Journal of Applied & Environmental Microbiology, 2015, Vol. 3, No. 1, 11-19
Available online at http://pubs.sciepub.com/jaem/3/1/3

DOI:10.12691/jaem-3-1-3

Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) Caused by *Aspergillus* Species in Ethiopia

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Received February 02, 2015; Revised February 12, 2015; Accepted February 26, 2015

Abstract Groundnut is an important cash crop for domestic markets as well as for foreign trade in several developing and developed countries. It is also one of the most valuable cash crops in eastern Ethiopia. However, its production is constrained by *Aspergillus* species, which cause quantitative losses and produce highly toxic and carcinogenic chemical substances known as aflatoxins. This article critically reviews Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) in Ethiopia and Its Management. Although groundnut has a huge potential as a cash crop to improve livelihoods of farmers and traders in various parts of Ethiopia, its market is declining and export of the crop has come to a standstill. This is due to aflatoxin contamination of the crop and the difficulty of meeting tolerance limits by importers and food processors, leading to rejection of the crop and reduction in market demand. Aflatoxin contamination is both a pre-harvest and postharvest problem. Therefore, management of aflatoxin contamination of groundnut in Ethiopia is very important using cultural practice such as habitat management, soil amendments and pre- and post-harvest managements, using physical control methods, using biological control methods, using resistance groundnut varieties and using chemical control methods.

Keywords: *Aflatoxin, Arachis hypogaea, Aspergillus flavus, groundnut, Ethiopia*

Cite This Article: Ephrem Guchi, "Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) Caused by *Aspergillus* Species in Ethiopia." *Journal of Applied & Environmental Microbiology*, vol. 3, no. 1 (2015): 11-19. doi: 10.12691/jaem-3-1-3.

1. Introduction

Groundnut (*Arachis hypogaea* L.), which is also known as peanut, earthnut, monkeynut and goobers, is an annual legume. It is one of the world's most important oilseed crops [1], ranking the 13th most important food crop and 4th most important oilseed crop of the world [2], being cultivated in more than 100 countries in six continents [3]. Groundnut kernels contain 40-50% fat, 20-50% protein and 10-20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron and potassium [4].

Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil. Oil pressings, seeds, and the haulms of groundnut are used as animal feed while the oilcakes are used as industrial raw material and fertilizer [5]. These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries.

Cultivated groundnut originated from South America [6]. Its cultivation is mostly confined to the tropical, subtropical, and warm temperate (zones) countries between 40^{o} N and 40^{o} S latitude. It is currently grown on 25.2 million hectares worldwide with a total production of 35.9 million metric tons, with developing countries in Asia (66%) and Africa (25%) as the major producers [7]. In 2009, China, India and the United States were the three largest producers of groundnut [8].

Groundnut is relatively new to Ethiopia. It was introduced from Eritrea to Hararghe in the early 1920s by Italian explorers [9]. Major groundnut producing areas in Ethiopia are Babile, Gursum, Beles, Didessa, Gambella and Pawe. Gamu Gofa, Illubabor, Gojam, Wello and Wellega are identified as potential production areas [9]. During the 2014, it was cultivated on 79943.03 ha of land and 112088.7 tons of groundnuts were produced, with average yield of 1.402 tons per ha [10].

Groundnut is affected by several diseases, such as late leaf spot (*Phaeoisariopsis personate* Berk and Curt), early leaf spot (*Cercospora arachidicola* Hori), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis* Speg), and bud necrosis (bud necrosis virus (BNV). Apart from these, infection of groundnut seed by molds mainly *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare can result in the contamination of the seed with aflatoxins, which are toxic fungal metabolites (mycotoxins). Aflatoxins are a group of structurally related toxic polyketide-derived secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus [11]*. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds targeting mainly the liver for toxicity and carcinogenicity [12].

Aflatoxin contamination of agricultural commodities poses considerable risk to human and livestock health and

has significant economic implication for the agricultural industry worldwide [13]. In the USA, it was reported that income losses due to aflatoxin contamination cost an average of more than US$100 million per year to US producers [14]. According to Cardwell *et al*., aflatoxin contamination of agricultural crops, such as groundnut and cereals, causes annual losses of more than $750 million in Africa [15].

Aflatoxins are the major mycotoxins that are most commonly associated with groundnuts [16]. Aflatoxin contamination of groundnut prevents groundnut producers from accessing bigger western markets, increases dependency on foreign food aid, stifles economic opportunities, and adversely affects consumer health. According to FAO, developing countries account for approximately 95% of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of aflatoxin contamination [17].

