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Aflatoxins: Mechanisms of Inhibition by Antagonistic Plants and Microorganisms

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1. Introduction

Aspergillus flavus and some other closely-related members of *Aspergillus* section *Flavi* especially *A. parasiticus* and *A. nomius* are common fungi that normally inhabit as saprobes in soils and on a wide variety of decaying organic matters (Samson, et al., 2000; Varga et al., 2010). Besides being an etiological agent of systemic aspergillosis and allergic reactions, *A. flavus* has received major attention due to its ability to produce the carcinogenic aflatoxins (AFs) (Hedayati et al., 2007). AFs are a group of structurally related compounds found worldwide in a wide array of food and feed crops including maize, peanuts, tree nuts and oilseeds. AF contamination of agricultural crops is a major concern due to economical losses resulting from inferior crop quality, reduced animal productivity and impacts on trade and public health. It has been estimated that about one-fourth of global food supply is contaminated annually with AFB_1, the most toxigenic among AFs. Hence, an action level of 20 ppb for total AFs in foods for human consumption has been imposed by Food and Drug Administration (FDA) in the United States and by regulatory agencies in many other countries. The biosynthetic pathway of AF is one of the best known pathways of secondary metabolism in microorganisms (Trail et al., 1995). Our current understanding about chemistry and molecular biology of AF biosynthesis is a direct consequence of bioconversion experiments using several blocked mutants as well as successful cloning and characterization of the majority of the AF biosynthetic pathway genes (Georgianna and Payne, 2009). About 30 genes and related intermediates are involved in the production of AFs. To ensure global safety on food and feed supplies, extensive researches have been carried out to effectively control and manage AF contamination of crops. Conventional procedures have been used to prevent contamination process in crops before and after harvest, the majority of which are expensive, time consuming and with limited success. These technologies include crop rotation, use of fungicides, and alteration in planting time. The rapid expansion in our knowledge about inhibition of AF biosynthesis by plants and

microorganisms has enable us to utilize them as potential AF biocontrol agents (Alinezhad et al., 2011; Holmes et al., 2008; Razzaghi-Abyaneh et al., 2008, 2009, 2010). A large number of compounds and extracts from natural sources including plants, bacteria, microalgae, fungi and actinomycetes have now been screened for the ability to inhibit toxigenic fungal growth and/or AF production. Substantial efforts have been carried out in identifying organisms inhibitory to AF biosynthesis through co-culture with AF-producing fungi with the aim of finding potential biocontrol agents as well as novel inhibitory metabolites. Despite the positive prospective in combating AF contamination, control of AF contamination of food and feed has not yet been achieved. Understanding the mechanisms by which plants and microorganisms and their bioactive metabolites affect AF biosynthesis is a major focus in the molecular biological study of AF. This endeavor would also help us to advance the knowledge about host plant-toxigenic fungus interactions, one of the most important aspects of AF contamination of crops. Genomics, proteomics and metabolomics studies have revealed novel inhibitory mechanisms on AF production by bioactive compounds from natural sources (Bhatnagar et al., 2008; Brown et al, 2010; Kim et al., 2008). In this review, we describe AF inhibitors from plants and microorganisms with an emphasis on their potential mechanisms of action at cellular and molecular levels. We highlight direct inhibition of AF biosynthesis via interfering with the signal transduction regulatory networks involved in gene expression and also by blocking activities of AF biosynthetic enzymes. We also address indirect inhibition of AF production by plants and organisms that affect toxin synthesis by the mechanisms including (i) down-regulation of fungal genes responsible for oxidative stress defense system which combats metabolic and environmental stressors, (ii) enhancement of plant defense mechanisms through genetic engineering and (iii) disruption of mitochondrial respiration which is critical for providing the first substrate of AF biosynthesis, acetyl-CoA.

2. *Aspergillus* species producing G-type aflatoxins in addition to B-type aflatoxins

Aspergilli that produce only B-type aflatoxins, i.e., AFB_1 and AFB_2 have been reported from species of three groups. They include many isolates of *A. flavus* and infrequent isolates of *A. pseudotamarii* in the section *Flavi*, rare isolates of *Emericella astellata* and *E. venezuelensis* (producing AFB_1 only) from the genus *Emericella* (anamorph: *Aspergillus* section *Nidulantes*) and rare isolates of *A. rambellii*, and *A. ochraceoroseus* from a newly proposed section *Ochraceorosei* (Cary et al., 2005; Frisvad et al., 2005). Several aspergilli besides producing B-type aflatoxins produce the G-type aflatoxins, AFG_1 and AFG_2. Species currently recognized to produce both B- and G-type aflatoxins belong exclusively to section *Flavi* and include *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. minisclerotigenes*, and *A. arachidicola* (Pildain et al., 2008; Varga et al., 2010). Isolates of *A. toxicarious* and *A. parvisclerotigenus* also have been mentioned in this category, but their species status needs further clarification.

3. Plants: Target sites of inhibitory action on AF production

The history of herbal medicine dates back thousands of years ago (Samuelsson 2004). Beneficial plants are widely distributed all over the world, and they are rich sources of useful secondary metabolites often as compounds with therapeutic roles in defense against a wide array of pathogens including viruses, bacteria and fungi, herbivores (both insects and

mammals) and environmental stresses like UV light and ozone (Bakkali et al. 2008; Korkina 2007). Plant bioactive metabolites can be divided into major groups including terpens (terpenoids, isoterpenoids), phenylpropanoids (flavonoids, tannins, glycosides, and lignins), phenolics and nitrogen-containing compounds (alkaloids and heterocyclic aromatics). Search of natural sources for novel inhibitors of AF biosynthesis has been a subject of intense study and a variety of bioactive AF inhibitory compounds have been reported from medicinal plants (Review by Razzaghi-Abyaneh et al. 2010 and references therein).

3.1 Phenylpropanoids from *Anethum graveolens* and *Petroselinum crispum*

Anethum graveolens L. (dill) is a short-lived annual herb cultivated as a native plant in southwest and central Asia including Iran. *Petroselinum crispum* (parsley) is a bright green hairless biennial herbaceous plant in temperate climates, an annual herb in sub-tropical and tropical areas. It is native to the central Mediterranean region including Iran, southern Italy, Algeria and Tunisia and widely cultivated as a herb, a spice and a vegetable. Several biological activities of both *A. graveolens* and *P. crispum* (Apiaceae family) have been attributed to major constituents of the whole plants including monoterpenes, flavonoids, furanocumarins and phenylpropanoids (Crowden et al. 1969). Phenylpropanoids are a large class of plant phenols with a three-carbon side chain and a phenyl ring derived from phenylalanine, an initial precursor, through shikimic acid pathway (Korkina 2007). A large number of plant-derived phenolics including flavonoids, cumarins and lignins are by-products of phenylpropanoid metabolism (MacRae & Towers, 1984). Phenylpropanoids are involved in plant defense against pathogenic and symbiotic microorganisms through cell wall strengthening and repair, direct antimicrobial activity and coordinating signaling and chemotaxis pathways against naturally occurring stressors. They are known for a wide range of biological activities from antimicrobial to adaptogenic, neurotropic, immunostimulatory, antioxidant, antiulcer, anticancer and antiproliferative properties (Korkina 2007 and references therein). In a recent study, we reported the isolation of a phenylpropanoid compound named dillapiol, from leaf essential oil of *A. graveolens* as specific inhibitor of AFG_1 production by *A. parasiticus* with an IC_{50} (50% inhibitory concentration) equal to 0.15 μM without obvious effect on fungal growth and AFB_1 synthesis (Razzaghi-Abyaneh et al. 2007). Another phenylpropanoid, apiol, isolated from the seed essential oil of *P. crispum* in the same study showed similar effects to dillapiol with an IC_{50} value of 0.24 μM for AFG_1. It is proposed that these phenylpropanoids may inhibit AFG_1 biosynthesis via inhibition of CypA, a cytochrome P450 dependent monooxygenase involved in conversion of *O*-methylsterigmatocystin to AFG_1 in AF biosynthetic pathway. More than 20 enzymes are involved in the formation of AFB_1, AFB_2, AFG_1, and AFG_2. Among them, six are P450 monooxygenases, which include OrdA, CypA, AvnA, CypX, VerA and VerB. AvnA is responsible for the conversion of averantin to 5′-hydroxyaverantin (Yu et al., 1997) and CypX for the conversion of averufin to hydroxyversicolorone (Wen et al., 2005). VerA and VerB are both involved in the conversion of versicolorin A to demethylsterigmatocystin (Keller et al., 1994; Keller et al., 1995). Cytochrome P450 monooxygenases belong to the superfamily of proteins that contain a heme cofactor. The active site of a P450 is a heme-iron center. The iron is tethered to the P450 protein via a thiolate ligand derived from a cysteine residue. This cysteine heme-iron ligand signature, F[S/G/E] XGXRXCXG, is present at the N terminal portion of the six P450s involved in AF biosynthesis. It is believed that OrdA and CypA may correspond to the microsomal enzymes and NadA the cytosol enzyme that are

