Chapter 1

Control of Aflatoxin Production Using Herbal Plant Extract

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Abstract

The aflatoxins are a group of chemically similar poisonous, carcinogenic fungal secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*, which are abundant in warm and humid regions of the world. They are probably the most intensively researched toxins in the world due to their carcinogenic and mutagenic effects. Aflatoxins have also been identified as a potential biological weapon for food and water contamination. The four major aflatoxins commonly isolated from different foods and feed stuffs are AFB1, AF B2, AFG1, and AFG2. Aflatoxin contamination of food and feed has gained global significance as a result of its deleterious effects on human as well as livestock health including gastrointestinal dysfunction, reduced feed utilization, anemia, jaundice, liver damage and immunity suppression. The profitability and marketing of various agricultural products are adversely affected by either contamination of aflatoxins or aflatoxin-producing fungi. The foods at highest risk of aflatoxin contamination are maize, chilies, peanuts, and cotton seeds. There are various physical, chemical, and natural methods investigated to prevent aflatoxin production and the growth of aflatoxin-producing fungus in various agricultural products. Here, we describe various natural plant extracts that would be potential source of controlling aflatoxin production in agricultural products.

Keywords: aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, plant extract, agricultural products

1. Introduction

Aflatoxins are poisonous, carcinogenic, mutagenic, immunosuppressive, and teratogenic secondary metabolites formed by *Aspergillus flavus*, *A. parasiticus* [1], and *A. nomius* [2]. These fungi are ubiquitous species and generally contaminate agricultural products such as rice, wheat, maize, barley, sorghum, black pepper, chili, ginger, coriander, turmeric, pistachios, almonds, walnuts, Brazil nuts, peanuts, oilseeds (cotton, sunflower, sesame, and soybean), milk, cheese, and animal feed [3–9]. The Food and Agriculture Organization (FAO) estimated that around 25% of the world's cereals are contaminated by mycotoxins, including aflatoxins [10]. Aflatoxins were first identified as causative agent of "Turkey X disease" in 1961. Due to this disease, about more than 100,000 young turkeys, ducks, and poultry birds died in England by eating contaminated Brazilian groundnut meal [11–15].

The aflatoxin was a combination of three words: first letter "A" from genus *Aspergillus*, next three letters "FLA" from species *flavus*, and the noun "TOXIN" [16]. Aflatoxins are quite stable and found resistant to degradation. Among the 18 different groups, aflatoxins B1, B2, G1, G2, M1, and M2 are the major classes and derivative of bifuranocoumarins. The aflatoxins B1 and B2 give blue color, while G1 and G2 give a yellowish green color under UV light. Aflatoxins M are hydroxylated derivatives of aflatoxins B and first isolated from milk. *A. flavus* produces only AFB1 and AFB2, but it is also able to synthesize cyclopiazonic acid. However, *A. parasiticus* produces AFBI, AFB2, AFG1, and AFG2 [17, 18].

The International Agency for Research on Cancer (IARC) classified AFB1 as class I human carcinogens [19] and have a positive association between dietary aflatoxins and liver cell cancer (LCC). This was the third leading reason of cancer death around the world [20]. The cytochrome p450 metabolized AFB1 in their epoxide form. Depurination occurs, when epoxide reacts with DNA or RNA. That will obstruct DNA and protein synthesis in active tissues of bone marrow, intestine, and liver. The order of toxicity of aflatoxins is AFB1 > AFB2 > AFG1 > AFG2 [21], and the critical point, which determined the biological activity of this group of mycotoxins, is terminal furan moiety of aflatoxin [22]. In cereal and their derivatives, maximum residual limits (MRLs) of aflatoxins are 2 μg kg^{1} for AFB1 and 4 μg kg^{-1} for the sum of four aflatoxins. In processed cereal-based foods and baby foods for infants and young children, the level of AFB1 is 0.1 μg kg^{-1}. These values were recommended by the European Union Commission Regulation (EC) [21]. According to the Food and Drug Administration (FDA), the safe limit of aflatoxins is 20 ppb (**Figure 1**) [23].

