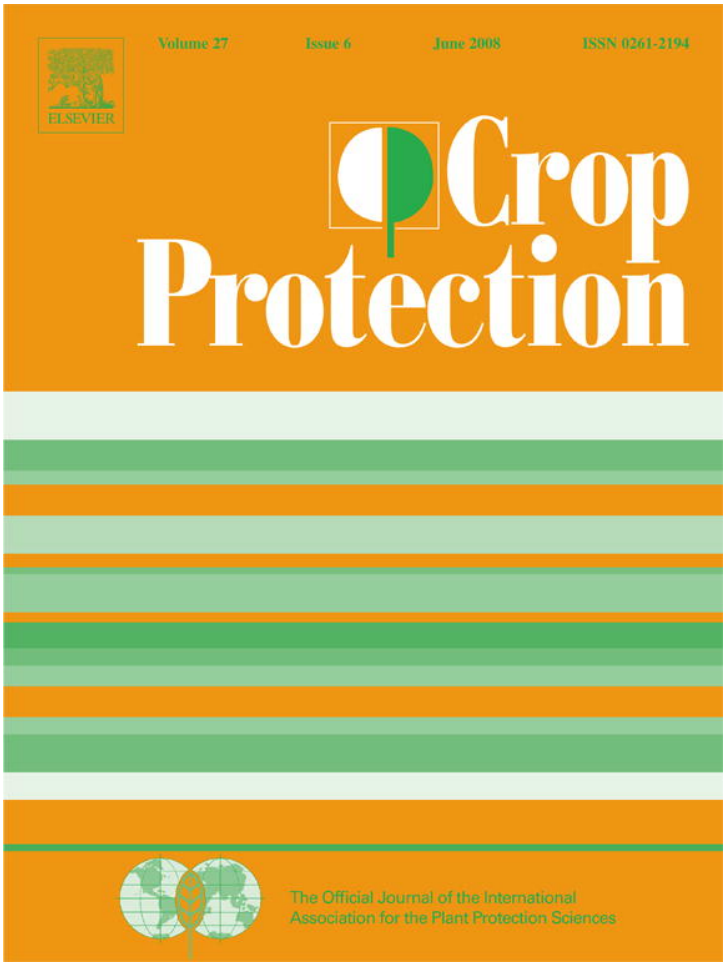

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Review

Mycotoxin research and mycoflora in some commercially important agricultural commodities

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Abstract

Mycotoxin contamination in certain agricultural systems has been a serious concern for human and animal health. Mycotoxins are toxic substances produced mostly as secondary metabolites by fungi that grow on seeds and feed in the field, or in storage. The major mycotoxin-producing fungi are species of *Aspergillus*, *Fusarium* and *Penicillium* and the important mycotoxins are aflatoxins, fumonisins, ochratoxins, cyclopiazonic acid, deoxynivalenol/nivalenol, patulin and zearalenone. The food-borne mycotoxins likely to be of greatest significance for human health in tropical developing countries are aflatoxins and fumonisins. This paper reviews the commodity-wise aetiology and contamination process of the major mycotoxins and the magnitude of contamination in commercially important agricultural commodities. This database would be useful as benchmark information for development and prioritization of future research programmes.

Keywords: Aflatoxins; *Aspergillus flavus*; Fumonisins; *Fusarium verticillioides*; Mycoflora; Mycotoxins

Contents

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1. Introduction

Mycotoxin contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and sub-tropics, where climatic conditions and agricultural and storage practices are conducive to fungal growth and toxin production. Mycotoxins are fungal secondary metabolites identified in many agricultural products screened for toxigenic moulds (Clevsrton and Ljunggren, 1985; CAST, 2003). Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic and dermatitic to a wide range of organisms, and known to cause hepatic carcinoma in man (Wary, 1981; Refai, 1988). There are many such compounds but only a few of them are regularly found in food and animal feedstuffs such as grains and seeds. Nevertheless, those that do occur in food have great significance in the health of humans and livestock. In a normal varied human diet, constant exposure to low levels of several toxins is possible. Very little is known about the effects of long-term low-level exposure, especially with regard to co-contamination with multiple mycotoxins. Since they are produced by fungi, mycotoxins are associated with diseased or mouldy crops, although the visible mould contamination can be superficial. The infection syptomatology of mycotoxin contamination is not obvious like other diseases where visible symptoms on plant parts are produced due to infection. With the increasing stringent regulations for mycotoxins, especially for aflatoxins imposed by the importing countries such as the European Union, the export industry of agricultural commodities is in jeopardy (Otsuki et al., 2001; Felicia, 2004). Over the last two decades various international inquiries on worldwide limits and regulations for mycotoxins were published. A study by the United Nations' Food and Agriculture Organization (FAO) on worldwide regulations for mycotoxins revealed that at least 77 countries now have specific regulations for mycotoxins (FAO, 2004).

The risk of contamination by mycotoxins is an important food safety concern for grains and other field crops. Mycotoxins affecting groundnuts/peanuts, cereals (maize, rice, sorghum, wheat, barley and oats), spices (black pepper, ginger and nutmeg) and chilli are considered to be of greater significance world over for human beings (Goyal, 1989; Bhat and Vasanthi, 2003; CAST, 2003; Bryden, 2007). The exposure to levels of aflatoxins from nanograms to micrograms per day occurs through consumption of maize and peanuts, which are dietary staples in several tropical countries, while fumonisins are found worldwide, primarily in maize and its products and sorghum. Human exposure of fumonisins is highest in regions like the former Transkei, South Africa, where mouldy home-grown maize damaged by insects is often consumed (Bhat and Vasanthi, 2003). The other vulnerable commodities to this menace are copra (dried coconut kernel), cottonseed, peppers and pistachio nuts. An attempt has been made here to review the risks of major mycotoxins, the associated mycoflora and contamination process, the magnitude of contamination, and the current status of research work being carried out worldwide in six high-risk agricultural commodities viz. groundnut, maize, rice, sorghum, spices and chilli.

2. Major mycotoxins

The most important groups of mycotoxins that occur quite often in food are: aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol), zearalenone and fumonisins. T-2 toxin is also found in a variety of grains but its occurrence, to date, is less frequent than the preceding five mycotoxins. The food-borne mycotoxins likely to be of greatest significance for human health in tropical developing countries are the aflatoxins and fumonisins (GASGA, 1997).

2.1. *Aflatoxins*

Aflatoxins are most potent carcinogens in animal and human populations (Squire, 1981) and interfere with the functioning of the immune system (Hsieh, 1988). A wide variety of animals, including fish, rodents, waterfowl, poultry, swine and cattle can be affected by aflatoxins (Robinson et al., 1982; Smith and Moss, 1985; Higgins et al., 1992). In 1993, the International Agency for Research on Cancer (IARC) assessed and classified naturally occurring mixtures of aflatoxins as a class 1 human carcinogen. Aflatoxins B and G found to occur in commodities have been detected in human sera as adducts. Biomonitoring of aflatoxins can be done by analysing for the presence of aflatoxin metabolites, blood protein adducts and DNA adducts (Sabbioni and Sepai, 1994). Residues of aflatoxin B and/or its metabolite, aflatoxin M, can occur in animal products, including milk. Aflatoxin M is also found in human milk if the mother consumes food containing aflatoxin B_1. During 1986, an outbreak of aflatoxicosis due to aflatoxin B_1 in feed occurred in an experimental pig farm in Meghalaya (Ghosh et al., 1988) and in ducklings in Tripura, India (Roy et al., 1989). Boyd et al. (1979) reported that a 20% cauliflower diet in rats significantly reduced serum α-fetoprotein (AFP) and increased hepatic mixed function oxidase activity giving protection against aflatoxin B_1-induced hepatocarcinoma. The addition of cabbage in the diet had similar effect on AFP and the effect on α-fetoprotein occurred long before any identifiable liver lesion could have developed (Boyd and Stoewsand, 1981).