involved in the formation of AFG_1 and AFG_2 (Yabe et al., 1999; Zeng et al., 2011). The CypA gene encodes a P450 monooxygenase, and its knockout in *A. parasiticus* abolished production of AFG_1 and AFG_2 but not AFB_1 and AFB_2. Fig. 1 shows that CypA likely catalyzes epoxidation of a closed ring intermediate of 370 Da (**2**) that is derived from HOMST epoxidized by OrdA followed by first cleavage of the A ring (Ehrlich et al., 2008). This separates AFG_1 formation from AFB_1 formation; the latter requires a second cleavage of the 370 Da intermediate to give an open-ring form (**3**, 388) followed by demethylation, reclosure, a decarboxylation/dehydration step (Udwary et al., 2002). Most recently, Zeng et al. (2011) confirmed in *A. parasiticus* that HOMST was converted to AFG_1 and AFB_1. Cai et al., 2008 showed that the *nadA* gene previously assigned to the adjacent sugar utilization gene cluster instead is required for the formation of AFG_1 and AFG_2. The *nadA* knockout mutants of *A. parasiticus* accumulate a new 360 Da precursor, NADA. As illustrated in the Fig. 1, OrdA performs two epoxidation reactions on the OMST aryl A-ring whereas CypA performs an epoxidation reaction on an alkene (non-aromatic) substrate. They likely have very different catalytic structures. The 370 Da (**2**) substrate has an *O*-methyl group situated next to the alkene where epoxidation by CypA occurs. This structure is similar to the moiety of dillapiol, apiol, and myristicin where *O*-methyl group(s) links to the planar methylenedioxyphenyl ring (Razzaghi-Abyaneh et al., 2007). These *O*-methyl containing phenylpropanoids like other polysubstrate monooxygenase inhibitors (Casida, 1970; MacRae & Towers, 1984) may be able to access and bind to the heme in the active site pocket of CypA but are unable to access or bind to the catalytic heme residue in OrdA, AvnA, CypX, VerA and VerB. This could explain why formation of AFG_1 and AFG_2 but not AFB_1 and AFB_2 was specifically inhibited by these types of compounds. The observed dosage effects may suggest that these inhibitory compounds compete for access with the 370 Da (**2**) substrate.

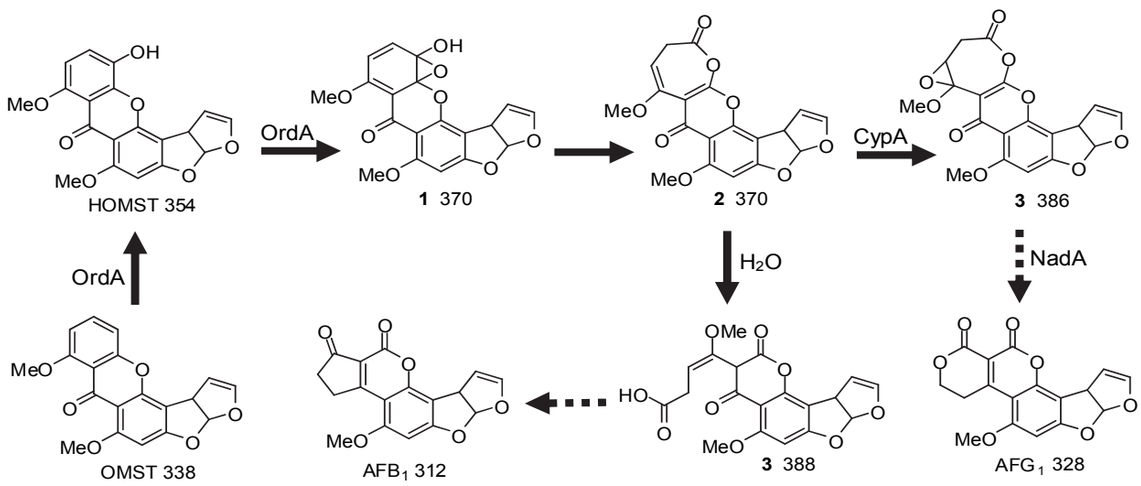

Fig. 1. Proposed pathway of aflatoxin G_1 formation from HOMST. It is believed that apiols exert their specific inhibition on formation of AFG toxins by affecting the pathway specific gene product i.e. CypA or that the inhibition is a result of structural similarities of apiols to some pathway intermediates, which enable apiols to inhibit CypA via binding to the heme in the active site pocket of the protein.

3.2 Spiroethers from *Matricaria recutita*

Matricaria recutita L. (syn: *M. chamomilla* L.; German chamomile) resides in the Asteraceae family and is one of the most widely used medicinal plants in the world (Salamon, 1992). It has a long history of application in herbal medicine which dates back ancient Greece and Rome periods where it was referenced by Hippocrates, Galen and Asclepius (Franke & Schilcher, 2005). The plant is an annual herb with erect branching and finely divided leaves growing to 50–90 cm tall. It has a stable natural monocyclic sesquiterpene alcohol named α-bisabolol as the main constituent (Tolouee et al., 2010). The plant has no reported toxic compounds and acute toxicity to human and animals. Consequently, it has been listed as generally recognized as safe (GRAS) by FDA (Bradley, 1993; Newall et al., 1996). A diverse range of pharmacological effects have been recognized for the plant including antimicrobial, anti-inflammatory, antioxidant, antispasmodic, antiviral, carminative, sedative and antiseptic properties. Other potentially active constituents are terpenoids, flavonoids, coumarins, and spiroethers which are believed to be responsible in part for the plant's wide range of biological activities (Newall et al., 1996). By screening 110 commercial essential oils from different plants using a microbioassay technique, Yoshinari et al. (2008) found a novel biological activity from *M. recutita* that completely inhibited AFG_1 production in *A. parasiticus* NRRL2999 at a concentration of 100 μg/ml. The components associated with the activity were identified as *(E)*- and *(Z)*-spiroethers (Martinez et al. 1987). Both compounds inhibited AFG_1 production with IC_{50} values of 2.8 and 20.6 μM without affecting fungal growth. However, they increased AFB_1 production in a concentration dependent manner. An *in vitro* feeding experiment with OMST as the substrate using *A. parasiticus* ATCC24690 (norsolorinic acid accumulating mutant) in the presence of spiroethers showed that these compounds specifically inhibited the pathway from OMST to AFG_1. In contrast, the mutant received only exogenous OMST produced both AFB_1 and AFG_1. Spiroethers have been implied to be able to inhibit human cytochrome P450 enzymes, CYP1A2 and CYP3A4 (Ganzera et al., 2005). Yoshinari et al. (2008) suggested that a possible inhibitory mechanism of spiroethers on AFG_1 production was on the activity of a cytochrome P450-dependent enzyme. In support of this notion, the authors further showed that spiroethers efficiently inhibited 3-acetyldeoxynivalenol by inhibiting TRI4, a key P450 monooxygenase involved in the early step of trichothecene biosynthesis. The target of spiroethers inhibition on AFG_1 formation likely is the aforementioned CypA, a P450 monooxygenase enzyme.

3.3 *Ageratum conyzoides*: Possible role for precocenes

Ageratum conyzoides is a species of invasive plants belonging to the family Asteraceae with high degree of environmental adaptability. It is native to tropical America, especially Brazil where it is commonly known as "Mentrasto". Several medicinal properties of this plant such as antimicrobial effects against different bacteria and fungi have been attributed to its main chemical constituents including terpenoids, flavonoids and phenolics (Kong et al., 2004). Nogueria et al. (2010) reported the complete inhibition of AFB_1 production by *A. flavus* IMI190 by the essential oil of the plant's aerial parts (concentrations ≥ 0.1 μg/ml) along with retarded fungal growth. Electron microscopic examination on oil-treated fungal structures revealed ultrastructural changes mainly in plasma membrane and memberanous organelles especially the mitochondria. In the excellent two-branch model presented for subcellular compartmentalization, translocation, AF gene expression and aflatoxisome biogenesis, Chanda et al. (2009) proposed that acetyl-CoA molecules necessary for early steps of AF biosynthesis are originated from β-oxidation of short chain fatty acids inside mitochondria.

