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Aflatoxin Contamination in Agricultural Crops and Their Eco-Friendly Management- A Review

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***Abstract*: -Background: Aflatoxins are a group of mycotoxins produced by *Aspergillus* species, including *A. flavus*, A. *parasiticus*, and *A. nomius*. A part of the world's food crops are estimated to be affected by mycotoxins; creating a large economical loss in the developed and developing countries. Aflatoxins represent the main threat worldwide.**

Result: There was an increased concern about the possibility of the presence of carcinogenic mold metabolites, particularly aflatoxins in food and animal feed products. Although aflatoxins are controlled by regulation in more than 80 countries, their legislation is not yet completely harmonized at the international level. Species of *Aspergillus* section Flavi including *A. flavus, A. parasiticus* and *A. nomius,* are known to cause about 25% yield loss in agriculture crops every year. Temperate and tropical conditions are favorable for growth and aflatoxin production by these fungi in stored products. Proper identification, characterization and accurate differentiation of aflatoxigenic and non-aflatoxigenic strains of Conclusion: A. flavus are necessary to understand diversity in order to develop proper management strategy. So far many national and international research groups have been tried in order to characterize and control *A. flavus* growth and aflatoxin production in various crops. In order to provide the current status of aflatoxigenic fungal infection and aflatoxin contamination in groundnut across different agro climatic conditions of India and to develop suitable eco-friendly management strategy

Key words: Aflatoxin, Agricultural crops, Aspergillus species, Aspergillus flavis

INTRODUCTION

Among more than few hundred known mycotoxins, aflatoxins represent the main threat worldwide. Aflatoxins, group of mycotoxins produced mainly by *A. flavus*, *A. parasiticus* and *A. nomius*. The word 'aflatoxin' came from *'Aspergillus flavus* toxin', because *A. flavus* and *A. parasiticus* are the predominant producers of aflatoxins (Yu *et al.*,2004).Aflatoxins were first discovered and characterized in the early 1960s when more than 1,00,000 turkey poults in England died of apparent poisoning from mold-contaminated groundnut meal. Initially, two toxic components of aflatoxin, AFB and AFG were identified on thin layer chromatography plates because of their blue or green fluorescence under ultraviolet light, respectively (Sargeant *et al.*, 1963). As many as 20 aflatoxins have been identified however only 4 of them B_1, B_2, G_1 and G_2 occur naturally. Chemically, these are crystalline substances, freely soluble in moderately polar organic solvents such as acetone, acetonitrile, Isopropanol, methanol and also dissolve in water to the extent of 10-20 mg/l. They are intensely fluorescent in ultraviolet light and are stable even at very high temperature of 260 °C (Bilgrami and Choudhary, 1998).

Almost all toxigenic isolates of *A. parasiticus* produce the B and G aflatoxins, whereas the toxigenic isolates of *A. flavus* produce only the B aflatoxins (Wilson and Payne, 1994). However, the strain and geographic location may have influence on this tendency. The most common aflatoxin-producing species, *A. flavus*, can be divided into S strains and L strains. The S strain produces numerous small sclerotia with an average diameter of < 400 µm and high levels of aflatoxins. In contrast, the L strain produces fewer, larger sclerotia and on average, less aflatoxin. Within the S strain, some isolates are termed as SB, which produce only B aflatoxins, while others, termed as SBG produce both B and G aflatoxins (Varga *et al.*, 2003).

Although aflatoxins B_1, B_2 and G_1 are common in the food sample, but AFB_1 is predominant (60-80% of the total aflatoxin content). Aflatoxin AFB_1 is the most potent toxic metabolite, which shows hepatotoxic, teratogenic and mutagenic properties and causes to mammals toxic hepatitis, hemorrhage, edema, immune suppression and hepatic carcinoma. Generally, AFB_2, AFG_1 and AFG_2 occur in the presence of AFB_1. In most cases AFG_1 is found in higher concentrations than AFB_2 and AFG_2 (Weidenborner, 2001). Mammalian metabolites of AFB_1

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and AFB_2 designated as M_1 and M_2 respectively were isolated from urine and milk.

Since aflatoxins are carcinogenic contaminants of food and feeds, frequently responsible for health and economic concerns in many countries (Yabe *et al.,* 1999; Bhatnagar *et al.,* 2003). It was estimated by United Nations' Food and Agriculture Organization (FAO), about 25% of the World's food is contaminated with mycotoxins (CAST, 1989) and much of this contamination is by aflatoxin.

Though several reviews have been reported regarding aflatoxins detection methods, degradation techniques aflatoxins prevalence, this review presents an update of different studies undertaken on aflatoxins contamination in agricultural crops, highlighting various eco-friendly strategies adopted for their management.

GLOBAL DISTRIBUTION OF *ASPERGILLUS FLAVUS* AND AFLATOXIN

Aspergillus section *Flavi* contains the major, economically important aflatoxin-producing fungi *A. flavus* and *A. parasiticus*. Less common aflatoxin producing species in this section are *A.nomius*, *A. pseudotamarii*, *A. bombysis* and *A. parvisclerotigenus* (Klich *et al.,* 1988). *Aspergillus ochraceoroseus*, *A. rambellii*, *Emericella venezuelensis* and *E. astellata* are four species which do not produce aflatoxin (Frisvad *et al*., 2005); the latter two species have *Aspergillus* anamorphs (asexual states). Section *Flavi* includes a number of other economically important species, such as the food fermentation or industrial species like *A. oryzae* and *A. sojae*.

Aspergillus species are extremely variable in mycotoxin production, with strains ranging from no producers to potent producers of aflatoxins (Horn and Dorner, 1999) which typically produce aflatoxins B1 and B2 and cyclopiazonic acid (Horn *et al*., 1996). In contrast, *A. parasiticus* is most prevalent in peanuts and synthesizes aflatoxins G1 and G2 in addition to B aflatoxins but not cyclopiazonic acid (Horn *et al*., 1996) and nonaflatoxigenic strains are rare (Horn *et al*., 1996; Tran-Dinh *et al*., 1999).