2.2. *Ochratoxins*

Ochratoxin A was discovered as a metabolite of *Aspergillus ochraceus* in 1965 during a large screen of fungal metabolites that was designed specifically to identify new mycotoxins (Van der Merwe et al., 1965). Shortly thereafter, it was isolated from a commercial corn sample

in the United States (Shotwell et al., 1969) and recognized as a potent nephrotoxin. Ochratoxin A is involved in endemic Balkan nephropathy that often is accompanied by upper urinary tract urothelial cancer (Hult et al., 1982; Grollman and Jelaković, 2007). In addition to this, animal studies indicate that ochratoxin A is a liver toxin, an immune suppressant, a potent teratogen, and a carcinogen (Kuiper-Goodman and Scott, 1989; Beardall and Miller, 1994). Ochratoxin A and B are produced by *Aspergillus* species Section Nigri and Circumdati (*A. ochraceus*, *Aspergillus melleus*, *Aspergillus sclerotiorum*, *Aspergillus osteanus*, *Aspergillus alliaceus* and *Aspergillus petrakii*) and *Penicillium* (*Penicillium viridicatum*, *Penicillium palitans*, *Penicillium commune*, *Penicillium cyclopium*, *Penicillium purpurescens* and *Penicillium variabile*). Ochratoxin A has been found in barley, oats, rye, wheat, coffee beans and other plant products, with barley having a particularly high likelihood of contamination. The toxin is also found to occur in groundnut and de-fatted groundnut cake, which is used as animal feed. Several detailed risk assessments have been conducted for ochratoxin A by Kuiper-Goodman and Scott (1989).

2.3. Trichothecenes

The trichothecenes constitute a family of more than 60 sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, *Trichothecium* and others (Cole and Cox, 1981; Scott, 1989). They are commonly found as food and feed contaminants and consumption of these mycotoxins can result in alimentary haemorrhage and vomiting (Joffe, 1986). Diacetoxyscirpenol, deoxynivalenol and T-2 are the best studied of the trichothecenes produced by *Fusarium* species. Deoxynivalenol is one of the most common mycotoxins found in grains. When ingested in high doses by agricultural animals, it causes nausea, vomiting and diarrhoea; at lower doses, pigs and other farm animals exhibit weight loss and food refusal (Rotter et al., 1996). For this reason, deoxynivalenol is sometimes called vomitoxin or food refusal factor. Although less toxic than many other major trichothecenes, it is the most prevalent and is commonly found in barley, corn, rye, safflower seeds, wheat and mixed feeds (Miller et al., 2001).

2.4. Fumonisins

Fumonisins were discovered and characterized in 1988 (Bezuidenhout et al., 1988). At least 15 related fumonisin compounds have been identified of which the fumonisin B (FB) group is predominant. The most abundantly produced member of the family is FB_1. Fumonisins are produced by a number of *Fusarium* species, notably *Fusarium verticillioides* (formerly *Fusarium moniliforme* = *Gibberella fujikuroi*), *Fusarium proliferatum* and *Fusarium nygamai*, as well as *Alternaria alternata* f. sp. *lycopersici* (Marasas et al., 2001; Rheeder et al., 2002). Fumonisins affect animals in different ways by interfering with sphingolipid metabolism (Merrill et al., 2001). They cause leukoencephalomalacia (hole in the head syndrome), a fatal disease in equines (Marasas et al., 1988) and hepatotoxic and carcinogenic effects in rats (Gelderblom et al., 1996). The occurrence of fumonisin B_1 is correlated with the occurrence of a higher incidence of oesophageal cancer in regions of Transkei (South Africa), China and northeast Italy (Peraica et al., 1999). The IARC has evaluated the cancer risk of fumonisins to humans and classified them as group 2B (probably carcinogenic) (Rheeder et al., 2002). The Codex Alimentarius Commission, a joint FAO and the World Health Organization (WHO) committee has produced documents on safety evaluations (WHO, 2001) of mycotoxins and offers advice on various aspects of prevention and control mycotoxins to countries worldwide. No restrictions have been currently placed on food because little is known about the effects of fumonisin on humans. Fumonisins have been found as a very common contaminant of maize-based food and feed in Africa, China, France, Indonesia, Italy, Philippines, South America, Thailand and the USA. Lessard (2003) studied the implication of fusariotoxins (trichothecenes, fumonisins and zearalenone) producing fungi on cereal grain quality and studied the key factors of toxinogenesis.

2.5. Zearalenone

Zearalenone is a non-steroidal oestrogenic mycotoxin produced by several *Fusarium* species (*Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti* and *Fusarium crookwellense*). All these species are regular contaminants of cereal crops worldwide (Hagler et al., 2001). It has been implicated in numerous mycotoxicoses in farm animals, causing infertility, abortion or other breeding problems especially in pigs. In blood, zearalenone and its metabolite, zearalanol bind to human sex hormone-binding globulin to some extent (Kuiper-Goodman et al., 1987; Eriksen and Alexander, 1998). As little as 0.1–5 $mg\,kg^{-1}$ zearalenone in a feed ration may produce oestrogenic syndrome in pigs. Also, uterine prolepses can occur in young pigs at concentrations as low as 1 $mg\,kg^{-1}$. However, there have been no limits or recommended levels for human consumption. It is found worldwide in a number of cereal crops such as maize, barley, oats, wheat, rice and sorghum (Kuiper-Goodman et al., 1987; Tanaka et al., 1988).

2.6. Cyclopiazonic acid

Cyclopiazonic acid (CPA), an indole tetramic acid, is mainly produced by *P. cyclopium* Westling (Holzapfel, 1968). Luk et al. (1977) discovered that isolates of *Aspergillus flavus* are capable of producing CPA, whereas Cole and Cox (1981) reported that species of *Aspergillus* not only produce aflatoxin but also a variety of other toxins

and toxic precursors to aflatoxin including CPA and sterigmatocystin. Cole (1986) suggested that CPA could have had a role in the etiology of 'Turkey X disease'. Ten more species of *Penicillium* and four species of *Aspergillus* viz. *A. flavus*, *Aspergillus versicolor*, *Aspergillus oryzae* and *Aspergillus tamarii* are known to produce this toxin. Analysis of commodities for the presence of CPA has been difficult because of laborious clean-up procedures that extracts require prior to the quantitative step.