Aflatoxin contamination of groundnut could occur before harvest while the crop is maturing in the field particularly favored by drought stress and high soil temperature, in storage and during marketing [16]. Aflatoxin contamination and associated fungi in groundnut continue to attract worldwide attention and have been reported from various countries. Aflatoxin contamination can occur on pods and seed in the soil near harvest, during harvest, and post-harvest in storage. Pre-harvest infection by *Aspergillus* spp. and the environmental factors that lead to colonization, infection of the seeds, plants, and aflatoxin accumulation have been reviewed in detail [18]. Outbreaks of acute aflatoxicosis from contaminated groundnut in humans have been documented in Kenya, India, Malaysia and Thailand [19]. One of the first major documented reports of aflatoxins in humans occurred in 150 villages of western India in 1974 where 397 persons were affected and 108 persons died [20].

In Ethiopia, an earlier report showed mean levels of aflatoxin B1 of 34.7 and 105 µg/kg in samples of groundnut and peanut butter, respectively [21]. Amare *et al.* reported aflatoxin levels of 5-250 µg/kg in groundnut seed from eastern Ethiopia [22]. Recently, Alemayehu *et al.*reported that total aflatoxin levels in *Aspergillus flavus* positive samples of groundnut seed varied between 15 and 11865 µg/kg [23]. These results indicated heavy aflatoxin contamination of groundnut samples from Ethiopia, at levels much higher than any international acceptable standards, e.g. FAO and WHO acceptable limit being 15 µg/kg.

Afltoxin contamination of groundnut could be minimized through various agronomic and seed handling practices [24,25,26]. Planting varieties that are resistant to fungal infection or that does not support high aflatoxin synthesis offers a sustainable, low cost approach for aflatoxin management that is suited for adoption by small scale groundnut producers. Amare *et al.* reported variations in terms of reaction to *A. flavus* infection among groundnut varieties widely cultivated in Ethiopia [22].

2. Importance of Groundnut Production in Ethiopia

Groundnut is the edible seed of the plant known as *Arachis hypogaea* L. Groundnut plays an important role in the livelihoods of the poor farmers and in the rural economy of many developing countries. The crop is relatively new to Ethiopia and was introduced from Eritrea to Hararghe in the early 1920s by the Italian explorers [9]. The major groundnut producing areas in Ethiopia are Babile, Beles, Chagni, Didessa, Gambella, Gursum; and Gamu Gofa, Gojam, Illubabor, Pawe, Wellega and Wello are also identified as potential production areas [9].

The country's requirement for groundnut is essentially met through domestic production. However, data on domestic production of the product is not readily available. According to Babile Agricultural and Rural Development Office, from the total farmland of 21,500 hectares, 8600 hectares (more than 40%) were covered by groundnut in the fiscal year 2009/2010. The other district near to Babile is Gursum and from its total land of agricultural processes, 35% (5,750 hectares) of the farm was covered by groundnut. According to these two district Agricultural offices, 1 tons per hectare groundnut seed is expected, which implies these two districts alone could produce more than 15,000 tons of groundnuts annually. On the other hand, other two districts, called Chagni and Pawe, are found in the northwestern part of Ethiopia and 3759 and 5000 hectares of land, respectively, were covered with groundnut in the year 2009/10 cropping season and produced about 17, 000 tons of groundnuts in total. In general, the abovementioned areas alone produced more than 65,000 tons of groundnuts per annum out of the country's total production [27].

The average yield for the years 2007/08, 2008/09 and 2009/10 nationwide was estimated to be 1.117, 1.123 and 1.112 tons per hectare, respectively. During the 2010/2011, the national coverage was estimated at 47,307.92 ha and 58265.288 tons of groundnuts were produced in Ethiopia. The average yield was estimated at 1.232 tons per hectare. During the 2014, it was cultivated on 79943.03 ha of land and 112088.7 tons of groundnuts were produced, with average yield of 1.402 tons per ha [10].

3. Aflatoxins

3.1. Overview of Aflatoxins

Aflatoxins are a group of structurally related polyketide mycotoxins that contaminate many agricultural commodities, such as almond, coffee, corn, cottonseed (*Gossypium* spp.), groundnut, pistachio, rice (*Oryza sativa* L.), soybean, sunflower, and wheat [28,29,30]. In addition, milk and milk products can be contaminated as a result of cows being fed on aflatoxin-contaminated feed. Aflatoxin B_1 (AFB_1) is the most common of the six forms of aflatoxins, AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2.

