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Aflatoxin contamination in groundnut (Arachis hypogaea L.); its causes and management

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ABSTRACT

Groundnut is a very important legume that is recognized worldwide for its benefits. It is grown in over 100 countries. The nutritional benefits such as its provision of important vitamins cannot be overemphasized. However, the production, marketing and consumption of groundnut is threatened by mycotoxins with aflatoxin being the major. Aflatoxin is produced by a difficult to control, complex pathogen, Aspergillus species that contaminate groundnut with this mycotoxin both in the field and during storage especially Aspergillus flavus which is the most common in nature. The response of moisture, temperature and even soil type all influence the proliferation of this pathogen and its subsequent aflatoxin contamination. In view of the many health challenges that this mycotoxin poses to humans and animal, various control measures involving biological, use of resistant groundnut genotypes, chemical strategies and physical separation have been proposed to be a remedy to this global issue.

Keywords: Mycotoxin, Aflatoxin, Aspergillus flavus, Health hazards, Groundnut.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume also known as peanut, earthnut, monkey nut and goobers and plays a very important role in human diets as one of the world's most valued oil seed crops (Dwivedi *et al.*, 2003). Groundnut occupies a very strategic position on the list of the world's cultivated crops; placing 13th in the world as well as being the 4th most important oil seed crop (Surendranatha *et al.*, 2013). More than 100 countries worldwide produce groundnut and developing countries constitute 94% of global production of the crop (Madhusudhana, 2013).

The kernel of the crop is a high deposit of nutrients including vitamin E, niacin, thiamine, falacin, riboflavin, calcium, phosphorus, magnesium, zinc, iron and potassium as well as considerable quantities of fat (40-50%), protein (20-50%), and carbohydrate (10-20%) (USDA, 2010). The benefits of the crop cannot be overemphasized in any setting as the entire crop is useful. Aside consuming the kernels in the boiled, roasted or raw state, a good

amount of oil can be extracted from them and the remaining oilcakes are used as fertilizer (Ayele, 2010). The entirety of groundnut usage leaves no waste in the system or to the farmer as every part of the crop is beneficial.

The origin of cultivated groundnut is South America (Weiss, 2000). According to FAOSTAT (2018), the world production of the crop was 4,097,498 tons. China is the leading producer of the crop with a yearly production of 17,150, 121 tons. Nigeria however is the 4th largest producer of the commodity in the world and the first in Africa. Ghana comes as the 17th largest producer of groundnut in the world producing up to 420,000 tons yearly.

The crop is affected by various diseases, from the leaves to the seeds. Diseases such as late leaf spot (Phaeoisariopsis personata Berk and Curt), early leaf spot (Cercospora arachidicola Hori), collar rot (Aspergillus niger Tiegh), rust (Puccinia arachidis Speg), and bud necrosis (bud necrosis virus (Ephrem, 2015). Groundnut seeds are also affected by molds of the Aspergillus family with the common in nature been Aspergillus flavus, it's responsible for the production of contamination (structurally aflatoxins related toxic polyketide-derived secondary metabolites) (Waliyar et al., 2006). The producers of aflatoxins major are Aspergillus flavus and Aspergillus parasiticus (Richard and Payne, 2003; Vargra et al., 2011). Contamination of groundnut with aflatoxins can occur at every stage of the cropping season and afterwards. Contamination can occur in the field before harvest, during harvest or after harvest. agricultural Several commodities are contaminated by aflatoxin producing fungi and consuming these produce may results in health threats to both human and livestocks (Kumar et al., 2017; Sarma et al., 2017; Ezikiel et al., 2019). Moreover, the contamination of crops especially groundnut by aflatoxins in developing countries leads to serious economic losses. According to Shephard (2003), aflatoxin contamination of stable crops like maize and groundnut poses major health challenges in Africa. Rural people who may not have other food options are forced to ingest aflatoxin contaminated crops daily. This moderate, chronic intake of aflatoxin through contaminated food can result in severe pathological conditions, such as liver cancer, immune system deficiency and impaired development of children (Wild *et al.*, 1992; Wild *et al.*, 1993; Gong *et al.*, 2004; Williams *et al.*, 2004).

