Aflatoxin contamination of groundnut (Arachis hypogaea L.): Predisposing factors and management interventions


ABSTRACT

Groundnut (Arachis hypogaea L.) is one of the most important oilseed crops in world agricultural trade. It is considered an important crop by virtue of its contribution to satisfying the protein needs of many households who cannot afford animal protein. Production and consumption of groundnuts are hampered among others, by Aspergillus flavus and Aspergillus parasiticus infection which subsequently contaminate groundnuts with aflatoxins. Aflatoxins are associated with acute and chronic toxicities in humans and animals causing induction of tumor, liver damage, liver cirrhosis, and carcinogenic, estrogenic, teratogenic, and immunosuppressive effects. Contaminated food crops expose millions of people to high risk of chronic aflatoxin exposure. Aflatoxin contamination can occur in the field before harvest, and after harvest during curing, storage and transportation. The major factors influencing A. flavus and A. parasiticus infection in groundnuts before harvest are insect damage to the developing seed/pod, drought and high soil temperatures. After harvest, environmental conditions such as high humidity and high temperatures promote fungal infection and aflatoxin accumulation. Agronomic practices such as crop rotation, use of resistant varieties, insect control, timely planting and harvesting, weed control, adequate fertilization and late season irrigation can reduce pre-harvest aflatoxin production. Additionally, atoxicogenic fungi can be applied in the field to competitively displace toxigenic fungi to reduce the population of toxigenic fungi in the soil. Post-harvest aflatoxin contamination of groundnuts can be minimized by rapid and proper drying following harvesting, proper transportation and packaging, sorting and post-harvest insect control. Sourcing information from different research and review articles, and book chapters, this paper provides extensive review on the predisposing factors and management of groundnut aflatoxin contamination before and after harvest.

1. Introduction

Groundnut (Arachis hypogaea L.) is an annual leguminous crop which has other names as peanut, earthnut, monkeynut and goobers. It is the 4th most important oilseed crop and 13th most important food crop worldwide (Reddy, Sudhakar, & Reddy, 2011). Throughout sub-Saharan Africa (SSA), groundnut is considered a valuable crop as it comprises 40% of the world’s groundnut production area, but contributes only 26% to groundnut production worldwide (ICRISAT, 2010). Apart from these constraints, infection of groundnut seed by molds mainly Aspergillus flavus and Aspergillus parasiticus can lead to contamination of the seed with aflatoxins (Guchi, 2015), which render groundnut unsafe for consumption and trade (Waliyar et al., 2016).

Aflatoxins are secondary metabolites produced by toxigenic strains of A. flavus and A. parasiticus under suitable environment during pre- or post-harvest operation (Reddy et al., 2011). Aflatoxin contamination results in downgrading of grains and oilseeds, and depletion of their 33.6–54.95% oil serving as an inexpensive source of high quality dietary protein and oil for both humans and animals (Asibu et al., 2008). Groundnut production is faced with numerous constraints, namely, drought, diseases such as rust, rosette, early and late leafspots, and pests such as leaf miners and aphids (Okello, Biruma, & Deom, 2010). Apart from these constraints, infection of groundnut seed by molds mainly Aspergillus flavus and Aspergillus parasiticus can lead to contamination of the seed with aflatoxins (Guchi, 2015), which render groundnut unsafe for consumption and trade (Waliyar et al., 2016).

Aflatoxins are secondary metabolites produced by toxigenic strains of A. flavus and A. parasiticus under suitable environment during pre- or post-harvest operation (Reddy et al., 2011). Aflatoxin contamination results in downgrading of grains and oilseeds, and depletion of their...
nutritional value (Jolly, Bayard, Awuah, Fialor, & Williams, 2009). Prolonged consumption of aflatoxins has been reported to cause impaired immune function, malnutrition and stunted growth in children, disabilities and death (Bbosa et al., 2013; Gong, Hounsa, & Egal, 2004). Aflatoxin is known to have adverse effect on reproductive health and also associated with liver cirrhosis, hepatitis B and C infections, and liver cancer (Bbosa et al., 2013).