2.7. Patulin

Patulin was first isolated as an antimicrobial active principle during the 1940s from *Penicillium patulum* (later called *Penicillium urticae*, now *Penicillium griseofulvum*). A number of early studies were directed towards harnessing its antibiotic activity, however, during 1960s, patulin was reclassified as a mycotoxin. Nowadays *Penicillium expansum*, the blue mould that causes soft rot of apples, pears, cherries and other fruits, is recognized as one of the most common offenders in patulin contamination. Patulin is regularly found in unfermented apple juice, although it does not survive the fermentation into cider products (Trucksess and Tang, 2001). The Joint FAO and WHO Expert Committee on Food Additives has established a provisional maximum tolerable daily intake for patulin of $0.4\,mg\,kg^{-1}$ of body weight per day (Trucksess and Tang, 2001).

3. Commodity-wise aetiology and contamination process

3.1. Groundnut

Aflatoxin contamination of groundnut (*Arachis hypogaea* L.), due to invasion by *A. flavus* and *Aspergillus parasiticus* is a major problem of rainfed agriculture in the semi-arid tropic environment. These fungi are widespread in light sandy soils most suitable for groundnut cultivation. Aflatoxin contamination of groundnut does not affect yield, but it causes serious health risks to human and cattle. Groundnut pods when in direct contact with spores of *A. flavus* in soil are frequently invaded before harvest (Hill et al., 1985; Horn et al., 1995). The mode and extent of invasion by *A. flavus* depends on soil population density of *A. flavus*, soil moisture and soil temperature during the pod development to maturity period. These fungi can invade and produce toxins in groundnut kernels before harvest, during drying and in storage.

Various surveys conducted in different parts of India (Tulpule et al., 1982; Ghewande et al., 1987; Rati and Shanta, 1994; Sharma et al. 1994; Bhat et al., 1996; Prashad et al., 1997; Sinha et al., 1999) have revealed that groundnuts and groundnut products are high-risk commodities for aflatoxin contamination. In three different surveys, conducted during 1965–1970 in Gujarat, Andhra Pradesh and Tamil Nadu states of India, 13–50% of the samples were contaminated with aflatoxins and the level of contamination ranged from 100 to $5000\,\mu g\,kg^{-1}$. Further, the contaminated samples were not uniformly distributed across the locations (Rao et al., 1965; Wagle, 1970). The majority of the groundnut samples collected in Uttar Pradesh (India) was contaminated by aflatoxins (Singh et al., 1982). Patil and Shinde (1985), at Rahuri (India), found 20% of the samples collected from different places contaminated with aflatoxins. Kshemkalyani and Patel (1988) reported that the storage conditions significantly influenced the aflatoxin contamination. Kumar et al. (2001) studied the prevalence of aflatoxin contamination in Tumkur district of Karnataka, India, during 2000 harvest season under the National Agricultural Technology Project, Aflatoxin contamination in groundnut: mapping and management in Gujarat, Andhra Pradesh and adjoining areas. Six villages in three *talukas* of Tumkur district were identified with a relatively higher population density of *A. flavus* and higher seed infection. Waliyar et al. (2003) collected various foods and feed samples including groundnut seed, maize, sorghum, soybean cake, groundnut cake, cotton cake, poultry feed, buffalo milk, cow milk and milk powders from farmers fields, farmers stores, oil millers storage, traders storage, retail shops and supermarkets. More than 2000 samples were analysed by ELISA and most of the commodities, with the exception of sorghum seed, contained high levels of aflatoxin. Groundnut cake was one of the major cattle feed ingredients in the peri-urban area and $>75\%$ of the samples contained $>100\,\mu g\,kg^{-1}$ aflatoxin leading to a high level of aflatoxin M_1, in milk samples.

Reddy and Reddy (1994) investigated the incidence of trichothecene-producing fungi in feed cakes (groundnut and sesame cakes) sampled in Andhra Pradesh. *Fusarium* was most commonly isolated followed by *Stachybotrys atra*, *Myrothecium roridum*, *Trichoderma viride* and *Trichothecium roseum*. The toxinogenic potential of individual isolates varied. Isolates of *Fusarium* spp. produced zearalenone, nivalenol, vomitoxin, diacetoxyscirpenol, T-2 toxin, neosolaniol and solaniol. *M. roridum* isolates produced roridin, *S. atra* isolates produced satratoxins and *T. roseum* produced trichothecin. Udagawa (1976) isolated *A. flavus*, *Aspergillus niger*, *Penicillium citrinum*, *P. cyclopium*, *Penicillium funiculosum*, *Penicillium paraherquei*, *Fusarium* and *Rhizopus* from groundnut samples in Papua New Guinea and *A. flavus, Aspergillus terreus*, *A. niger* and *Mucor* from maize. From 40 groundnut seed samples collected throughout Egypt, 43 species of fungi, belonging to 16 genera were isolated (El-Magraby et al., 1988). The most dominant genera were *Aspergillus*, *Penicillium* and *Fusarium. Aspergillus fumigatus* (62% of samples), *A. flavus* (57%), *A. niger* (55%), *Penicillium chrysogenum* (52%) and *Fusarium oxysporum* (55%) were the most frequently isolated species. Mycotoxins identified were aflatoxins B_1, B_2, G_1, and G_2, citrinin, fumagillin, diacetoxyscirpenol, T-2 toxin, satratoxin H, and zearalenone. Ranjan and Sinha (1991) investigated the occurrence of mycotoxigenic fungi and mycotoxins in animal feed

from Bihar, India. He found that out of 385 *A. flavus* group isolates 53% were capable of producing aflatoxins in sucrose–magnesium sulfate–potasium nitrate–yeast extract (SMKY) (Diener and Davis, 1966) liquid medium. The toxigenic strains of other mycotoxigenic fungi were *A. ochraceus*, *A. versicolor*, *P. citrinum* and *F. verticillioides*. Aflatoxins were present in 139 samples and zearalenone, ochratoxin A and citrinin were also identified alone or as co-contaminants. Abbas et al. (2005) studied the relationships between aflatoxin production and sclerotia formation among isolates of *Aspergillus* section Flavi from the Mississippi Delta. They observed that about 50% of the isolates from corn, soil and peanut produced large sclerotia, while only 20% of the rice isolates produced large sclerotia. The isolates that did not produce sclerotia were significantly less likely to be toxigenic than strains that produced large sclerotia.

Environmental conditions required to induce pre-harvest aflatoxin contamination of groundnuts was studied by Cole et al. (1989). They showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of invasion levels of up to 80% by *A. flavus* and *A. parasiticus*. Also, larger, more mature groundnut kernels required considerably more drought stress to become contaminated than did smaller, more immature kernels. The role of environmental stress in predisposition of groundnuts to aflatoxin contamination was demonstrated by several workers (Sanders et al., 1985; Thai et al., 1990). Although, roots did not suffer drought stress, when pods suffered stress, the risk of aflatoxin contamination increased (Sanders et al., 1993). The rainy season crop is often subjected to drought, particularly end-of-season drought, in most of the areas in the major groundnut-producing regions in India. This encourages *A. flavus* infection and aflatoxin contamination (Ghewande et al., 1987; Bhat and Rao, 1990; Ghewande, 1997). Since the development of genetic transformations systems for *A. flavus* and *A. parasiticus*, 10 genes have been isolated in the aflatoxin pathway and nine enzymatic conversions have been elucidated or confirmed (Payne and Brown, 1998; Bhatnagar et al., 2003).

