www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 5/3 (2012) 123-133

Review article/Derleme

Mycotoxins as health hazard

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Abstract

A mycotoxin is a toxic secondary metabolite produced by an organism of the fungus kingdom, including mushrooms, molds, and yeasts. Mycotoxins are non-volatile, relatively low-molecular weight secondary metabolites of certain fungi that are toxic to human beings, plants and animals. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. Mycotoxicosis is the poisoning by ingestion of mycotoxins through food contaminated by toxigenic fungi. Mycotoxins comprise a structurally diverse and chemically complex group of fungal metabolites and many of which have been implicated as significant health hazards on a world wide scale.

Key words: Mycotoxins, Health hazard, Secondary metabolite

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Sağlık açısından bir tehlike olarak mikotosinler

Özet

Bir mikotoksin; mantar, küf ve maya da dahil olmak üzere, mantar aleminden bir organizma tarafından üretilen toksik sekonder metabolitidir. Mikotoksinler, insan, bitki ve hayvanlar için toksik olan belirli mantarların uçucu olmayan, göreceli olarak düşük molekül ağırlıklı ikincil metabolitlerdir. Toksin üretimi iç ve dış ortamlarda bağlıdır ve toksin bulaşmış organizma ve duyarlılık, metabolizma ve savunma mekanizmalarına bağlı olarak, onların şiddeti büyük ölçüde değişir. Mikotoksikoz toksijenik mantarlar tarafından kontamine yiyecek yoluyla mikotoksinler tüketilmesi ile zehirlenmesidir. Mikotoksinler fungal metabolitlerin yapısal olarak farklı ve kimyasal karmaşık bir grup oluşturan ve bir çoğu dünya çapında bir ölçekte önemli sağlık tehlikesi olarak sorumlu tutulmuştur.

Anahtar kelimeler: Mikotoksin, Sekonder metabolit, Sağlık açısından tehlike

1. Introduction

A mycotoxin (from Greek - mykes means "fungus" and Latin - toxicum means “poison") is a toxic secondary metabolite produced by an organism of the fungus kingdom, including mushrooms, molds, and yeasts (Turner et al., 2009; Richard, 2007). Mycotoxins are non-volatile, relatively low-molecular weight secondary metabolites of certain fungi that are toxic to human beings, plants and animals. Chemically mycotoxins are non-antigenic compounds of low molecular weight. These toxins are generally detected in milk, cheese, corn, peanuts, cotton seeds, wheat grains, pulses copra, almond, figs, spices and other foods and feeds. They are fungal secondary metabolites are formed by consecutive series of enzyme catalyse reactions from a few biochemically simple intermediates of primary metabolites such as acetate, mevalonate, malonate and certain amino acids. Mycotoxins are toxic, secondary metabolites of low molecular

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weight produced by naturally occurring fungi (Chu, 1992). Uraguchi and Yamazaki (1978) defined mycotoxins as "secondary fungal metabolites capable of causing pathological changes or physiological abnormalities in man and warm blooded animals." In the present context, the term ''mycotoxin'' may be defined as "a group of chemically unrelated secondary fungal metabolites which are detrimental to living organisms and cause illness and death in human and animals and in certain cases are also harmful to green plants." The reason for the production of mycotoxins is not yet known; they are neither necessary for growth nor the development of the fungi (Fox and Howlett, 2008). The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms.

Mycotoxicosis is the poisoning by ingestion of mycotoxins through food contaminated by toxigenic fungi. Historically, mycotoxicosis has been known for hundreds of years (9^{th} and 18^{th} century) due to death of thousands of people in Europe caused by food-borne and a toxins producing fungus (*Claviceps purpurea*) in rye grains.

The existence of mycotoxins was not documented until 1960. Before 1900, in Italy, researchers there believed consumption of moldy corn by children led to the development of illness (Christensen, 1975). Some experiments, done at that time, included the isolation, and growth of the suspected fungus in pure culture, and isolation of toxic compounds from the fungus that the researchers believed to be the cause of the illness. Burnside et al (1957) studied an extensive outbreak of moldy corn disease in the southeastern United States in the early 1950's where hundreds of wild pigs foraging in cultivated corn fields became ill and many died. Teams of veterinarians and mycologists collaborated to determine the cause of the deaths of these pigs. They isolated a number of different fungi from the moldy corn and inoculated each fungus on moist corn that had been sterilized and then fed them to pigs. The consumption of corn inoculated with *Aspergillus flavus* caused outward signs and inward lesions found in other cases of the so-called moldy corn disease. However, since there was no toxin(s) isolated, there was little attention paid to the article since it still seemed like old news, i.e. domestic animals poisoned by eating moldy corn.

It would not be until 1960, when approximately 100.000 turkeys and a lesser number of other domestic birds died in England, causing losses of approximately several hundred thousand dollars, before the first mycotoxin was isolated and identified. The first appearance of aflatoxins is often dated to a shipment of contaminated groundnut (peanut) meal delivered to Britain from Brazil in 1959. The meal was used in poultry feed that killed turkeys, ducklings and game birds (pheasant and partridge). The syndrome was called "Turkey X disease" and was characterized by extensive liver damage including fatty change and subcutaneous hemorrhage. Further imports of contaminated meal killed calves and pigs in Britain. Contaminated cottonseed meal causing liver cancer in farm-raised trout was found to be due to the same agent. Reexamination of these earlier cases and a number of mass kills of livestock, especially of farmed fish in the US in earlier years suggested that the same agent had played a role there too. Using the toxicity to ducklings to monitor purification, the toxin was obtained from *Aspergillus flavus* found in groundnuts from Uganda and was dubbed an aflatoxin.