Aflatoxin and its origin

Aflatoxin is a secondary metabolite of the fungus Aspergillus species and is a group of highly toxic, cancer-causing mycotoxins produced by several members of the fungal genus, Aspergillus (IARC, 2012). Aflatoxins carcinogenic, teratogenic are and immunosuppressive secondary metabolites produced by several Aspergillus species (Frisvad et al., 2019). A. flavus is the most common aflatoxin producing spp. in nature (Amaike and Keller, 2011) but depending on locations/time, A. parasiticus, A. nomius, and other species may be significant contributing aflatoxin agents to contamination (Probst et al.. 2014: Kachapulula et al., 2017; Kumar et al., 2017). The name aflatoxin was derived from a toxin producing fungus which caused a disease referred to as "Turkey X disease" around 1960 in England (Forgaes, 1962). The disease resulted in the death of 100,000 young turkeys. The fungus was later identified as Aspergillus flavus in 1961 by Blout and the toxin produced was named aflatoxin due to origin (letter "A" for the genus Aspergillus, the next set of three letters, "FLA", for the species flavus, and the noun Toxin, means poison). The fungus Aspergillus sp., belongs to the class Deuteromycetes (Fungi Imperfecti; sub-

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class Hyphomycetes); their teleomorphs can be found in the Ascomycetes (Akrobortu, 2018). The fungi adapts easily on food commodities as good substrate for growth because of the large number of enzymes it secretes for substrate digestion during its development (Hell, 1997). Pelczar et al. (1993) indicated that the Ascomycete (ascospores) produce sexual spores differentiated endogenously in a well the ascocarp while Deuteromycetes reproduce vegetatively by conidia. Hell (1997) stated that aflatoxins are only produced by two related species: A. flavus and A. parasiticus, with the latter species producing specifically the G-type of aflatoxin. However, recent studies indicate that nine different species of Aspergillus and two different Emericella species produce aflatoxin (Frisvad et al., 2006).

Based on chromatographic and fluorescence characteristics, all aflatoxins known to date can be classified into 18 different types. The

major ones are aflatoxin B1, B2, G1 and G2 (Figure 1), as well as M1and M2 (Lerda, 2010). Other aflatoxins have less commonly been found in nature since they are metabolic derivatives mostly found in pure cultures (Franco et al., 1998). Among these compounds, B1 is normally predominant in concentrations in cultures as well as in food products (Oliviera and Germano, 1997). Aflatoxin M1 and M2 are hydroxylated forms of B1 and B2 (Dors et al., 2011). When B1 in contaminated feed or foodstuffs is ingested by domestic animals, such as dairy cows, the toxin undergoes liver biotransformation and is converted into aflatoxin M1. Aflatoxin M1 then becomes the hydroxylated form of B1. Aflatoxin M1 is subsequently excreted in milk, tissues and biological fluids of these animals (Oatley et al., 2000; Peltonem et al., 2001). Aflatoxins are carcinogens and genotoxins that directly influence the structure of DNA (Williams et al., 2004).



FIGURE 1. The major groups of aflatoxins



Economic importance of aflatoxin in groundnut

A. flavus and A. parasiticus are the two main fungi responsible for the production of aflatoxins. Aflatoxins are extremely toxic substances that can cause severe health challenges in both humans and animals. This toxic substance has been found to be the cause of different types of cancers particularly the liver cancer and hepatitis B and C. In Africa and some part of the world, aflatoxin is believed to be involved in malnutrition (protein insufficiency) in children (Turner, 2013; Ephrem, 2015; Benkerroum, 2020). Moreover, it is reported that children with kwashiorkor (childhood malnutrition from protein insufficiency) were detected with high levels of aflatoxin in the Sudan, South Africa and Nigeria. Ninety-three percent (93%) of children between 6-9 years old were tested and found to be positive for aflatoxin albumin adducts in Gambia (Turner, 2013; Ephrem, 2015). Aflatoxins are pervasive in warm and humid regions which makes most of the commodities from these areas especially groundnut contaminated with aflatoxin (Dohlman, 2003). Throughout the world, aflatoxin contamination has caused diverse health challenges and economic risks. Aflatoxin contamination of crops leads in economic losses to farmers and to countries due to rejection of the produces (Bryden, 2012). India being the second largest producer of groundnuts has low export of 800,000 tons per year due to aflatoxin contamination (Suneja, 2019). It is reported that. Africa loses more than \$750 million annually due to aflatoxin contamination of agricultural crops and commodities (Pandey et al., 2019). Interestingly, the greatest worldwide of groundnut proportion production is from developing countries amounting up to 95%. Meanwhile they are unable to meet the standards of the international market to sell the commodity due to aflatoxin contamination (Ephrem, 2015).

