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REVIEW

Aspergilllus and mycotoxin contamination of stored food grains -a review

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Deterioration of the quality and quantity of the food materials is a major problem in storage systems .Food security is a major challenge for developing countries. Mycotoxins are secondary metabolites produced by filamentous fungi that have deleterious effects on human and animal consumers. Mycotoxins are structurally diverse, deriving from a number of biosynthetic pathways and their effect upon consumers is equally diverse ranging from acutely toxic to immunosuppressive or carcinogenic. Aflatoxins are probably the most significant mycotoxins worldwide, produced by several members of the *Aspergillus* section Flavi. Some strains of *A. flavus* and most strains of A. *parasiticus* and *A. nomius* produce aflatoxins with *A. parasiticus* and *A. flavus* being the two most agriculturally important species. This paper provides a brief review of sources of aflatoxin, factors promoting food contamination, occurrence of aflatoxins, novel approaches and technologies for aflatoxin analysis and detection, effects and consequences of contamination, and detoxication and control of aflatoxins. Further detection of toxin producing molds in food and feed are being given serious consideration. This review also focuses on methods for detection of fungi and mycotoxins in agriculture products in Kashmir.

Key words: Mycotoxins, aflatoxins, *Aspergillus* species, detoxification, natural compounds

INTRODUCTION

Post-harvest spoilage by filamentous fungi is one of the most important threats associated with processed and stored food products worldwide. Discoloration, quality deterioration, reduction incommercial value and mycotoxin production has been linked to moldy contaminated foods.

This situation is made worse in the tropics where the warm and humid climates provide these micro organisms with favorable conditions for their spread and subsequent establishment in numerous substrates. Three genera viz. *Fusarium, Penicillium* and *Aspergillus* which are all potential mycotoxin producers, could be considered the most significant toxigenic fungi growing in processed and stored foods. Fungal deterioration of stored seeds and grains is a chronic problem in the Indian storage system because of the tropical hot and humid climate. Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins. There is a general increase in consumption of contaminated grain with mycotoxins which causes different health problems including death.

Mycotoxins are relatively high molecular weight fungal metabolites that contain one or more oxygenated alicyclic rings. The mycotoxins of the greatest significance in foods and feeds are aflatoxins which have carcinogenic, mutagenic, teratogenic and immunosuppressive effects on the health of humans and animals(Jouany *et al*., 2005) of these mycotoxins, aflatoxins are the most lethal ones and composed of approximately twenty fungal metabolites secreted by *Aspergillus flavus*, *A. parasiticus*, *A. mominus* and *A. pseudotamarii*. The major aflatoxins are known as B1, B2, G1, and G2 (Banu and Mathumary, 2010; Liu and Wu, 2010). Aflatoxins are the most potent natural carcinogenic mycotoxins and they have been linked with a higher preva

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lence of hepatocellular cancer in Africa (Strosnider *et al*., 2006). There is a very high risk of Hepatitis B and Hepatitis C carriers to develop liver cancer when they are exposed to aflatoxin. There have been recent outbreaks of acute aflatoxicosis in Kenya (Probst *et al*., 2007), but chronic exposure to aflatoxins has much wider health effects than these rare acute poisonings (Williams *et al*., 2004). Aflatoxins are highly toxic and children in areas of high aflatoxin exposure have been found to have stunted growth (Gong *et al*., 2004). Prolonged intake of moderate to low concentrations of these mycotoxins may result in serious problems. There are more of these mycotoxins in food items but concentrations beyond that risk the human health. Kashmir being the main producer of walnuts, almonds, saffron etc. in India and with temperate climate of the region being favourable for the growth of the aflatoxigenic fungi the stored food items are subjected to fungal damage.Thus the population of the region are more subjected to the such toxins leading to health related problems.