The detrimental effects of aflatoxins have prompted many countries to set maximum levels of total aflatoxin in groundnuts and groundnut products intended for human and animal consumption (Van Egmond, Schothorst, & Jonker, 2007). For example, maximum limits of 4 ppb for European Union (EC, 2010), 20 ppb for USA (Torres, Barros, Palacios, Chulze, & Battilani, 2014) and Ghana (Masters, Ghosh, Daniels, & Sarpong, 2013), 10 ppb for Kenya (Wagacha, Mutegi, Karanja, Kimani, & Christie, 2013) and 15 ppb for Malaysia (Leong, Ismail, Latif, & Ahmad, 2010) have been documented. However, these permissible levels are often impossible to achieve, especially in developing countries such as sub-Saharan countries, due to poor practices and fungi-favoured environmental conditions that prevail during production and storage of groundnuts (Bbosa et al., 2013). It is against this background that this paper seeks to review the predisposing factors for groundnut aflatoxin contamination and current approaches for its management in order to instill cautionness in producers and consumers during groundnut cultivation and storage.

2. Aflatoxinigenic fungi

The large genus, Aspergillus, is subdivided into 7 subgenera which are further divided into sections (Klich, 2002). Aspergillus section Flavi, commonly referred to as Aspergillus flavus group, belonging to the subgenus Circumdati has gained worldwide attention due to its ability to produce toxins. Aspergillus oryzae, A. sojae and A. tamarii form the non-aflatoxinigenic group whereas A. flavus, A. parasiticus and A. nomius constitute the aflatoxinigenic group of the section Flavi (Rodrigues et al., 2007). A. nomius is not of practical importance (Pitt, 2000). A. flavus and A. parasiticus are the most economically and agronomically important species that infect and produce aflatoxins in agricultural crops prior to harvest or during storage (Yu, Whitelaw, Nierman, Bhatnagar, & Cleveland, 2004).

2.1. Description of Aspergillus flavus and Aspergillus parasiticus

A. flavus and A. parasiticus are soil borne imperfect filamentous fungi that grow on living and decaying plant matter with strong affinity for oilseeds and nuts such as groundnuts, maize and cottonseeds (Pitt, 2000). The fungi overwinter as mycelia in plant tissues or as sclerotia or conidia in the soil. The sclerotia have the ability to survive in the soil under harsh environmental conditions and produce conidia or mycelia resulting in population increase under hot and dry conditions. Conidia can further be dispersed by air, rain splash or through the soil. Conidia dispersed through air infects aboveground crops such as maize while infection of underground crops such as groundnut is facilitated by conidia movement through the soil and rain splash (Amaike & Keller, 2011; Gupta, 2012; Hedayati, Pasqualotto, Warn, Bowyer, & Denning, 2007). A. flavus exhibits two sclerotial morphotypes on the basis of its aflatoxin-producing ability (Jeffrey, Ehrlich, & Ehrlich, 2006) and sclerotial characteristics (Hedayati et al., 2007) when cultured in vitro on laboratory growth media. The sclerotial morphotypes are the L strain that produces few but large sclerotia usually larger than 400 μm in diameter whereas the S strain which is also described as A. flavus var. parvisclerotigenus produces numerous small sclerotia usually less than 400 μm in diameter. The S strain has a very stable aflatoxinigenic trait and produces higher amounts of aflatoxins than isolates of L strain on the same media (Gnonlonfin et al., 2013).

3. Factors promoting the growth of aflatoxinigenic fungi and aflatoxin production

Aflatoxinigenic fungi can invade and produce aflatoxin in pods and seeds of groundnut prior to harvest, during harvest and after harvest. Inoculation and colonization of A. flavus and A. parasiticus with initial inoculum from the soil are dependent on fungal population, temperature, relative humidity, insect infestation and moisture content of the soil (Gnonlonfin et al., 2013; Guchi, 2015). However, Gnonlonfin et al. (2013) indicated that complex interactions among the different factors occur to result in significant invasion of groundnut seeds and subsequent aflatoxin production by aflatoxinigenic fungi. High soil temperature and late season drought stress are the two most important factors that occur concurrently to promote pre-harvest infection and aflatoxin contamination of groundnut seeds. Post-harvest aflatoxin contamination of groundnut on the other hand is influenced by high humidity and high temperature during storage (Guchi, 2015).