Awareness of aflatoxins

and Khlangwiset (2010) viewed Wu awareness and education among farmers, government functionaries, and the public as crucial to allocating finances to fight aflatoxin contamination of food. Awareness the health effects aflatoxinof of contaminated food is low among the public in Ghana. It is reported that aflatoxin awareness is low among producers, buyers well-educated consumers and even (Florkowski and Kollavalli. 2013) According to Agbetiameh et al. (2018), majority of Ghanaians were not aware of the aflatoxins and its harmful health impact. Smallholder farmers manually sort discoloured kernels or infected kernels, then sell the clean kernels at higher price and often consumed the sorted kernel due to lack of knowledge of the health effects of aflatoxins (Matacic, 2016). In some part of West Africa such as Nigeria, education is very important to consumer awareness of aflatoxin contamination (Ezikiel et al., 2018). It was reported that 85% of consumers lack awareness of aflatoxin contamination in the snacks and possible health risks associated with it ingestion. Findings revealed that socio-economic characteristics such as education level, participation in social and extension services. market orientation, economic motivation and general perception has effect farmers knowledge of aflatoxin on contamination. Farmer do not consider management of mycotoxin as a challenge in agriculture because there is no price incentives for uncontaminated products (Udomkun et al., 2017). According to Jolly

et al. (2009), "Awareness of Aflatoxin contamination of groundnuts had a positive effect on Ghanaian professionals' abilities to take action to reduce its level of contamination." Therefore there is need for continual education of the general public on the effects of aflatoxin contamination on the health especially children.

Status of aflatoxin contamination of groundnut in Africa

comparison among Α some African countries on aflatoxin levels in groundnut and groundnut products showed varying results which means that aflatoxin contamination in groundnut is a canker. Some Africa countries have regulatory measures for management of aflatoxin contamination. Maximum risk limit of aflatoxin contamination for Ghana is 20 ppb (Torres et al., 2014), Kenya is 10 µg/kg (Ncube and Maphosa, 2020), Zimbabwe is 5 ppm (Nziramasanga, 2014), South Africa is 10 ppb (Njoroge, 2018), and Malawi is 5 ppb (FAO, 2004). Aflatoxin levels in groundnut in Mali between 2009 and 2010 were found to be more than 10 ppb in 35-61% of samples from farmers' fields and 39-91% samples from farmer's stores. Peanut paste in Mali had high aflatoxin level of more than 300 ppb. In Nigeria, aflatoxin levels in groundnut oil had 500 µg/g (Alakonya and Monda, 2013). In Ghana, aflatoxin levels in groundnut were reported to be 216 µg/g while groundnut paste had 3,278 μ g/g and groundnut sauce 943 μ g/g (Alakonya and Monda, 2013). According to Bediako et al. (2019), findings shown that aflatoxin contamination in Ghana exceeded the acceptable limit of 20 ppb set by Ghana Standard Authority. Moreover, a study in Kenya also indicated that, 37% of groundnuts and its products did not meet the total aflatoxin limit set by Kenya Bureau Standard of 10 µg/kg (Ncube and Maphosa, 2020). Also, because of aflatoxin contamination, groundnuts from Malawi cannot access more limiting markets such as those in the EU (Njoroge, 2018). These show that immediate strategies must be developed to curb this challenge in Africa.