In developing countries, about 4.5 billion people are chronically exposed to uncontrolled amounts of aflatoxins [24]. Consumption of contaminated products causes aflatoxicosis in humans and animals. Aflatoxicosis may be acute and chronic. Acute condition caused death, while chronic condition results in immune suppression and cancer. In human, it is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart [25]. Due to aflatoxicosis, in Kenya about 215 people died in 2004 [26–28]. In animal, aflatoxicosis is characterized by gastrointestinal dysfunction, reduced feed utilization, anemia, jaundice, liver damage, decreased milk and egg production, and immunity suppression [29]. In plants, AFs retarded

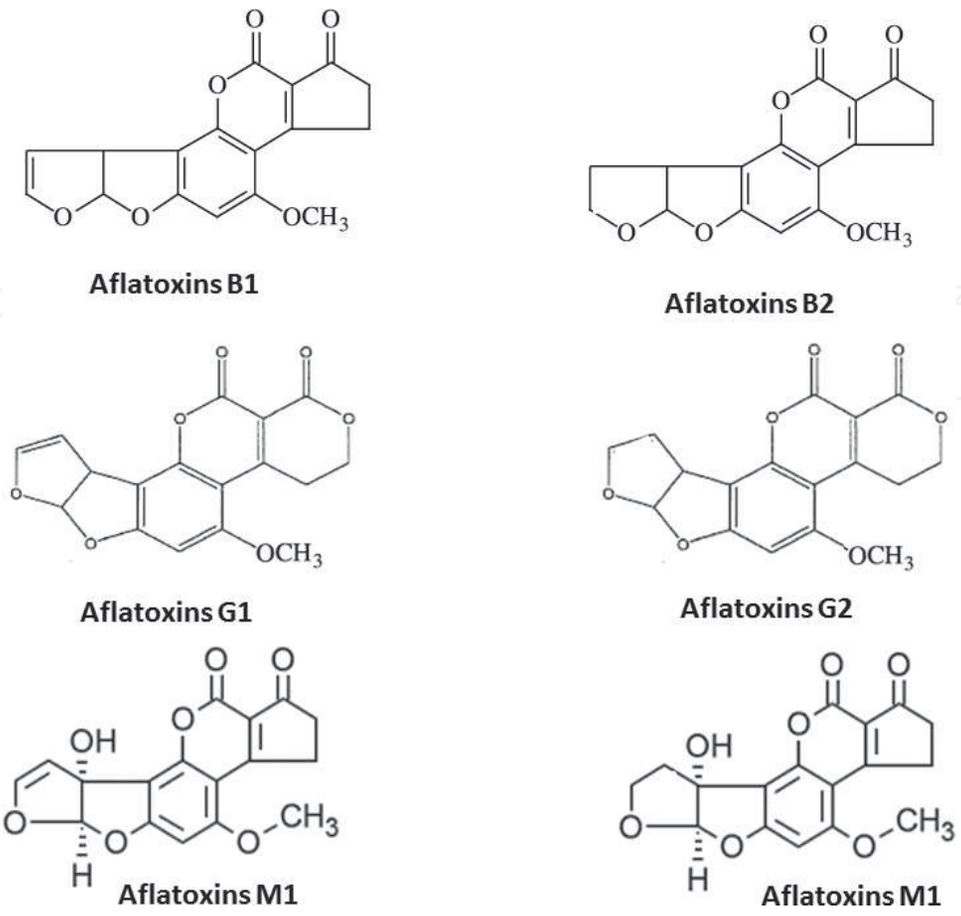

Figure 1. Major classes of aflatoxins.

seed germination, seedling growth, and root elongation. It also inhibits chlorophyll, carotenoid, and some enzymes synthesis [30].

Although *A. parasiticus* and *A. flavus* are related fungi, they are different from each other on the basis of their color and length of conidiophore. Sterigmata were the main characteristics, which differentiate the two *Aspergillus* species. The sterigmata of *A. flavus* were biseriate as compared to *A. parasiticus*, which has uniseriate sterigmata [31]. In 2006, Cary and Ehrlich reported that about 12 *A. flavus* groups are 96% similar to *A. parasiticus*. Another character that distinguishes the two fungi species is their adapted environment. The *A. flavus* acclimated to aerial and foliar environment, mostly prominent in tree nuts, corn, and cottonseed, while the *A. parasiticus* adapted to soil environment and dominated in peanuts [32]. *A. flavus* exists in two forms: one is the S type, while the other is the L type on the basis of morphological, physiological, and genetic characteristics [33]. On average, S-strain isolates produce much more aflatoxins than L-strain isolates [34]. S strain synthesized frequently small sclerotia that are less than 400 μm and processes lesser conidia as compared to L-strain isolates whose sclerotia sizes are greater than 400 μm [34, 35].The members of genus *Aspergillus* mostly contaminate agriculture commodities in tropic and sub-tropic region. Contamination may occur at different stages such as in pre-harvesting stage, harvesting stage, post-harvesting stage, or in storage and transportation stage. In pre-harvesting, the field fungi attack on growing

crop because of different reasons. It may be the environmental stress (hot and dry condition and soil moisture), mechanical damage (by arthropods, birds, rodents, and nematodes), or delayed harvesting. While in post-harvesting, contamination occurred due to improper drying, storage in polythene bags, damage during shelling, or storage in poorly ventilated warm environment.