3. Results and discussion

2.1 Types of mycotoxins

Mostly all the fungi are equipped with toxin producing ability depending on environmental conditions. There are different types of mycotoxins produced by different fungi like, *Aspergillus, Penicillium, Fusarium, Stachybotrys, Alternaria, Claviceps, Boletus, Amanita, Coprinus, Myrothecium, and Pithomyces* etc.

2.1.1. Aflatoxins

Aflatoxins are a group of related difurano coumarin secondary metabolites produced by certain strains of *Aspergillus flavus* and *A. parasiticus*. The mycotoxin isolated was named aflatoxin, the "a" from *Aspergillus* and "fla" from *flavus*. Feeding test of food containing aflatoxin, with various laboratory animals, demonstrated that to varying degrees, all animals tested were sensitive to aflatoxin. These compounds were isolated from groundnut milk following outbreak of liver diseases among chicks, turkies, pigs, and calves etc. during 1960, 1961 in Great Britain. Further, cows eating contaminated feed will produce milk containing the slightly modified aflatoxins M.

Four different types of aflatoxins were identified and their chemical structures were determined. These are - Aflatoxin B_1, B_2, G_1 and G_2 (Martins et al., 2001) (Figure 1). The former two show characteristic blue fluorescence and the latter two bluish-green or green fluorescence under ultraviolet light which forms the basis of the most simple chemical assay procedure used at present to detect aflatoxins in food and feed. B_1 and G_1 are the most abundant forms in nature than their dihydroderivatives B_2 and G_2 occurring in lesser amount. Milk from cow feed on aflatoxin contaminated feed showed a different form of aflatoxin called M_1 and M_2 (Figure 2). Aflatoxin B_1 is one of the most potent carcinogens known, being capable of inducing liver cancer at concentration below 1 $\mu g\ kg^{-1}$ body weight. Aflatoxins may come out into eggs or may be accumulated in muscles of hen or in milk of cow, meat of cow etc. thus entering easily into human food chain. Moreover, mycotoxins are highly thermostable often withstanding a temperature of cooking and thus poisoning the food product and thereby poses a serious threat to human population. They are most

oftenly produced in groundnut but can also be produced in breads, cakes and other bakery products. The optimum temperature for the growth of aflatoxin producing strains of *Aspergillus* is 30 °C with a relative humidity 75% to 80% or even more. They are soluble in chloroform, ethanol, benzene, methanol and other polar solvents but not soluble in water.

Aflatoxins are acutely toxic to most of the mammals although there are considerable variations in their toxicity towards different groups of mammalian species. Depending upon the concentration aflatoxins may be carcinogenic, mutagenic, teratogenic or oestrogenic with the results of reduced immune responses and acute disease syndrome. The organs most commonly affected by aflatoxins and some other mycotoxins are livers, but other organs like kidney, adrenal glands, ovary, stomach etc. also show acute damage and develop bleeding lesions and cancers. Hepatic carcinoma is the main symptom of aflatoxin hazards and almost 50% cases of liver cancer is caused due to aflatoxin contamination. Only 0.3-ppm concentration of aflatoxin has been proved to be the potent dosage (minimum level) for causing hepatic carcinoma. It has been reported that dietary aflatoxin B1 (AFB_1) adversely affected growth performance, feed conversion, apparent digestibility coefficients, and cause physiological disorders and histological changes, in particular on hepatopancreatic tissue (Boonyaratpalin et al., 2001; Burgos-Hernadez et al., 2005).

Conversely, AFB_1 levels between 50–100 ppb showed no effect on growth in juvenile black tiger shrimp (*Penaeus monodon*). Nevertheless, growth was reduced when AFB_1 concentrations were elevated to 500–2500 ppb. Survival dropped to 26.32% when 2500 ppb AFB_1 was given, whereas concentrations of 50–1000 ppb had no effect on survival. There were marked histological changes in the hepatopancreas of shrimp fed diet containing AFB_1 at a concentration of 100–2500 ppb for 8 weeks, as noted by atrophic changes, followed by necrosis of the tubular epithelial cells. Severe degeneration of hepatopancreatic tubules was common in shrimp fed high concentrations of AFB_1 (Boonyaratpalin et al., 2001).