3.2. History of Aflatoxins

Aflatoxins were discovered in 1960 when more than 100,000 young turkeys died in England over the course of a few months from an apparently new disease that was termed "Turkey-X disease". It was soon found that the mortality was not limited to turkeys. Ducklings and young pheasants were also affected. After a careful survey of the outbreaks, the disease was found to be associated with the Brazilian groundnut meal. An intensive study of that groundnut meal revealed its toxic nature as it produced

typical symptoms of Turkey-X disease when consumed by poultry and ducklings. A study on the nature of the toxin suggested its origin from the fungus *Aspergillus flavus*. Thus, the toxin was named "aflatoxin" by virtue of its origin from *A. flavus* (Guo *et al*., 2008). This was the event which stimulated scientific interest and gave rise to modern mycotoxicology. Research on aflatoxins led to a "golden age" of mycotoxin research during which several new mycotoxins were discovered [31]. Other important mycotoxins produced by *Aspergillus*, *Fusarium* and *Penicillium* include ochratoxin, patulin and fumonisins [31]. Among all mycotoxins and polyketide compounds synthesized by fungal species, aflatoxins (the most potent hepatotoxic and carcinogenic metabolites) continue to receive major attention and are most intensely studied.

3.3. Types of Aflatoxins

There are four major types of aflatoxins (namely AFB_1, AFB_2, AFG_1 and AFG_2) among at least 18 structurally related mycotoxins [32]. Aflatoxins designated by B_1 and B_2 show strong blue fluorescence under UV light, whereas the G_1 and G_2 forms show greenish yellow fluorescence. *A. flavus* produces aflatoxin B_1 and B_2. Other toxic compounds produced by *A. flavus* are cyclopionic acid, kojic acid, nitropropionic acid, aspenoxin, aflam and aspergillic acid. *A. parasiticus* produces aflatoxin G_1 and G_2 in addition to B_1 and B_2, but not cyclopionic acid [33]. Aflatoxin B_1 is considered to be the most important of the four because it is the most toxic and has been classified by the International Agency for Research on Cancer a probable human carcinogen [34]. Level of carcinogenicity is $B_1>G_1>B_2>G_2$ in that order. Aflatoxin B_1 is predominant, the most toxic and most potent hepatocarcinogenic natural compound ever characterized. Aflatoxin M_1 and M_2 are other significant members of the aflatoxin family and are oxidative forms of aflatoxin B_1, which are modified in the digestive tract of some animals and humans and can be isolated from milk.

Aflatoxins, like any other mycotoxins, are a subclass of substances which originated as a result of secondary metabolism of fungi. Unlike primary metabolites, these secondary metabolites are not essential for the growth of the fungi but have survival functions in nature [35]. Genes required for aflatoxin production have persisted in fungi for more than 100 million years [36]. Expression of secondary metabolite biosynthesis genes does not occur at high growth rates, which indicates that the synthesis of these metabolites occurs during growth repression.

3.4. Implications of Aflatoxins for Human and Animal Health

Numerous epidemiological studies have established the connection between aflatoxin consumption and incidence of liver cancer in humans [37]. Acute aflatoxin poisoning is rare, 25% of these cases are fatal, and chronic exposure rates are high, especially in developing counties. As such, many countries established legal limits for aflatoxin concentrations allowed in foods, specifically groundnuts have intended for human consumption [38]. Developing countries have especially high incidences of aflatoxicosis because regulation and testing is prohibitively expensive, and food is scarce because uncontaminated alternatives are not available.

Gong *et al*. demonstrated that in Benin and Togo, children exposed to the highest level of aflatoxin had a 2 cm lower height gain than those exposed to the lowest level [39]. Aflatoxins suppress the immune system of susceptible populations in humans, such as young children and HIV/AIDS patients. In a study in Gambia, it was found that secretory immunoglobulin-A in saliva may be reduced by dietary levels of aflatoxin in children resulting in reduced levels of antibodies. In Ghana, changes in the percentages of immune cell subsets following aflatoxin B_1 exposure reduced cell immunity, decreased human resistance to infections and reduced immune responses to vaccinations. Aflatoxin is a carcinogen and acute aflatoxicosis results in human deaths. Aflatoxins also cause oxidative stress, liver necrosis, haemorrhage and death in broiler chickens, pigs and cattle [40,41].

The carcinogenic effects of aflatoxin in animals are well established and highly species dependent. Since aflatoxin, specifically aflatoxin B_1 (AFB_1), is one of the most carcinogenic chemicals studied, the FDA, World Health Organization (WHO), and European Union (EU) limit permissible concentrations in foods (groundnut feeds) intended for animal feed as follows: for dairy animals (20 g/kg), immature animals (20 g/kg), breeding beef cattle, breeding swine, or mature poultry (100 g/kg) and finishing swine of 100 lbs or greater (200 g/kg) [42].