Soil is the reservoir for the primary inoculum which is responsible for the infection of crops susceptible to aflatoxin contamination. The manner of infection of *Aspergillus* species to the aerial fruiting of crops such as corn, cotton differs compared to the subterranean fruiting of peanuts (Payne, 1998). The hot humid climate and lack of proper storage facilities are congenial for the development of species of *Aspergillus*, *Fusarium* and *Penicillium* to proliferate and produce mycotoxins profusely in developing country like India (Sashidhar *et al*., 1992).

Aflatoxin producing fungi are native to warm arid, semi-arid, and tropical regions with changes in climate resulting in large fluctuation in the quantity of aflatoxin producers (Bock *et al*., 2004). Maize and groundnuts are the main crop, which are exposed to human as well as to aflatoxin because they are extensively consumed worldwide and unfortunately are also the most susceptible crops to aflatoxin contamination (Wu and Khlangwiset, 2010). About 40 % of the productivity loss due to diseases in developing countries is also exacerbated by aflatoxins (Wagacha and Muthomi, 2008).

FACTORS AFFECTING AFLATOXIN PRODUCTION

The crops grown under warm climatic conditions have greater chances of infection by aflatoxin producing fungi (Sanders *et al.,* 1984; Schmitt and Harburgh, 1989). Levels of *A. flavus* infection and aflatoxin production are related primarily to environmental conditions and hence cannot be directly correlated (Davidson *et al.,* 1982).

Higher yield of aflatoxins are associated with rich carbohydrate content, such as wheat, rice and to a lesser extent oilseeds such as cottonseed, soybean and groundnuts. Groundnut seeds under field conditions are most susceptible for aflatoxin contamination when the water activities are between 0.90-0.95 (Dorner *et al.,* 1989). Aflatoxin formation requires nutrients, such as minerals (especially zinc), vitamins, fatty acids, amino acids and starch as energy source (Wyatt, 1991). Aflatoxin B_1 production gets stimulated at higher temperatures relative to aflatoxin G_1. AFB_1 production was maximum between24-28 °C whereas 23 °C was most favorable for AFG_1 production.

REGULATIONS WITH AFLATOXINS

Aflatoxins represent the main threat worldwide. After 1975 there was an increased concern about the possibility of the presence of carcinogenic mold metabolites, particularly aflatoxins in food and animal feed products. Although aflatoxins are controlled by regulation in more than 80 countries, their legislation is not yet completely harmonized at the international level (Cucci *et al*., 2007). Several institutions around the world have classified and regulated aflatoxins in food. The European Union has the most rigorous regulations concerning mycotoxins in food. The limits of AFB1 and total AF in foods are 5 and 10 µg/kg, respectively, in more than 75 countries around the world whilst they are 2 and 4 µg/kg in the European Union (Herzallah, 2009). The International Agency for Research on Cancer (IARC) classified aflatoxins as Group 1 of human carcinogens (Alcaide-Molina *et al*., 2009). In USA, the U.S. Department of Agriculture and the U.S. Food and Drug Administration (FDA) have established an "actionable" level of 15-20 ppb of AFs in animal feed products.

CROPS CONTAMINATED WITH ASPERGILLUS FLAVUS AND AFLATOXIN

The main crops susceptible to fungal growth and consequently to mycotoxins' production include peanuts (raw, roasted, sweet and infrosted), corn (popcorn, hominy and grains), wheat, rice, nuts, walnuts, hazelnuts, cashew, almond, dried fruits, spices, cotton seed, cassava, vegetable oils, cocoa and others that are normally used in the composition of foods and feeds. In horticultural crops, fruits and vegetables appear to be of minor sources of mycotoxins and are primarily associated with dried fruits (figs and prunes), certain processed products (apple and grape juice) and are probably apples and grapes

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(Dombrink-Kurtzman, 2008). Most reports concerning aflatoxin formation on fruits refer to figs or citrus fruits (Drusch and Ragab, 2003).

Different species of *Aspergillus*; *A. candidus*, *A. flavus*, *A. niger*, *A.terreus*, and *A. ruber* were dominant during storage of maize, groundnut and soybean seeds as recorded by Bhattacharya and Raha (2002). Similar observations were reported by Singh *et al.* (2004) in groundnut and Kumar *et al.* (2004) in pigeon pea. The fungi responsible for the rot disease in infected groundnut seeds yielded three different fungi, *A. niger*, *A. versicolor* and *A. fresen* (Ihejirika *et al.*, 2005). Similarly, the seed mycoflora of Brazil nut pods (*Bertholletia excelsa*) dominated by *Penicillia* and *A. flavus* Arrus *et al*. (2005)The surface disinfected seeds of cowpea, lupin and bean showed lesser incidence of seed-borne storage mycoflora compared to non-disinfected seeds (Embaby and Abdel-Galil, 2006). The most frequently isolated fungi from these seed samples include *A. niger*, *A. parasiticus*, *Aspergillus* spp. *F. oxysporum*, *Fusarium* sp. and *Trichodermaspp*.

Similarly, the most frequently isolated fungi from stored nuts including pistachio, peanut, hazelnut and almond nuts were species of *Aspergillus*, *Penicillum*, *Mucor* and *Fusarium* reported from different regions of Tehran (Khosravi *et al*., 2007).