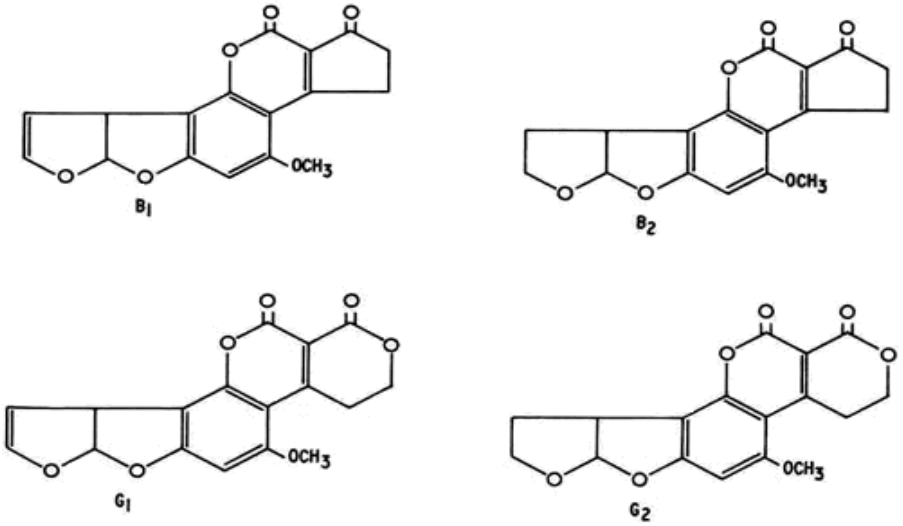

Figure 1. Structures of aflatoxins B_1, B_2, G_1, and G_2

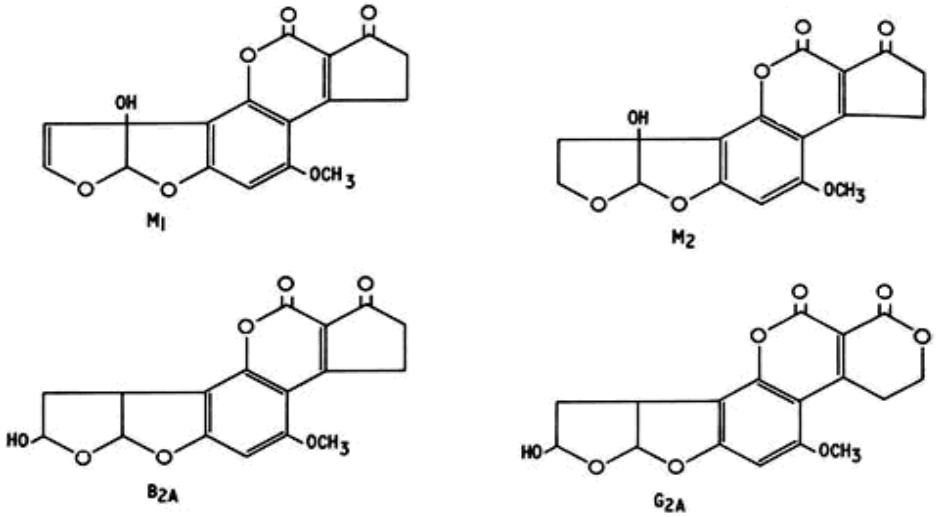

Figure 2. Structures of Aflatoxins M_1, M_2, B_{2A}, and G_{2A}

2.1.2 Fusarium toxin

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize (Schaafsma and Hooker, 2007). Several species of *Fusarium* produce different types of mycotoxins liks – moniliformin, fumonisins, Trichothecenes and Zearelenone. Trichothecenes is also known as T–2 mycotoxin that is deoxyninalenol. Zearelenone is also known as F-2 mycotoxin. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins (Desjardins and Proctor, 2007).

2.1.3 Zearalenone (Zear.)

It is naturally occurring mycotoxin produced by *Fusarium moniliforme, F. roseum, F. oxysporum, F. rtrichinous* and *F. graminearum* which results in estrogenic syndromes involving swelling of vulva, rectal and vaginal prolapse, enlargement of uterus etc (Figure 3). Zearelenone is found to be toxic to swine population and also to the other mammals including human beings. It causes bleeding lesions on genital organs and develops cancer in ovaries, uterus etc. This toxin develops most of the cervical cancers. It causes non-functioning in ovaries, abortion and abnormal piglets. Sometimes the male swine shows the symptoms of feminization. It may also cause dermatitis, oesophageal cancer, digestive disorders and promote cancer in urinary bladder.

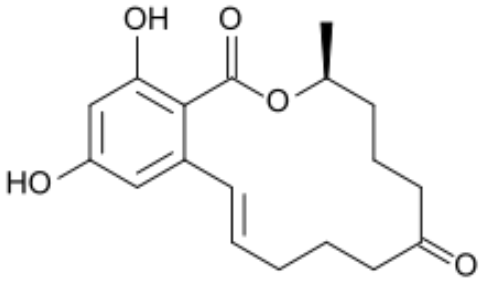

Figure 3. Structure of Zearalenone

Figure 4. Structure of Fumonisins

Fumonisins (Figure 4) causes leukoencephalomalacia and moniliformin (Figure 5). Fumonisins isolated from *F. moniliforme* and are found to cause diseases in horses. It has also been reported to have cancer promoting activity in rat (Roy, 1996). Fumonisins also produced by maize pathogen *F. verticillioides* and *F. proliferatum* and at very low levels, by *Alternaria* in black end stem rot in tomatoes, asparagus and garlic (Seefelder et al., 2002). *Fusarium* toxins are mostly produced in rice, corn, wheat, wheat flour, corn flakes etc. Trichothecenes produce inactivity, degeneration of cells in bone marrow and intestinal cerosis, degeneration of bone cells and malformation of bones and cartilages even diarrhoea, haemorrhage and death. The report of using trichothecenes in biological war was published during Vietnam War. The yellow rain occur in certain parts of Vietnam were chemically analysed by different European scientists and was found to contain trichothecenes group of chemicals.

2.1.4 Trichothecenes

The effects of the first trichothecene toxin, T-2, documented was in the 1940s where it was associated with an outbreak of alimentary toxic aleukia (ATA). At its peak, in 1944, the population in the Orenbury District and other districts of the then USSR suffered enormous casualties, more than 10 percent of the population was affected and many fatalities occurred. The term alimentary toxic refers to the toxin being consumed in foods and aleukia refers to the reduced number of leucocytes or white blood cells in the affected person. Other symptoms included bleeding from nose and throat, multiple, subcutaneous hemorrhages.