In an UNDP-sponsored project on "Promising Groundnut as Food Crop for Sustained Nutritional Security" Basu (2001) demonstrated the strength of integrating pre- and post-harvest factors in reducing aflatoxin risk through farmers' participatory research mode. Combination of critical pre- and post-harvest factors at soil, plant and storage levels reduced aflatoxin risk substantially (0–5 $\mu g\,kg^{-1}$) in large number samples and 78% were made safe to eat even in a high-risk area. The storage aspects of the produce at farmers level and aflatoxin build up under ordinary storage condition over a period of 3 months were monitored in Anantapur district and various storage practices were studied to keep aflatoxin B_1 within the prescribed limit from the health point of view (Basu, 2001). Kumar et al. (2002) evaluated an integrated management package to reduced pre-harvest seed infection by *A. flavus* in groundnut. Seed infection studies revealed a predominance of *A. flavus* infection in plots under farmers practice (10%) compared with that under an integrated aflatoxin management package (2%). Desai and Basu (2002) have analysed the significance of aflatoxin contamination as a non-tariff trade barrier. An integrated approach giving handy guidelines for farmers, traders and processors to safeguard groundnut from aflatoxin contamination was described by Kumar et al. (2005).

3.2. Maize

Maize (*Zea mays* L.) grain is a good substrate for mould infection and production of potentially dangerous mycotoxins harmful to both humans and animals. A large number of fungi are associated with grain mouldiness, but the most common are *A. flavus*, *A. parasiticus*, *F. graminearum*, *F. verticillioides*, *Penicillium* spp. and *Diplodia maydis* (Kpodo et al., 2000; Gonzáslez, et al., 2003). These fungi produce different types of mycotoxins, consumption of which can even lead to death in acute cases. An aflatoxin epidemic was first reported from India from Banswara (Rajasthan) and Panchmahals (Gujarat) in 1975 among *Bhils* (the largest and most widely distributed tribal group in India) who had consumed maize heavily contaminated with *A. flavus*. The epidemic was characterized by jaundice, rapidly developing ascites and portal hypertension. Approximately 400 persons were affected by the epidemic.

A mycotoxin problem in standing maize crops was first reported from India by Bilgrami et al. (1978). Kernels of a standing maize crop in Bihar were found to be infested with aflatoxin-producing strains of *A. flavus*. Prakash and Siradhana (1978) reported that incubation period of 12 d at 25 °C, and 100% RH under normal daylight were optimum for production of aflatoxin B_1 on inoculated maize grains. Maize grains with 15% moisture content did not support mould growth even after 45 d of storage in modified atmosphere storage (MAS) systems and dry matter loss was also significantly reduced under 60% CO_2-modified atmosphere in maize grains with 20% moisture content (Janardhana et al., 1998). Aflatoxin B_1 treatment of germinating maize suppressed RNA, protein and DNA synthesis. Inhibition of RNA synthesis was considered to be caused by interference with RNA polymerase. Exposure of artificially infected maize to sunlight for 7 h reduced aflatoxin B_1 by 80%. Increasing the exposure time to sunlight had no effect on further reduction in toxin content (Waghray et al., 1995). A regular survey of some maize-growing areas of Bihar state, India, for 3 consecutive years (September 1984–1986) revealed heavy infestations of mycotoxin-producing fungi with different maize samples (Sinha, 1990). Aflatoxin-producing fungi had the highest frequency of occurrence in all cases and aflatoxins were the most common mycotoxins produced by these fungi. Maize samples of the *kharif* (rainy season) crop had a greater incidence of aflatoxins (47%)

than samples of the *rabi* (summer season) crop (17%). Stored maize grains also had a high incidence of aflatoxins (43%). Most of the contaminated samples contained aflatoxins at levels above 20 $\mu g\,kg^{-1}$. Janardhana et al. (1999) studied the mycotoxin contamination of maize grains grown in Karnataka, India. The 197 maize samples analysed representing different cultivars, collected from various agro-climatic regions of Karnataka revealed the association of 24 diverse species of both field and storage moulds belonging to 14 genera. Munkvold (2003a) studied the epidemiology of *Fusarium* ear rot (or pink ear rot) and *Gibberella* ear rot (or red ear rot), both of which resulted in mycotoxin contamination of maize grain. Aspects of the epidemiology of both diseases were studied and efforts were made to synthesize the information into comprehensive models of disease development. The primary mycotoxins produced by these fungi, fumonisins and deoxynivalenol, had differing roles in the disease-cycle, which are not completely understood, especially in the case of fumonisins (Munkvold, 2003a). Velluti et al. (2001) studied the production of fumonisin B_1, zearalenone and deoxynivalenol by *F. verticillioides*, *F. proliferatum* and *F. graminearum* in mixed cultures on irradiated maize kernels. The presence of *F. graminearum* decreased the fungal populations of *F. verticillioides* and *F. proliferatum* under almost all conditions tested. Gutema et al. (2000) analysed the food-grade corn and corn-based food products intended for human consumption from different locations in the USA for fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and moniliformin. Among 100 food-grade commercial corn samples, 71% contained FB_1 with concentrations ranging from 43 to 1642 $\mu g\,kg^{-1}$. None of the samples contained FB_2. Fifty-percent of the samples contained moniliformin with concentrations ranging from 26 to 774 $\mu g\,kg^{-1}$. All samples were infected by *Fusarium* with infection rates ranging from 8% to 88%. The simultaneous occurrence of FB_1 and moniliformin was observed in 34% of corn samples and 53% of corn-based food products. Maragos et al. (2001) described a rapid, portable fluorescence polarization-based assay for fumonisins in maize with a limit of detection for FB_1 of 0.5 $\mu g\,kg^{-1}$ in spiked maize. Forty-eight samples of field-contaminated maize were tested by fluorescence polarization and an established HPLC method, with a good correlation between the two. De Girolamo et al. (2001) developed a new analytical method for the determination of fumonisins in maize and maize-based food products like maize flour, corn flakes, extruded maize and infant formula by the use of acetonitrile (ACN)-water (1 + 1, $v + v$) as extraction solvent and immunoaffinity column for clean-up.

The cells of *Lactobacillus* spp. were found effective in preventing growth of the mould and bacterial metabolites were effective in reducing the amount of aflatoxin produced, although the growth was not much affected (Karunaratne et al., 1990). Wicklow et al. (2005) reported a protective endophyte of maize, *Acremonium zeae,* which produced antibiotics inhibitory to kernel rotting and the mycotoxin-producing fungi *A. flavus* and *F. verticillioides* in cultural tests for antagonism. Chemical studies of an organic extract from maize kernel fermentations of *A. zeae* (NRRL 13540) displayed significant antifungal activity against *A. flavus* and *F. verticillioides* and the metabolites accounting for this activity were two newly reported antibiotics pyrrocidines A and B.

3.3. Rice

Mycotoxin contamination often occurs in the field prior to harvest. Post-harvest contamination can occur if the drying is delayed and during storage of the crop if moisture is allowed to exceed critical values for the mould growth. Delayed harvest in rainy weather frequently leads to grain sprouting on the panicle, particularly for non-dormant *japonica* rice. Often unseasonal rainfall or dew deposition can cause mycotoxin contamination. In addition to this, rice is often consumed as parboiled all along the coastal areas of India, particularly from Kerala to North coastal Andhra Pradesh, Orissa, West Bengal, Assam and other north-eastern states. The parboiling process generally leads to softening of the grain and opening of the hull, the conditions that are more suitable for the storage fungi to enter, if later drying is not adequate. Of the major fungi, *A. flavus* and *A. parasiticus* have been identified as the quality deterrent, producing aflatoxin-contaminated seeds when stored.