About 1200 rice samples consisting of paddy (675) and rice grains (525) were screened which were collected from 20 rice growing states in India covering 43 locations. The screening method showed that all samples recorded *Aspergillus* spp. incidence which included *A. flavus* (1002), *A. niger* (910), *A. ochraceus* (154) and *A. parasiticus* (331) (Reddy *et al*., 2009). Similarly, Freitas - Silva *et al.* (2011) reported *Aspergillus* section *Flavi* isolate was the predominant fungus found in Brazil nut followed by *Aspergillus* section *nigri* and *circumdati* and other *Fusarium* species. Freier *et al*. (2001) also reported that among 17 species of *Aspergillus*, *A. flavus* was the predominant one followed by *A. niger*, *Penicillium citrunum* and *P. glabrum* in brazil nut.

EFFECT OF AFLATOXIN ON HUMAN AND LIVESTOCK

Aflatoxins, AFB1 are potent protein synthesis inhibitors, impair differentiation in sensitive primordial cells (Moss and Smith, 1985) and lead to teratogenic effect on ducklings, hamsters, rats, trout, rabbits and a number of other vertebrates. In India, aflatoxin contaminated groundnut cake and maize meal contributed to the death of more than 2,00,000 broiler chickens and 2000 baby chickens in Andhra Pradesh and Karnataka respectively (Jelinek *et al.*, 1989; Vasanthi and Bhat, 1998).

Toxicity of AFB1 is ten times that of potassium cyanide, 68 times higher than arsenic and 416 times greater than melamine. Furthermore, their carcinogenicity is over 70 times than that of dimethyl-nitrosamine and 10000 times than that of benzene hexachloride (BHC) and international agency for research on Cancer (IARC) of the World Health Organization (WHO) has accepted that aflatoxin should be classified as a Group 1 carcinogen in 1987, and then AFB1 was classified as Group 1 (carcinogenic to humans) by the WHO-IARC in 1993 (Li et al., 2009).

Aflatoxins are biosynthetic inhibitors both *in vivo* and *in vitro*, with large doses causing total inhibition of biochemical processes and lower doses affecting different metabolic pathways. Aflatoxin inhibit oxygen uptake in tissues by acting on adenosine tryphosphatase enzyme of electron transport chain resulting in decreased production of ATP. Aflatoxin also reduces hepatic glycogen level, probably by inhibiting glycogenesis or depression of glucose transport to liver cells or acceleration of glycogenilysis (Moss and Smith, 1985).

There are two general forms of the disease caused by exposure to aflatoxin, aflatoxicosis, poisoning resulting from ingestion of moderate to high levels of aflatoxins in contaminated food or feed. Acute aflatoxicosis results in rapid progressive jaundice, edema of the limbs, pain, vomiting necrosis, cirrhosis or in severe cases, acute liver failure and death (Fung and Clark, 2004; Lewis *et al.*, 2005). Outbreaks of acute aflatoxicosis from contaminated food in humans have been documented in Kenya, India, Malaysia and Thailand (CAST 2003). However, the most widely spread outbreak of aflatoxicosis in humans occurred in more than 150 villages in western India in 1974 where 397 peoples were affected and 108 people died (Krishnamachari et al., 1975). In July 2004, an incident of aflatoxin poisoning in Kenya had occurred involving 317 cases and 125 deaths due to consumption of aflatoxin contaminated maize, the largest and most severe outbreaks of acute aflatoxicosis documented worldwide (Centre for Disease Control and Prevention, 2004;Lewis, 2005; Strosnider et al., 2006).

Chronic aflatoxicosis causes cancer with the liver as the primary target organ, besides immune suppression, teratogenicity and other symptoms (Bennett and Klich, 2003). There is also evidence that respiratory exposure to aflatoxin increases the occurrence of respiratory and other cancers (Dvorackova, 1990). Hepatocellular carcinoma (HCC) is the third-leading cause of cancer death globally according to WHO (2008), with 550,000–600,000 new cases each year. Eighty-three percent of these deaths occur in East Asia and sub-Saharan Africa (Parkin et al., 2005; Strosnider et al., 2006; Kirk et al., 2006). According to researches by University of Pittsburgh, aflatoxin may play causative role in 4.6–28.2% of all global HCC cases (Liu and Wu, 2010). Liver cancer has an increasing incidence that parallels the rise in chronic hepatitis B (HBV) and hepatitis C (HCV) infection (Liu and Wu 2010). HBV infection and aflatoxin synergize to produce ~30-fold higher liver cancer risk in HBV-positive, aflatoxin-exposed persons, as compared to HBV-negative persons (Groopman et al., 2008; Wu and Khlangwiset, 2010; Liu and Wu, 2010). In addition to liver cancer, aflatoxin has also been linked to stunted growth in children and immune system disorders (Gong et al., 2004; Jolly et al., 2008; Khlangwiset et al., 2011).

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AFLATOXIN AND PLANT HEALTH

Earlier to 1980s, aflatoxin problem was considered as a postharvest problem, and research was focused only on postharvest problems. However, severe pre-harvest aflatoxin contamination was reported in Australia, and in several countries of Asia and Africa. Surveys conducted in different parts of India have revealed that groundnuts and their products are high-risk commodities for aflatoxin contamination (Ghewande *et al.*, 1989; Sahay and Rajan, 1990; Kolhe *et al.*, 1994). Research work carried out in India has shown that seed infection by *A.flavus* and aflatoxin contamination increase with advancing maturity of pods, indicating the importance of harvesting the crop at optimum maturity.

Aflatoxin contamination in crop plants can be divided into two distinct phases. The first phase takes place in the developing crop where the infection occurs through the wounds caused either mechanically by birds, mammals, insects or due to the stress resulting from hot dry conditions (Sommer *et al.*, 1986; Odvody *et al.*, 1997; Dowd, 1998; Guo *et al.*, 2003). The second phase of contamination may occur at any time from crop maturation to consumption (Cotty, 2001). It occurs when the mature crop is exposed to warm, moist conditions either in the field or during transportation/storage (Cotty, 1991).