The infected food in this case was millet, which made up a great part of the diet of the people in the region, and at times, during WWII, it was not uncommon to allow the millet to be left standing in the fields over winter because bad weather in the fall prevented its harvest at the proper time. During the late winter and early spring the millet would become infected with a variety of fungi, including *Fusarium tricinctum*, and when the people gathered and ate this fungus, many came down with what was diagnosed as ATA. Thousands were affected, and many died. Locally, Joffe, a plant pathologist determined the outbreak of ATA was caused by consumption of a toxin, present in the millet, which had been contaminated by *F. tricinctum.* This was a remarkable conclusion since this was 20 years before aflatoxin was discovered. However, Joffe did not isolate or identify the toxin involved and as a result his work remained unknown until about 1965 when he presented a summary of his research at a symposium on mycotoxins. The mycotoxin involved

was later given the common name T-2, and classified as one of several trichothecenes. Fed orally to rats, it has an LD50 of 3.8mg/kg, which is lower than that of aflatoxin, but still toxic enough.

The trichothecenes are a very large family of chemically related toxins produced by various species of *Fusarium, Trichoderma, Verticimonosporium, Myrothecium*, etc. The distinguishing chemical feature of trichothecenes is the presence of a trichothecene ring, which contains an olefinic bond at C-9, 10; and an epoxide group at C-12, 12 (Figure 6). There are several naturally occurring trichothecenes in food and feeds elaborated by *Fusarium* spp including T-2 mycotoxin and deoxynivalenol. T-2 mycotoxin has been studied most extensively and is a natural contaminant of food and feed.

All trichothecenes are mycotoxins, but not all mycotoxins are trichothecenes. This family of mycotoxins causes multiorgan effects including emesis, cardiovascular alterations, immunodepression, skin toxicity and bone marrow damage. The tichothecenes are nonvolatile, low molecular weight (MW 250-550) compounds (Ueno, 1989). This group of mycotoxins is relatively insoluble in water but highly soluble in acetone, ethyl acetate, chloroform, dimethyl sulfoxide, ethanol, methanol and propylene glycol. Purified trichothecenes generally have a low vapor pressure, but they do vaporize when heated in organic solvents. Extraction of trichothecenes from fungal cultures with organic solvents yields a yellow-brown liquid, which, if allowed to evaporate, forms a greasy, yellow crystalline product. Some experts believe this extract to be the yellow contaminate of yellow rain. In contrast, highly purified toxins form white, crystalline products that have characteristic melting points (Mirocha et al., 1983; Watson et al., 1984). When maintained as either crystalline powders or liquid solutions, trichothecene mycotoxin compounds are stable when exposed to air, light, or both.

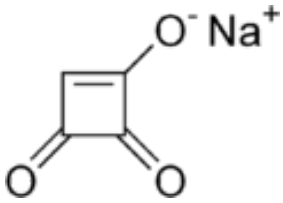

Figure 5. Structure of Moniliformin

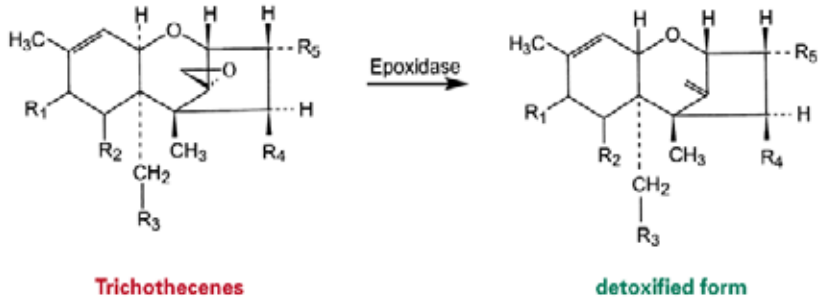

Figure 6. Detoxification of trichotecenes by microbial enzymes. The change of structure deactivates its toxic effects.

2.1.5 Vomitoxin

Vomitoxin, also known as deoxynivalenol (DON), is a type B trichothecene, an epoxy-sesquiterpeneoid (Figure 7). This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize, and less often in rice, sorghum, and triticale. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum*, both of which are important plant pathogens which cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize. A direct relationship between the incidence of *Fusarium* head blight and contamination of wheat with deoxynivalenol has been established. The incidence of Fusarium head blight is strongly associated with moisture at the time of flowering (anthesis), and the timing of rainfall, rather than the amount, is the most critical factor. Furthermore, deoxynivalenol contents are significantly affected by the susceptibility of cultivars towards *Fusarium* species, previous crop, tillage practices, and fungicide use (Beyer et al., 2006). Vomitoxin and other type B trichothecenes are produced by *Fusarium sp.* and can be an important contaminant of wheat. Deoxynivalenol levels of 200, 500, and 1000 ppb in the diet significantly reduced body weight and growth rate in white shrimp *Litopenaeus vannamei* (Trigo-Stockli et al., 2000).

When compared to other trichothecene mycotoxins which can form in grains and forages, vomitoxin is relatively mild. Vomitoxin belongs to a class of mycotoxin (tricothecenes) which are strong protein inhibitors. Inhibition of protein synthesis following exposure to vomitoxin causes the brain to increase its uptake of the amino acid tryptophan and, in turn, its synthesis of serotonin. Vomitoxin is not a known carcinogen as with aflatoxin. Large amounts of grain with vomitoxin would have to be consumed to pose a health risk to humans. The U.S. Food and Drug Administration has established a level of 1ppm (parts per million) restriction of vomitoxin.