The incidence of the heavy rains (cyclones) during the harvesting season in India correlates with aflatoxin contamination of the rice crop (Tulpule et al., 1982). Bhatt and Krishnamachari (1978) reported a fairly high amount of aflatoxin contamination in wide variety of food viz. parboiled rice, maize, groundnut, etc., which can have serious implications for human health. Pande et al. (1990) reported that the quantity of aflatoxin was higher in rice samples compared with wheat and maize. Fungal infection was more frequent in parboiled dried paddy and milled parboiled rice. Of the various stages, rice at the drying stage and the stage preceding milling were shown to contain aflatoxins. Three samples were aflatoxin-positive and showed levels of aflatoxins ranging from 20 to100 $\mu g\,kg^{-1}$ at the drying stage and 25–120 $\mu g\,kg^{-1}$ after parboiling. Prasad et al. (1987) tested 56 samples of stored rice and 12 were positive for aflatoxin. Three of these came from containers of woven rice straw, two each from earthen pots, iron bins and gunny bags. Levels of aflatoxins ranged from 184 to 2830 $\mu g\,kg^{-1}$. *Aspergillus* species are common contaminants in stored rice and its incidence increases with the infestation of rice weevil (*Sitophilus oryzae*) (Prasad et al., 1987; Choudhury et al., 1999). Studies by Prasad et al. (1986) and Pawan et al. (1990) showed that a number of samples of stored paddy rice were contaminated with aflatoxin B_1, G_1 and G_2. Higher moisture content of the grains at the time of storage increased the *Aspergillus* infection and aflatoxin contamination (Nandi and Haggblom, 1984). A survey on the

prevalence of aflatoxin B_1 (AFB_1) in rice bran in coastal and interior districts of Tamil Nadu and Andhra Pradesh, India revealed that 62% of the samples contained AFB_1 and the levels far exceeded the permissible limit of $50\,\mu g\,kg^{-1}$ (Elangovan et al., 1999). Jayaraman and Kalyansundaram (1990) reported that 35% of the samples of raw rice bran and parboiled rice bran showed the presence of aflatoxin B_1. However, it was shown that bran of parboiled rice supports higher aflatoxin production than bran of raw rice (Jayaraman and Kalyansundaram, 1994). In a study from Sri Lanka, Bandara et al. (1991) reported that in almost all the samples of parboiled rice, AFB_1 and AFG_1 contents were significantly higher than the raw milled rice. Cultivar differences in the amount of aflatoxin B_1 and G_1 was showed by Sinha and Dubey (1991). The aleurone layer contains high levels of lipids and it is the preferred site of colonization by *A. flavus* (Gajapathy and Kalyansundaram, 1986). Kim and Lee (1996) using ELISA and immunohistochemical staining revealed that the edible portion of inoculated grains exhibited significantly higher levels of toxin than did the rice hulls, and the embryo contained a higher proportion of toxins than the endosperm. Udagawa (1976) isolated *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. versicolor*, *Chaetomium globosum* and *P. citrinum* from milled rice from Malaysia. Fouzia and Samajpati (2000) isolated mycotoxin-producing fungi from contaminated grains of rice sold in the local markets of Calcutta, India. It was found that aflatoxin B_1 was produced by *A. flavus* and *A. parasiticus*, aflatoxin G_1 by *A. flavus*, ochratoxin by *A. ochraceus*, sterigmatocystin by *Aspergillus japonicus* and citrinin by *P. citrinum*.

The work at Directorate of Rice Research, Hyderabad, India on the samples collected from flood-affected paddy fields as well as from the farmers storage structures in Andhra Pradesh, revealed that the predominant fungi were *A. flavus*, *Aspergillus nidulans*, *A. fumigatus* and *A. candidus*. It was also observed that the frequency of occurrence of *A. flavus* increased after storage. Grain samples of flood-affected paddy cultivar NLR 9672 collected from the standing crop, threshing floors and storage sites in Andhra Pradesh, India showed germination to the extent of 73%, 65% and 78%, respectively, with *Aspergillus sydowii* as one of the most predominant fungus associated with the rice seeds (Reddy, 1990). The residual bran after extraction of oil generally goes as cattle feed and this contained some aflatoxin (Jayaraman and Kalyansundaram, 1996). In another study with rice bran samples taken from rice mills in coastal and interior districts of Andhra Pradesh, Karnataka and Tamil Nadu, nearly 62% samples showed aflatoxin B_1 at low levels. One-third was in the $50–500\,\mu g\,kg^{-1}$ range, while another third of the samples had up to $2000\,\mu g\,kg^{-1}$ (Elangovan et al., 1999). Poor infrastructure, hygiene and sanitary conditions at the mills were identified as the reasons for aflatoxin B_1 contamination in rice bran samples, which are more prone to invasion by the *Aspergillus* spp. In seed samples from the threshing floor, where *A. flavus* was detected after storage for 1 year, aflatoxin B_1 was estimated at $32\,\mu g\,kg^{-1}$. In the dehusked seed samples, the concentration of aflatoxin B_1 increased from $30\,\mu g\,kg^{-1}$ prior to storage to $60\,\mu g\,kg^{-1}$ after storage (Waghray et al., 1988). A preliminary survey of some samples of raw and parboiled rice bran showed that 35% of the samples contained aflatoxin B_1 (Jayaraman and Kalyansundaram, 1990). When the oil was extracted from contaminated bran, a considerable amount of aflatoxin was present in the crude oil, but refined oil was free of toxin.

A survey conducted by Food Standards Agency, UK in 2002 to determine the levels of mycotoxins present in a range of rice (long grain rice, easy cook rice, basmati rice, specialty rice, brown rice, short grain rice, flaked and ground rice) available at retail outlets in the UK indicated the absence of Ochratoxin A, sterigmatocystin, fumonisin B_1, B_2, B_3 and zearalenone. Aflatoxins in the rice samples analysed were at levels ranging from 0.2 to $1.8\,\mu g\,kg^{-1}$. All levels found were below the EC legislative limits of $2\,\mu g\,kg^{-1}$ aflatoxin B_1 and $4\,\mu g\,kg^{-1}$ total aflatoxin in cereal products for direct human consumption. A low level of deoxynivalenol ($12\,\mu g\,kg^{-1}$) was detected in one sample of rice.

The reduction of aflatoxin content of rice through bio-control agents is an eco-friendly, safe and economic approach. Tsubouchi et al. (1981) observed inhibitory effects of non-aflatoxigenic fungi on aflatoxin production in rice cultures (culture media) by *A. flavus* (on pepper fruit). Dorner et al. (1998) found that bio-control agents were non-toxigenic colour mutants of *A. flavus* and *A. parasiticus* that were grown on rice for use as soil inoculum. Lee and Kim (1989) observed inhibition of aflatoxin accumulation by *A. flavus* in rice by *Aspergillus kawachii* and *Aspergillus shirousamii* so that the rate of toxin accumulation and the maximum concentration of accumulated aflatoxins were considerably reduced, although the initiation of aflatoxin accumulation was not affected.