The economic impact of *A. flavus* invasion and aflatoxin production includes reduced seed germination rates and compromised product quality such as mold growth, discoloration and unpleasant odor, chemical and nutritional alterations (Paster and Bullerman, 1988). Aflatoxin affects amylase activity in germinating seeds causing inhibition of starch hydrolysis and consequent nonavailability of sucrose to the embryonic axis during imbibitions. The embryos of aflatoxin contaminated seeds remain alive with fairly high dehydrogenase activity and are capable of growth in culture when supplemented with sucrose (Chatterjee, 1988).

The aflatoxins are produced as the fungi grow on food grains and in other agricultural products. Some macroscopic observations show that aflatoxins affect the plants by inhibiting seed germination, and elongation of the hypocotyls or roots of seedlings. The production of aflatoxins in food grains interferes with protein synthesis by inhibiting the incorporation of amino acids into protein, resulting in non-germination of embryo. Proteins, lipids and free fatty acids are reported to change significantly when peanuts are infected with *Aspergillus flavus* or *A. parasiticus* (Deshpande and Pancholy, 1979). Changes in soluble proteins and enzymes as indicators of infection of some nuts with *A. flavus* also have been reported (Deshpande and Pancholy, 1979).

Aflatoxin inhibits chlorophyll synthesis which results in the albinism of the affected plants (Reiss, 1971). Electron microscopic studies also revealed the inhibition of grana formation in chloroplasts of maize leaves treated with aflatoxins. Aflatoxin inhibition of chlorophyll-a, chlorophyll-b and proto-chlorophyll in the expanding cotyledons of cucumber (Cucumis sativa) has been studied. It also binds to DNA and thus prevents RNA synthesis (Crisan, 1970).Tripathi and Mishra (1981) recorded the inhibition in chromatin bound DNA dependent polymerase activity in germinating maize seeds treated with aflatoxins.

Inhibition of seed germination and seedling growth by aflatoxin B1 has been studied earlier in other crop plants (Crisan, 1973). Mehan and Chohan (1974) also recorded 100 % inhibition in seed germination of *Phaseolus aureus* due to the toxins present in the culture filtrate of the toxigenic strain of *Aspergillus flavus*. Hell K (2002) recorded inhibition in seedling growth of maize at a higher concentration (600 µg/l) of aflatoxin B1.

Llewellyn et al. (1982) studied the effect of aflatoxin level on *Pimpinella anisum* (anise) and *Cuminum cyminum* (cumin) seeds by treating with AFB1 (2.5 and 5.0 µg/ml concentrations) and observed higher toxin concentration caused more severe degree of retardation in root elongation in both seed types

Aflatoxin also causes physiological abnormalities during seed growth. AflatoxinB1 significantly reduced the seed germination and seedling growth of mung bean also reduction in root and shoot length was also recorded with AflatoxinB1 concentration at 1000 µg/1.(Sinha and Kumari, 1990).

In cotton, *A. flavus* affected quality of fiber by causing boll rot, infection of the fiber is known as yellow spot disease (Marsh et al., 1955). Inhibition or significant reduction in seed germination, seedling growth as well as chlorophyll and carotenoid contents of mustard and gram seeds were observed with different concentrations of aflatoxin B1 (Sinha *et al.*, 1992).

Aflatoxin B1 significantly lowered the contents of nucleic acids and protein (both quantitative and qualitative) of germinating seeds of maize and also the contents of chlorophylls and carotenoid in seedlings (Prasad *et al.*, 1996). Similar observations were reported where aflatoxin from *A. flavus* induced albinism in corn and citrus seedlings and lightening of chlorophyll coloration at 100 µg of aflatoxin was observed by Reiss *et al.* (1971) when *Lepidium* seeds were treated at different concentrations of aflatoxins.

Prasad (1997) observed maize seeds treated with combined concentrations of aflatoxin and citrininin the ratio of3:1 and 1:3 (v/v) inhibited seed germination, seedling growth, chlorophyll, carotenoid, sugar, protein and nucleic acid contents, ∝-amylase activity and respiratory quotient. Embaby and Abdel-gail, (2006) were with opinion that the surface borne storage moulds affect seed germination. Lupinseeds recorded higher rate of germination after surface disinfection when compared to non-disinfected seeds. In a similar kind of study adverse effect on germination, shoot length and root growth in five genotypes of soybean seeds was observed when soaked in culture filtrate of aflatoxin producing strain (Ahmmed et al., 2008).Reduction in seed germination, shoot growth and suppression in the activity of cellulase and amylase enzymes was observed with increase in concentration of aflatoxin in bean, red gram, green gram, and black gram (Janardhan et al., 2011). In another study, the effect of crude toxic metabolite AFB1 of *A. flavus* was tested on seed germination, seedling vigor and chlorophyll content in three cultivars of maize. All the parameters significantly

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reduced in aflatoxin treated seeds (Shirurkar and Wahegonkar, 2012).

MANAGEMENT STRATEGIES

Aflatoxin production by aflatoxigenic fungi in crops varies from year to year. Prevention of fungal attack and aflatoxin contamination is possible by using healthy seeds, proper irrigation, rotation of crops, harvesting after full maturity, drying the harvested crops within time and storing them under proper atmosphere and preventing insect or other damages to the crop (Cotty, 1991). However, even in spite of careful management practices, unacceptable aflatoxin levels may occur from exposure of the developing crop or from exposure of the mature crop either prior to harvest or during storage in modules, handling, transportation or even while use.

Chemicals (fungicides) have been reported to inhibit the growth of aflatoxigenic fungi and subsequent aflatoxin production. Today, there are strict regulations on chemical pesticides use, and there is an attempt to restrict the most hazardous chemicals from the market (Pal and Gardener, 2006). Hence, it is necessary to replace chemical pesticides or fungicides to overcome possible environmental pollution and health problems.