Therefore, destruction of mitochondria resulting from exposure to *A. conyzoides* may account in part for its inhibitory effects on AFB_1 production. Two dimethylchromenes i.e. precocenes I and II were identified as the main constituents in the essential oil of *A. conyzoides* (Nogueria et al., 2010). These compounds purified also from *Matricaria recutita* essential oil have been recently reported as potent inhibitors of trichothecene biosynthesis by *Fusarium graminearum* (Yaghchi et al., 2009). Inhibition of 3-acetyldeoxynivalenol, a precursor of deoxynivalenol, by precocene II might be a consequence of decreased mRNA levels of *Tri4* and some other genes encoding proteins required for deoxynivalenol biosynthesis. Whether a direct inhibition of AFB_1 production by *A. conyzoides* is at the gene expression level by precocene II warrants further investigation.

3.4 Caffeic acid: An example of antioxidant-based inhibition of AF biosynthesis

Oxidative stress is an imbalanced state where excessive amounts of reactive oxygen species (ROS) overcome endogenous cell scavenging (antioxidant) capacity, leading to oxidation of a wide array of macromolecules such as enzymes, proteins, nucleic acids and lipids (Dai & Mumper, 2010). It has been shown that oxidative stress is a prerequisite and stimulatory factor for AF biosynthesis (Jayashree and Subramanyam, 2000; Reverberi et al., 2005). Oxidative stress is a result of exposure of fungal cells to ROS such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) and lipoperoxides (LOOH), which are formed from unsaturated fatty acids. These molecules can be generated as by-products of cell respiration or as a consequence of fungal response to environmental stressors like infectious microorganisms. ROS production at physiological concentrations plays an important role in fungal developmental processes such as conidiation and differentiation (Reverberi et al., 2008 and references therein). If the ROS level exceeds the cell-scavenging capacity, it can damage cell membranes and cell metabolism. Under such conditions, fungal cells preserve themselves by activating free radical scavenging system composed of the antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX). It is believed that AF and its precursors produced by toxigenic fungi as a defense mechanism in response to abnormally elevated levels of ROS (Narasaiah et al., 2006; Reverberi et al., 2008). Reverberi et al. (2008) first described the role of a gene Ap*yapA* from *A. parasiticus* in regulating cell differentiation and aflatoxin biosynthesis following ROS formation and the activation of antioxidant defense mechanisms. This gene is an orthologue of *Yap1* from *Saccharomyces cereviciae* which modulates the expression of many antioxidant related genes. The authors proposed that consecutive events starting from *A. parasiticus* growth followed by oxidative burst, triggered the expression of Ap*yapA,* modulation of antioxidant defense and finally led to initiation of AF biosynthesis. Many plants have antioxidant properties. Hence, they may exert inhibitory effects on AF biosynthesis by affecting oxidative stress responses in toxigenic fungi. Caffeic acid is one of the most important naturally occurring plant secondary metabolites with well known antioxidant activity. It decreases lipid peroxidation and GSH depletion resulting in reduced cell death (Lima et al., 2006). Caffeic acid is found in all plants because it is a key intermediate in the lignin biosynthesis. Recently, it has been used as a marker for the elucidation of antioxidant-based inhibition of AF biosynthesis (Kim et al. 2008). Addition of caffeic acid at a final concentration of 12 mM to fat-riched growth media reduced by >95% of AF production by *A. flavus* NRRL3357 without any obvious effect on fungal growth. Microarray-based gene expression profiling showed that expression of all genes in the AF biosynthetic cluster except for *norB* and the AF pathway regulatory gene, *aflJ*, were down-

regulated in caffeic acid-treated *A. flavus* compared with non-treated controls. Further microarray analysis of a number of genes involved in lipid metabolism, cell wall integrity and transport, and oxidative/antioxidative activities suggested a combination of events for the caffeic acid-induced inhibition of AF production by *A. flavus* (Kim et al., 2008).

3.5 Plant-based naturally occurring phenolics

Phenolics are the most abundant secondary metabolites of plants with more than 8,000 known structures ranging from simple compounds such as phenolic acids to complex structures such as tannins (Dai & Mumper, 2010). Phenolic acids, flavonoids, tannins, stilbenes and lignans are the main classes of plant phenolics. Besides the role in defense against UV, pathogens, parasites and predators, plant polyphenols have received special attention regarding their potent antioxidant properties which make them promising in suppressing oxidative stress associated disorders such as cancer. Inhibition of fungal growth and AF production by phenolics has been a subject of many studies (Hua et al., 1999; Kim et al., 2005, 2006; Razzaghi-Abyaneh et al., 2008). Hua et al. (1999) showed that plant phenolics i.e. acetosyringone, syringaldehyde and sinapinic acid efficiently inhibited the biosynthesis of AFB_1 in *A. flavus*. Using a norsolorinic acid (NOR) accumulating mutant of *A. flavus*, they proposed that these phenolics exert their inhibitory effects on AFB_1 biosynthesis at one or more early steps in the AF biosynthetic pathway. Razzaghi-Abyaneh et al. (2008) showed a novel biological activity from leaf essential oil of *Satureja hortensis* L. as strong inhibition of AF biosynthesis by *A. parasiticus* NRRL2999. The active substances purified by column chromatography were identified as phenolics, thymol and carvacrol. Bioassay with HPLC purified fractions revealed that both carvacrol and thymol effectively inhibited fungal growth. Inhibition of AFB_1 and AFG_1 production by these phenolics exhibited a dose-dependent manner at concentrations of 0.041 to 1.32 mM throughout all two-fold dilutions. The IC_{50} values of the compounds for AFB_1 and AFG_1 was as 0.50 and 0.06 mM. Since these phenolics are potent antioxidants, they likely exert their inhibitory activities on AF production through mediation of oxidative stress levels in the fungus. Kim et al. (2006), using a *S. cerevisiae* model system, demonstrated an effective synergism of natural phenolics with known antifungal chemicals such as carboxin and strobilurin. They showed that growth inhibition of *A. flavus* by the phenolics salicylic acid, thymol, vanillyl acetone, vanillin and cinnamic acid is via targeting the mitochondrial oxidative stress defense system. Since mitochondria are responsible for providing acetyl-CoA which is a main precursor for AF biosynthesis, disruption of mitochondrial respiration chain may account in part for the inhibitory effects of antifungal phenolics on AF production.

3.6 *Azadirachta indica*: A global tree for global problems

Azadirachta indica A. Juss (syn. *Melia azadirachta* L., Neem, Margosa) is a subtropical tree native to the drier areas of Asia and Africa. The plant is known for its medicinal, spermicidal, antiviral, antibacterial, antiprotozoal, insecticidal, insect repellent, antifungal and antinematode properties (Allameh et al., 2001; Bhatnagar et al., 1988). It is indigenous to the Indian subcontinent where it has been used in agriculture, medicine and cosmetics. Several active substances from different parts of the plant have been identified. Extracts from different parts contain terpenoids, desactylimbin, quercetin and sitosterol. It has been shown that aqueous extracts of leaves and seeds inhibit AF production by *A. parasiticus* at concentrations higher than 10% (v/v) without affecting fungal growth. Studies suggested that the inhibitory components of these extracts are non-volatile substances that affect the

synthesis of enzymes in the early steps of AF biosynthetic pathway (Bhatnagar et al., 1988; Zeringue and Bhatnagar, 1990). Allameh et al. (2001) did not find a positive correlation between AF production and the activity of fatty acid synthase, a key enzyme involved in AF production on neem-treated *A. parasiticus*. Razzaghi-Abyaneh et al. (2005) showed that AF production at 96 h in cultures containing 50% neem leaf and seed extracts was inhibited by 90 and 65%, respectively. Electron microscopy examination of treated fungus and non-treated controls revealed an association between decreased AF production and morphological changes suggesting that the integrity of cell barriers particularly cell wall is crucial in the regulation of AF biosynthesis and excretion.

3.7 Caffeine: An alkaloid from cocoa and coffee beans

Caffeine is a xanthine alkaloid which was isolated from coffee in 1820 by a German chemist, Friedlieb Ferdinand Runge. This compound also is found in different quantities in the beans, leaves and fruits of some plants, and acts as a natural pesticide against plant pathogens. Caffeine has been reported to inhibit fungal growth and mycotoxin (sterigmatocystin, citrinin, patulin and ochratoxin A) production by some *Aspergillus* and *Penicillium* species (Buchanan & Lewis, 1984 and references therein). Its mechanism of action was elucidated by Buchanan & Lewis (1984). They observed nearly complete inhibition of AF production along with a marked suppression (80-90%) in growth of *A. parasiticus* in submerged cultures containing 2 mg/ml caffeine. Based on the results of the feeding experiments with [U-C^{14}] glucose and enzymatic assays, Buchanan & Levis proposed that caffeine blocks AF production by affecting respiratory system of fungal cells and by inhibiting glucose uptake which is necessary for the production of acetyl-CoA, the building block of AFs. It seems that caffeine inhibits glucose uptake by directly affecting glucose transport system rather than altering the level or activity of enzymes associated with the glucose metabolism.