3.1. Pre-harvest factors

Aflatoxin contamination does not always correlate positively with the incidence of invasion by Aspergillus flavus (Sanders, Cole, Blankenship, & Hill, 1985). The main factors influencing pre-harvest infection of groundnuts by A. flavus and A. parasiticus are insect damage to the pod and developing seed, and plant stress due to high soil temperatures and drought (Torres et al., 2014). Cole, Sanders, Hill, and Blankenship (1985) reported that following the invasion of aflatoxinigenic fungi, growth of fungi and subsequent aflatoxin production occurred as a result of breakdown of natural resistance mechanism due to environmental stresses mainly high temperature and drought. Temperature and drought stresses are well known factors for aflatoxin contamination of groundnuts (Cole, Blankenship, Hill, & Sanders, 1984; Graufud, Prasad, Waliyar, & Taheri, 2006; Diao et al., 2015; Sanders, Cole, Blankenship, & Dorner, 1993). High temperature and drought stresses affect the physiology of plants with the likelihood of plants exhibiting susceptibility to infection or aflatoxin production in response to the stresses (Klich, 2007). Pitt, Taniwaki, and Cole (2013) confirmed that drought stress reduces plant’s natural defenses against infection, the water activity in the soil, growth and activities of bacteria, amoeboae and competing fungi and promotes growth of xerophilous A. flavus and A. parasiticus. Payne and Hagler (1983) observed a significant increase in proline, an aflatoxin production enhancer, due to drought stress. Drought stress has been reported to inhibit the production of phytoalexins, antimicrobial compounds produced by some plants. For instance, Dorner, Cole, Sanders, and Blankenship (1989) observed no aflatoxin in immature groundnuts until phytoalexin production ceased due to drought stress. Excessive drought strains groundnut pods and kernel seed coat resulting in establishment of entry points for infection (Okello et al., 2010). Drought stress for a period of 85–100 days at a soil temperature of 29 °C produced the greatest number of colonized edible grade groundnut and high aflatoxin levels (Cole et al., 1984). Higher aflatoxin contamination has been reported in groundnuts grown under rain-fed conditions than those grown under irrigation (Reddy, Sulochanamma, Subramanyam, & Balaguravaiah, 2003). Sufficient moisture is therefore required to control or reduce aflatoxin production in groundnut (Diao et al., 2015).

3.2. Harvest factors

Timely harvesting of groundnuts is necessary to reduce aflatoxin contamination. Delays in harvesting of groundnuts will yield poor quality seeds/pods due to mold infections including A. flavus and A. parasiticus and subsequent aflatoxin contamination of the seeds/pods (Torres et al., 2014). Infestation of groundnut pods by pests, and mechanical damage to pods or kernels during digging and threshing of groundnuts, provide entry points for invasion by aflatoxinogenic fungi.
(Waliyar, Kumar, Traore, & Kodio, 2008). A confirmation by Sobolev, Gou, Holbrook, and Lynch (2007) indicated that insect injury to groundnut pods or kernels might result in aflatoxin contamination of groundnuts under conditions that rarely support fungal infection and aflatoxin production.

3.3. Post-harvest factors

Post-harvest aflatoxin contamination of groundnut is highly influenced by transportation and storage conditions (Diao et al., 2015). The importance of sanitation, moisture content and environmental temperature in reducing the incidence of aflatoxin contamination during storage and transportation cannot be overemphasized. High kernel moisture during storage can result from leaky roofs, condensation arising from improper ventilation in the storeroom, high-moisture foreign material associated with stored groundnuts, and improper post-harvest drying of groundnuts (Davidson, Whitaker, & Dickens, 1982).

Post-harvest aflatoxin contamination of groundnut can most effectively be reduced by sorting to remove contaminated groundnuts before storage (Dorner, 2008).