3.4. Sorghum

The mycotoxigenic fungi *Aspergillus*, *Penicillium* and *Fusarium* were shown to be associated with grain moulds in sorghum and pearl millet (Waliyar et al., 2004). Prevalence of toxigenic strains of *A. flavus* in sorghum fields and aflatoxin B_1 contamination of sorghum grain has been reported. Infection by *A. flavus* in sorghum and production of aflatoxin causing bile duct proliferation and death was observed. Traditionally, the disease pellagra has been associated with corn consumption and niacin deficiency, and has presently been recognized as a multiple factor nutritional syndrome. In the recent past, it has been suggested that consumption of mycotoxin-contaminated sorghum/corn may be involved in the development of pellagra in a sorghum/corn-eating population. Sashidhar et al. (1991) assessed the levels of mycotoxins (aflatoxin B_1

and T-2 toxin) in sorghum collected from a traditionally sorghum-eating population and found that despite 25% fungal contamination in the sorghum samples collected, the levels of mycotoxins were minimal (1.4%) and there was no incidence of pellagra in the survey area. The absence of pellagra in the survey areas may be attributed to the changing dietary pattern.

Besides aflatoxins, other toxins detected in sorghum are tricothecenes, zearalenone, fumonisin B_1 and B_2 produced by the fungus *Fusarium*. Among these, T-2 toxin is the most important toxin. Extracts of mouldy sorghum when applied externally to the shaved skin of guinea pigs, rats and rabbits the skin showed marked redness, inflammation and crust formation (Rukmini and Bhat, 1978). Other signs included pinpoint haemorrhage, scab formation and subdermal haemorrhage. Shotwell et al. (1980) reported more than $1000\,\mu g\,kg^{-1}$ zearalenone in 18% of the samples. Blaney (1985) reviewed mycotoxin contamination of pre- and post-harvest sorghum. Four cases of suspected mycotoxicosis in commercial swine were reported, two due to aflatoxins another due to aflatoxins and ochratoxin A, and the fourth due to zearalenone. Very high concentrations of the mycotoxins viz. aflatoxin ($<9.6\,mg\,kg^{-1}$), ochratoxin ($<0.1\,mg\,kg^{-1}$) and zearalenone ($<8\,mg\,kg^{-1}$) were detected in sorghum harvested and improperly stored. Elegbede et al. (1982) reported the field and storage fungi, including *Aspergillus*, *Fusarium*, *Penicillium*, *Curvularia*, *Phoma*, *Alternaria*, *Chaetomium* and *Helminthsoporium* spp. from sorghum grains. Mycotoxins detected in the samples were zearalenone, ochratoxins and sterigmatocystin but no aflatoxins. The level of contamination was below $50\,\mu g\,kg^{-1}$ except for zearalenone in two samples (100 and $143\,\mu g\,kg^{-1}$), ochratoxin A in one sample ($52\,\mu g\,kg^{-1}$) and ochratoxin B in two samples (52 and $60\,\mu g\,kg^{-1}$). Reddy et al. (1985) studied the aflatoxin and other mycotoxin contamination in sorghum under field conditions. Samples collected from different fields after a heavy rainfall was observed to be infested with numerous fungi predominantly *A. flavus, Curvularia lunata* and *F. verticillioides*. Compared with the cultivars with loose panicles, the sorghum seeds in compact heads were heavily contaminated with moulds. Levels of aflatoxin B_1 and B_2 were higher than those of zearalenone, T-2 toxin and aflatoxin G_1.

In Nigeria, Salifu (1981) studied mould invasion and mycotoxin contamination in developing grains of sorghum where aflatoxin ranged from 10 to $80\,\mu g\,g^{-1}$. Aflatoxin B_1, B_2, G_1 and G_2 had been shown in the isolate of *A. flavus* from sorghum ears (Tripathi, 1973). The production of aflatoxin by *A. flavus* on 20 sorghum cultivars were recorded as very high ($1\,mg\,kg^{-1}$), high ($0.25–1.0\,mg\,kg^{-1}$), medium ($0.05–0.25\,mg\,kg^{-1}$) and low (less than $0.05\,mg\,kg^{-1}$). Forbes et al. (1992) reviewed the grain mould in sorghum. Reddy and Nusrath (1986) had reported the pre-harvest fungal infection of sorghum fields and contamination of aflatoxin. The post-harvest handling of sorghum grain during threshing caused AFB_1 contamination of sorghum grain. *Fusarium* toxins, especially fumonisin B_1, was detected more than the toxic limits in the market samples of sorghum (Shetty and Bhat, 1997). Mukherjee and Lakshminarasimham (1995) studied aflatoxin contamination of sorghum seeds during storage under controlled conditions. The results indicated that 20 °C and 73.5% relative humidity (RH) were safe storage conditions. Maximum aflatoxin levels were observed at 31 °C and 81.0% RH. Although *A. flavus* grew well at 40 °C and all humidity levels tested, aflatoxin production was comparatively lower but reached a hazardous level after 5 months of storage. Bhat et al. (2000) reported the outbreak of fumonisins and aflatoxins in humans fed on mould-infected sorghum. In India, sorghum was stored in traditional storage structures viz. "*kotlu*" (storage rooms) earthenware pots, gunny bags and reed baskets. Among the structures, "*kotlu*" form of storage was most susceptible to fungal attack (Sashidhar et al., 1992). *Aspergillus* and *Fusarium* were the major fungal genera seen under storage. Naturally occurring phenols offer a detoxification mechanism to fumonisin B_1 (Beekrum et al., 2003).

The response of different coloured sorghums to *Aspergillus* infection and aflatoxin production was studied by Ratnavathi and Sashidhar (2003). The chemical quality of the grain was more deteriorated in white sorghums compared with red and yellow sorghums. The indication of resistance in red sorghums was shown by the delayed production of aflatoxins and it is mostly attributed to high phenolic content. The increased concentration of hydrolases also known for their antifungal activity was observed in red, yellow and white sorghums (Ratnavathi and Sashidhar, 2000).

3.5. Spices

For the export of spices and spice products, the exporting countries have to comply with the specifications laid down by the regulatory agencies in importing countries. The most popular specification for spices and herbs world over is the "ASTA (ASTA = American Spice Trade Association) Cleanliness Specifications for Spices, Seeds and Herbs". In addition to the cleanliness specification, the importing countries insist on the specification for parameters like pesticide residues, aflatoxin, trace metal and microbial contamination. European Union has prescribed limits for aflatoxin as $5\,\mu g\,kg^{-1}$ for aflatoxin B_1 and $l0\,\mu g\,kg^{-1}$ for total aflatoxin. Member countries in the European Union and others have fixed limits for aflatoxin varying from 1 to $20\,\mu g\,kg^{-1}$.