Pre-harvest factors influencing aflatoxin contamination of groundnut

Climate change and weather conditions

Climate change is unfriendly to agriculture production which threatening food and feed security in many parts the world, including Sub-Saharan Africa (Wheeler and Von Braun. 2013). Occurrences of severe aflatoxin contamination both in regions prone to aflatoxin contamination and regions that as of now are relatively unaffected are expected to increase as a result of hotter, dryer conditions influence by climate change (Magan et al., 2011; Battilani et al., 2016). Moreover, drought stress during plant growth development is among the most important weather conditions that gives favourable condition for the growth of Aspergillus spp. (Torres et al., 2014). Finding by Njoroge (2018) indicated that, at preharvest, aflatoxin contamination occurs when toxigenic fungi infects the developing pods or seeds by drought and high temperature. It was also reported that, on the field groundnuts are susceptible to aflatoxin contamination when the plant is stressed by high temperature and drought (Jordan et al., 2018).

Soil type

The type of soil in which groundnut seeds are grown is able to influence the proliferation of *A. flavus* and the subsequent aflatoxin contamination of groundnut in that area. Light sandy soil promotes the rapid spread of *A. flavus*, especially under dry conditions in the later growth and development period. Moreover, reduction in the level of aflatoxin contamination in groundnut seeds can occur in heavier soil because it have a higher water-holding capacity (CAC, 2004; Torres *et al.*, 2014).

Maturity

Aflatoxin contamination of groundnut is prominent in smaller and immature seeds that matured seeds. Several studies have proven that both *A. flavus* invasion and aflatoxin contamination are higher in smaller, more immature kernels than in mature kernels (Sanders *et al.*, 1981; Cole *et al.*, 1982; Hill *et al.*, 1983; Cole *et al.*, 1985; Sanders *et al.*, 1985). Mature groundnut seeds possess a certain level of sustained resistance to aflatoxin contamination (Dorner *et al.*, 1989).

Factors during harvesting

Late and mechanical harvesting of groundnut can cause aflatoxin contamination (Heathcote and Hibbert, 1978; Torres et al., 2014). The time of harvest however depends on the maturity of the crop and the weather prevailing conditions during harvesting. Over mature or immature pods at harvest can all lead to high contamination of aflatoxins in the final products. Harvesting in very humid weather conditions lead to high aflatoxin contaminations than in the dry weather (Kabak et al., 2006). The damage groundnut caused seeds during to mechanical harvesting makes them prone to infection by A. flavus and subsequent aflatoxin contamination (Heathcote and Hibbert, 1978).

Post-harvest factors influencing aflatoxin contamination of groundnut

The moisture level of groundnut seeds after harvest influences their contamination with aflatoxins. Kernel moisture contents of 10% or higher after harvest is likely to be more prone to aflatoxin contamination. Drying at the appropriate time and keeping the moisture content at the required level can be effective in controlling aflatoxin contamination of groundnut after harvest (Torres *et al.*, 2014). The practice of sorting seeds before storage is effective in reducing aflatoxin in groundnut (Cole *et al.*, 1995; Dorner, 2008). However, it is also important to check the temperature and moisture in the storage area to prevent contamination during storage (Dickens, 1977; CAC, 2004).

Management of aflatoxin in groundnut

Aflatoxin infection by Aspergillus sp. increases basically with a subsequent increase in temperature, humidity, insect damage and nitrogen deficiency. The effects of temperature and humidity on aflatoxin infection are therefore important in aflatoxin management. Aflatoxin contamination can be higher under stress condition such as drought (CAC, 2005). Therefore, early planting and also use of drought resistance groundnut varieties is recommended for management of aflatoxin contamination. Prevention or management procedures of aflatoxin contamination should be pointed to both the process of contamination and the fungi causing contamination. The process of contamination can be divided into two stages based on crop maturity (Cotty et al., 2001). The first stage occurs during the development of the crop and this stage is usually associated with physical damage to the crop typically by either physiologic stress or insect activity (Russell, 1982; Cotty al.. 2001). Crop mechanisms et contaminated during the first phase often fluoresce a bright green-yellow as a result of kojic acid production in crop tissue by the aflatoxin-producing fungi (Zeringue et al., 1999). After maturity, the crops remain vulnerable to contamination, providing a space during which a second phase of contamination may occur (Cotty et al., 2001; Bock and Cotty, 1999). Proper management of aflatoxin contamination in agricultural crops especially groundnut starts in the farmer's field and continues through harvest, drying, storage and processing (Vincelli and

Parker, 2002). The various strategies that have been designed to manage aflatoxins should adhere to the following: a) aflatoxin must be transformed to non-toxic products, b) fungal spores and mycelia should be destroyed to prevent formation of new toxins, c) the food or feed material should retain its nutritive value and palatability, d) the physical properties of raw material should not change significantly and it must be cost efficient (Rustom, 1997; Awad *et al.*, 2010).