2.1.8 Ochratoxin A

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochatoxin A (Bayman and Baker, 2006).

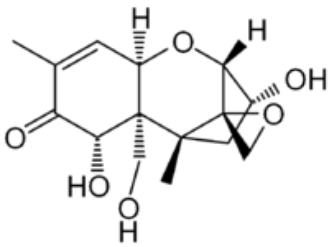

Figure 7. Structure of Vomitoxin

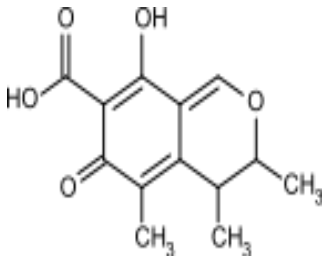

Figure 8. Structure of Citrinin

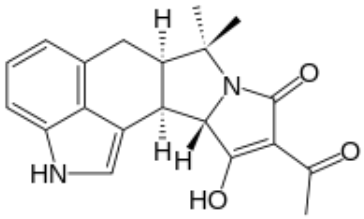

Figure 9. Structure of Cyclopiazonic acid

2.1.6 Penicillium toxins

Several species of *Penicillium* produce toxins like Patulin, Citrinin, Citriviridin, Cyclopiazonic acid, Penicillic acid etc. These toxins are mainly produced in mouldy peanut, dry fruits, rice, corn, cheese, chestnut, cider, apple juices and different fruit juices made from fruits infected with P*enicillium* spp., molded bakery products, wine, jam, jelly, syrup, scented supari, bean etc. These toxins are responsible for cardiac beriberi, kidney damage, capillary damage, oedema, bloody diarrhoea and death. Rubratoxin and Ochratoxin are produced by species groups of *Penicillium*. These toxins cause linear diseases, degeneration and necrosis of liver. They can persist in cooked foods also and thereby possessing a public health problem.

Citrinin is a methylated heterocyclic compound produced mainly by the toxigenic strains of *P. citrinum* (Figure 8). It is associated with yellow rice disease in Japan and acts as a nephrotoxin in all animal species tested. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys (Bennett and Klich, 2003). Cyclopiazonic acid was originally isolated from the culture of *P. cyclopium.* (Figure 9). There are evidences that cyclopiazonic acid might have been involved along with the aflatoxins in the "Turkey X" syndrome in England in 1960 (Roy, 1996).

2.1.7 Citreoviridin

Several species of *Penicillium* (e.g. *Citro viride*) and also species of *Aspergillus* have been reported to produce Citreoviridin in food and feedstuffs (Figure 10). There. It causes paralysis, cardiomuscular disturbances and loss of eyesight in experimental animals.

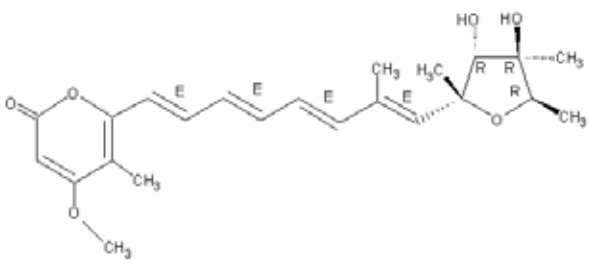

Figure 10. Structure of Citreoviridin

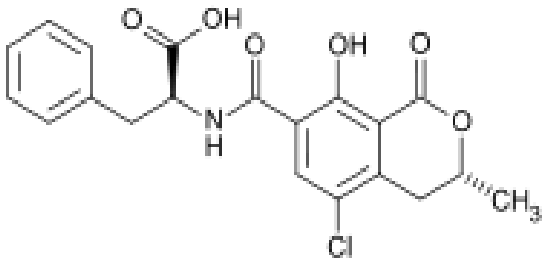

Figure 11. Structure of Ochratoxin A

Ochratoxin A is a pentaketide/ amino acid hybrid molecule which is produced by numerous species of P*enicillium* and *Aspergillus*, especially *P. verrucosum*, which is common on cereals in temperate climates and *A. ochraceus* and *A. carbonarius* which grow on the flesh of coffee berries during drying (Figure 11). There. Coffee can, therefore, be contaminated with Ochratoxin A, mercifully much of it is destroyed during roasting. It is highly nephrotoxic and has been implicated in a degenerative human kidney disorder called 'Balkan endemic nephropathy', it is also suspected to cause cancer of the gall bladder. Further, Ochratoxin A causes a renal degenerative disorder of farm animals, especially pigs. A chemically closely related mycotoxin is citrinin.

Production of ochratoxin, by *Aspergillus chraceus*, was first described in South Africa by Theron et al., (1966), where it was isolated along with a number of other fungi. In experiments done with this isolate, he LD 50 of ochratoxin for rats is 22mg/kg, but a lesser amount will result in severe liver damage. A single dose of 12.5 mg/kg was administered to pregnant rats on the tenth day of gestation, and of the 88 fetuses involved, 72, or 81.8% died or were resorbed. Ducklings seem to be equally sensitive to ochratoxin as they are to aflatoxin.