3.8 Gallic acid from walnuts

Gallic acid is a phenolic compound and a key component of hydrolysable tannins found in different plant species such as walnuts, oat bark and tea leaves. It is synthesized from an early intermediate named 5-dehydroshikimate in shikimate pathway. Among diverse biological activities reported for gallic acid, antimicrobial, antioxidant and antitumor properties are involved in plant defense against environmental stressors and pathogens. Inhibition of AF prduction by gallic acid without obvious effect on fungal growth was first described by Cary et al. (2003). Investigation on the mechanisms of action of gallic acid has shown that the compound affects AF biosynthesis by i) inhibition of the expression of AF biosynthetic pathway genes *nor1* and *ver1* without affecting transcription of the regulatory gene i.e. *aflR*, ii) disruption of the signal transduction pathway of oxidative stress system and iii) suppression of expression of regulatory genes of AF biosynthetic pathway such as *laeA*, whose expression is triggered by oxidative stress (Cary et al., 2003; Kim et al., 2005; Mahoney & Molyneux, 2004).

3.9 Salicylaldehyde: A volatile natural plant compound

Salicylaldehyde is an aroma compound of *Fagopyrum esculentum* and other buckwheat which acts as a key precursor of a variety of chelating agents with commercial importance. Little has been documented about physiological roles and biological properties of this volatile compound. Recently, Kim et al. (2010) showed that salicylaldehyde inhibits AF

production in *A. flavus* and *A. parasiticus* by 13-45% at a concentration of 9.5 mM; it also caused retardation in fungal growth. Using the model of yeast gene deletion mutants, they suggested that the fungal antioxidant system is the molecular target of salicylaldehyde and that vacuolar detoxification plays an important role in fungal resistance to the inhibitory effects of salicylaldehyde.

3.10 Carotenoids from maize and other plants

A large number of plant carotenoids have been reported as inhibitors of AF biosynthesis (Norton, 1997 and references therein). Norton (1997) studied the effects of maize carotenoids on AF biosynthesis by *A. flavus* and found all 11 carotenoids tested except α-tocopherol markedly suppressed AFB_1 production. The compounds containing the α-ionone ring i.e. α-carotene, lutein and α-ionone were the most active carotenoids capable of inhibiting >90% AFB_1 production. Exposure of a norsolorinic acid (NOR) accumulating mutant of *A. parasiticus* SRRC162 to α-carotene resulted in production of low levels of both NOR and AF, indicating that a target site(s) of α-carotene likely are at early steps of AF pathway before NOR formation. Comparative analysis of chemical structures of tested carotenoids showed the conjugated tail and the double-bond arrangement of the ring to be the determinants of the AF inhibitory activities. Based on the structure/activity data, modification of cell membranes that indirectly affect cytosolic polyketide synthase and specific interaction with hydrophobic domains of AF pathway enzymes were postulated to result in the observed inhibition (Norton, 1997).

3.11 Resistance associated proteins from maize kernel (RAPs)

The information derived from genomics, proteomics and metabolomics has provided us a better understanding of how the AF producing fungi survive in the field and how they invade host plants and produce AF (Bhatnagar et al., 2008; Brown et al., 2010; Kim et al., 2007; Rajasekaran et al., 2006). Published studies revealed that plants respond to fungal invasion and infection through: i) producing inhibitors to fungal cell wall degrading enzymes, ii) producing specific inhibitors against fungal growth and/or AF production, iii) producing ROS and stress responsive proteins, iv) increasing lignification and cell wall cross-linking and v) triggering host cell death at the site of infection (Bhatnagar et al., 2008; Liang et al., 2006). Among crops susceptible to AF contamination, maize has been the subject of several studies because of its importance as human staple and as animal feeds worldwide. Natural resistance to AF contamination has been noticed in maize genotypes during field screening. Comparative proteomics studies have identified maize kernel resistance associated proteins (RAPs) as promising breeding markers (Bhatnagar et al., 2008; Brown et al., 2010). RAPs were classified in three major groups including antifungal, storage and stress-responsive proteins. A RAP from maize kernel, the 14 kDa trypsin inhibitor, in resistance to fungal invasion and AF contamination has been confirmed (Brown et al., 2010 and references therein). This trypsin inhibitor indirectly suppresses AF production by inhibiting α-amylase of *A. flavus*, a fungal pathogenesis factor (Chen et al., 1998; Fakhoury & Woloshuk, 1999). Extracellular hydrolases of *A. flavus* including α-amylase are responsible for degrading starch to glucose and maltose used for fungal growth. Fakhoury & Woloshuk (1999) first described *Amy1* as α-amylase gene of *A. flavus* and confirmed the role of α-amylase in AF biosynthesis. They showed that α-amylase produced by *A. flavus* generated sugar concentrations sufficient to induce AF biosynthesis. How different classes of RAPs

contribute to resistance of maize to AF accumulation is a key question remains to be answered.

4. Microorganisms and their bioactive metabolites

Beneficial microorganisms especially bioactive fungi, bacteria and actinomycetes are cell factories that can produce a wide array of biologically active substances inhibitory to AF production. It has been reported that, on average, two or three antibiotics derived from microorganisms enter into the market each year (Clark, 1996). Hundreds of antifungal compounds also have been isolated from different fungi, bacteria and actinomycetes. Terrestrial actinomycetes especially those classified in the genus *Streptomyces* are rich sources of antifungal and AF inhibitory metabolites (Deshpande et al., 1988). Most recently, the roles of mycoviruses and RNA silencing in relation to AF control have gained special attention (Hammond et al., 2008; Schmidt 2009).

4.1 Bacteria and actinomycetes

4.1.1 Cyclo(L-Leucyl-L-Prolyl); A cyclic dipeptide from *Achromobacter xylosoxidans*

Achromobacter xylosoxidans is a non-fermentative, gram-negative bacillus belonging to the family Alcaligenaceae. It has been associated with a variety of clinical cases ranging from superficial sepsis to potentially fatal nosocomial infections. *A. xylosoxidans* is a newly emerging microorganism isolated with increased frequency from the lungs of patients with cystic fibrosis, but information about its clinical relevance is limited (Saiman & Siegel, 2004). Yan et al. (2004) reported a new biological activity for an environmental strain of *A. xylosoxidans* that inhibited AF production by *A. parasiticus*. The inhibitory metabolite was successfully isolated by a combination of chromatographic techniques and identified as a heat and chemical resistant cyclic dipeptide, "cyclo(L-Leucyl-L-Prolyl)". This compound has also been reported from different organisms including *Streptomyces* sp., an ascomycete (*Rosellinia necatrix*), a marine sponge (*Rhaphisia pallida*) and *Halobacillus litoralis*, a marine bacterium (Yan et al., 2004 and references therein). By using a tip culture method, the IC_{50} value of the compound on AFB_1 production was determined to be 200 μg/ml. It was inhibitory to *A. parasiticus* growth at a high concentration of 6000 μg/ml. RT-PCR analyses showed that cyclo(L-Leucyl-L-Prolyl) inhibited AF biosynthesis by repressing transcription of AF pathway genes *aflR*, *hexB*, *pksL1* and *dmtA*. The feeding experiment for conversion of sterigmatocystin (ST) to AF in the presence of the compound showed the loss of the most enzymes involved in the pathway from ST to AF. Cyclo(L-Leucyl-L-Prolyl) may direct or indirect affect the expression of the pathway regulatory gene, *aflR*. Further studies are needed in order to elucidate the underlying mechanisms of the inhibition.

4.1.2 Dioctatin A from *Streptomyces* sp. SA-2581

Dioctatin A was first isolated from *Streptomyces* sp. SA-2581 by Takeuchi et al. (1991) as an inhibitor of human dipeptidyl peptidase II, a property which accounts for immunosuppressive effects of the compound. Dioctatin A is a white powder with molecular formula of $C_{12}H_{39}N_3O_4$, molecular mass of 397.6 Da and a melting point of 263-265 ^oC. Yoshinari et al. (2007) demonstrated that Dioctatin A also is a strong inhibitor of AF production by *A. parasiticus*. AF production was inhibited with an IC_{50} value at 4.0 μM without any obvious effect on fungal growth. Using RT-PCR, they showed that dioctatin A

inhibited the transcription of *pksA*, *ver1* and *omtA* and significantly repressed the pathway regulatory gene, *aflR*. Besides inhibition in AFB_1 and AFG_1 production, an efficient suppression of conidiation was observed on solid medium accompanied by a concomitant reduction in the mRNA level of *brlA*, which encodes a conidiation-specific transcription factor. Based on the data about inhibition of ST biosynthesis and conidia formation in *A. nidulans*, they proposed that dioctatin A may target the G protein signaling pathway and thus results in inhibition of AF biosynthesis. Dioctatin A may be a good bioactive agent for the control of AF contamination based on several proven benefits including simple structure, no toxicity to mammals, inhibiting AF production in a model infection system on raw peanuts, inhibition of AF and conidiogenesis without affecting fungal growth (lowering the chance for spread of resistance), and targeting only secondary but not primary metabolism.