Alternatively, during recent years biological control methods are an important alternative to synthetic chemicals. Biological control is a promising approach for reducing both aflatoxin contamination and *A. flavus* infection under pre-harvest and postharvest conditions in groundnut (Dorner and Cole, 2002; Dorner *et al.*, 2003). A number of biological agents (bacteria, yeasts, actinomycetes, algae) (Mishra and Das, 2003) and non-aflatoxigenic strains of *A. flavus* and *A. parasiticus* (Dorner, 2004) have been tried for biological control of aflatoxin contamination. Aflatoxin reduction has been achieved by applying non-aflatoxigenic strains of *A. flavus* and *A. parasiticus* to soil around developing plants (Brown *et al.*, 1991; Cotty, 1994). Geocarposphere (pod-zone) bacterial strains were reported to be reducing groundnut pod colonization by *A. flavus* (Mickler *et al.*, 1995; Anjaiah *et al.*, 2006). In addition, several edible botanical extracts have been reported to have good inhibitory effects on both growth of *A. flavus* and *A. parasiticus* and aflatoxin production *in vitro* (Bhatnagar *et al.*, 1990; Pradeep *et al.*, 2003).

Good agricultural practices are basically the fundamental prevention strategies, with particular attention to correct manuring and irrigation; need based water supply is considered another important preventive action in peanuts (Dorner *et al.*, 1989). It was also suggested that late season irrigations could increase soil moisture and decrease soil temperature and thereby used as a promising way to lower aflatoxin content in mature seeds (Wilson *et al.*, 2002).

Various physical, chemical, and biological methods to reduce the aflatoxin level in foods and feeds have been tried for many years. It is hoped that understanding the biosynthesis of aflatoxin will facilitate development of control strategies and provide an understanding of how and why aflatoxin was evolved. The method that has received utmost attention is the treatment of aflatoxin-contaminated feeds (primarily cottonseed, corn, and peanut products) with ammonia (i.e. ammoniation) (Smith and Moss, 1985).

In addition to chemical method, plant products have also been found useful in inhibiting *A. flavus* invasion and aflatoxin contamination during postharvest processing and storage. Thepropionic acid (3ml/g) treatment of groundnuts, stored at 90% relative humidity, has been found to reduce *A. flavus* infection considerably (Patker *et al.*, 1995).

There are two possibilities to control fungal contamination *i.e.* heat treatment or chemical treatment, but it is necessary to replace chemical pesticides or fungicides to avoid soil pollution and health problems. Alternatively, antifungal agents produced by microorganisms may be used as biocontrol agent (Chitarra *et al.*, 2003). Biological control offers an important alternative to synthetic chemicals.

MANAGEMENT WITH MICROORGANISMS

Control of phyto pathogens by biological means was environmentally advantageous in comparison to chemical control which has many risks on human health and environment (Nautiyal, 2001). So far a number of fungi, bacteria (Kimura and Hirano, 1988; Karunaratne *et al.*, 1990) and yeast (Paster *et al.*, 1993) have been shown to inhibit the growth of *A. flavus in vitro*. Aflatoxins cannot be readily removed from contaminated foods by detoxification. Therefore, there is interest in developing a biological control method that can increase crop safety by decreasing toxin content and that is based on the displacement of toxigenic isolates using atoxigenic isolates of the same species. It has been reported that aflatoxin production is inhibited by lactic acid bacteria, *Bacillus subtilis* and many molds. This inhibition may result from many factors including competition for space and nutrients required for aflatoxin production but not for growth and production of aflatoxigenic metabolites by co existing microorganisms.

Many microorganisms including bacteria, yeasts, moulds, actinomycetes and algae are able to remove or degrade aflatoxins in foods and feeds (Line and Brackett, 1995). Some strains of lactic acid bacteria have been reported to be effective in removing aflatoxin B1 from contaminated liquid media (El-Nezami *et al.*, 1998). Nazazato *et al*. (1990) reported four fungal strains, *A. niger*, *Eurotium herbariorum*, *Rhizopus* sp. and non aflatoxin producing *A. flavus* were able to convert AFB1 to aflatoxicol (AFL). These fungi could convert AFB1 to aflatoxicol-A (AFL-A). Then by actions of medium components or organic acids produced from the fungi, AFL-A was converted to aflatoxicol-B (AFL-B). Other microorganisms were also tested for their possible ability to degrade aflatoxins. The strain *Nocardia asteroids* reduces AFB1 by biotransformation to another fluorescent product (Arai *et al.*, 1967). Shapira (2004) reported *Cornebacterium rubrum* has the ability to degrade aflatoxin. Significant reduction of AFB1 was observed by *Rhodococcus erythropolis*; only 17% residual AFB1 was

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left after 48 h and only 3-6% was detectable after 72h (Tenoila *et al*., 2005).

BACTERIAL STRAINS

In the 1960s researchers screened over 1000 microorganisms for their ability to remove aflatoxin from solution. Of these, bacterium *Flavobacterium aurantiacum* which could irreversibly removed aflatoxin from various foods. Fazeli (2009) demonstrated that the bacterium actually metabolized the toxin to water soluble degradation products and CO_2. They concluded that mechanism of detoxifying aflatoxin was mineralization. The association of fluorescent *Pseudomonas* with crop roots and proliferation in the rhizosphere has been found to be beneficial to both cereals and legumes. These bacteria help the development of a healthy root system by virtue of their quick colonization of the rhizosphere which leads to the plant growth promoting effects, prevention of pathogen establishment in the rhizosphere through antibiotics or siderophore production and stimulation of plant defense mechanisms (Bolton *et al.*, 1990; Thomashow *et al.*, 1990; Loper and Buyer, 1991; Vanpeer *et al.*, 1991; Bonsall *et al.*, 1997).