3.5. Economic Importance of Aflatoxins

Aflatoxins are naturally occurring mycotoxins that are predominatly produced by two species of fungi: *Aspergillus flavus* and *parasiticus*. Aflatoxins are some of the most potent toxic substances found in foods and feeds. Since the 1960s, when they were first discovered as responsible for the death of 100,000 turkeys in England, aflatoxins have been a subject of concern of many studies. Aflatoxins are highly toxic and can cause serious harm to human and animal health. Numerous studies have linked aflatoxins to various diseases, such as cancer of liver and hepatitis B and C. High levels of aflatoxin were detected in children with *kwashiorkor* (childhood malnutrition from protein insufficiency) in the Sudan, Durban, South Africa and Nigeria. In Gambia, 93% of sampled children (6-9 years old) were tested and found to be positive for aflatoxin albumin adducts. Aflatoxins are ubiquitous but are commonly found in warm and humid climates [16]. Hence, most commodities from tropical countries, especially groundnut and maize, are easily contaminated with aflatoxins.

Aflatoxin contamination of human and animal feeds poses serious health and economic risks worldwide. FAO estimates that 25% of the world food crops are affected by mycotoxins each year and constitute a loss at post-harvest [44]. According to Cardwell *et al*. aflatoxin contamination of agricultural crops causes annual losses of more than $750 million in Africa [15]. In the US, it was reported that income losses due to aflatoxin contamination cost an average of more than US$100 million per year to US producers [14]. Aflatoxin due to the invasion by *Aspergillus flavus* of the groundnut pod is a serious problem in the international groundnut market and has seriously hampered the export business of the developing countries [17].

To restrict exposure of groundnut to aflatoxin contamination, many countries and governmental agencies

have set safety regulations, limiting the average concentration of aflatoxin on groundnut and groundnut products. For instance, in 1974 the Food and Drug Administration (FDA) proposed a tolerance level at 15 parts per billion (ppb) for aflatoxin in groundnut products. These regulations on food crops due to aflatoxin toxicity have a considerable economic impact on crop production and consequently on farm organization. According to FAO, developing countries account for approximately 95% of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of aflatoxin contamination [17].

4. Aflatoxin Producing Fungi

4.1. Ecology and Biology of Aflatoxigenic Fungi

Aflatoxins are produced by four *Aspergillus* species. These include *Aspergillus flavus* Link ex Fr, *Aspergillus nomius* Kurtzman, Horn and Hesseltine, *Aspergillus parasiticus* Speare, and *Aspergillus tamarii [44]*. The agronomically and economically most important aflatoxin producers are the closely related *A. flavus*, hence the name afla-toxin, and *A. parasiticus*. Both species are soil-borne fungi that grow on living and decaying plant matter. These fungi produce aflatoxins on various commodities, but they are a concern on corn, groundnut and cottonseed. *A. flavus* can be distinguished from *A. parasiticus* by its smooth spores and yellow-green colonies on potato dextrose agar (PDA) medium [45]. *A. parasiticus* produces dark yellow-green conidia with nearly spherical vesicles that produce roughened conidia. It can be readily distinguished from *A. flavus* by its rough-walled conidia [45].

Dominant aflatoxins produced by *A. flavus* are B_1 and B_2, whereas *A. parasiticus* produces two additional aflatoxins G_1 and G_2 [18]. *A. flavus* of the section Flavi is the most common species involved in pre-harvest aflatoxin contamination of crops and causes aflaroot or yellow mould. *A. flavus* is the most common mycotoxin-producing fungus found in groundnuts; this is true across various climates and geographic regions.

Aflatoxigenic fungi are soil-borne imperfect filamentous fungi, which are saprophytic during most of their life cycle, and grow on wide variety of substrates, including decaying plant and animal debris. Two major factors that influence soil populations of these fungi are soil moisture and soil temperature. These storage fungi can grow at temperatures ranging from 12 to 48 ^{o}C, with optimum of 25 to 42 ^{o}C, and at water potentials as low as -35 MPa. Under high soil temperatures and low moisture, which are associated with drought stress, these fungi become highly competitive and dominant, produce abundant inocula, and outcompete other microflora on corn, cotton, and groundnut [18].

Neither *A. flavus* nor *A. parasiticus* has a known sexual stage; they reproduce only by asexual means, but undergo genetic recombination through a parasexual cycle. Morphology of the conidiophore, which bears asexual spores, is the most important taxonomic character in the identification of *Aspergillus*. Other important morphological structures used in identification are cleistothecia, hulle cells, and sclerotia [31]. These fungi can survive either as mycelium or as resistant structures known as sclerotia [46]. *A. flavus* type fungi are genetically and phenotypically diverse. There are of two types, L isolates producing abundant conidiophores, large sclerotia, and variable amounts of aflatoxin, while the S isolates produce abundant, small sclerotia, fewer conidiophores, and high levels of aflatoxins.

Aflatoxigenic fungi are ubiquitous in nature and have important roles in natural ecosystems and human economy. *Aspergillus* species are capable of recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some species of *Aspergillus* degrade more refractory compounds, such as fats, oils, chitin and keratin. Maximum decomposition occurs in the presence of sufficient nitrogen, phosphorus and other essential inorganic nutrients. Foods utilized by humans and domestic animals are also good nutritional sources for *Aspergillus* species [31].