4.1.3 Aflastatin A from *Streptomyces* sp. MRI142

Aflastatin A was first isolated from solvent extracts of the mycelial cake of a soil isolate of *Streptomyces* sp. MRI142 (Ono et al., 1997). Using NMR and chemical degradation analyses, it was revealed that the compound has a skeleton of tetramic acid derivative with a highly oxygenated long alkyl chain (Ono et al., 1998). It is active against different yeasts, mycelial fungi and gram-positive but not gram-negative bacteria (Ono et al., 1997). Aflastatin A completely inhibited AF production by *A. parasiticus* NRRL2999 in liquid and solid cultures at a concentration of 0.5 μg/ml without affecting fungal growth (Ono et al., 1997). Its inhibitory mechanism was studied by evaluating the effect on AF biosynthetic pathway and glucose metabolism in *A. parasiticus* (Kondo et al., 2001). Inhibition of NOR production was observed when *A. parasiticus* ATCC24690 was cultured on potato dextrose agar plates in the presence of 0.1% (v/v) of aflastatin A. Glucose metabolism and ethanol accumulation were accelerated with a marked suppression in transcription of related genes *aldA* and *facA*. RT-PCR of the AF biosynthesis genes showed a significant reduction in transcription of *pksA*, *ver1*, *omtA* and *aflR* when cells were exposed to this compound. Aflastatin A may suppress AF biosynthesis either directly via affecting *aflR* transcription or indirectly by causing a marked disturbance in the regulatory machinery of carbon metabolism.

4.1.4 Blasticidins A and S from *Streptomyces griseochromogenes*

Blasticidin A, a peptidyl nucleoside antibiotic, was first reported by Fukunaga et al. (1958) as an anti-phytopathogenic substance and its absolute configuration was determined by Sakuda et al. (2007). It is a potent inhibitor of AF biosynthesis by *A. parasiticus* (Sakuda et al., 2000). Using two-dimensional differential gel electrophoresis (2D-DIGE) Yoshinari et al. (2010) showed that blasticidin A inhibited AF (total of B_1 and G_1) production and fungal growth with IC_{50} values of 0.25 and 1.6 μM, respectively. MALDI-TOF MS analysis of protein spots on the 2D-DIGE gel of blasticidin A-treated *A. flavus* showed decreased amounts of AF biosynthetic enzymes including Vbs, OmtB, OmtA, NorA, Ver-1 and Nor-1 after 36 h treatment. Levels of other proteins with unknown functions were also decreased. It was suggested that protein synthesis in toxigenic fungi maybe the possible target site of blasticidin A. Blasticidin S, another peptidyl nucleoside antibiotic highly similar to blasticidin A, was reported in the same study as an inhibitor of AF production (IC_{50} = 28 μM) with weak inhibitory effect on fungal growth (IC_{50} >1000 μM).

4.2 Mushrooms and microfungi

4.2.1 β-glucans and culture filtrates from *Lentinula edodes* and *Trametes versicolor*

Mushrooms have received major attention with regard to their biological properties including healing effects against different diseases, antioxidant, anticancer, antiviral, and antibacterial properties and hepatoprotective effects against AF (Zjalic et al., 2006 and references therein). Recently, mushrooms have been explored as potential control agents for AF contamination (Reverberi et al., 2005; Zjalic et al., 2006). Reverberi et al. (2005) concluded that culture filtrate and purified β-glucans from *Lentinula edodes*, an edible basidiomycetous mushroom native to East Asia, significantly inhibited AF production by *A. parasiticus* without affecting fungal growth. Their RT-PCR analyses of treated *A. parasiticus* mycelia showed a delay in activation of AF biosynthetic pathway genes *aflR* and *norA* as well as a simultaneous activation of *hsf2*-like, a transcription factor involved in oxidative stress responses. They suggested that AF production inhibition by β-glucans and culture filtrate of *L. edodes* resulted from a stimulation of fungal anti-oxidant system that activates antioxidant enzymes such as SOD, catalase and glutathione peroxidase. As a consequence, a delay in AF gene transcription leads to a marked suppression of AF biosynthesis. Mushroom constituents have some advantages over chemicals and plants extracts including low toxicity, simple extraction procedures and easy production on waste materials. Therefore, *L. edodes* culture filtrate is a promising tool to control pre- and post-harvest AF contamination of crops. Zjalic et al. (2006) examined another industrially important mushroom, *Trametes versicolor*, for its antifungal activity against an AF producing *A. parasiticus*. They showed that lyophilized culture filtrates and purified exopolysaccharides of different strains of *T. versicolor* efficiently inhibited AF (B and G series) production in the range of 40-90% in submerged cultures and on maize and wheat seeds without affecting fungal growth. Antioxidant activity of *T. versicolor* culture filtrate possibly is associated with β-glucan, a free radical scavenging agent that suppresses AF biosynthesis. RT-PCR analyses of AF biosynthetic genes showed that *T. versicolor* filtrate also significantly inhibited expression of *norA* and markedly delayed *aflR* transcription.

4.2.2 Wortmannin from *Penicillium* and other microfungi

Wortmannin is a furanosteroid fungal metabolite produced by different mycelial fungi especially *Penicillium funiculosum* and *Talaromyces wortmannii* (Bräse et al., 2009). It is well known for its biological activity as a specific covalent inhibitor of phosphoinositide 3-kinases. These group of enzymes are responsible for regulating various cell survival signaling pathways including growth and proliferation, receptor mediated endocytosis, apoptosis and membrane trafficking in mammalian cells (Shepherd et al., 1988). Recently, Lee et al. (2007) showed that wortmannin at a concentration of 1 μM inhibited fungal growth, asexual sporulation, AF production and expression of AF pathway genes *ver1* and *nor1*. The inhibition on AF biosynthesis appears to interfere the phopsphatidyl inositol 3-kinase-mediated signaling pathway similar to that described for mammalian hepatocytes by Rondione et al. (2000). In the model proposed, a cascade of events including blocking of the phopsphatidylinositol 3-kinase activity, inhibition of phosphodiesterase activation, accumulation of cAMP levels to higher than the physiological state, reduction of *aflR* expression and activity, and reduction of promoter activity of *nor1* and *ver1* genes occur in wortmannin-treated *A. parasiticus* which concertedly led to strong inhibition of AF production.

4.3 dsRNA viruses; RNA silencing as a mechanism for AF suppression

Viruses, the fundamental component of life, are involved in modulating intracellular gene activities. They are unique among microorganisms in terms of adaptability, propagation of genomic materials and cellular metabolism. The history of interaction between viruses and aflatoxigenic fungi dates back more than 20 years ago when Schmidt and co-workers (1986) described the effects of viruses on AF production by *A. flavus*. Transfection experiments with naked and complete dsRNA virus from *Penicillium chrysogenum* (*PcV*) which shared similarities in structure and size to dsRNA materials of a non-toxigenic strain of *A. flavus* resulted in a stable suppression of AF biosynthesis by toxigenic *A. flavus*. The recent descriptions of RNA interference (Schmidt 2004) and the interaction of *Aspergillus* mycoviruses with their host via RNA interference (Hammond & Keller, 2008; Hammond et al., 2008) suggest that dsRNA virus from *P. chrysogenum* may degrades transcripts of AF genes by the RNA interference mechanism (Schmidt et al., 2009). However, further experiments using morphological traits revealed that the *PcV*-gene suppressing effect on AF biosynthesis is probably nonspecific because it also affected genes involved in both morphogenesis and secondary metabolism (Schmidt, 2004). Schmidt (2009) proposed that suppression of *veA* gene by *PcV*-induced siRNAs eventually led to the blocking of AF biosynthesis in the virus transfected *A. flavus*.