Pre- harvest management strategies

The adoption of good agronomical and cultural practices (selection of appropriate planting date, and seed dressing with systemic fungicides), can minimize aflatoxin contamination in groundnuts (Njoroge, 2018; Jordan et al., 2018). Use of resistant cultivar is essential and recommended if aflatoxin contamination must be reduced (Waliyar et al., 2013). Use of non aflatoxigenic strains as biological control can reduced aflatoxin levels (Bandyopadhyay and Cotty, 2013). Planting at the onset of the rains can also reduce aflatoxin contamination (Chalwe et al., 2016). Moreover, during preharvest stages of the crop, aflatoxin contamination can be reduced by adequate soil water management during pods development in groundnuts (Chalwe *et al.*, 2016). Therefore, to minimizing pre-harvest aflatoxin contamination in groundnuts, there should be adequate soil moisture and planting on set of rains.

Biological control

The biological strategy used in controlling the aflatoxin producing fungi involves the application of atoxigenic *A. flavus* strains to groundnut fields (Bandyopadhyay and Cotty, 2013). Dorner (2009) reported that the application of the nontoxigenic strains significantly reduce aflatoxin contamination. The use of biocontrol agents as seed dressing and soil application aided in the control of the population of *A. flavus* in the geocarposphere and subsequently on preharvest kernel infection of groundnut (Anjaiah *et al.*, 2006).

Use of resistant groundnut genotypes

Aflatoxin reduction in groundnut can be achieved through planting A. flavus-resistant genotypes. Aflatoxin reduction genetic manipulation is an old strategy that has been attempted in several groundnut-producing countries since the late 1960s. Breeding resistant cultivars can be achieved when sources of stability and high-level groundnut resistance is readily available. Research scientists, especially plant breeders have identified groundnut germplasm resistant to pre-harvest and post-harvest aflatoxin contamination. Studies have shown the use of RNAi to suppress A. flavus growth and aflatoxin production in groundnut (Pandey et al., 2019). Also, some aflatoxin resistant genotypes have been identified for various resistance mechanism. Examples of resistant genotypes include 12CS-104 and 73-33 (Dieme et al. 2018), ICG 12625 and ICG 4750 (Jiang et al., 2010); ICGV 98305, ICGV 98348, ICGV 98353 and Tifton 8 (Girdthai et al., 2010); Igola, Serenut 1, Serenut 2 and entry 99527 (Olwari et al., 2013); ICG 1471, NC3033, ICGV 88145 and GT-C20 (Korani et al., 2018). However, availability of resistant varieties will be the best solution for farmers to minimize aflatoxins contamination.