4.2. Life Cycle of *Aspergillus* Species

Aspergillus flavus is a saprophytic fungus that survives on dead plant tissue and sometimes behaves as a weak and opportunistic pathogen [47]. The sources of inocula for *A. flavus* and *A. parasiticus* are sporogenic sclerotia, conidia and mycelia that over-winter in plant debris [48]. In fields repeatedly cropped to groundnut or rotated between groundnut, maize and cotton, conidia from sporogenic sclerotia are the primary source of *A. flavus* inocula. Conidia adjacent to the developing groundnut pods germinate in the soil following the release of carbon and nitrogen substrates by injured groundnut pegs and result in colonization of the pods. Hot humid conditions favor the release of spores on plant residues, and these spores are dispersed by wind through the field [49]. Conidia that adhere to insect bodies are physically moved to plant parts and flowers in groundnut. Smaller, generally immature kernels are more easily infected in a shorter period of time than kernels in more mature pods. Infections of groundnut kernels at other maturity stages are relative to the survival of the fungus and not necessarily to a new infection at a later stage of maturity. *Aspergillus flavus* does not always establish a successful systemic infection in groundnut plants.

5. Occurrence of Aflatoxins in Foods in Ethiopia

In Ethiopia, the investigation on fungi associated with grain, and the extent of the mycotoxins they cause is very limited. A preliminary survey on prevalence of *Aspergillus flavus* on cereal grains in Ethiopia was conducted three decades ago [49]. In the study, mycological examination was made and the *A. flavus* isolate potential to produce mycotoxin was determined. The result showed that the fungus is associated more with sorghum and maize than with teff and barley. From the isolated *A. flavus*, 80% of the isolates were capable of producing aflatoxin *in vitro*. The favorable conditions for the fungus and associated toxin production included high relative humidity and temperature of the growing area. Bisrat and Gebre also reported that aflatoxins were identified from ground pepper (*berbere*) and ground roasted pea (*shiro*) although the detection was much lower than various international

standards and its frequency was also lower [21]. Unpublished works of various investigators showed that aflatoxins are prevalent in maize, sorghum, barley, different staples and leguminous foods of Ethiopia.

Amare *et al.* in his study of mycoflora, aflatoxins using thin layer chromatography, and resistance of groundnut cultivars from eastern Ethiopia, showed that 42% of the samples he collected contained detectable aflatoxins at levels ranging from 5 to 250 µg/kg. About 85% of the isolates of *A. flavus* were also capable of producing aflatoxins in culture [22]. Apart from the abovementioned investigation, some other studies showed that aflatoxins are prevalent despite limited available information. According to Amare *et al*. there is occurrence of *Aspergillus* and *Fusarium* mycotoxins in Ethiopian barley, sorghum, wheat and teff [50]. There is a need to further extend the database on the nature and extent of mycotoxin contamination and the associated fungi in the region, because such information is a prerequisite for assessing the risk to consumer health and for managing the mycotoxin problems.

Amare collected maize grain samples from Dire Dawa (eastern Ethiopia), Adama, and Ambo, and identified 15 species of fungi from 17 samples [51]. Amongst these, *Aspergillus* spp. occurred in 94% of the samples, *Fusarium* spp. occurred in 76.5% of the samples, while *Penicillium* spp. were found in 64% of the samples. He also detected aflatoxins, fumonisins, deoxynivalenol and nivalenol with varying concentrations. In this study, it was concluded that toxin-producing fungi occurred ubiquitously so that further monitoring of mycotoxins in maize in Ethiopia is justifiable considering the different agro-ecological zones of maize-producing areas (hot, humid western and southwestern regions of the country) with detailed analysis of the pre-harvest and post-harvest storage factors.

Eshetu conducted a study on the aflatoxin content of groundnut (*Arachis hypogaea*) in relation to shelling and storage practices of farmers in Ethiopia [27]. The finding showed that *Aspergillus flavus* was the most potent aflatoxigenic strains infecting 80.69% of the total samples analyzed. Eighty-five percent of the tested isolates of *A. flavus* were capable of producing aflatoxins. Alemayehu *et al*. also reported that the total aflatoxin levels in the positive samples of groundnut seed varied between 15 and 11865 µg/kg [23]. Eshetu suggested that researches by investigators on the aflatoxin content of groundnut and other cereals in pre-harvest and spatial distribution of it are needed to fulfill the aflatoxin information gap at the country level [27].