5. Concluding remarks and future prospective

Despite the rapid growth of our knowledge in genetics and molecular biology of AF biosynthesis in recent years, little has been documented on how we can practically combat the global problem of AF contamination of crops and agricultural commodities. The information derived from genomic resources such as whole genome sequence and expressed sequence tag (EST) of *A. flavus*, as well as from proteomics and metabolomics studies will provide us a better understanding of how AF-producing fungi survive in the field and how they invade host plants and produce the carcinogenic AFs. A large number of compounds originated from plants and microorganisms have been proven as strong inhibitors of AF biosynthesis. Recent advances in the identification of the target sites of these inhibitors have shown that they may act via i) interfering with the signal transduction regulatory networks involved in AF gene expression, ii) blocking activities of AF biosynthetic enzymes, iii) down-regulating fungal genes of the oxidative stress defense system that combats metabolic and environmental stressors, iv) inhibiting fungal pathogenesis factors and v) disrupting mitochondrial respiration, a critical process that provides acetyl-CoA for AF biosynthesis. Elucidation of the underlying mechanisms by which plants and microorganisms and their bioactive metabolites affect AF biosynthesis is a major focus in the current molecular biological studies of AF biosynthesis. This endeavor also will advance the knowledge on the complex host plant-toxigenic fungus interactions, which is one of the most important aspects in solving the AF contamination problem.

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7. References

Alinezhad, S.; Kamalzadeh, A.; Shams-Ghahfarokhi, M.; Rezaee, M. B.; Jaimand, K.; Kawachi, M.; Zamani, Z.; Tolouei, R. & Razzaghi-Abyaneh, M. (2011). Search for novel antifungals from 49 indigenous medicinal plants: *Foeniculum vulgare* and *Platycladus orientalis* as strong inhibitors of aflatoxin production by *Aspergillus parasiticus*. *Annals of Microbiology*, doi: 10.1007/s13213-010-0194-1.

Allameh, A.; Razzaghi-Abyaneh, M.; Shams, M.; Rezaee, M. B. & Jaimand, K. (2001). Effects of neem leaf extract on production of aflatoxins and activities of fatty acid synthetase, isocitrate dehydrogenase and glutathione S-transferase in *Aspergillus parasiticus*. *Mycopathologia*, 154, 79-84.

Bakkali, F.; Averbeck, S.; Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils-A review. *Food and Chemical Toxicology*, 46, 446-475.

Bhatnagar, D. & McCormic, S. P. (1988). The inhibitory effect of neem (*Azadirachta indica*) leaf extracts on aflatoxin synthesis in *Aspergillus parasiticus*. *Journal of the American Oil Chemists Society*, 65, 1166–1168.

Bhatnagar, D.; Rajasekaran, K.; Payne, G. A.; Brown, R. L.; Yu, J. & Cleveland, T. E. (2008). The 'omics' tools: genomics, proteomics, metabolomics and their potential for solving the aflatoxin contamination problem. *World Mycotoxin Journal*, 1, 3-12.

Bradley, P. (1993). *The British Herbal Compendium: Vol. 1: A Handbook of Scientific Information on Widely Used Plant Drugs.* British Herbal Medicine Association, London, UK.

Bräse, S.; Encinas, A.; Keck, J. & Nising, C. F. (2009). Chemistry and biology of mycotoxins and related fungal metabolites. *Chemical Reviews*, 109, 3903-3990.

Brown, R. L.; Chen, Z. –Y.; Warburton, M.; Luo, M.; Menkir, A.; Fakhoury, A. & Bhatnagar, D. (2010). Discovery and characterization of proteins associated with aflatoxin-resistance: Evaluation their potential as breeding markers. *Toxins*, 2, 919-933.

Brown, R. L.; Cotty, P. J. & Cleveland, T. E. (1991). Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. *Journal of Food Protection*, 54, 623-626.

Buchanan, R. l. & Lewis, D. F. (1984). Caffeine inhibition of aflatoxin synthesis: Probable site of action. *Applied and Environmental Microbiology*, 47, 1216-1220.

Cai, J.; Zeng, H.; Shima, Y.; Hatabayashi, H.; Nakagawa, H.; Ito, Y.; Adachi, Y.; Nakajima, H. & Yabe, K. (2008). Involvement of the *nadA* gene in formation of G-group aflatoxins in *Aspergillus parasiticus*. *Fungal Genetics and Biology*, 45, 1081-1093.

Cary, J. F.; Harris, P. Y.; Molyneux, R. J. & Mahoney, N.E. (2003). Inhibition of aflatoxin biosynthesis by gallic acid. In: Proceedings of the 3rd Fungal Genomics, 4th Fumonisin, and 16th Aflatoxin Elimination Workshop. 13–15 October, 2003, Savannah, GA., p 51.

Cary, J. W.; Klich, M. A. & Beltz, S. B. (2005). Characterization of aflatoxin-producing fungi outside of *Aspergillus* section *Flavi*. *Mycologia* 97, 425-432.

Chanda, A.; Roze, L. V.; Kang, S.; Artymovich, K. A.; Hicks, G. R.; Raikhel, N. V.; Calvo, A. M. & Linz, J. E. (2009). A key role for vesicles in fungal secondary metabolism. *Proceedings of the National Academy of Sciences USA*, 106, 19533-19538.

Chang, P. –K. & Hua, S. –S. T. (2007). Nonaflatoxigenic *Aspergillus flavus* TX9-8 competitively prevents aflatoxin accumulation by *A. flavus* isolates of large and small sclerotial morphotypes. *International Journal of Food Microbiology*, 114. 275-279.

Chen, Z. -Y.; Brown, R. L.; Lax, A. R.; Guo, B. Z.; Cleveland, T. E.; & Russin, J. S. (1998). Resistance to *Aspergillus flavus* in corn kernels is associated with a 14-kDa protein. *Phytopathology*, 88, 276-281.

Clark, A. M. (1996). Natural products as a resource for new drugs, *Pharmaceutical Research*, 13, 1133-1141.

Crowden, R. K.; Harborne, J. B. & Heywood, V. H. (1969). Chemosystematics of the Umbelliferae–A general survey. *Phytochemistry*, 8, 1963-1984.

Dai, J. & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352.

Deshpande, B. S.; Ambedkar, S. S. & Shewale, J. G. (1988). Biologically active secondary metabolites from *Streptomyces*. *Enzyme and Microbial Technology*, 10, 455-473.

Donner, M.; Atehnkeng, J.; Sikora, R. A.; Bandyopadhyay, R. & Cotty, P. J. (2010). Molecular characterization of atoxigenic strains for biological control of aflatoxins in Nigeria. *Food Additives and Contaminants*, 27, 576-590.

Dorner, J. W. (2004). Biological control of aflatoxin contamination of crops. *Journal of Toxicology and Toxin Reviews*, 23, 425–450.

Ehrlich, K. C.; Scharfenstein, L. L. Jr.; Montalbano, B. G. & Chang, P. K. (2008). Are the genes *nadA* and *norB* involved in formation of aflatoxin G_1? *International Journal of Molecular Sciences*, 9, 1717-1729.

Fakhoury A. M. & Woloshuk, C. P. (1999). *Amy1*, the α-amylase gene of *Aspergillus flavus*: involvement in aflatoxin biosynthesis in maize kernels. *Phytopathology*, 89, 908-914.

Franke, R. & Schilcher, H. (2005). Chamomile: Industrial Profiles (Medicinal and Aromatic Plants). CRC Press, Boca Raton, FL, USA.

Frisvad, J. C.; Skouboe, P. & Samson, R. A. (2005). Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B_1, sterigmatocystin and 3-*O*-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. *Systematics and Applied Microbiology*, 28, 442-453.

Fukunaga, K.; Misato, T.; Ishii, I. & Asakawa, M. (1958). Blasticidin, a new anti-phytopathogenic fungal substance. *Part I. Bulletin of the Chemical Society of Japan*, 19, 181–188.

Ganzera, M.; Schneider, P. & Stuppner, H. (2005). Inhibitory effects of the essential oil of chamomile (*Matricaria recutita* L.) and its major constituents on human cytochrome P450 enzymes. *Life Sciences,* 78, 856–861.

Garber, R. K. & Cotty, P. J. (1997). Formation of sclerotia and aflatoxins in developing cotton bolls infected by the S strain of *Aspergillus flavus* and potential for biocontrol with an atoxigenic strain. *Phytopathology*, 87, 940–945.

Georgianna, D. R. & Payne, G. A. (2009). Genetic regulation of aflatoxin biosynthesis: From gene to genome. *Fungal Genetics and Biology*, 46, 113-125.

Hammond, T. M.; Andrewski, M.D.; Roossninck, M. J. & Keller, N. P. (2008). *Aspergillus* mycoviruses are targets and suppressors of RNA silencing. *Eukaryotic Cell*, 7, 350–357.

Hammond, T. M. & Keller, N. P. (2008). RNA silencing in the Aspergilli. *Mycology Series*, 26, 197–209.

Hedayati, M. T.; Pasqualetto, A. C.; Warn, P. A.; Bowyer, P. & Denning, D. W. (2007). *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology*, 153, 1677-1692.