Christensen (1975), over a period of several years, examined 100 different samples of black pepper from all over the world. In dilution cultures of these samples, the number of fungus colonies in whole or ground black pepper averaged 52,000/g and the upper range was over half a million per gram. These colonies were mostly of *A. flavus*, *A. ochraceus* and *A. versicolor*. Some samples of ground pepper were caked lightly with fungus mycelium

when first opened in the laboratory and with time, a number of these became solidly caked with mycelium. Takatori et al. (1977) and Ayres et al. (1980) found the *Aspergillus* and *Penicillium* spp. to be dominant in all examined spices samples. *Aspergillus* and *Penicillium* spp. were the main components of cardamom, cinnamon, fennel, coriander, cumin, black cumin and white pepper, all of which are common in the food industry. They found a high degree of contamination in all samples. Misra (1981) and Roy et al. (1988) isolated *A. flavus*, *A. niger*, *A. fumigatus*, *A. orchaceus*, *A. candidus*, *A. sydowii*, *Chaetomium dolicholrichum*, *F. verticillioides*, *Penicillium oxalicum*, *Alternaria, Curvularia* and *Rhizopus* from the seeds of *Amomum subulatum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Piper nigrum*, *Cinnamomum zeylanicum*, and from the bark of *Acacia catechu*, all of which are commonly used medicinal plants. Rani and Singh (1990) found that 89% of samples of fennel, coriander and cumin were contaminated with aflatoxin B_1 at the levels 3000, 1640 and 1580 $\mu g\,kg^{-1}$, respectively. In addition, Roy et al. (1988) and Roy and Chourasia (1990) determined that the seeds of *P. nigrum* and *Mucuna prurians*, and the barks of *A. catechu*, *C. sativum*, and *Elettaria cardamomun* were contaminated with aflatoxin B_1 at levels below 20 $\mu g\,kg^{-1}$. Aziz and Youssef (1991) isolated *A. flavus* and *A. parasiticus* with a high tendency for aflatoxin production from some common herbal drugs and spices. Aziz et al. (1998) studied contamination of some common medicinal plant samples and spices and their mycotoxins. Ten fungal genera viz. *A. flavus*, *A. parasiticus*, *A. niger*, *F. oxysporum* and *P. viridicatum* occurred most often on the medicinal plant samples. Direct determination of mycotoxins in medicinal plant samples revealed aflatoxin B_1 in 17 samples at an average of from 10 to 160 $\mu g\,kg^{-1}$, ochratoxin A in three samples at an average of from 20 to 80 $\mu g\,kg^{-1}$, and no detection of penicillic acid, zearalenone or T-toxin. *A. flavus*, *A. parasiticus* and *A. oryzae* were aflatoxin producers, whereas, *A. ochraceus*, *P. viridicatum* and *P. variable* were ochratoxin A producers. In addition, *P. viridicatum*, *P. chrysogenum* and *P. commune* were penicillic acid producers.

A. ochraceus was reported to be one of the major organisms producing ochratoxin A (Aziz, 1987). Leistner and Pitt (1977) found that, out of 442 *Penicillium* isolates, 44 synthesized penicillic acid, 17 ochratoxin A, 11 penitrem, 10 citrinin, 6 patulin and 3 produced both patulin and citrinin. Overy and Frisvad (2005) studied the mycotoxin production and post-harvest storage rot of ginger (*Zingiber officinale*). He found that *Penicillium brevicompactum* to be the predominant species isolated from 85% of the samples. Mycophenolic acid was identified from corresponding tissue extracts.

Aflatoxin production at various stages of development in cardamom and black pepper was reported by Banerjee et al. (1993). The toxin was assessed using MAB-based ELISA. All *A. flavus* isolates tested produced aflatoxin B_1 in amounts ranging from 65 to 3000 $ng\,ml^{-1}$. In another study by Geetha and Reddy (1990), *A. flavus* was indicated in the production of carcinogenic aflatoxin, mainly in ginger, mustard, garlic and pepper. The highest fungal counts were observed in black pepper and the lowest in curry leaves. When three spices—coriander, fennel and ginger collected from Bihar were screened for aflatoxin-producing fungi, *A. flavus* predominated and most isolates produced only aflatoxin B_1 in varying amounts (Prasad et al., 1984). A literature review on the incidence of mycotoxins as contaminants of various seasonings indicated the presence of aflatoxins (Vrabcheva, 2000), which are more frequently found in red peppers (paprika, chilli and capsicum), nutmeg, mustard and ginger. High concentrations of aflatoxins are frequently detected in nutmeg, particularly, aflatoxin B_1 and B_2. Freire et al. (2000) isolated a wide range of field and storage fungi, totalling 42 species from black pepper, white pepper and Brazil nut. Chaetocin, penitrem A and xanthocillin were identified only from black pepper, while tenuazonic acid was identified from both black and white pepper. Aflatoxin G_2, chaetoglobosin C and spinulosin were identified from poor quality Brazil nuts.

3.6. Chilli

Significant levels of contamination with *A. flavus* are common in red peppers (*Capsicum annuum*), which are not usually subjected to processing except drying and grinding. During humid weather red chilli gets infected by *A. flavus* and seeds are contaminated by the fungus. Besides aflatoxins, Ochratoxin A was found as simultaneous contaminants. Ath-Har et al. (1988) reported that *A. flavus*, *A. niger*, *A. nidulans*, *A. sydowii*, *A. ochraceus*, *Penicillium* and *Rhizopus* spp. were most frequently isolated from *Capsicum frutescens* and other spices. Sixteen isolates of *A. flavus* produced B_1 aflatoxin among which 25% of the isolates were responsible for aflatoxin production in chilli. A survey of the peppers entering storage at several localities in 1977–1978 showed that the total mould count exceeded the permissible levels for finished products in importing countries and insect infestation was common. Insects cause a small percentage of pods to become completely filled with *Aspergillus* before storage. The predominant insect species found were *Tribolium castaneum*, *Tribolium confusum* and *Sitotroga cerealella. Aspergillus*-filled pods showed no surface growth of mould but could readily be identified by their yellow discoloration. They acted as reservoirs of infection. It was shown that the beetles and the moth carry conidia of *Aspergillus* and other fungi on their body surfaces, and intact conidia were found in the digestive tract of larvae of *Tribolium* (Seenappa et al., 1979). Seenappa et al. (1980) further reported that several *Aspergillus* spp. occurred on the surface of dried *C. annuum* fruits. When stored at 70% RH and 28 °C for 10 d only members of the *A. glaucus* group developed.

A. flavus and *A. ochraceus* predominated at 85% RH and *A. flavus* alone at 95% RH. Extent of colonization of the surface of pods was related to the RH of storage and growth was observed at 85% and 95% but not at 70% RH. Subrahmanyam and Rao (1973) reported high levels of aflatoxin in a culture filtrate of *A. flavus* isolated from chilli powder. Aflatoxins, ochratoxin A and fumonisins were found in chilli paste. The highest mycotoxin levels and frequency of occurrence were in chilli powder, curry powder and ginger (Patel et al., 1996). Out of 157 retail samples including curry powders, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon and coriander, almost 95% of samples contained $<10\,\mu g\,kg^{-1}$ total aflatoxins and only nine samples had higher levels. The highest concentration in a retail sample was $48\,\mu g\,kg^{-1}$ in a chilli powder. Cooking experiments showed that aflatoxin levels in spiced sauces are not reduced by domestic cooking with either microwave or conventional gas oven heating (Macdonald and Castle, 1996). Klieber (2001) studied the aflatoxin contamination level in various chilli products like chilli powder, paprika spices, dried fruit and sauces in retail stores in Adelaide, Australia and its management. The average aflatoxin level ranged between 0 and $89\,\mu g\,kg^{-1}$ and only 9% passed Australian requirements of levels below $5\,\mu g\,kg^{-1}$. The efficacy of different methods of extraction, purification and estimation for aflatoxin B_1 in chilli powder was investigated by Shantha (1999) and found the percent recovery ranged from 65 to 108. He also reported that the toxin recovered was low (0–10%) if the contamination level was $<40\,\mu g\,kg^{-1}$ in the equilibrated samples. Improved recoveries were obtained when the added aflatoxin levels were 50, 70 and $100\,\mu g\,kg^{-1}$. Hexane or petroleum ether solvents seemed to bind lower levels of aflatoxin B_1.