The current research results of Alemayehu *et al.* reported heavy contamination of groundnut samples in Ethiopia at a level much higher than any international, FAO and WHO-standards (which is 15 µg/kg) [23]. They also suggested that investigation of groundnut contamination by toxigenic fungi and associated mycotoxins should continue in groundnut producing regions across the country to come up with a complete picture of grain contamination both temporally and spatially. Such studies will serve as important basis to understand the full extent of the problem and also to work on appropriate management measures, and also serves as a wakeup call to create awareness on the aflatoxin problem in the country and possible remedies.

6. Occurrence of Aflatoxin Contamination in Groundnut

Aflatoxin contamination can occur in pods and seed in the soil near harvest, during harvest, and post-harvest in storage. Pre-harvest infection by *Aspergillus* spp. and the environmental factors that lead to colonization, infection of the seeds, plants, and aflatoxin accumulation have been reviewed in detail [18]. Initial inoculum in groundnuts most likely originates in the soil. Inoculation and colonization are dependent on soil pathogen population, temperature and moisture content of the soil. The two most important conditions that favor pre-harvest invasion and aflatoxin contamination of groundnut seeds are the simultaneous occurrence of excessively high soil temperature and late season drought stress. Neither heat nor drought alone can induce high levels of pre-harvest contamination. Insect and mechanical damage to the pod increase the opportunity for invasion by *Aspergillus* and the consequent accumulation of aflatoxins. Similarly, post-harvest aflatoxin contamination is most attributable to improper storage of the pods and seed. Conditions important for aflatoxin formation during storage are high humidity and high temperature.

7. Management of Aflatoxin Contamination of Groundnut

7.1. Overview of Aflatoxin Management

Management of aflatoxin contamination in groundnuts is complex. Both preventative and curative procedures may be necessary. Aflatoxin occurrence and severity in field crops is largely a matter of uncontrollable natural events. The complete elimination of aflatoxin from human food, while desirable, is almost impossible to achieve, as aflatoxin is distributed in a wide range of agricultural products where it is an unavoidable natural contaminant.

However, certain practices such as the “farm to fork” policy of the European Union (EU) can be put in place to reduce aflatoxin levels. Pre-harvest and post-harvest management strategies employed to reduce aflatoxin in food result in lower productivity, but better quality. This is confirmed by Hameeda *et al*. who state that commodities contaminated with aflatoxin have a lower market value and cannot be exported [52]. The authors also reported that animals fed with groundnut meal contaminated with aflatoxin have lower productivity and slower growth. Likewise, Magan *et al*. concluded that poor post-harvest management can immediately result in rapid loss of quality and encourage aflatoxin growth [53]. Research on the aflatoxin problem has linked aflatoxin production to poor farming practices. In a survey of 300 samples collected from Benin’s farmers, Hameeda *et al*. analyzed the relationship between the level of aflatoxin and storage practices [52]. It was reported that aflatoxin levels at the beginning of storage were less than the ones found in maize stored for six months.

Dohlman proposed a strategy to reduce both health risks and the economic costs associated with mycotoxins. Food producers must be more aware of mycotoxin effect, and therefore, handling practices that would minimize

mycotoxin contamination. Moreover, another solution would be to encourage the adoption of process-based guidelines (good agricultural practices (GAPs) or good manufacturing practices (GMPs)) [16].

Hazard analysis and critical control points (HACCP) are employed to reduce unacceptable aflatoxin levels from insect damage to the developing crop in the field or when the mature crop is exposed to moisture prior to harvest or during handling, transportation and storage. However, small-scale industries, subsistence production and food insecurity make the economics and enforcement of aflatoxin regulations impractical [54]. Current management of aflatoxin contamination starts in the farmer's field and continues through harvest, drying, storage and processing [54].

7.2. Cultural Practices Used in the Management of Aflatoxins

Habitat Management: Certain cultural practices, such as: summer ploughing, selection of appropriate planting date to take advantage of periods of rainfall to avoid end-season drought effects, seed dressing with systemic fungicides or biocontrol agents, maintaining good plant density in the fields, removal of premature dead plants, managing insect pests and diseases, timely harvesting, exclusion of damaged and immature pods, quick pod drying, controlling storage insect pests and storing the pod/seed with less than 10% moisture content can prevent fungal infection and proliferation [24,25,26]. The use of safe and efficient mechanical threshers, and seed storage bins are other cultural practices for reducing aflatoxin in groundnuts.

Although most of the options are cost-effective and practical under subsistence farming conditions, they remain largely un-adopted by farmers. Researcher efforts resulted in devising appropriate combination of practices (timely harvesting, windrow drying and threshing) that are more compatible with socio-economic profiles and farming practices which contribute to good adoption of the farmers of a particular region. Late season irrigation to alleviate drought stress of plants is effective in reducing aflatoxin contamination in the field. Choice of cultivar is important, especially on location where irrigation is not available. The cultivar should mature before late season drought stress occurs, and where it can be harvested and dried in conditions not favorable post-harvest contamination. Control of pod-feeding insects through application of recommended insecticides and use of insect-resistant cultivars should be an integral part of the strategy to eliminate pre-harvest aflatoxin contamination [24,25,26].

