxins**

When the risk management of aflatoxin through elimination is not possible/successful or as an alternative, we can apply inactivation or detoxification of mycotoxin either through physical or chemical method, which becomes inevitable.

### **Physical methods**

#### **Thermal inactivation**

Thermal inactivation of aflatoxin is achieved by cooking, roasting, frying, spray drying, baking etc. Different rice cooking methods used by Indians were compared and it was found that pressure cooking at 15 psi for 5 minutes gave maximum destruction (72%) of aflatoxin to the method of ordinary cooking (50%) and cooking with excess water (50%) (Rehana et al, 1979). They recommended pressure cooking of rice not only because of destroying maximum amount of aflatoxins but also for preserving nutrients of rice. Microwave roasting destroy aflatoxins almost completely. Unfortunately, this process would involve an increase in processing cost, making it commercially impractical. Thereby, microwave roasting cannot be a good solution.

Aflatoxin B<sub>1</sub> is moderately stable in heated peanut, corn oil and coconut oil. Therefore, frying in unrefined oil could add aflatoxin during processing (Dwarkanath et al, 1975). Fortunately, oils are not of concern because only a small percentage of aflatoxin

B<sub>1</sub> present in oilseed passes into extracted or pressed oil as refining and bleaching operations eliminates it.

Fumonisin are fairly heat stable compounds. It has been reported that the loss of FB<sub>1</sub> and FB<sub>2</sub> is more rapid and extensive in alkaline or acidic environments than at neutral pH. Thermal processing operations such as boiling or retorting, which occur at temperatures < 125°C, have little effect on fumonisin content of food, however, foods that reach temperature > 150°C during processing may have significant losses of fumonisins (Scott and Lawrence, 1994). Some investigations revealed that in thermal decomposition of fumonisins the toxic potential is not necessarily eliminated. Ochratoxin A appears to be more readily destroyed in dry cereal than in the presence of water (unlike aflatoxin B<sub>1</sub> and patulin). This was also evident in a study reported by Osborne (1979), where ochratoxin A was not degraded during bread making but 62% was lost after baking of biscuits, which have lower water content. DON has proven to be more heat - stable during food processing than any other mycotoxin tested.

#### **Inactivation through light / irradiation**

Aflatoxin destroy in chloroform solution to the extent of 99% when exposed to UV - light. Before the degradation of the aflatoxin B<sub>1</sub> molecule, photodimerization of the coumarin moiety takes place. Exposure to sunlight of aflatoxin contaminated vegetable oils, revealed encouraging results. Unrefined groundnut oil containing aflatoxin (> 100 mg/kg) was exposed to bright sunlight, gas filled tungsten lamp or longwave UV light (Santha and Sreenivasamurthy, 1977). Sunlight destroyed 99% of the aflatoxin present in 15 min, whereas, tungsten lamp light and ultraviolet light destroyed 82% to 85% of aflatoxin in 18 hours and 30% to 40% of aflatoxin in 2 hours, respectively.

Complete destruction of aflatoxin was also obtained when aflatoxin contaminated peanut oil kept in glass containers was exposed to direct sun light (50,000 Lux) for one hour (Santha and Sreenivasamurthy, 1980). Photodestructed aflatoxin was not

regenerated in the oil during storage for six months in dark.

Santamarina et al, (1995) concluded that gamma irradiation in combination with other methods could be employed to achieve removal of mycotoxins. A significant reduction was recorded in the levels of T-2, DON and zearalenone at doses above 7.5 KGy (Hooshand and Klopfenstein, 1995). However, significant losses in the levels of some of the essential aminoacids were also observed due to this irradiation. Detoxification at the level of 70% to 90% of some trichothecenes has also been observed in the contaminated corn by applying ultra sonication (Lindner and Hasenhurti, 1996).

#### **Chemical methods**

Various chemicals viz. chlorine, ozone, hydrochloric acid, benzoyl peroxide, ammonia, sodium hypochloride and ethanolamine have shown to destroy pure aflatoxin or aflatoxin in contaminated materials. However, the detoxification process must satisfy following criteria:

- (i) It must destroy or detoxify the aflatoxin.
- (ii) It must not produce or leave any toxic or carcinogenic residues in the final product.
- (iii) It should destroy fungal spores and mycelium as under suitable conditions they could grow and recontaminate the product.
- (iv) It should preserve, as far as possible, the nutritive value and palatability of the starting material.
- (v) It should not alter significantly the important technological properties of the starting material.

Unfortunately, many of the chemicals either decrease significantly the nutritive value or produce toxic products or have undesirable side effects.