Generally, moisture content of 10% or higher after harvest predisposes groundnuts to aflatoxin contamination. Therefore, timely drying and maintenance of safe moisture levels would achieve effective control of post-harvest aflatoxin contamination of groundnuts (Torres et al., 2014). A. flavus infection was observed in 70% of high moisture grains (> 18%) with a positive correlation between the rate of infection and aflatoxin production (Mora & Lacey, 1997). Positive correlation between kernel moisture content and aflatoxin production was found in groundnut (Kaaya, Kyamuhangire, & Kyamanywa, 2006), cowpea (Houssou et al., 2009) and maize (Hell, Cardwell, Setamou, & Gou, Holbrook, and Lynch, 2007) indicated that insect injury to groundnuts under conditions that rarely support fungal infection and aflatoxin production (Kaaya, Kyamuhangire, & Kyamanywa, 2006). Water activity (a_w), is the amount of free humidity in a crop or product and is the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature. Water activity beyond 0.95 at 25 °C provides a conducive environment for fungal growth and spore germination. Fungal growth slows down below 0.85 a_w with inhibition of growth occurring between 0.70 and 0.75 a_w (Hassane et al., 2017). Gqaleni, Smith, Lacey, and Gettinby (1997) discovered the optimum water activity (a_w) as 0.996 and 0.80–0.82 as minimum a_w for growth of A. flavus. Northolt, van Egmond, and Paulsche (1977) reported that at higher water activities (0.98–0.99), higher amounts of aflatoxins are produced but production of aflatoxin ceases at or near a_w 0.85. Diener and Davis (1970) found that aflatoxin production could be prevented in groundnuts by rapidly drying to or below a_w of 0.83 after harvest.

The effect of temperature on the growth of A. flavus and A. parasiticus and subsequent aflatoxin production in a number of different agricultural commodities and on artificial media has been established. Diener and Davis (1967) reported that the optimum temperatures for aflatoxin production by A. flavus and A. parasiticus are 25 °C and 25–30 °C respectively in groundnuts. They further noticed a change in aflatoxins B_1 and G_3 levels produced by A. parasiticus, with a reduction in aflatoxin G_1 as temperatures increased. Molina and Giannuzzi (2002) used laboratory media and mathematical modelling to detect the optimum temperatures of 27.84 °C and 27.32 °C at pH of 5.9 and 5.5 respectively for aflatoxin production by A. parasiticus.

4. Management of aflatoxin contamination of groundnut

Aflatoxin contamination is often a continuous process, beginning in the field and accelerating during harvest, drying, and storage. Fungal infection and subsequent aflatoxin contamination are influenced by extrinsic factors, intrinsic nutritional factors, processing factors and microbial factors both at pre-harvest and post-harvest (Mejía-teniente, Chapa-oliver, Vazquez-cruz, Torres-pacheco, & Guevara-gonzález, 2011). Approaches to address aflatoxin contamination employ both pre-harvest and post-harvest techniques to stop the activities of toxigenic fungi (Dorner, 2004). Pre-harvest control measures include use of biocontrol agents, adoption of good agricultural practices and the development of resistant varieties. Pre-harvest prevention particularly through host plant resistance is the best and most widely explored strategy for aflatoxin management. Post-harvest control is a corrective method aimed at eliminating or inactivating aflatoxins in kernels (Mejía-teniente et al., 2011).

4.1. Pre-harvest aflatoxin prevention strategies

Prevention of Aspergillus infection before harvest is an important step in reducing aflatoxin contamination by virtue of the fact that effective post-harvest interventions depend to a large extent on successful pre-harvest infection control (Bhatnagar-Mathur, Sunkara, Bhatnagar-Panwar, Waliyar, & Sharma, 2015; Sharma et al., 2017). Pre-harvest aflatoxin management employs strategies that degrade the cell wall of toxigenic fungi, interfere with fungal growth and development, and prevent aflatoxin biosynthesis. It manipulates host-plant-fungi interaction and regulates environmental and agronomic factors that predispose groundnut pods or kernels to field Aspergillus infection (Bhatnagar-Mathur et al., 2015; Hell & Mutegi, 2011; Torres et al., 2014). Strategies such as biological control, good agricultural practices, plant breeding and biotechnology have been employed to prevent and/or reduce pre-harvest aflatoxin contamination in groundnut (Bhatnagar-Mathur et al., 2015).