Aflatoxin Management Through Soil Amendments: Soil amendments with gypsum (as source of calcium), farmyard manure and cereal crop residues applied either singly or in various combinations at different cropping stages would contribute to reduction in the pre-harvest *A. flavus* infection and aflatoxin contamination in groundnut [11]. Application of gypsum and farmyard manure at the time of sowing was found to be most effective in reducing seed infection and aflatoxin contamination (mean reduction of 80% compared to controls). This managements options is feasible because lime and farmyard manure are cheap and easily available in most developing countries, including SSA.

Post-harvest Aflatoxin Management Practices: Several studies in West Africa have shown that aflatoxin contamination increased with the delay in pod removal after lifting the plants and during storage [26]. In addition, traditional heap drying enhanced rapid fungal proliferation and toxin production. Small and immature seeds (beans) contain the highest toxin and segregating such seeds would reduce the contamination in the final product. Replacement of farmers' traditional practice of 'heap' drying with windrow drying of lifted plants has dramatically reduced the contamination. In Andhra Pradesh State, India, mechanical threshers were introduced for rapid threshing of pods to avoid the heaping.

However, on-farm and household storage conditions in Asia and SSA are not adequate to store groundnut safely (lack of clean storage bins, frequent pest infestation) and create conditions conducive for aflatoxin contamination even in a healthy produce from the field. Rapid post-harvest drying can prevent the further invasion of groundnut seeds by *Aspergillus* spp. Early harvesting and the rapid drying of groundnut kernels to moisture levels below 15% effectively stop aflatoxin accumulation.

Storage conditions at low temperatures and reduced humidity are important components for reduced *A. flavus* growth and aflatoxin production. Storage facilities must be regularly monitored to ensure early detection and management of insect infestations. Old grain residue must not be mixed with new grain and storage areas must be sanitized before new grain is stored **(http://ec.europa.eu)**. According to Smith, good warehousing practices largely prevent further increase of post-harvest aflatoxin contamination [55]. The most important factor in preventing aflatoxin contamination in groundnut in storage is moisture control. A good warehouse should have a double roof and sidewalls and be adequately cooled and properly ventilated to prevent wetting of the groundnuts. Pods should be dried to less than 9% moisture prior to storage. Controlled atmosphere (CA) in storage with high CO_2 and low O_2 appears to inhibit the *Aspergillus* growth. Hence hermetic atmosphere storage seems desirable in preserving the groundnut quantity and quality.

According to Cole *et al*., post-harvest screening to remove contaminated seed appears to be a promising means to reduce or to eliminate aflatoxin [56]. When aflatoxin contamination occurs, there are usually only a few highly contaminated seeds irregularly distributed in the groundnut lots. Most of the harvested seeds are free of contamination. Early removal of high-risk seeds, *e.g.*, those that are damaged, immature, or discolored, should be an effective way to prevent further contamination and increase the groundnuts' value. Practical methods include manual sorting, seed size and density separation, or electronic color sorting. Electronic color sorting has proved to be the most effective aflatoxin management strategy available in the processing phase.

In conclusion, the recommended cultural practices include optimizing irrigation; avoiding mechanical and insect pest damage during cultivation, harvesting, storage and processing; optimizing harvest time; rapid post-

harvest drying; and storage at low temperature, and managing humidity and seed moisture.

7.3. Physical Control of Aflatoxin Production

Sanitation practices, such as mechanical or hand sorting, can reduce aflatoxin levels by removing low-density mould-infected kernels [57]. Prado *et al*. found that gamma irradiation at doses of 15, 20, 25, and 30 kilo Gray (kGy) resulted in a 55-74% aflatoxin B_1 reduction in groundnut kernels [58]. Ogunsanwo *et al*. (2004) found positive correlations between loss of aflatoxin and dry roasting conditions. Roasting at 140 ^{o}C for 40 minutes reduced aflatoxin B_1 and G_1 by 58.8 and 64.5%, respectively, while roasting at 150 ^{o}C for 30 minutes resulted in 70 and 79.8% reduction in aflatoxin B_1 and G_1, respectively. Cooking and steaming for 1 hour under pressure reduces aflatoxin by up to 60% [57]. This is because high temperature breaks the ring chemical structure of aflatoxin.