Faraj *et al*. (1993) showed that *Bacillus subtilis* to inhibited *Aspergillus* growth and *B. stearothermophilus* inhibited aflatoxin production by *A. flavus* and *A. parasiticus*. Mixing *B. subtilis* with groundnuts reduced the damage caused by *A. flavus*. Mass screening of bacteria for antagonistic activity against *A. flavus* was done by Mishagi *et al*. (1995). They reported that there was 60-100% reduction in *A. flavus* incidence by *P. cepacia* isolate in synthetic media and the same isolate showed 41-100% reduction in damaged locules in field conditions. However, this is the first report of a bacterial antagonist that is capable of reducing *A. flavus* induced damage to a plant in the field.

Complete inhibition of aflatoxin production as well as growth of toxigenic strains of *A. flavus* was observed by Dorner (2004) on PDA plates. The bacterial strain *Bacillus subtilis* succeeded in the inhibition of growth of *A. parasiticus* and *A. flavus* in corn and peanut. The antifungal metabolites produced by *Bacillis pumilus* grown on potato glucose broth have inhibited the mycelial growth of *Aspergillus*, *Penicillium* and *Fusarium* spp. and aflatoxin production by *A. parasiticus* (Munimbazi and Bullerman, 1998b).

The *Pseudomonas* species isolated from the rhizosphere of green gram have wide range of antifungal activity against phytopathogenic fungi like *Aspergillus* spp., *Curvularia* spp., *Fusariumoxysporum* and *Rhizoctoniasolani* in culture and they also proved to be effective in nodule promotion which in turn leads to symbiotic nitrogen fixation and plant growth (Sindhu *et al*. 1999). Kong *et al*. (2010) first made an attempt to investigate the efficacy of marine strains of *Bacillus megaterium* isolated from yellow sea of east China against growth of *A. flavus* in groundnut kernels under *in vitro* and *in vivo*.

NONTOXIGENIC ASPERGILLUS FLAVUS

As much as 40% of soil isolates of *A. flavus* are incapable of producing aflatoxins (Cotty *et al*., 1994). Addition of atoxigenic strains of *A. flavus* to the soil of susceptible crops to dilute toxin producing strains is being used to remediate aflatoxin contamination of cotton and peanuts (Cotty and Bayman 1993, Horn *et al*., 2000 Initial studies with biocontrol strategy in 1987 using naturally occurring strain of *A. parasiticus* (NRRL 18991) was carried out by Dorner *et al*., 1992). The nonaflatoxin strains of *A. flavus* used or proposed for biological control of mycotoxins contamination of agricultural crops have been isolated from soil (Cotty 1994; Dorner *et al*., 1999; Abbas *et al*., 2006).Not only does application to soil of nontoxigenic strains of *Aspergillus* reduced levels of preharvest aflatoxin contamination in peanuts, but it also showed a carryover effect, reducing contamination that may occur during long term bulk storage (Dorner and Cole, 2002).

Cotty and Bayman (1993) reported that atoxic *Aspergillus* species competed successfully with toxic isolates in a mixed culture condition, but the competition mechanism is not well elucidated. Application of nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* in the field is a very successful strategy to reduce aflatoxin contamination of preharvest crops (Dorner, 2005). The non aflatoxin producing strains occupy the same niche as the natural toxigenic strains. The method of competitive exclusion has been successfully applied to cotton, peanut and corn fields (Cotty *et al*., 1996; Dorner, 2005). In a comparative study with three types of nontoxigenic *A. flavus* formulations an average of 92% reduction in aflatoxin contamination was reported in peanut plots during second year of treatment (Dorner *et al*., 2003). Later, Dorner and Horn (2007) observed the efficacy of nontoxigenic strains in controlling aflatoxin contamination in peanutsby applying natuarlly occurring nontoxigenic strains of *A. flavus* and a UV induced mutant of *A. parasiticus* in separate and in combination.

TRICHODERMA SPECIES

Trichoderma species are free-living fungi which are highly interactive in root, soil and foliar environments. Considered to be eager colonizers and particularly invasive fungi they work against fungal phyto pathogens either indirectly by competing for nutrients and space, modifying environmental conditions or promoting plant growth and enhancing plant defense mechanisms and antibiosis or directly through mechanisms such as myco-parasitism. This dominance is achieved by biosynthesizing a wide array of secondary metabolites, transforming a great variety of natural and xenobiotic compounds and producing varied degradative enzymes such as chitinase. Gachomo (2008) demonstrated that volatiles from *Trichoderma* species were able to arrest the hyphal growth of different fungal pathogens on agar plates. Calistru and McLean (1997) reported that two isolates of *T. harzianum* and two of *T. viride* were capable of inhibiting the growth of *A. flavus* by morphological alteration of micro heads. There was reduction in *A. flavus* contamination from 32% to 8% and

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aflatoxin contamination from 92 µg/kg to 26 µg/kg when seeds of groundnut were treated with *Trichoderma viride* and *Trichoderma harzianum* (Waliyar *et al*., 2005).

Antagonistic activity of culture filtrate of *Trichoderma* isolate against *Aspergillus parasiticus* showed inhibition zone of 33.75mm and this may be due to production of some biological metabolites (Thanaboripat *et al*., 2006). Similar kind of investigation culture filtrates of *Trichoderma viride* and *T. harzianum* were found inhibitory to*A. flavus* (Rifi *et al*., 1969).