4.1.1. Biological control

Several strains of A. flavus and A. parasiticus exist but not all strains produce aflatoxin. Some strains produce aflatoxin (toxigenic strains) while others do not produce aflatoxin (atoxigenic strains) (Dorner, 2009). Biological control of pre- and post-harvest aflatoxin contamination in crops has been attained by the application of competitive non-toxigenic strains of A. flavus and/ or A. parasiticus. Atoxigenic strains of A. flavus and A. parasiticus are predominantly asexual, genetically stable, competitively aggressive and are unable to recombine with native toxigenic strains of A. flavus and A. parasiticus, hence their use as biocontrol agents (Bhatnagar-Mathur et al., 2015). Atoxigenic strains competitively overcome natural toxigenic strains in the same niche and compete with toxigenic strains for space, crop substrates and nutrients required for aflatoxin production. Furthermore, inoculation with atoxigenic strains provides a carryover effect and may protect groundnuts from post-harvest aflatoxin contamination (Dorner, 2004; Torres et al., 2014). Atoxigenic strains of Aspergillus have reduced aflatoxin contamination by 70–90% in groundnut and cotton field experiments (Dorner, 2008). Afla-Guard™ brand obtained from a non-toxigenic A. flavus strain NRRL 21882 has been commercialized for biological control of toxigenic A. flavus strains in peanuts (Horn & Dorner, 2009). Atoxigenic A. flavus based afla-guard® and AF36® have been commercialized in the United States for biological control of aflatoxin contamination in peanut, maize, and cottonseed (Bhatnagar-Mathur et al., 2015; Dorner, 2009). Atoxigenic strain of A. flavus that has the ability to displace toxigenic strains in the soil by competitive exclusion has been isolated from Nigerian soils (Atehking et al., 2008; Gnolonfin et al., 2013). In Africa, atoxigenic A. flavus strains have shown reduction in aflatoxin contamination (70–99%) in both laboratory and field trials through competitive elimination of toxigenic fungi from groundnut and maize fields (Bhatnagar-Mathur et al., 2015). Dorner, Cole, and Blankenship (1998) revealed that introduction of atoxigenic strains of A. flavus and A. parasiticus to soils of developing groundnuts resulted in 74.3%–99.9% reduction in aflatoxin contamination in the United States. By applying atoxigenic strains of A. flavus and A. parasiticus in the field, 95.9% reduction of post-harvest aflatoxin contamination has been achieved (Dorner & Cole, 2002).

Although some level of aflatoxin control has been achieved with the use of atoxigenic strains, there exist a high potential for “super-competitors” to emerge through repeated applications over many

63

K. Asare Bediako et al.

Food Control 98 (2019) 61-67
4.1.2. Good agricultural practices

Another way of managing aflatoxin contamination in groundnut fields is the adoption of good agricultural practices such as maintenance of optimal plant densities, timely planting, avoidance of drought stress, adequate weed control, supply of sufficient plant nutrition, control of plant pathogens and insect pests, sound harvesting practices, crop rotation and management of crop residues (Bhatnagar-Mathur et al., 2015).

The use of insect resistant cultivars coupled with the control of pod-feeding insects by application of recommended insecticides constitute an integral part of pre-harvest aflatoxin contamination reduction (Waliyar, Kumar, Natre, Traore, & Kodio, 2007). Insects create infection wounds through their feeding habits and cause kernels to dry out to moisture content conducive for A. flavus growth and aflatoxin production (Wagacha & Mathomi, 2008). Thus, proper management of insect pests through chemical application can provide adequate kernel protection and consequently reduce aflatoxin contamination in groundnuts.

The application of cereal crop residues, lime and farmyard manure improves the water-holding capacity of the soil and as a consequence, lessens the effect of late season moisture stress, and reduce fungal invasion and aflatoxin accumulation in the groundnut seeds. A report by Waliyar et al. (2008) suggests that soil amendments such as use of cereal crop residues, lime application, and application of farm yard manure can provide effective control of A. flavus infection and reduce aflatoxin levels in groundnuts by 50–90%. The authors further indicated that the use of lime as a calcium source enhances pod filling and thickens the groundnut cell wall while the application of manure promotes growth of microorganisms that suppress soil infections. Mean aflatoxin contamination reduction of 80% compared to controls was achieved with the application of farmyard manure and gypsum at planting. The use of farmyard manure and gypsum was noted as the most effective strategy in reducing seed infection and aflatoxin contamination by toxigenic fungi (Guchi, 2015).