SOURCE OF AFLATOXINS

Mycotoxins are toxic metabolites of fungi which, if ingested, can cause acute or chronic toxic effects such as carcinogenic, mutagenic, teratogenic, atherogenic and oestrogenic effects in humans and animals.The illnesses caused by mycotoxins are called mycotoxicosis. The mycotoxins are not only hazardous to consumer health but also affect food quality resulting in huge economic losses. Of these mycotoxins, aflatoxins are the most lethal ones. Most of the aflatoxin producing fungal species infecting the stored food grains mainly belong to the genus *Aspergillus*. The genus *Aspergillus*, a member of the phylum Ascomycota, includes over 185 known species. Several members of Aspergillus section Flavi produce aflatoxin. These includes, *Aspergillus flavus* and *Aspergillus parasiticus*, as well as several less common taxa including *Aspergillus nomius, A. tamarii, A. pseudotamarii, A. minisclerotigenes* and *A. bombycis*. Most of these crops are cereals, fruits, vegetables and oil seeds that are highly susceptible to fungal growth and mycotoxin production.The general list of various fungal species producing mycotoxins is given below. The most potent of the four naturally occurring aflatoxins is aflatoxin B1 (AFB1) which is highly toxic, mutagenic and hepatocarcinogenic secondary metabolites (El-Nagerabi *et al*., 2012). Aflatoxins have been detected in cereal grains, oil seeds, fermented beverages made from grains, milk, cheese, meat, nut products, fruit juice and numerous of the agricultural commodities. Experiments conducted on the contaminated Brazilian peanut meal resulted in the isolation of *A. flavus*, and when the fungus was inoculated into untainted peanut meal, the fungus produced toxins similar to those found in the contaminated meal. The numbers of microorganisms on most dried fruits vary from a few hundred per gram of fruits to thousands and they are mostly on the outer surfaces. Spores of bacteria and molds are likely to be the most numerous. In 1990, post harvest losses in the United States were estimated to be $500 million per year. The United States estimates that in developed countries the average minimum overall losses from biological degradation are 10%, while in developing countries that estimate may be up to 20%. High environmental temperatures and moisture, along with dockage and broken kernels, provide conditions that accelerate mold and insect development within the grain mass, increasing grain losses.

OCCURRENCE AND FACTORS PROMOTING AFLATOXIN CONTAMINATION

Fungi which grow and produce toxins in grains during storage are influenced by several factors. Some of this factors are related to; inadequate moisture and temperature, combined with long residence time in warehouses, which are stressful situations and originate toxigenic potential outbreak (Dilkin, 2002). Growth of *Aspergillus* species, spore and/or toxins production is affected by temperature, pH, water activity (aw) availability of air, and nutritional factors. The most important factors that help predict the occurrence of aflatoxins in food include weather conditions (temperature and atmospheric humidity), agricultural practices (crop rotation and soil cultivation) and internal factors of the food chain (drying and storage conditions).

Due to the great health concern in relation to aflatoxin contaminated food ingestion, studies are being conducted worldwide to verify the occurrence of aflatoxins. The main food products susceptible to fungal growth and consequently to mycotoxins' production include: peanuts (raw, roasted, sweet and infrosted), corn (popcorn, hominy and grains), wheat, rice, nut, walnuts, hazelnuts, cashews, almonds, dried fruits, spices, cotton seed, cassava, vegetable oils, cocoa and others that are normally used in the composition of foods and feeds.

The occurrence of AFB1, B2, G1 and G2 in animal feeds and ingredients, comprising corn, soybean meal, mixed meal, sunflower, wheat, canola, palm kernel, copra meals was verified by Khayoon *et al*. (2010). The results showed that 19% of the tested samples were contaminated with aflatoxins. Reiter *et al*. (2010) evaluated eighty-one rice samples purchased from different markets. The results revealed that AFB1 could be quantified in 15 samples and AFB2 (1.5 μg kg–1) in one sample. Dors *et al*. (2011) conducted survey of mycotoxins in parboiled and whole rice. From the samples analyzed, 9% were contaminated with AFB1 in levels ranging from 11 to 74 μg kg–1. Aflatoxin and ochratoxin A migration during rice parboiling process under different conditions of soaking, autoclaving and drying was studied. It was noted that there was mycotoxin migration from the husk to the starchy endosperm in the following proportions: 32% AFB1, 44% AFB2, 36% AFG1 and 22% AFG2.