4. Conclusion

Mycotoxins are a chemically diverse group of fungal metabolites that have a wide variety of toxic effects. Though a large number of fungi are associated with groundnut kernel, maize, rice, and sorghum grain, chilli, and various spices, the most common mycotoxin-producing fungi are *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Fusarium verticillioides*, *Fusarium graminearum* and *Penicillium* spp. While maize and groundnut have been identified as very high-risk commodities for mycotoxin contamination, many other commodities have also been identified as vulnerable to this menace. In a normal varied human diet, constant exposure to low levels of several toxins is possible. Very little is known about the effects of long-term low-level exposure, especially with regard to co-contamination with multiple mycotoxins. Various combinations of the mycotoxins have been identified by Lopez-Garcia (1998) indicating that their behaviour in such cases is altered. Sedmikova et al. (2001) found that ochratoxin A could increase the mutagenicity of aflatoxin B_1 in the case of their simultaneous occurrence in the same substrate. Also, due to the heterogeneity of mycotoxin contamination and the potential for sampling regions with elevated toxin levels ("hot spots"), consistent sampling and analysis is difficult (Lopez-Garcia et al., 1999).

A systematic approach to understand the problem is required to combat the adverse effects of these mycotoxins and to protect consumers and animal health. One possible approach to management of the risks associated with mycotoxin contamination is the use of an integrated system based on the Hazard Analysis and Critical Control Point (HACCP) approach (Lopez-Garcia et al., 1999) and should involve strategies for prevention, control, good manufacturing practices and quality control at all stages of production, from the field to the final consumer. The concept behind an integrated management system is similar to a "hurdle" effect, where at each phase of production, i.e. pre-harvest, harvest and post-harvest processing the risks are minimized.

Some of the future line of research in this direction would be:

- Cultural practices
  - Development of good agricultural practices during pre- and post-harvest stages including appropriate drying techniques and storage.
  - Understanding temporal and spatial dynamics of mycotoxigenic fungi populations in different production systems and characterization of the strains for their toxigenicity for development of location-specific modules for management of mycotoxin contamination.
  - Development of efficient pre- and post-harvest antagonists as a component to be integrated into management package. A culture bank for mycotoxigenic fungi may be developed among which there is a scope for obtaining non-toxigenic cultures for deployment as bio-control tools.
  - Development of simple, efficient, cost-effective sampling and analytical methods suitable for screening and segregation of contaminated lots of commodities early in the marketing chain and for control during processing. With several novel approaches being developed, such as molecular imprint polymers (Weiss et al., 2003) and immuno- (De Saeger et al., 2002) and bio-assays (Widestrand et al., 2003), adoption of such methods may be possible.
  - Additional research is needed to develop the ability to predict when and where environmental conditions may make mycotoxin contamination probable.
- Host resistance
  - Breeding for increased tolerance and reduced mycotoxin levels is being practiced but the amount of resistance achievable may be limited due to complicated genetics and/or linkage to undesirable agronomic traits. Molecular markers can be employed to speed up the incorporation of chromosomal regions that have a quantitative effect on

resistance (quantitative trait loci). Research directed towards identifying groundnut lines with resistance to aflatoxins is continuing and it appears to show some promise, but this is a long-term approach and no lines have yet been released. Results of these efforts at genetic control of aflatoxin will surely guide analogous efforts with other mycotoxins.

- Transgenic approaches
  - Working out the resistance mechanism and identification of the candidate gene for antifungal enzymes in the host crop. For example, groundnut produces a stilbene phytoalexin (Sobolev et al., 1995; Murphy et al., 2006) in response to fungal infection. Enhancing the production of stilbenes in groundnut seeds by gene transfer may make them less prone to fungal infection.
  - The lipoxygenase enzymes (LOXs), recently characterized and cloned, are suspected of playing an important role in the Aspergillus seed interaction. The studies demonstrated that Aspergillus infections induce seed lipoxygenase expression leading to generation of bioactive oxylipins (Burow et al., 2000; Wilson et al., 2001; Tsitsigiannis et al., 2005). Conversely, seed oxylipins stimulate sporulation and mycotoxin synthesis in Aspergillus nidulans, A. flavus and A. parasiticus (Burow et al., 1997; Calvo et al., 1999). Further studies exploring the nature of seed/fungal oxylipin signalling and its role in regulating mycotoxin production may permit interception of such signalling and thereby, a novel method for preventing mycotoxin contamination of food and feeds (Keller, 1998). Further, the study of LOXs expression might provide some support to screen groundnut genotypes against A. flavus. Identification of cultivars with grain hardness correlated to high prolamin content and polyphenols in sorghum is a good proposition in this direction.
  - Another option is to enhance expression of such a compound by the existing gene, thereby capitalizing on the plant's own defense mechanisms. For example, enzymes that catalyze production of antifungal compounds could be targeted for expression. Alternatively, genetic engineering methods to increase production of enzymes that degrade mycotoxins are also being pursued (Duvick, 2001; Munkvold, 2003b). Brigitte et al. (2003) isolated and characterized a gene from Arabidopsis thaliana encoding a UDP-glycosyltransferase that detoxifies deoxynivalenol. The constitutive over expression of such genes in the host crop may lead to enhanced tolerance against deoxynivalenol. Transgenic maize has been patented for fumonisin-degrading corn for swine consumption (Duvick and Rood, 1998). Efforts are also under way to engineer plants to produce compounds that disrupt mycotoxin synthesis. For example, enhanced expression of a α-amylase inhibitor in Aspergillus spp. could result in significantly reduced aflatoxin levels (Duvick, 2001; Munkvold, 2003b).
  - Transgenic approaches to genetically enhanced resistance to insect feeding may be another approach to prevention of mycotoxins in grain. Insects play an important role in the proliferation of mould growth in the field and in storage. Resistance developed through the use of several Bt (Bacillus thermophilus) genes in corn wheat, and other cereal grains to minimize insect damage has led to effective reduction in Fusarium ear rot (F. verticillioides and F. proliferatum) mycotoxin levels in grain. Munkvold (2003b) cited 19 reports on Bt hybrids, with 12 of these demonstrating reduced mycotoxin production compared with the parent corn.

In conclusion, the prevention of mycotoxin contamination of human foods could have a significant effect on public health in low-income countries, and deserves significant attention. The food industry should take the lead in these efforts, because it will lead to improved economic sustainability of the industry, enhanced food safety efforts, enhanced international trade efforts and improved public health.

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