Generally, prolonged moisture deficit during seed formation coupled with high soil temperatures (above 22°C) promote A. flavus and A. parasiticus infection and facilitate aflatoxin production (Horn, 2005; Torres et al., 2014). Maintenance of high water activity in groundnut kernels until time of harvest is necessary to preserve the natural defense mechanism (phytoalexin production) of peanuts against growth of aflatoxicogenic fungi, even after fungal invasion (Dorner, 2008). Therefore, Torres et al. (2014) recommended end-of-season irrigation as a strategy to combat heat and drought stress. However, the practicality of late season irrigation remains a challenge in semi-arid and arid areas where water supplies are limited.

4.1.3. Plant breeding

Breeding for resistance to A. flavus and A. parasiticus infection and/or aflatoxin production plays a significant role in the management of aflatoxin contamination. It is one of the most promising long-term approaches for aflatoxin management in Africa (Gnonlonfin et al., 2013). However, the effectiveness of plant breeding in reducing aflatoxin contamination depends upon availability of genetic variability for resistance, the level and stability of available sources of resistance, and accessibility to reliable and efficient screening techniques. Aspergillus penetrates groundnut pod walls and seed coat with its hyphae and finally gains access to the nutritious coryledons of groundnut seeds. Genetic resistance is achieved at pod wall level (pod infection), seed coat level (seed invasion and colonization), and at the cotyledon level (resistance to aflatoxin production) (Bhatnagar-Mathur et al., 2015; Nigam et al., 2009). The pod shell structure acts as a physical barrier to confer resistance to pod infection while the resistance to seed invasion and colonization is attributed to presence of wax layers, density and thickness of palisade cell layers, phenolic compounds, and absence of microscopic fissures and cavities (Bhatnagar-Mathur et al., 2015; Upadhyaya, Waliyar, & Nigam, 2002).

Resistance to aflatoxin contamination in groundnut operates at three levels, resistance to in vitro seed colonization (IVSC) by aflatoxigenic fungi, resistance to pre-harvest seed infection and resistance to aflatoxin production (Liang, Luo, & Guo, 2006; Nigam et al., 2009). Lack of significant relationships among the three resistance mechanisms has been detected and attributed probably to different genes controlling the different resistances or to differential gene function in different environments (G × E interaction) (Nigam et al., 2009; Upadhyaya et al., 2002; Waliyar et al., 2016). Breeding for resistance to aflatoxin contamination faces limitations such as significant genotype x environment (G x E) interactions and the absence of correlation between A. flavus and A. parasiticus resistance under laboratory conditions and under field conditions (Kisyombe, Beute, & Payne, 1985; Upadhyaya et al., 2002). In spite of significant G × E interaction reported for resistance to natural seed infection, some groundnut accessions have shown consistently high levels of resistance against pre-harvest seed infection, IVSC, and aflatoxin production across tests and locations, which make them good candidates for use in a resistance breeding programme (Nigam et al., 2009; Waliyar et al., 2016). Several hundred breeding lines with proven performance have been identified when tested for yield and resistance to IVSC. Six breeding lines have been developed through efforts by researchers to transfer IVSC resistance to different genetic backgrounds (Mixon, 1983). Sources of resistance to both A. flavus and aflatoxin accumulation were identified and released in Uganda as commercial varieties (Okello, Monyo, Deom, Ininda, & Oloka, 2013). Several groundnut aflatoxin resistant lines have been released at different locations in West Africa following evaluation of 472 groundnut lines for pre-harvest seed infection and yield (Upadhyaya, Nigam, Mehan, Reddy, & Yellaiah, 2001). Holbrook, Wilson, and Matheron (2002) identified 14 accessions of the United States groundnut germplasm core collection with an average of 70% reduction in pre-harvest aflatoxin contamination. Further report from the authors indicates that six of the 14 accessions showed over 90% reduction in multiple years of testing.

4.1.4. Biotechnological approaches

Advances in genomics and genetic engineering technology have resulted in various modifications of the host-pathogen interaction which when combined with good agricultural and post-harvest practices could reduce aflatoxin contamination significantly (Bhatnagar-Mathur et al., 2015).