2.1.9 Patulin

Patulin is a toxin produced by the *Aspergillus*, *Penicillium*, *Byssochlamys nivea* and *Paecilomyces* fungal species (Figure 12). *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs. It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moss, 2008). Patulin is a small (tetraketide-derived) molecule, its biosynthesis is complex, involving the formation and subsequent cleavage of an aromatic ring.

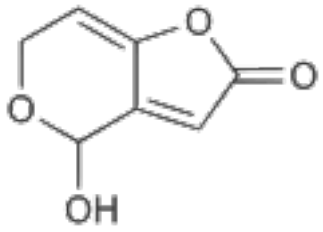

Figure 12. Structure of Patulin

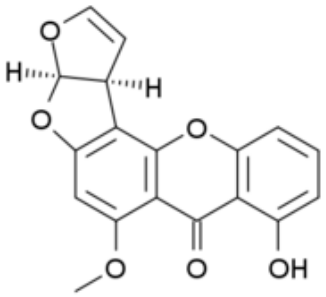

Figure 13. Structure of Sterigmatocystin

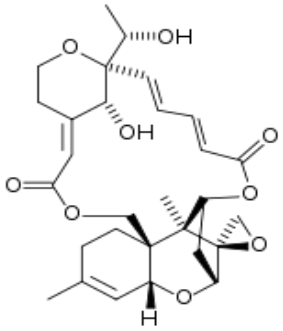

Figure 14. Structure of Satratoxin H

2.1.10 Sterigmatocystin

This toxin is produced by *Aspergillus versicolor*, *Penicillium lateum* and *Bipolaris* spp is responsible for hepatic carcinoma and cancer of renal track and renal tumour (Figure 13). It is produced in cereals and legumes. It is a precursor in the biosynthesis of aflatoxins. *Aspergillus versicolor*, under the right conditions, produces sterigmatocystin, a toxic compound given the name because the fungus once was called *Sterigmatocystis*. The toxin is known to cause lung, liver and kidney tumors in laboratory animals and has been implicated as the cause of disease in calves that have consumed feed heavily invaded by *A. versicolor*. The toxin has also been detected in moldy coffee beans in Africa, but no evidence indicates that even if these beans were used to brew coffee that the toxin would be in the drink (Bokhari and Aly, 2009).

Aspergillus fumigatus is known to be an animal pathogen. Infection occurs through inhalation of spores and affects the lungs. Infection may also occur in eggs and the fetuses of cows. However, it also produces a metabolic product that may be considered a toxin or an antibiotic. This species is said to be thermophilic, that is, it is found in substrate where there are extremely high temperatures, up to 122 ºF (=50 ºC). Under the proper conditions, *A. fumigatus* produces fumagillin. This compound is used as an amoebicide that is, as a means to rid the body of amoebae that are human pathogens and has been used effectively in honey bees as well. However, the correct dosage of this compound is critical. A little bit more than you need to get rid of the amoebae and you will be getting rid of the patient as well. *A. fumigatus* is known to produce various immunosuppressive mycotoxins including gliotoxin. Gliotoxin was found to be detectable in the sera of aspergillosis mice and aspergillosis patients (Kamei and Watanabe, 2005).

2.1.11 Satratoxin H

Satratoxin H is produced by *Stachybotrys chartarum* and caused stachybotrotoxicosis (Figure 14). Stachybotrotoxicosis also arose in the Soviet Union. The first outbreak was reported in 1931 when a number of horses were affected by a syndrome that typically began with an irritation of the mouth, throat, nose and lips accompanied by swollen glands that often progressed to death. Necropsies showed extensive internal hemorrhaging, especially on the digestive tract, but with all major organs affected. These symptoms were typical of small intakes of contaminated feed. The syndrome was not transmissible, indicating a poisoning and it was eventually traced to hay contaminated with the fungus *Stachybotrys chartarum*. The fungus produces a number of toxic compounds of which satratoxin H is the most abundant. In recent years, stachybotrotoxicosis has become recognized as a public health concern in the United States. This was first brought to public notice in the 1990's when cases of young children with bleeding in the lungs began appearing in Cleveland, Ohio.

Tremorgens (literally 'tremor producing') are produced by species of *Penicillium, Aspergillus*, *Claviceps*, and *Acremonium.*

2.2 Effect and mode of action of mycotoxins

Most of the mycotoxins that have the potential to reduce growth and health status of animals consuming contaminated feed are produced by *Aspergillus, Penicillium* and *Fusarium sp.* These toxic substances are known to be

either carcinogenic (e.g. aflatoxin B1, ochratoxin A, fumonisin B1), estrogenic (zearalenone), neurotoxic (fumonisin B1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes) or immunosuppressive (aflatoxin B1, ochratoxin A and T-2 toxin).

Mycotoxicoses is the term used for poisoning associated with exposures to mycotoxins. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxics have been poorly studied. Therefore it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins (Bennett and Klich, 2003). In turn, mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lyphmpatic system, they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin.

After ingestion mycotoxins enter in human body and encounter various molecules. Toxins interact with gastrointestinal microflora, epithelial cells of intestine liver, bile, blood, kidney, reproductive and nervous systems, skin and lungs. Some of the effects of mycotoxins are briefly discussed below.

2.2.1 Effect on energy production

There are several mycotoxins, which inhibit certain enzymes involved in Krebs's cycle. Moniliformin, a toxin of *Fusarium*, inhibits the oxidation of pyruvate and α-ketoglutarate. It also causes disturbances in intracellular osmoregulation.