Contamination rate of aflatoxin depends upon humidity, temperature, storage, and soil conditions [36]. Optimum condition for fungal growth in cereal is moisture content about 18% (equal to 85% relative humidity) and temperature about 12–42°C with an optimum at 27–30°C in tropical and sub-tropical areas [37]. An important point to be considered was the time of incubation that effects the production of toxin by *Aspergillus* species [38]. Optimum duration for the production of aflatoxins was 14 days of incubation at 30°C. When the length of incubation time increased, there will be reduction in aflatoxin level because of re-adsorption or degradation by fungus [39]. The fungal growth is effected by 20% CO_2 and 10% O_2 level [40]. The metals such as manganese and zinc are crucial for aflatoxin production. But the mixture of cadmium and iron mixture reduces the mold growth and aflatoxin synthesis [41].

The infectious cycle of *Aspergillus* species is mostly dependent upon host species. Overwinter fungus developed either mycelium or sclerotia (resistant structure) that have the ability to grow on soil surface [42, 43]. Under favorable condition (high temperature and moisture level) in summer, it either produced hyphae or conidia (asexual spores). Through air or insects, conidia spread in soil and on silk and kernels and contaminate agriculture commodities (**Figure 2**) [44].

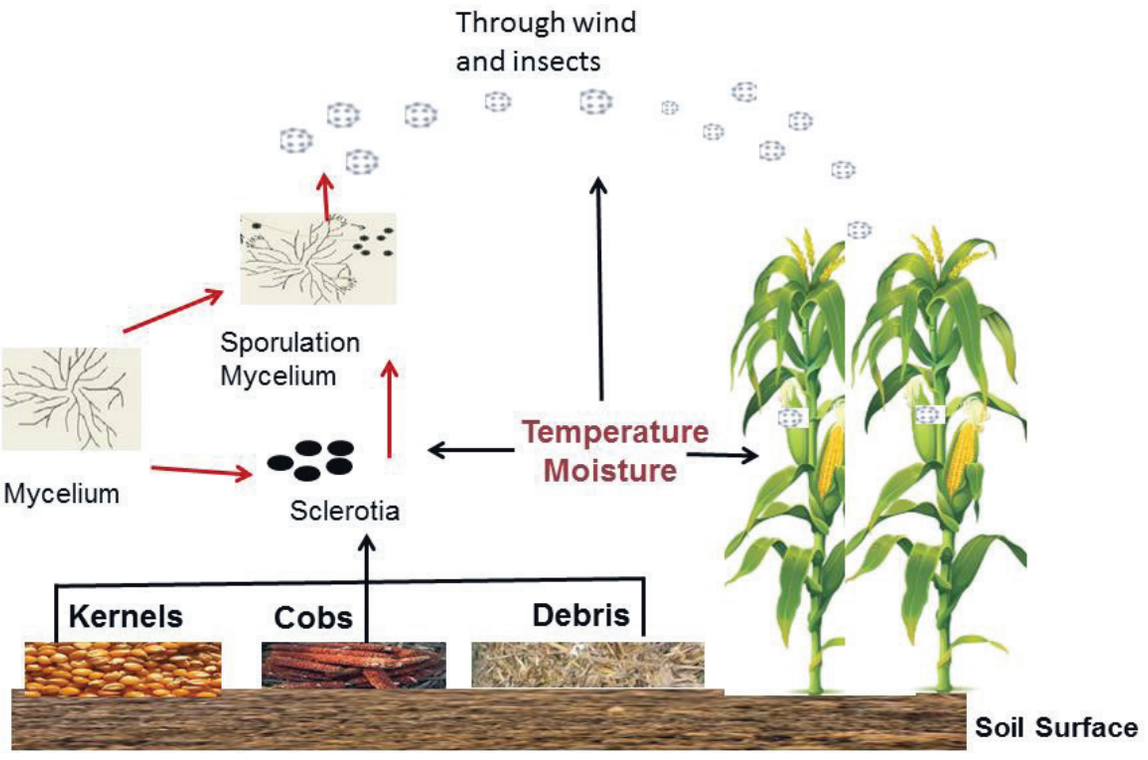

Figure 2. Life cycle of *A. flavus* in field.