Ramos *et al*. (2008) detected the presence of *Aspergillus* spp. and aflatoxin contamination among grain samples and the result were correlated with the greatest amount of rain during harvest. Levels of contamination ranged from “not detected” (nd) to 277.8 μg kg^{-1},for AFB1; from 0.7 to 14 μg kg–1for AFB2; and from nd to 34.1 μg kg–1for AFG2 Aflatoxin contamination in 70% of maize samples from criollo varieties, which have not undergone genetic intervention, at levels ranging from 1 to 2.6 μg kg^{-1},was found by Oliveira *et al*. (2010). In Kashmir the temperate climate poor storage conditions, poor financial conditions of farmers etc. together count for the fungal damage of the stored food items.

SEPARATION AND DETECTION OF AFLATOXINS

Different techniques have been found for the determination of aflatoxins in the last few years. Aflatoxins separation has been performed for many years by HPLC, using mainly reversed phase columns, with mobile phases composed of water, methanol and acetonitrile mixtures.

Chromatographic performance has improved with column technology, particularly with reduced size of the column packing material (Shephard, 2009). The AFs are named due to their properties under UV-irradiation, where AFB1 and AFB2 emit blue fluorescence (350 nm), AFG1 and AFG2 green fluorescence (350 nm). These important features can be used for rapid identification and detection (Reiter *et al*., 2009).

The coupling of HPLC to mass spectrometry is the more commonly employed detection technique in the last years. The ionization sources employed based on atmospheric pressure ionization techniques such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) has resulted in a range of new methods (Sulyok *et al*., 2007; Beltrán *et al*., 2011). The advantages of LC-MS techniques lie in the improved detection limits, the confirmation provided by mass spectral fragmentation and the ability to filter out by mass any impurities that interfere in spectrophotometric detectors.

The application of aflatoxin-specific antibodies has produced a range of immunoassay analytical methods (Acharya and Dhar, 2008). A number of commercial enzyme-linked immunosorbent assays (ELISAs) are well established and available. The essential principle of these assays is the immobilization on a suitable surface of antibody or antigen and the establishment of a competitive process involving this resource and components of the analytical solution (Shepard, 2009). Piermarini et al. (2009) developed a method, called ELIME-array (Enzyme-Linked-Immuno-Magnetic-Electrochemical-array) for the determination of AFB1 in corn samples. In order to determine AFB1 at a level of regulatory relevance, a sample treatment that employs extraction, cleanup and concentration steps, was selected. The recovery of the ELIME-array was calculated by analyzing replicates of four certificate reference materials (CRMs). Analytical approaches for screening and quantification of aflatoxins are given below (Fig.1). Analysing the presence of aflatoxins is the major criteria in food safety. Various methods adopted for their detection are broadly classified into two type mainly cultural and analytical methods.

HEALTH EFFECTS OF AFLATOXIN CONTAMINATION

A sizeable portion of the world population living below poverty line in the developing and underdeveloped countries of Asia and Africa are suffering from health problems associated with consuming mycotoxin contaminated grains and cereals (ref needed on the cases linking the mycotoxins and other health factors. It is estimated that about 35%