2.2.2 Inhibition of synthesis of DNA, RNA etc.

Aflatoxin B_1 inhibits DNA synthesis in liver cells. This is caused due to covalent binding of aflatoxin B_1 to DNA and proteins. This is also known to inhibit nuclear RNA synthesis in liver cells of rat. Aflatoxin B_1 causes delay in interferon production in turkeys and at high doses, it reduces IgG and IgA in chicks with consequences of decreased acquired immunity. Aflatoxin also reduces cell-mediated immune response in animals. Ochratoxin inhibits the activity of phenylalanine – tRNA synthetase which is required in the first step of protein synthesis. It also reduces the renal mRNA coding for certain enzymes such as phosphoenolpyruvate carboxylase.

2.2.3 Effects on nervous system

Mycotoxins are grouped into three categories on the basis of mode of action

a) Mycotoxins causing paralysis and inhibition in respiratory systems e.g., citrioviridin. They kill the nerve cells disrupting energy supply as they inhibit ATP-ase activity.
b) Mycotoxins inducing trembling in animals, e.g., fumitremorgin A, penitrem A which alter the functional states of the neurotransmitters and disrupts the nervous system.
c) Mycotoxins causing vomiting in animals, e.g., Trichothecenes, Phallodin, Amatoxin etc. They act on chemoreceptor trigger the zone in medulla oblongata and change the biogenic amines.

2.2.4 Effect on hormonal activities

In target cells, steroid hormones regulate the functions. Steroid forms the complexes with receptors, which are then activated and transported to the nucleus. They bind with the activator sites of chromatin and induce protein synthesis selectively. Aflatoxin B_1 binds covalently with acceptor sites of chromatin and thus reduces the nuclear acceptor sites of hormone receptor complex (HRC) and consequently hormonal activities are reduced.

2.2.5 Carcinogenic effects

Aflatoxins (B_1, G_1, M_1, B_2 and G_2), Sterigmatocystin, Versicolorin A, Luteoskyrin are known as carcinogenic mycotoxins. These are also known as genotoxins. The chemicals which cause gentle damage and initiates the carcinogenic processes are known as genotoxic or initiator chemicals whereas those which promote transformation of genetically modified cells to cancerous cells are known as promoters. Aflatoxin B_1 binds with the guanine base of DNA and forms a large bulky adduct which dis-stabilize the DNA double helix and mislead for DNA replication. When these bulky adducts get accumulated, they form cancers.

2.2.6 Effect on reproductive systems

Urogenital system of swine cattle and poultry birds are known to be affected by zearalemone which at 1 ppm produces hyperstrogenism in pigs. In young male swine it produces testicular atrophy and mammary gland enlargement,

infertility. Ergot ingestion may cause abortion in animals. Moreover, ergot is also associated with reduced weight gain and milk production in animals (Dubey, 2006).

2.2.7 Effect on immune system

Mycotoxins that impair the immune system include AFB_1, T-2 toxin, OTA, DON and fumonisin. Most of this toxins cause impairment of the immune system by inhibiting the synthesis of key proteins associated with the immune function.

Consumption of trichotecene mycotoxins causes suppression of immune response by reducing both phagocytic activity and chemotaxis by macrophages (Manning, 2001).

Total hemocyte, granulocyte and phenoloxidase activity were reduced in shrimp fed with T-2 toxin and zearalenone (Supamattaya et al., 2006). Aflatoxin, suppresses phagocytosis by macrophages, which alters subsequent processing and presentation of antigen to B lymphocytes with consequential reductions in disease resistance (Manning, 2001).

2.3 Control and management of mycotoxins

Although the presence of mycotoxins in feed represents an increase threat to aquaculture operations there are a number of options available to feed manufacturers and farmers to prevent or reduce the risk of mycotoxicosis associated with mycotoxin contamination. These range from careful selection of raw materials, maintaining good storage conditions for feeds and raw materials, and using an effective mycotoxin deactivator product to combat the widest possible range of different mycotoxins that may be present.

Binders or adsorbents have been used to neutralize the effects of mycotoxins by preventing their absorption from the animal's digestive tract. Unfortunately, different mycotoxin groups are different in their chemical structure and therefore it is impossible to equally deactivate all mycotoxins using only one single strategy. Adsorption works perfectly for aflatoxin but less, or non-absorbable mycotoxins (like ochratoxins, zearalenone and the whole group of trichothecenes) have to be deactivated by using a different approach.

Biotransformation is defined as detoxification of mycotoxins using microorganisms or enzymes which specifically degrade the toxic structures to non-toxic metabolites. BBSH 797, a *Eubacterium* species, patented by Biomin®, produces enzymes, so-called de-epoxidases, which degrade the toxic epoxide ring of trichothecenes.

Mycofix®Plus is a mycotoxin deactivator which combines adsorption and bio-inactivation to break functional groups of mycotoxins such as trichothecenes, ochratoxin A and zearalenone, and also includes immune-stimulation with addition of selected plant extracts.

It is a very complicated problem and it is unlikely that an acceptable overall solution will be developed in near future. Several attempts or methods have been developed to detoxify the aflatoxin and other mycotoxin contaminations in food and feed by using various physical and chemical and biological means.

2.3.1 Chemical methods

Of the several chemical methods so far developed using H_2O_2, Ammonia and O_3. Ammoniation (use of ammonia gas) resulted in significant reduction in levels of aflatoxins in contaminated peanuts, cottonseed meals, corn etc. But this method has some disadvantages like possible presence of toxic derivatives of aflatoxin.