Aflatoxin contamination is inescapable due to health hazards in human and animal, crops deterioration, and economical loses. In the past, many strategies (physical, chemical, and biological) are used to avoid aflatoxin contamination. Physical strategies usually used are rodent-proof room, cold storage of feeds with less than 100-g/kg moisture level, use rapid drying and gamma radiation, and so on. In chemical strategies, propionic acid, acetic acid, benzoic acid, citric acid, hydrogen peroxide, copper sulfate, and ammonium hydroxide are used to inhibit the growth of fungi and aflatoxin production. But the formation of toxic residue by chemical treatment was the main concern that causes potent health problems. As compared to chemical, physical practice is a healthier option but it is slow processes. Other strategies used were the biological control in which different microorganisms such as bacteria, yeast, and non-toxic stain of *A. flavus* and *A. parasiticus* were used to detoxify aflatoxins by microbial binding and biotransformation [45–48]. This is a laborious and costly process. Therefore, to avoid potential risk, the use of safe, renewable, and biodegradable natural plant extracts to remove aflatoxin contamination [49] is required.

2. Effect of active ingredients of medicinal plants on aflatoxins producing fungus

Modern research found that phytophenols as plant secondary metabolite existed above 8000 structures. These structures resemble with tannin and phenolic acid [50]. Phytophenols showed antiallergenic, antioxidant, anti-inflammatory, antimicrobial, antiorthrogenic, and antithrombotic activity [51]. These plant compounds exhibited key biological activity in the degradation of many microorganisms [52]. Plants, herbs, essential oils, and spices in powder or extracts form are used to detoxify microbes due to the presence of flavonoids, betalain, phenolics, phytoalexins, and thiosulfonates. But mostly antimicrobial and antioxidant activities of plant extracts were due to their phenolic alignments [53].

A recent study exposed the antifungal and antiaflatoxigenic nature of phenolic components of plant extracts [54–56]. The syringaldehyde, sinapic acid, and acetosyringone were the plant phenolic compounds that inhibited the production of aflatoxin B1 [57]. However, salicylic acid, thymol, vanillyl acetone, cinnamic acid, and vanillin were phenolic compounds that ceased *A. flavus* growth by targeting oxidative mitochondrial stress as defense system [58].

Medicinal plants have been used from centuries for the treatment of various diseases. There are about 53,000 medicinal plants around the world [59]. In developing countries, according to World Health Organization, about 70–95% people used medicinal plants as primary health care for the treatment of diseases [20]. In current scenario, 70% of synthetic medicines are derived from plants [60]. Medicinal plants have antifungal, antimicrobial, anthelmintic, antibiotic, antiviral, anti-inflammatory, antiarthritic, antirheumatic, and antihemorrhoidal properties.

The various medicinal plants native to Southeast Asia including bitter cucumber (*Momordica charantia*), Asiatic pennywort (*Centella asiatica*), betel nut (*Areca catechu*), betel vine (*Piper betle)*, Chaa Phluu (*Piper sarmentosum*), false coriander (*Eryngium foetidum*), Chinese radish (*Raphanus sativus*), clove (*Syzygium aromaticum*), Eucalyptus (*Eucalyptus globules*), Indian mulberry (*Morinda citrifolia*), Madagascar periwinkle (*Catharanthus roseus*),bmangosteen (*Garcinia mangostana*), mandarin (*Citrus reticulate*), onion (*Allium cepa*), pepper (*P. nigrum*), pomegranate

(*Punica granatum*), tomato (*Lycopersicon esculentum*), hedge flower (*Lantanacamara*), roselle (*Hibiscus sabdariffa*), Non Taai Yaak (*Stemona tuberosa*), Raang Chuet (*Thunbergia laurifolia*), Saab Sue (*Chromolaena odorata*), turmeric (*Curcuma longa*), water primrose (*Jussiaeda repens*), and wishing tree (*Cassia bakeriana*) were tested for their ability to control aflatoxins producing fungus [61]. The above study found that ethanolic extracts of some medicinal plant showed the inhibition of aflatoxins producing fungus.

The highest activity was showed by betel vine, a traditional Thai medicine, followed by false coriander, Indian mulberry, Chaa Phluu, Chinese radish, and clove. The leaf of betel vine is used topically for urticaria, contains eugenol and chavicol, and mostly chewed by mouth as antiflatulent, antimicrobial, and antipruritic [62].

Crude ethanolic extract of olive callus in different ratios was used to inhibit the aflatoxins synthesis [63] by the addition of appropriate amounts of extracts onto potato dextrose agar (PDA) to obtain the final concentration of 0.5 and 1%, and *Aspergillus* was then point-inoculated into PDA. The results showed that ethanolic extract of olive callus had no inhibitory effect on fungal growth but it reduced 90% of aflatoxin synthesis. The main compounds in olive callus are reported as caffeic acid, coumarin; o-, p-, or m-coumaric acid and catechin which facilitate the reduction of aflatoxin. Only o-coumaric acid and caffeic acid showed antifungal and antibacterial activity.