Ammoniation of corn, peanuts, cottonseed and meals to alter the toxic and carcinogenic effects of aflatoxin contamination has been intensively investigated. Efficacy and safety of ammoniation as a practical solution to

aflatoxin decontamination in animal feeds have been demonstrated. Use of ammonia treatment to reduce risks posed by aflatoxin contamination, particularly in the high temperature / high pressure treatment has been recommended. With the ambient temperature / atmospheric pressure method, caution must be exercised to assure even distribution of ammonia gas / water or aqueous ammonia solution in the product. This process is limited to whole kernel seed / nut products and has not been successful for ground or meal products due to inability of ammonia to penetrate commodity. For another toxin, fumonisins, Park and co-workers (1992) found that atmospheric pressure/ambient temperature ammoniation reduced fumonisin content of *F.moniliforme* but did not reduce the toxicity of material when fed to animals.

Traditional alkaline heat treatment (Nixtamalization) of corn used in the manufacture of tortillas reduces significantly the levels of aflatoxin (Ulloa and Herrera, 1970). Hondrich and co-workers (1993) showed that nixtamalization greatly reduces FB<sub>1</sub> content. However, when nixtamalized corn was fed to weaning rats, the toxicity of the inoculated corn was not reduced. Modified 'nixtamalization' procedure involves the addition of hydrogen peroxide and sodium bicarbonate to traditional process. Results from this study show 40% reduction of brine shrimp mortality compared to traditional nixtamalization.

In oil seed meal oxidative destruction of aflatoxin by ozone has also been considered as a practical method (Dwarkanath et al, 1968). Total aflatoxin B<sub>1</sub> was found to be inactivated after incubation with ozone for two hours at 100°C in cottonseed and peanut meals having 22 per cent and 30 per cent moisture. Since ozone reacts only with the olefinic bonds of B<sub>1</sub> and G<sub>1</sub>, its usefulness as detoxifying agent is reduced in later stages (Lillehoj et al, 1987). Sodium bisulfite has also shown to form water soluble products after reaction with major aflatoxins viz. B<sub>1</sub>, G<sub>1</sub>, M<sub>1</sub> and aflatoxicol under various experimental conditions (Doyle and Marth, 1978). More than 45% reduction in the level of aflatoxin M<sub>1</sub> has been recorded due to

addition of 0.04 g potassium bisulfite per 10 ml milk (Doyle et al, 1982). Sodium chloride has a marked influence on loss of aflatoxin in artificially contaminated unshelled peanuts following cooking in water in a pressure cooker for 0.5 h. Loss in concentration was 80 per cent to 100 percent with 5 per cent sodium chloride but only 35 per cent without the salt (Scott, 1984).

Hydrogen peroxide in different concentrations (3% , 5 % and 10%) has its efficacy in destruction of zearalenone in contaminated corn (Abdalla, 1996) However, the percentage of destruction was dependent on the concentration of hydrogen peroxide, temperature, period of exposure, maximum destruction was recorded when the contaminated corn was treated with 10% hydrogen peroxide at 80°C for 16 hours. Calcium hydroxide monomethylamine effectively decontaminated feed containing T2 toxin and zearalenone. Patulin and penicillic acid are moderately stable in apple juice with a half-life of several weeks (Pohland and Allen, 1970). The rate of disappearance of these toxin increases markedly with the addition of ascorbate or a mixture of ascorbate and ascorbic acid to juice.

Concern about chemicals and toxic residues in the food chain is a matter of great concern. Processing of food not only alters the food but also adds new conditions and ingredients. For risk management there is a need to explore the application of new food additives to control mycotoxins as an integral part of the process.

## 5. Conclusion

Complete elimination of mycotoxin contamination seems to be practically impossible. However, risks associated with mycotoxin contaminated commodities can be reduced by following an integrated mycotoxin prevention and control management. The most effective and practical procedure include good cultural practices, use of resistant crops (developed through RAP or new biotechnological processes), biological control, physical removal of damaged or incomplete kernels/seeds, chemical inactivation such as

ammoniation procedure and use of additional chemical agent normally used in industrial processes (nixtamalization). However, more information is needed to develop decontamination treatments, also to determine the safety of the final processed products and also the prevention of recontamination during storage.

Contaminated grains and feeds may contain a wide variety of different mycotoxins of differing chemical characteristics including heat stability, solubility, and absorbent affinity. Human exposure to the contaminants should be considered without affecting the marketability of the product i.e. processors and consumers risk. Risk of mycotoxin contamination in food exists from the crop grown in the field until the final product is consumed. One of the approaches for reducing the levels of mycotoxins in food supply can be to encourage the diversion of moulds and contaminated grains to non-food use or processing industries which recover one or more mycotoxin free products. In this case also we have to consider the relationship between concentration of mycotoxins in the ration fed to meat, dairy animals and poultry and the concentration of mycotoxins or its metabolites that appear as residues in muscle, adipose and tissue organs, milk or eggs.

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