of human cancers are directly related to diet, and the presence of aflatoxins in foods is considered an important factor in the formation of liver cancer, mainly in tropical countries. Aflatoxins pose serious health concerns to animals as well as human beings. Malabsorption syndrome and decreased bone strength have been associated with aflatoxin consumption. The overall toxicity of aflatoxin in an animal appears to be determined by the rate of formation of the reactive intermediate, its binding to the largest macromolecules (DNA, RNA) and the rate of detoxification and other competing reactions.These toxins have been incriminated as the cause of high mortality in livestock and in some cases of death in human beings (Murjani, 2003). Aflatoxin B1 is known to be the most significant form that causes serious risk to animals and human health. The carcinogenic effect of aflatoxin B1 has been studied in fishes such as; salmonid, rainbow trout, channel catfish, tilapia, guppy and Indian major carps carps and *Penaeus monodon*. There is little on the effect of aflatoxin on Nile tilapia *Oreochromis niloticus*.

The poisoning occurs as a result of ingesting foods containing AFs, and has been identified in two forms: either as acute or severe intoxication, resulting in direct liver damage, or chronic subsymptomatic exposure, which could result in a range of consequences such as; acute illness, nutritional immunologic consequence, and an accumulative effect on the risk of liver cancer.

DETOXIFICATION AND CONTROL

The reduction of population exposure to aflatoxin, and the consequent reduction of health risks will only be possible with a job with the food producers and efficient actions of sanitary vigilance. Aflatoxins can be detoxified or removed from contaminated food and nutrients by physical, chemical or biological methods. The inactivation of these compounds by physical and chemical methods have not proved to be effective and economically viable (**Mishra and Das, 2003**). However, biological degradation offers an attractive alternative to eliminate these toxins retaining food nutritional value.

The control of fungi and of aflatoxin biosynthesis is extremely important for agriculture and public health. To overcome these problems, the usual practice is to fumigate or treat the stored commodities using different synthetic preservatives. However, none of these methods has solved the problem. The increase of demand for safe and organic food, without chemical preservatives, incites many researchers to investigate the antimicrobial effects of natural compounds. Many strategies are taken intending to prevent fungal growth and further mycotoxin production and food contamination, including chemical, physical or biological treatments which require sophisticated equipment and expensive chemicals or reagents (Reddy *et al.,* 2010). The use of natural plant extracts provides an opportunity to avoid chemical preservation, thus the search for new antifungal material natural sources for food preservation has increased (Soliman and Badea, 2002).

The first written report of medicinal use of plants was discovered at Mesopotamia and dated from about 2600 B.C (Cragg and Newman, 2005). In recent years a lot of efforts have been employed to identify novel molecules derived from natural sources that exhibit a wide range of clinical and pharmacological activities. This led to an extensive research on organic substances synthesized by various plants and microorganisms growing in diverse habitats. Thus popular medicinal plants that have an ethno-botanical history should be screened for active extracts against aflotoxins produced by various *Aspergillus* species.

Plants have been used as a remedy since ancient times. The natural plant extracts may provide an alternative way to prevent fungal contamination of food or feed. Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides (**Zhang *et al*., 2005**). Recently, the antimicrobial activity of some higher plant products that are biodegradable and safe to human health (Kumar *et al*., 2008) has attracted the attention of microbiologists in the control of plant disease, but the actual use of these products for the control of post harvest pathogens of fruits generally, and in particular for citrus pathogens is, however, still limited.

CONCLUSION

Chronic consumption of aflatoxin-contaminated foods is a common problem in both humans and animals worldwide especially in poor developing nations of south East Asia and sub-Saharan Africa. In this regions there are poor handling of food during harvesting, processing and storage of food and

food products thus allowing the growth of mold on them. Aflatoxins are also responsible for the malabsorption of various nutrients thus leading to nutritional deficiencies, impaired immune function, malnutrition and stunted growth and hence the development of kwashiorkor and marasmus in infants. Aflatoxins also can affect almost all the different body systems and hence the health of the affected individuals especially in poor developing nations of south East Asia and sub-saharan Africa where there is poor food harvesting, processing and storage thus allowing the growth of mold on them. With Kashmir being the main producer of walnuts and saffron the chances incidence of aflatoxication are much more.

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