Various concentrations (0, 2, 4, 6, 8, and 10% (w/v)) of clove, garlic, and carrot's crude aqueous extracts were tested for their possible inhibitory effect on *Aspergillus* growth and aflatoxin production in 50 g of rice. The results showed that garlic and clove at 10% (w/v) and carrot at 2% inhibited the *Aspergillus* growth and also reduced the level of aflatoxin production in rice [64]. Crude extracts of garlic, eugenol, and onion were used to reduce *A. flavus* growth as well as aflatoxin synthesis in maize and SKMY liquid medium [65]. The study showed that garlic extract inhibited 61.94% fungus growth. However, onion extract ceased about 60.44% aflatoxin synthesis. While on maize grain, eugenol extract reduced 60.35% aflatoxins synthesis. Hussain and Ali [48] compared the antifungal activity of some herbal spices, chemicals, and plants to inhibit the growth of aflatoxins producing fungus like *A. flavus and A. parasiticus*. They found that benzoic and propionic acid showed complete inhibition of *A. flavus* at (0.1–0.5%) and *A. parasiticus* at (0.2–0.5%), while clove (0.5%), garlic (0.5%), and onion (0.5%) showed complete inhibition of both *Aspergillus*.

The aqueous and phenolic extracts of several other natural and medicinal plants have been tested against *Aspergillus* [66]. Aqueous extracts of *Lupinus albus* (Leguminosae), *Ammi visnaga* (Umbelliferae), and *Xanthium pungens* (Compositae) were found to cease the growth of *A. flavus* and also the production of aflatoxin [67]. It was also found that the inhibitory effect was proportional to the applied concentration.

3. Role of essential oils on the inhibition of aflatoxins producing fungus and its production

The search for naturally occurring compounds or metabolites having bioactivity against aflatoxins producing fungi has been the target of interest in the search for ecologically friendly

products [68]. There are many essential oils produced by medicinal plants that have been tested for their inhibiting ability of aflatoxin production [69, 70].

Essential oils were extracted from 16 aromatic plants, that is, safflower (*Carthamus tinctorius*), marigold (*Tagetes erecta*), coriander (*Coriandrum sativum*), pomelo (*C. maxima*), mangosteen (*G. mangostana*), *Kaempferia parviflora*, ginger (*Zingiber officinale*), pepper (*P. nigrum*), Boraphet (*Tinospora crispa*), aloe (*Aloe vera*), lavender (*Lavendula officinalis*), rosemary (*Rosemarinus officinalis*), cinnamon (*Cinnamomum cassia*), eucalyptus (*E. globules*), thyme (*Thymus vulgaris*), and white wood (*Melaleuca cajuputi*), and their ability to inhibit the *Aspergillus* on PDA by agar diffusion test [71].

Different ratios (50, 25, 12.5, 6.25%) of each essential oil were placed onto a cylinder cup (6 mm dia) on agar plate streaked with *A. flavus*. It was observed that the essential oil extracted from white wood showed the highest inhibition followed by the essential oils of cinnamon and lavender, respectively. Sindhu et al. [72] used *Curcuma longa* leaves essential oil of 0.01, 0.05, 0.1, 0.5, 0.75, 1, and 1.5% concentration in YES broth that was inoculated with *A. flavus* spores. *C. longa* oil of 1 and 1.5% concentration reduced 95.3 and 100% aflatoxin (AFB1, AFG1) synthesis, respectively. They analyzed α-phellandrene, terpinolene, and p-cymene as an active compound in turmeric leave oil extract by gas chromatography-mass spectrometry (GC-MS). Mahmoud [73] also used 0.01% of five essential oils namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde), and thymol (phenolic ketone) to suppress the *Aspergillus* growth. The result showed the complete inhibition of *A. flavus* growth.

4. Conclusions

Despite all efforts, it has been very difficult to control the exposure of man and animals to aflatoxins, because of their natural occurrence in the environment. Although the prevention of aflatoxin contamination by inhibiting the fungal growth in food and feeds is the best practice, other measures are also necessary. The advantage of using active compound based on natural plant is that they are safer, ecologically friendly than any chemical compounds, and synthetically produced antimicrobial agents. Other procedures such as the removal or decomposition of aflatoxins are also necessary as the prevention of contamination alone may not always be successful.

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