A number of candidate genes associated with resistance to Aspergillus infection and aflatoxin production have been identified using cDNA libraries and proteomics (Bhatnagar-Mathur et al., 2015; Guo et al., 2008; Wang, Zhang, Chen, Li, & Liang, 2010). A total of 21,777 expressed sequence tags (ESTs) generated from developing groundnut...
seeds have been found to be involved in defense reaction against Aspergillus infection and subsequent aflatoxin contamination. These ESTs provide useful information for identification of resistance-related genes, for gene expression profiling and for microarray design (Guo et al., 2008). In a gene expression profiling study using resistant and susceptible cultivars of groundnut infected with a mixture of A. flavus and A. parasiticus spores, up-regulation of 62 genes was observed in resistant cultivars in response to Aspergillus infection (Guo et al., 2011). Additional 22 putative Aspergillus-resistance genes were over-expressed in the resistant cultivar in comparison with the susceptible cultivar. These genes could be exploited to develop Aspergillus-resistant groundnut cultivars through marker-assisted breeding and genetic engineering. Tremendous increase in β-1,3-glucanase activity observed in the resistant lines following inoculation with A. flavus could be a defense mechanism against A. flavus infection (Liang, Holbrook, Lynch, & Guo, 2005). A study to identify resistance-related proteins in response to A. flavus infection under drought stress resulted in five proteins (Oso7g0179400, PII protein, CDK1, Oxalate oxidase and SAP domain-containing protein) expressed exclusively in the resistant cultivar. Under drought stress, six proteins (heat shock protein precursor, RIO kinase, β-ascorbate peroxidase, iso-Ara h3, 50 S ribosomal protein L22 and putative 30 S ribosomal S9) were up-regulated in the resistant cultivar inoculated with A. flavus (Wang et al., 2010). These proteins could be functionally grouped into four namely; defense response, signaling components, regulation of transcription and storage proteins. Genetic resistance of groundnut to A. flavus infection has been achieved by over-expression of antifungal plant defensins, MsDef1 and MsDef4.2 (Sharma et al., 2017). The plant defensins are activated upon attack by A. flavus to either prevent infection or cease growth of the fungus following infection. In the same study, inhibition of aflatoxin production by different A. flavus strains through host-induced silencing of aflM and aflP genes in the aflatoxin biosynthetic pathway has been reported. Where infection proceeds normally, groundnut seeds produce RNA molecules to silence genes responsible for the synthesis of aflatoxin in the fungus. Consequently, durable A. flavus resistant groundnut lines with very low aflatoxin levels have been generated. Pathogenesis-related (PR) proteins play a significant role in disease resistance by protecting host plants from fungal infection. The role of chitinase in fungal cell or sclerotial wall lysis and degradation has been reported (Bhatnagar-Mathur et al., 2015). This knowledge was applied to develop a transgenic groundnut containing a rice chitinase gene with enhanced resistance to A. flavus (Prasad, Bhatnagar-Muthur, Waliyar, & Sharma, 2013). Likewise, a transgenic groundnut engineered with a tobacco β-1,3-glucanase gene proved resistant to IVSC with no aflatoxin contamination (Sundaresha et al., 2010).

4.2. Post-harvest aflatoxin control strategies

Proper handling and processing of groundnuts after harvest can reduce fungal infection and subsequent aflatoxin production. Poor post-harvest management practices encourage growth of toxigenic fungi leading to rapid loss of quality due to aflatoxin contamination (Magan, Hope, Cairns, & Aldred, 2003). Aflatoxin contamination in West Africa has been linked to delay in pod removal after uprooting the plants and during storage. Very often in Africa, groundnut pods are heaped in the field after uprooting the plants for about four weeks for partial sun drying prior to home drying. The high relative humidity conditions experienced by most African countries during traditional heaping expose groundnut pods to rapid fungal invasion and aflatoxin production (Guchi, 2015). Mestres et al. (2004) observed that complete drying is not achieved even under dry conditions before loading grains into stores resulting in aflatoxin contamination during storage.