Holmes, R. A.; Boston, R. S. & Payne, G.A. (2008). Diverse inhibitors of aflatoxin biosynthesis. *Applied Microbiology and Biotechnology*, 78, 559-572.

Hua, S. -S. T. (2004). Application of a yeast, *Pichia anomala* strain WRL-076 to control *Aspergillus flavus* for reducing aflatoxin in pistachio and almond. *Bulletin of International Organization for Biological and Integrated Control of Noxious Animals and Plants* 27, 291–294.

Hua, S. –S. T.; Grosjean, O.-K. & Baker, J. L. (1999). Inhibition of aflatoxin biosynthesis by phenolic compounds. *Letters in Applied Micobiology*, 29, 289-291.

Jayashree, T. & Subramanyam, C. (2000). Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus*. *Free Radical Biology & Medicine*, 29, 981-985.

Keller, N. P.; Kantz, N. J. & Adams, T. H. (1994). *Aspergillus nidulans verA* is required for production of the mycotoxin sterigmatocystin. *Applied and Environmental Microbiology*, 60, 1444-1450.

Keller, N. P.; Segner, S.; Bhatnagar, D. & Adams, T. H. (1995). stcS, a putative P-450 monooxygenase, is required for the conversion of versicolorin A to sterigmatocystin in *Aspergillus nidulans*. *Applied and Environmental Microbiology*, 61, 3628-3632.

Kim, J. H.; Campbell, B. C.; Yu, J.; Mahoney, N.; Chan, K. L.; Molyneux, R. J.; Bhatnagar, D. & Cleveland, T.E. (2005). Examination of fungal stress response genes using *Saccharomyces cerevisiae* as a model system: targeting genes affecting aflatoxin biosynthesis by *Aspergillus flavus* Link. *Applied Microbiology and Biotechnology*, 67, 807–815.

Kim, J. H.; Mahoney, N.; Chan, K. L.; Molyneux, R. & Campbell, b. C. (2006) Controlling Food-contaminating fungi by targeting antioxidant stress-response system with natural phenolic compounds. *Applied Microbiology and Biotechnology*, 70, 735-739.

Kim, J. H.; Yu, J.; Mahoney, N.; Chan, K. L.; Molyneux, R. J.; Varga, J.; Bhatnagar, D.; Cleveland, T. E.; Nierman, W. C. & Campbell, B. C. (2008). Elucidation of the functional genomics of antioxidant-based inhibition of aflatoxin biosynthesis. *International Journal of Food Microbiology*, 122, 49-60.

Kim, J. H.; Campbell, B. C.; Mahoney, N.; Chan, K. L. & Molyneux, R. J. (2010). Chemosensitization of aflatoxigenic fungi to antimycin A and strobilurin using salicylaldehyde, a volatile natural compound targeting cellular antioxidant system. *Mycopathologia*, doi: 10.1007/s11046-010-9356-8.

Kim, Y. H.; Woloshuk, C. P.; Cho, E. H.; Bae, J. M.; Song, Y.-S. & Huh, G. H. (2007) Cloning and functional expression of the gene encoding an inhibitor against *Aspergillus flavus* α-amylase, a novel seed lectin from *Lablab purpureus* (*Dolichos lablab*).*Plant Cell Reports*, 26, 395-405.

Kondo, T., Sakurada, M., Okamoto, S., Ono, M., Tsukigi, H., Suzuki, A., Nagasawa, H. & Sakuda, S. (2001). Effects of aflastatin A, an inhibitor of aflatoxin production, on aflatoxin biosynthetic pathway and glucose metabolism in *Aspergillus parasiticus*. *The Journal of Antibiotics (Tokyo)*, 54, 650–657.

Kong, C.; Hu, F.; Xu, X.; Liang, W. & Zhang, C. (2004). Allelopathic plants. XV. *Ageratum conyzoides* L. *Allelopathy Journal*, 14, 1–12.

Korkina, L. G. (2007). Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. *Cellular and Molecular Biology*, 53, 15-25.

Lee, J. –W.; Roze, L. V. & Linz, J. E. (2007). Evidence that a wortmannin-sensitive signal transduction pathway regulates aflatoxin biosynthesis. *Mycologia*, 99, 562-568.

Liang, X. Q.; Luo, M. & Guo, B. Z. (2006). Resistance mechanisms to *Aspergillus flavus* infection and aflatoxin contamination in peanut (*Arachis hypogaea*). *Plant Pathology Journal*, 5, 115-124.

Lima, C.F.; Fernandes-Ferreira, M. & Pereira-Wilson, C. (2006). Phenolic compounds protect HepG2 cells from oxidative damage: relevance of glutathione levels. *Life Sciences*, 79, 2056–2068.

MacRae, W. D. & Towers, G. H. N. (1984). Biological activities of lignans. *Phytochemistry* 23, 1207-1220.

Mahoney N, Molyneux RJ (2004) Phytochemical inhibition of aflatoxigenicity in *Aspergillus flavus* by constituents of walnut (*Juglans regia*). *Journal of Agricultural and Food Chemistry*, 52, 1882–1889.

Martinez, V.; Barbera, O.; Sanchez-Parareda, J. & Marco, J. A. (1987). Phenolic and acetylenic metabolites from *Artemisia assoana*. *Phytochemistry*, 26, 2619–2624.

Narasaiah, K. W.; Sashidhar, R. B. & Subramanyam, C. (2006) Biochemical analysis of oxidative stress in the production of aflatoxin and its precursor intermediates. *Mycopathologia*, 162, 179–189.

Newall, C. A.; Anderson, L. A. & Phillipson, J. D. (1996). *Herbal Medicines: a guide for healthcare professionals*. Pharmaceutical Press, London.

Nogueira, J. H. C.; Gonçalez, E.; Galleti, S. R.; Facanali, R.; Marques, M. O. M. & Felício, J. D. (2010) *Ageatum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. *International Journal of Food Microbiology*, 137, 55-60.

Norton, R. A. (1997). Effect of carotenoids on aflatoxin B_1 synthesis by *Aspergillus flavus*. *Phytopathology*, 87, 814-821.

Ono, M.; Sakuda, S.; Suzuki, A. & Isogai, A. (1997). Aflastatin A, a novel inhibitor of aflatoxin production by aflatoxigenic fungi. *The Journal of Antibiotics (Tokyo)*, 50, 111–118.

Ono, M.; Sakuda, S.; Ikeda, H.; Furihata, K.; Nakayama, J.; Suzuki, A. & Isogai, A. (1998). Structures and biosynthesis of aflastatins: novel inhibitors of aflatoxin production by *Aspergillus parasiticus*. *The Journal of Antibiotics (Tokyo)*, 51, 1019–1028.

Pildain, M. B.; Frisvad, J. C.; Vaamonde, G.; Cabral, D.; Varga, J. & Samson, R. A. (2008). Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. I*nternational Journal of Systematic and Evolutionary Microbiology*, 58, 725-735.

Rajasekaran, K.; Cary, J. W. & Cleveland, T. E. (2006) Prevention of preharvest aflatoxin contamination through genetic engineering of crops. *Mycotoxin Research*, 22, 118-124.

Razzaghi-Abyaneh, M,; Allameh, A,; Tiraihi, T,; Shams-Ghahfarokhi, M. & Ghorbanian, M. (2005). Morphological alterations in toxigenic *Aspergillus parasiticus* exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. *Mycopathologia*, 159, 565-570.

Razzaghi-Abyaneh, M.; Yoshinari, T.; Shams-Ghahfarokhi, M.; Rezaee, M. B.; Nagasawa, H. & Sakuda, S. (2007). Dillapiol and apiol as specific inhibitors of the biosynthesis of aflatoxin G_1 in *Aspergillus parasiticus*. *Bioscience, Biotechnology and Biochemistry*, 71, 2329-2332.

Razzaghi-Abyaneh, M.; Shams-Ghahfarokhi, M,; Yoshinari, T,; Rezaee, M. B,; Nagasawa, H. & Sakuda, S. (2008). Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. *International Journal of Food Microbiology*, 123, 228-233.

Razzaghi-Abyaneh, M.; Shams-Ghahfarokhi, M.; Rezaee, M. B.; Jaimand, S.; Alinezhad, S.; Saberi, R. & Yoshinari, T. (2009). Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. *Food Control*, 20, 1018-1024.

Razzaghi-Abyaneh, M.; Shams-Ghahfarokhi, M.; Rezaee, M. B. & Sakuda, S. (2010). Natural Aflatoxin Inhibitors From Medicinal Plants, In: *Mycotoxins in Food, Feed and Bioweapons*, Rai, M. & Varma, A. (ed.), 329-352, Springer-Verlag, Berlin, Heidelberg.