Enzyme activities of *Trichoderma* are effective in suppressing the growth of peanut mouldsincluding *Fusarium* species, *A. niger*, *A. flavus*, *A. parasiticus* and *A. ochraceus* by antagonistic assay on PDA plates (Gachomo and Kotchoni, 2008). Further, the *Trichoderma* isolates were inoculated to inhibit aflatoxin production by molds infecting peanut. Suppression in growth of aflatoxigenic fungi by *Trichoderma* species was observed which in turn resulted in lesser production of aflatoxins by smaller colonies. Considerable decrease in the production of aflatoxin was reported with pre-sterilized volatile compounds of *T. harzianum* (Augero *et al*., 2008). The ability of spore suspensions of *T. harzianum* and *T. viride* to efficiently suppress the growth of *A. flavus* and*A. parasiticus* and also to reduce aflatoxins in peanut kernels was demonstrated by Gachomo *et al*. (2008).Potential*Trichoderma* isolates can be used for pre-harvest aflatoxin contamination of groundnutSrilakshmi *et al*. (2001).An *in vitro* antagonistic activity against was exhibited by *Trichoderma* isolate and its extract against post-harvest pathogens *A. niger*, *A. flavus*, *A. fumigatus*, *Fusrium* sp. and *Penicillium* in dual culture assay and disc diffusion assay (Rajendran, 2010).

MEDICINAL PLANT EXTRACTS

Even though effective and efficient control of seed-borne fungi of seeds can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Frank *et al*., 1987; Gassner *et al*., 1997; Wodageneh and Wulp, 1997; Harris *et al*., 2001). Thus, there is a need to search for alternative approaches that are eco-friendly and not capital-intensive to store grains/cereals for human consumption without toxicity problems. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties in laboratory trails (Meepagala *et al*., 2002;Pokhrel *et al*., 2002;Sang *et al*., 2002; Chowdhury *et al*., 2003;Garg and Jain, 2003; Parcha *et al*., 2003; Wilkinson *et al*., 2003; Dukic *et al*., 2004; Lee *et al*., 2004; Svetaz *et al*., 2004). Plant metabolites and plant-based pesticides appear to be better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999).

Green plants appear to be reservoir of biotoxicants and constitute inexhaustible source of number of pesticides. Srivastava (2003) have mentioned that natural fungicides are free from environmental toxicity as compared to synthetic compounds. Natural compounds are less phytotoxic, easily biodegradable and more systematic (Saxena *et al*., 2005). Plant essential oil for controlling fungus in food preservation has been used for hundreds of years (Bullerman *et al*., 1977). Morozumi (1978) isolated o-methoxycynamaldehyde from cinnamon and demonstrated that this compound highly effective against *A. flavus* and *A. parasiticus.*

The biosynthesis of aflatoxin B1 can be inhibited by extracts of certain plants that are toxic to fungi and may be useful in controlling the fungal growth and mycotoxins production. TLC analysis showed significant decrease in the concentration of aflatoxin B1 production and a reduction in *A. flavus* biomass when aqueous extract of *Polymnia sonchifolia* was amended with YES broth medium (Pinto *et al*., 2001). Extracts of plants such as garlic and onion, effectively retarded the growth and aflatoxin production (Chen, 1999). Natural compounds such as flavonoids, stilbene, essential oils and others were also active in inhibition of aflatoxin production (Sobolev *et al*., 2006). Ethanolic extracts of olive callus tissues inhibited aflatoxin production by 90% in BNM *A. flavus* without inhibiting the fungal growth (Parter *et al*., 1988).

Donil and Dauda, 2003 recommended aqueous moringa seed extract (AMSE)as biofungicide which potentially reduced the growth of*A. niger*, *A. flavus*, *Rhizoctonia stolonifer* and *Mucor* species in groundnut..

An ecofriendly management of *A. flavus* infection and aflatoxin contamination in peanut was carried out by Sandosskumar *et al*. (2007) where significant reduction (95%) in the accumulation of AFB1 and 56% decrease in *A. flavus* incidence were recorded when peanut was intercropped with zimmu.

A significant decrease ingrowth and/or sporulation of *A. flavus* and two *A. parasciticus*and also aflatoxin B1 production was recorded when assayed with different concentrations of essential oils of clove, poleo, mountain thyme and eucalyptus (Bluma *et al*., 2008).

Dry formulations of natural plant products serve as a good tool for safer storage of soybean seed for sowing purpose and also to overcome *A. flavus* concentration and ultimately to check mycotoxins production. Significant decrease in the incidence of *A. flavus* and an elevation in the germination percentage were recorded by monthly evaluation of six months stored soybean seeds treated with leaf powder of *W. somnifera*, *H. suavelens*, *E. citriodara* and peel powder of *P. granatum* as compared to untreated control (Krishnamurthy *et al*., 2008). Best inhibition of *Aspergillus flavus* was recorded with methanolic extracts *Saussurealappa* (Parekh and Chanda, 2008).

Srichana *et al*. (2009) suggested that high concentration of betel leaf extract inhibited the activity of *A. flavus*. The first report on inhibitory effect of essential oil of *Aegel mermelos* on aflatoxin production and fungal growth in stored commodities was reported by Singh *et al*. (2009).The essential oil of *A. mermelos* completely inhibited the aflatoxin B1 production and at 750 µl/l concentration it completely stopped even the growth of fungi.in Czapek's medium

Distilled herbal waters of herbal and aromatic plants; Oregano, Pickling herb, Savory and Thyme (black)

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samples exhibited complete inhibitionof *A. flavus* in CZ media by Ozkalp and Ozcan (2009).

Ethanol extract of *Centilla asciatica* (L) suppressed the growth of *A. flavus*comparison with water and petroleum ether and antibiotic ciprofloxacin (Jagtap *et al*., 2009).