4.2.1. Rapid post-harvest drying and proper storage

Rapid drying has been reported to reduce the moisture level of agricultural crops including groundnuts. This practice creates unfavourable conditions for fungal growth and invasion thereby promoting safe storage over extended periods (Lanyasunya, Wamai, Musa, Olowofeso, & Lokwaleput, 2005). In groundnuts, windrow drying of harvested plants as opposed to farmers’ traditional practice of heap drying yielded significant reduction of aflatoxin contamination (Guchi, 2015). Low moisture content during transportation, storage and marketing of groundnuts can be maintained by avoiding leaking roofs and condensation arising from inadequate ventilation (Wagacha & Muthomi, 2008). Proper storage of groundnut according to Torres et al. (2014) is one maintained under clean, dry conditions with low kernel moisture content (about 8%) and at low temperature, and with protection from insect infestation to avoid moldiness and consequent risk of aflatoxin contamination. A study conducted on drying and proper storage of groundnuts in some farming communities in Guinea resulted in 50% reduction in aflatoxin exposure relative to control communities (Turner et al., 2005). The post-harvest methods tested in the intervention communities included drying on mats, complete sun drying, storage in jute bags and on wooden pallets, and the use of insecticide. Awuah and Ellis (2002) observed that groundnuts dried to a moisture content of 6.6% were devoid of fungal infection for 6 months irrespective of the storage method while at 12% moisture content; only jute bags with 3% (w/w) Syzygium aromaticum powder as a protectant effectively inhibited A. parasiticus infection. Further increase of moisture content to 18.5%, however rendered this method ineffective. For post-harvest management of aflatoxin, maximum moisture contents of 9% for unsheled groundnuts and 7% for shelled groundnuts should be maintained during storage. At these moisture contents, relative humidity of 70% and temperature of 25–27 °C ensure safe storage of groundnuts for approximately one year (Torres et al., 2014; Waliyar et al., 2008).

4.2.2. Sorting

Aflatoxin contamination of groundnut begins with only a few highly contaminated seeds irregularly distributed in the groundnut lots. Cole, Dorner, and Holbrook (1995) revealed that post-harvest screening to remove contaminated seeds presents a promising strategy to reduce or eliminate aflatoxin contamination. High levels of aflatoxin are usually found in heavily molded and or damaged kernels (Turner et al., 2005; Whitaker et al., 2006) and segregation of such seeds would reduce aflatoxin contamination in the final product. Davidson et al. (1982) further confirmed that about 80% of aflatoxin contaminated groundnuts are associated with the small and shriveled seeds. Aflatoxin levels according to Park (2002) have been reduced by 40–80% following removal of physically damaged and infected grains from the whole lot on the basis of their colour, disfigured shapes, shriveled and reduced size. Segregation is achieved through manual sorting, seed size and density separation, or electronic colour sorting. However, the most effective method which has reduced aflatoxin by 70% in commercial shelling plants is electronic colour sorting (Dorner, 2008). The efficiency of the electronic colour sorting technology has improved in recent times but since not all aflatoxin-contaminated kernels are discoloured the technology is never 100% effective in aflatoxin removal. Dorner (2008) suggested that blanching followed by photoelectric colour sorting and hand-picking offer the best method for effective reduction of aflatoxin in shelled groundnut lots.

5. Conclusion

Aspergillus flavus and Aspergillus parasiticus are widespread in nature and can under favourable conditions infect agricultural crops and contaminate them with aflatoxins. Groundnut being a subterranean crop is vulnerable to aflatoxin contamination due to its direct contact with the soil which serves as a primary reservoir for A. flavus and A. parasiticus. Following aflatoxin contamination, groundnuts are rejected on the international markets and those that enter the food chain expose consumers to serious health hazards and even death. This review has
highlighted a number of pre- and post-harvest interventions for aflatoxin management in groundnuts. It is however worth noting that no one strategy is completely effective against A. flavus and A. parasiticus infection and subsequent aflatoxin contamination. Integrated adoption of these interventions is therefore essential for sustainable reduction of aflatoxin contamination in groundnuts. This will result in the availability of wholesome groundnuts fit for human and animal consumption and acceptable for trade in high-value markets. Additionally, this paper would serve as a useful guide for researchers and organizations rolling out aflatoxin intervention programmes across the globe.

Acknowledgement

The authors gratefully acknowledge the financial support from the DAAD. We appreciate the assistance of Dr. Jacob Ulzen, Mr. Maxwell Lampety and Mr. Maurice K. Amooh in reviewing the paper.

References