Reverberi, M.; Fabbri, A. A.; Zjalic, S.; Ricelli, A.; Punelli, F. & Fanelli, C. (2005). Antioxidant enzymes stimulation in *Aspergillus parasiticus* by *Lentinula edodes* inhibits aflatoxin production. *Applied Microbiology and Biotechnology*, 69, 207–215.

Reverberi, M.; Zjalic, s.; Ricelli, A.; Punelli, F.; Camera, E.; Fabbri, C.; Picardo, M.; Fanelli, C. & Fabbri, A. A. (2008). Modulation of antioxidant defence in *Aspergillus parasiticus* is involved in aflatoxin biosynthesis: a role for the Ap*yap*A gene. *Eukaryotic Cell*, 7, 988-1000.

Rondinone, C. M.; Carvalho, E.; Rahn, T.; Manganiello, V. C.; Degerman, E. & Smith, U. P. (2000). Phosphorylation of PDE3B by phosphatidylinositol 3-kinase associated with the insulin receptor. *Journal of Biological Chemistry*, 275, 10093–10098.

Saiman, L. & Siegel J. (2004). Infection control in cystic fibrosis. *Clinical Microbiology Reviews*, 17, 57–71.

Sakuda, S.; Ono, M.; Ikeda, H.; Nakamura, T.; Inagaki, Y.; Kawachi, R.; Nakayama, J.; Suzuki, A.; Isogai, A. & Nagasawa, H. (2000a). Blasticidin A as an inhibitor of aflatoxin production by *Aspergillus parasiticus*. *The Journal of Antibiotics (Tokyo)*, 53, 1265–1271.

Salamon, I. (1992). Chamomile, a medicinal plant. *The Herb, Spice, and Medicinal Plant Digest*, 10, 1–4.

Samson, A. R., Hoekstra, E. S.; Frisvad, J. C. & Filtenborg, O. (2000). *Introduction to Food and Airborne Fungi*, (Sixth edn.), CBS-Utrecht, The Netherlands.

Samuelsson, G. (2004). *Drugs of Natural Origin: a Textbook of Pharmacognosy*, 5th Swedish Pharmaceutical Press, Stockholm.

Schmidt, F. R.; Davis, N. D.; Diener, U. L. & Lemke, P. A. (1983). Cycloheximide induction of aflatoxin synthesis in a nontoxigenic strain of *Aspergillus flavus*. *Nature Biotechnology*, 1, 794–795.

Schmidt, F. R.; Lemke, P. A. & Esser, K. (1986). Viral influences on aflatoxin formation by *Aspergillus flavus*. *Applied Microbiology and Biotechnology*, 24, 248–258.

Schmidt, F. R. (2004). RNA interference detected 20 years ago? *Nature Biotechnology*, 22, 267–268.

Schmidt, F. R. (2009). The RNA interference-virus interplay: tools of nature for gene modulation, morphogenesis, evolution and a possible mean for aflatoxin control. *Applied Microbiology and Biotechnology*, 83, 611-615.

Shepherd, P. R.; Withers, D. J. & Siddle, K. (1988). Phosphoinositide 3-kinase: the key switch mechanism in insulin signaling. *Biochemical Journal*, 333, 471–490.

Takeuchi, T.; Aoyanagi, T.; Okami, Y.; Osanawa, H.; Iinuma, H. & Ogawa, K. (1991). Novel aminopeptidase-inhibiting dioctatins and their manufacture with *Streptomyces* species. Japanese Patent JP 03077857 A2 19910403 Heisei.

Tolouee, M.; Alinezhad, S.; Saberi, R.; Eslamifar, A.; Zad, S. J.; Jaimand, K.; Taeb, J.; Rezaee, M. B.; Kawachi, M.; Shams-Ghahfarokhi, M. & Razzaghi-Abyaneh, M. (2010). Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. *International Journal of Food Microbiology*, 139, 127-133.

Trail, F.; Mahanti, N. & Linz, J. (1995). Molecular biology of aflatoxin biosynthesis. *Microbiology*, 141, 755-765.

Udwary, D. W.; Casillas, L. K. & Townsend, C. A. (2002). Synthesis of 11-hydroxyl O-methylsterigmatocystin and the role of a cytochrome P-450 in the final step of aflatoxin biosynthesis. *Journal of the American Chemical Society*, 124, 5294-5303.

Varga, J.; Frisvad, J. C. & Samson, R. A. (2010). A reappraisal of fungi producing aflatoxins. *World Mycotoxin Journal*, 2, 263-277.

Wen, Y.; Hatabayashi, H.; Arai, H.; Kitamoto, H. K. & Yabe, K. (2005). Function of the *cypX* and *moxY* genes in aflatoxin biosynthesis in *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, 71, 3192-3198.

Yabe, K.; Nakamura, M. & Hamasaki, T. (1999). Enzymatic formation of G-group aflatoxins and biosynthetic relationship between G- and B-group aflatoxins. *Applied and Environmental Microbiology*, 65, 3867-3872.

Yaguchi, A.; Yoshinari, T.; Tsuyuki, R.; Takahashi, H.; Nakajima, T.; Sugita-Konishi, Y.; Nagasawa, H. & Sakuda, S. (2009). Isolation and identification of precocenes and piperitone from essential oils as specific inhibitors of trichothecene production by *Fusarium graminearum*. *Journal of Agricultural and Food Chemistry*, 57, 846-851.

Yan, P. –S.; Song, Y.; Sakuno, E.; Nakajima, H.; Nakagawa, H. & Yabe, K. (2004). Cyclo(L-Leucyl-L-Prolyl) produced by *Achromobacter xylosoxidans* inhibits aflatoxin production by *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, 70, 7466-7473.

Yoshinari, T.; Akiyama, T.; Nakamura, K.; Kondo, T.; Takahashi, Y.; Muraoka, Y.; Nonomura, Y.; Nagasawa, H. & Sakuda, S. (2007). Dioctatin A is a strong inhibitor of aflatoxin production by *Aspergillus parasiticus*. *Microbiology*, 153, 2774-2780.

Yoshinari, T.; Yaguchi, A.; Takahashi-Ando, N.; Kimura, M.; Takahashi, H.; Nakajima, T.; Sugita-Konichi, Y.; Nagasawa, H. & Sakuda, S. (2008). Spiroethers of German chamomile inhibit production of aflatoxin G and trichothecene mycotoxin by inhibiting cytochrome P450 monooxygenases involved in their biosynthesis. *FEMS Microbiology Letters*, 284, 184-190.

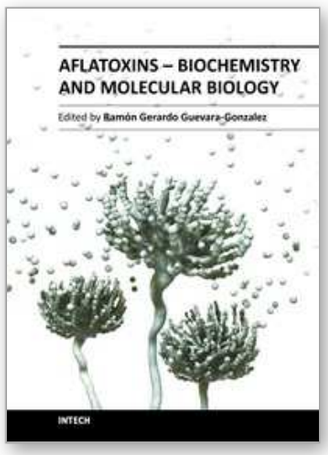
Yoshinari, T.; Noda, Y.; Yoda, K.; Sezaki, H.; Nagasawa, H. & Sakuda, A. (2010). Inhibitory activity of blasticidin A, a strong aflatoxin production inhibitor, on protein synthesis of yeast: selective inhibition of aflatoxin production by protein synthesis inhibitors. *The Journal of Antibiotics*, 63, 309-314.

Yu, J.; Chang, P. -K.; Cary, J. W.; Bhatnagar, D. & Cleveland, T. E. (1997). *avnA*, a gene encoding a cytochrome P-450 monooxygenase, is involved in the conversion of averantin to averufin in aflatoxin biosynthesis in *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, 63, 1349-1356.

Zeng, H.; Hatabayashi, H.; Nakagawa, H.; Cai, J.; Suzuki, R.; Sakuno, E.; Tanaka, T.; Ito, Y.; Ehrlich, K. C.; Nakajima, H. & Yabe, K. (2011). Conversion of 11-hydroxy-O-methylsterigmatocystin to aflatoxin G_1 in *Aspergillus parasiticus*. *Applied Microbiology and Biotechnology*. Doi: 10.1007/s00253-010-2999-z.

Zeringue, H. J. Jr. & Bhatnagar, D. (1990). Inhibition of aflatoxin production in *Aspergillus flavus* infected cotton bolls after treatment with neem (*Azadirachta indica*) leaf extracts. *Journal of the Americal Oil Chemists Society*, 67, 215–216.

Zjalic, S.; Reverberi, M.; Ricelli, A.; Mario-Granito, V.; Fanelli, K. & Fabbri, A. A. (2006). *Trametes versicolor*: A possible tool for aflatoxin control. *International Journal of Food Microbiology*, 107, 243-249.



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Aflatoxins â€“ Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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