2.3.2 Physical method

There are several methods for detoxifying aflatoxin and other mycotoxin contaminations like-

a) Heat treatment (Sun dried)
b) Removal of infected seeds or portions of the seeds from seed lots (Electronic or hand sorting).
c) Adsorption
d) Filtration
e) Density gradient separation
f) Exposure to sunlight and other radiations
g) Solvent extraction method etc.
i) Crop rotation should be adopted to lower the primary inoculum of toxigenic fungi.

2.3.3 Biological method

Bioremediation of aflatoxin contamination is an another dimension of research by using some microorganisms like *Trichoderma, Pseudomonas* etc. but it requires some high skilled genetic engineering technology. Biological

method of detoxification should be adopted by using certain strains of yeasts, moulds or bacteria e.g. *Flavobacterium aurantiacum* (NRRL B-184) from the liquid medium. These catalyse the hydration of aflatoxins.

2.4. Use of trichothecenes as a biological weapon

During the mid 1970s, when Vietnam was invading Laos, there were stories of "yellow rain" in areas where entire villages were killed. One eye witness account of such an event was told by a Hmong refugee, in Thailand. While tending his poppies, outside of his village, he and his family witnessed the bombing of their village by the Vietnamese, in MIGS, with a yellow powder that came down like yellow rain. Returning to the village, he found all of the animals and most of the people were dead. The bodies were bleeding from the nose and ears and their skin were blistered and yellowed. The few people left alive, when he arrived, were "jerking like fish when you take them out of the water". These people also eventually died. The witness took his family away from the village, but as they left they felt shortness of breath and sick to their stomach. This story is similar to other stories that were heard concerning yellow rain.

It was believed by the United States at that time that the Soviet Union was somehow involved in what occurred in the Hmong village, and medical teams were sent to investigate. However, because of the remoteness of these villages, news of such attacks normally took 4 to 6 weeks to reach someone who could notify the medical teams. By the time investigators reached a village, there was no evidence as to what happened. It would not be until 1980 that a Defense Department chemist recognized the symptoms described by victims of the bombing as similar to trichothecene mycotoxicosis. Samples from victims and from vegetation in the areas were tested and some were found to contain trichothecenes. With this information, President Ronald Reagan accused the Soviet Union of violating the Geneva Convention and Biological Weapons Convention, which of course they denied. However, these accusations would continue for three more years.

While the accusations and denials were aired, the media and scientific community gave a more critical examination of the yellow rain story. The analysis that demonstrated Trichothecenes were being used was initially based on a single leaf, collected where one of the chemical attacks occurred. Subsequent specimens were collected later that also showed Trichothecenes were present, but the ratio of trichothecenes differed where it was found and was entirely absent in some samples. In addition, little fanfare was given to the over one hundred samples analyzed by the United States Army, which did not find any indication of trichothecenes. The eye witness accounts also came into question. Although it was implied that many villages were attacked with yellow rain, all of the witnesses were from a single refugee camp in Thailand, and even these accounts were thought to be unreliable. For example in relating a story of the bombing, one villager had initially said that 213 villagers were killed, but in a later retelling, there were only thirteen people killed and then forty. Further erosion of the government's yellow rain story came about when a Yale University entomologist, whose expertise was in Southeast Asian bees, examined yellow rain samples and observed that they contained pollen from the native plants in the area. Based on the appearance of these samples, it was concluded that they were feces of bees. In one species of bees, present in the area, there is a tendency for the bees to swarm when they defecated, as a cleansing ritual, which could give the appearance of yellow rain falling. News of such chemical attacks soon stopped and many civilian scientists were convinced that the entire yellow rain incident was a hoax that was carried out by the military to increase funding for defensive chemical and biological weapons.

2.5 Suggestions to prevent mycotoxin contamination of feedstuffs

- Control the environmental factors that influence fungal growth
  Moisture content of grain (<14%), Relative humidity (<70%), Temperature (-2.2 ºC), Oxygen availability
- Control the physical condition of the grain
  Minimize grain damage during harvest
  Screen grain to reduce broken kernels
- Clean storage system regularly, *****Use mold inhibitors and anti-caking additives
- Ammoniation-to reduce aflatoxin concentrations, *Floating separation-*Fusarium* – infected kernels are lighter than sound kernels, *Wash,wet or dry milling and heating process (roasting, boiling, baking and frying), *Addition of 0.5% hydrated sodium calcium aluminosilicate in formulated feed.

4. Conclusions

The experience of the past four decades indicates that it is possible to maintain present food consumption levels by increasing overall food supplies in quantitative terms. However, in terms of providing food of the right quality that is nutritious and free from mycotoxins, the task ahead is challenging, particularly in developing nations of the world. Many research institutes, including Directorate of Rice Research, India, have carried out on mycotoxin contamination and developed technologies (viz. use of botanicals and microbiologicals) that can significantly reduce contamination, but these technologies are not adopted by the farmers due to lack of awareness. Hence this review aimed to document the level of knowledge and extent of adoption of mycotoxin management practices of rice and constraints

faced by farmers in adoption of this technology through various programs. Finally, recent advances in mycotoxicology have made it possible to use this research for improving safe consumption of food that is free of mycotoxins..

Acknowledgements

First author Selima Khatun acknowledges the UGC Meritorious Fellowship, New Delhi, Government of India, for financial assistance.

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(Received for publication 10July, 2012; The date of publication 15 December 2012)