Methyleugenol (4-allyl 1,2 dimethoxybenzene) is a naturally occurring substance usually present in many essential oils and fruits. Sudakar *et al*. (2009) for the first time reported its activity in complete inhibition of *A. flavus* colonization and AFB1 production in peanut by spraying 0.5% methyleugenol on pods and kernels. This study also revealed that prophylactic treatment or post infection spray of 0.5% methyleugenol can prevent post harvest *A. flavus* colonization and aflatoxin synthesis. Similar work was carried out by Gouramma *et al*. (1997) using cinnamom and clove oils which contain cinnamic aldehyde and eugenol. Velazhahan *et al*. (2010) studied the effect of seed extracts of *Trachyspermumammi* (Ajowan) in detoxifying aflatoxins. Among aqueous extracts of leaves or seeds of medicinal plants belonging to 17 families, only seed extract of ajowan showed maximum degradation of AFG1 (65%). Further it was found that dialyzed *T. ammi* extract was more effective (90% degradation of aflatoxin G1) than the crude and which also showed detoxification of other aflatoxins like AFB1, AFB2 and AFG2 with respective degradation of 61, 54 and 46%.

Mohana *et al*. (2011) recommended high concentrations of plant extracts such as *Acacia nilotica*, *Caesalpinia coriaria*, *Decalepis hamiltonii*, *Emblica officinalis*, *Lawsonia innermis* and *Mimosops elengi*to manage the seed borne pathogenic fungiincluding *Alternaria alternata*, *A. flavus*, *Curvularia lunata*, *Drechslera oryzae*, *D. halodes*, *Fusarium monoliforme*, *Pyricularia oryzae* and *Trichoconis padwickii* as alternative to fungicides.

Hexane and chloroform extracts from dried fruit rinds of *Garcinia cowa* and *G. pedunculata* were effectivein inhibiting the *A. flavus* growth and reducing aflatoxin production (Joseph *et al*., 2005).

Essentialoil of *Ammomumsubulatum* was found as an effective suppressor in inhibiting not only the mycelia growth of toxigenic strains of *A. flavus* but also completely inhibited aflatoxin B1 production (Singh *et al*., 2008).

Essential oils of nutmeg, cinnamom, clove and eucalyptus showed inhibitory activity against *A. flavus*, *A. niger*, *A.terreus*, *A. oryzae*, *A. fumigatus*, *F. moniliforme*, *F.solani* and *Penicillium* (Shirurkar and Wahegonkar, 2012).

CONCLUSION

Aspergillus flavus is an opportunistic pathogen with broad host range and its toxic secondary metabolite aflatoxin contamination is major devastating issue of many agricultural crops including oil yielding crops, cereals, millets and others. However their contamination occurs under field conditions they cause severe losses during storage period due to improper handling and poor transportation facilities. The present review revealed the occurrence of aflatoxins, and their management through eco-friendly approach.

Species of *Aspergillus* section Flavi including *A. flavus*, *A. parasiticus* and *A. nomius*, are known to cause about 25% yield loss in agriculture crops every year (FAO, 2008). Temperate and tropical conditions are favorable for growth and aflatoxin production by these fungi in stored products. Proper identification, characterization and accurate differentiation of aflatoxigenic and non-aflatoxigenic strains of A. flavus are necessary to understand diversity in order to develop proper management strategy. So far many national and international research groups have been tried in order to characterize and control *A. flavus* growth and aflatoxin production in various crops. In order to provide the current status of aflatoxigenic fungal infection and aflatoxin contamination in groundnut across different agro climatic conditions of India and to develop suitable eco-friendly management strategy

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CONFLICT OF INTEREST STATEMENT

We declare that no conflict of interest.

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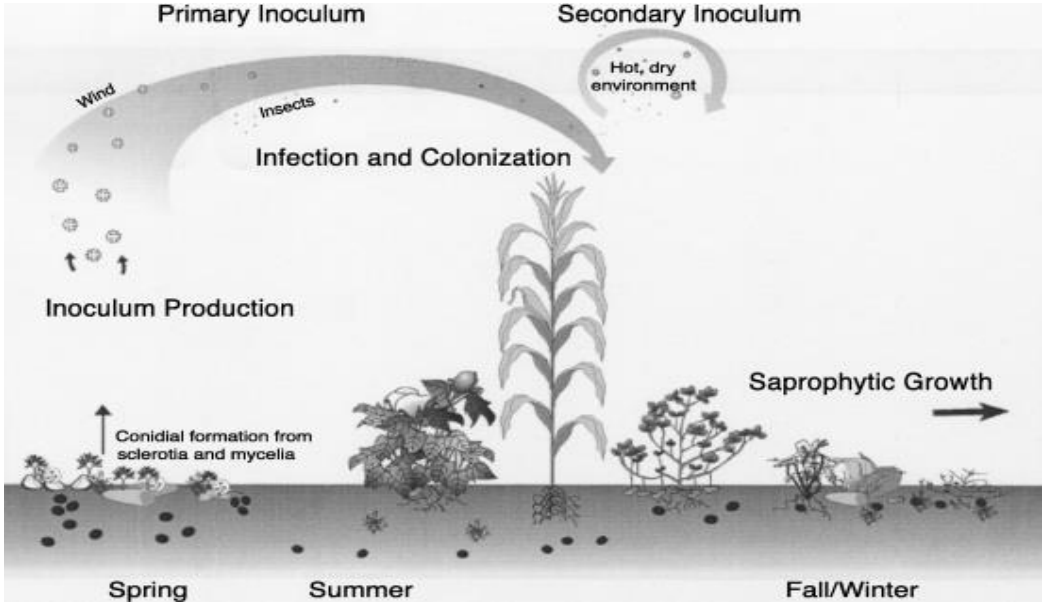

Figure 1.Pre-harvest infection of cotton, corn and peanuts by *Aspergillus flavus*. Sclerotia and conidia produced by *A. flavus* growing on crop debris and in the soil serve asprimary inoculum for young plants in the spring. Later in the growing season, conidia produced on crop debris or on infected plants provide high levels of secondary inoculum when environmental conditions are conducive for disease development. (Copyright Marcel Dekker Inc.) (Scheidegger *et al*., 2003).