7.4. Biological Control of Aflatoxin Production

Biological control of toxigenic *A. flavus* strains can be achieved by the application of atoxigenic *A. flavus* strains to maize, groundnut and cotton fields [59]. Dorner *et al*. reported that field application of the nontoxigenic strains not only reduced aflatoxin contamination in the field but also reduced aflatoxin contamination that occurred in storage [60]. Biocontrol agents were used as seed dressing and soil application to determine their effects on population dynamics of *A. flavus* in the geocarposphere and subsequently on pre-harvest kernel infection of groundnut. Application of 11 kg/ha of an atoxigenic *A. flavus* strain with a food source such as wheat (*Triticum aestivum* L.) or sorghum (*Sorghum bicolor* L.) once a year resulted in the displacement of over 80% of aflatoxin-producing *Aspergillus* spp. in Arizona and Texas [61].

Similarly, significant reduction (20-90%) in *A. flavus* seed infection was recorded with several treatments over the control [62]. These practices have been applied with success in developed countries but have been of limited use in developing countries. In spite of precautions, damage and contamination can still occur, even on undamaged pod and seed, which led to application of various curative methods to eliminate or lower the contamination. According to Thakur and Waliyar (2005), selected isolates of *Trichoderma* (*T. viride* (Tv 47), *T. harzianum* (Th 23), *T. harzianum* (Th 20), *T. koningii* (Tk 83)), geocarposphere bacterial strains of *Pseudomonas* (*P. aeruginosa* CDB35, *P. cepacia* and *P. fluorescens*) and Actinomycetes (strain CDA19)) have been used for reducing groundnut seed colonization by competitive exclusion/ inhabitation of *Aspergilli*. Aflatoxin production is also inhibited by lactic acid bacteria (*Bacillus subtilis)* and many other molds. This inhibition may result from many factors, including competition for space and nutrients in general, competition for nutrients required for aflatoxin production but not for growth, and production of anti aflatoxigenic metabolites by co-existing microorganisms. For instance, *Bacillus subtilis*, a bacterium isolated from groundnuts, was found to inhibit the growth of *A. flavus* in groundnuts.

7.5. Use of Resistant Groundnut Genotypes

Besides adopting certain cultural harvest and storage practices, planting *A. flavus*-resistant genotypes is an effective and low-cost part and parcel of an integrated aflatoxin management. Alleviation of aflatoxin contamination through genetic manipulation has been attempted in several groundnut-producing countries since the late 1960s. Breeding resistant cultivars is possible only when there are available sources of stablity and high-level groundnut resistance. Groundnut cultivar also affects contamination levels; newer cultivars are more resistant to fungal growth while native, or unimproved, cultivars are more susceptible to contamination [63]. Resistance to aflatoxin contamination in groundnut is probably due to resistance to pod infection, resistance to seed invasion and colonisation by the fungus, and resistance to aflatoxin production. However, Cantonwine *et al*. found that seed colonisation by *A. flavus* does not appear to be a significant plant trait that affects the field resistance of groundnut to aflatoxin contamination [64].

Drought-tolerant groundnut genotypes have reduced aflatoxin levels and resulted in higher yields than drought-susceptible genotypes when subjected to late-season planting and drought stress [65]. Under drought conditions, the receding groundnut foliar canopy allows solar radiation to strike the bare soil surface, raising the temperature of the geocarposphere, thus promoting the production of aflatoxin.

7.6. Chemical Control of Aflatoxin Contamination

Ring opening of the aflatoxin chemical structure occurs at 100°C, followed by decarboxylation, leading to the loss of the methoxy group from the aromatic ring of the aflatoxin molecule. Aflatoxin G1 and G2 are more susceptible to chemical hydrolysis than aflatoxin B1 and B2 because of the presence of two ether linkages in the G group compared to the B group which possess a single ether linkage [66]. Ammonia at 0.5-7% coupled with long exposure time, ambient temperature and pressure has been successfully used to inactivate aflatoxin in contaminated commodities, such as groundnut meal, cottonseed and maize. This process has been approved by safety and regulatory agencies, such as Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) [67].

Aflatoxin bioavailability was reduced in the gastrointestinal tract of animals by chemisorbents, such as activated carbon, bentonite, clays, and aluminosilicates. A Nova Silclay (NS) can act as a selective enterosorbent of aflatoxins when mixed with animal feed at inclusion rates as low as 0.25% (w/w) [61]. At this inclusion rate, NS sequesters aflatoxin resulting in reduced aflatoxin in the gastrointestinal tract and neutralization of its toxic effects. It does, however, not interfere with vitamin and micronutrient uptake. Long-term studies at Texas A & M University and in a Phase I Adverse Events trial at Texas Technology University confirmed the relative safety of NS in rodents [61]. NS will be a promising novel, inexpensive and easily disseminated remedy to aflatoxin management in Africa once Phase II human studies are completed to determine its efficacy [61].

Acknowledgments

I acknowledge Dr. Mashilla Dejene from Department of Plant Pathology, Haramaya University for his critical comments of this review article.

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