Post-harvest management strategies

Storage

In Africa, smallholder farmers conventionally store groundnuts in containers that are usually made of wood, bamboo, thatch, or mud placed on raised platforms and covered with thatch or metal roofing sheets (Hell and Mutegi, 2011). More improved storage containers such as polypropylene bags are now being used, but because these are not airtight, groundnut pods are still susceptible to fungal and aflatoxin contamination (Hell et al., 2000; Udoh et al., 2000). In bag storage, a major precaution is making sure that the bags are clean when reusing them. Reused bags have been identified to be associated with spores of A. flavus which makes it important to clean them before reuse (Hell et al., 2000). Bulaong and Dharmaputra (2002) reported that grain moisture content, mould growth, aflatoxins, and free fatty acid content were significantly higher in groundnut pods which were stored in jute bags than in those stored in polyethylene-doubled jute bags. The use of hermetic triple-layer bags (PICS, Purdue Improved Crop Storage) for grain and kennel storage has proved effective against aflatoxin contamination over traditional storage devices (Murdock et al., 2003; Hell et al., 2010). Studies on hermetic packaging have showed that hermetic packages and containers are capable of protecting groundnuts from moulds and aflatoxin contamination (Paramawati et al., 2006). Apart from the storage containers, the condition of the kernels and the prevailing conditions in the storage structure are important factors that can influence aflatoxin contamination. Kernel moisture of 7.5%, temperature of 10 °C, and relative humidity of 65% are optimal bulk storage conditions for groundnut, allowing storage of up to one year (Pattee and Young, 1982). Groundnut seeds with moisture content of 10% and above should be avoided to prevent mouldy growth (Diener and Davis, 1977). Reports by Awuah and Ellis (2002) shows that groundnuts dried to 6.6% moisture levels are free of fungi for six months notwithstanding the storage protectant used. These safe moisture levels are applicable to both unshelled and shelled groundnuts. The maximum moisture content for storage of

unshelled groundnuts is 9%, higher than that for shelled groundnuts (7%). At these moisture levels, if the relative humidity is maintained at 70% and temperature at 25-27 °C, groundnuts can be stored for one year (Odogola, 1994). Community-based intervention studies in Guinea, West Africa, showed that proper drying of groundnut seeds and proper storage conditions is one sure way to combat aflatoxin contamination of this crop (Turner et al., 2005). Maintaining low moisture levels during storage, transportation, and sales is very important as well. This can be achieved by avoiding other moisture sources such as leaking roofs and condensation arising from inadequate ventilation (Wagacha and Muthomi, 2008). Biological activity during storage should be minimized to preserve grain quality by adequate drying to <10%moisture, elimination of insect activity, low temperature, and inert atmosphere (Turner et al., 2005; Lanyasunya et al., 2005). Storage of dry pods in airy, dry, and clean rooms reduces aflatoxin accumulation (Rahmianna et al., 2007). Sanitation measures such as cleaning storage structures prior to bringing in new produce can lead to a reduction in aflatoxin levels (Hell et al., 2000).

Chemical control of aflatoxins

The use of ammonia at 0.5-7% coupled with long exposure time, ambient temperature and pressure has proved effective in successfully deactivating aflatoxin in contaminated commodities, such as groundnut meal, cottonseed and maize. This chemical control method has been tested and endorsed by leading safety and regulatory agencies, such as Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) (Ephrem, 2015). The use of antifungal materials from natural sources and chemical preservatives is a solid practice to prevent post-harvest aflatoxin contamination in groundnuts (Onyeagba *et al.*, 2004; Haciseferogullary *et al.*, 2005). Although effective, the application of eugenol which is the active ingredient of these oils is expensive. However, methyleugenol (4allyl-1,2-dimethoxybenzene) can be a costeffective derivative of eugenol and can be applied for post-harvest protection of groundnut pods and kernels from *A. flavus* and aflatoxin contamination when sprayed at 0.5% concentration (Sudhakar *et al.*, 2009).

Physical separation

Harvesting groundnuts with hoes leads to a significant damage to shells and kernels, thus predisposing them to fungal infection during storage (Kaaya and Warren, 2005). About 80% of aflatoxin contamination of groundnut results from small, shrivelled seeds (Davidson et al., 1982), mouldy and stained seeds (Fandohan et al., 2008), and damaged seeds (Hamid, 1997). Therefore, it is an essential practice to sort kernels and to remove discoloured or damaged/shrivelled pods to reduce aflatoxin levels (Awuah and Kpodo, 1996; Park, 2002; Afolabi et al., 2006). Groundnuts of low quality have considerably higher aflatoxin levels than groundnuts of high quality (Mutegi et al., 2009). Sorting can be done by physical characteristics (colour, size, density) and by

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near-infrared reflectance (DeMello and Scussel, 2009). Floating and density segregation also reduces aflatoxins in storage units; kernels that float in tap water contain up to 95% aflatoxins (Kirskey et al., 1989; Phillips et al., 1994). Electronic sorting and hand-picking methods are also adopted to minimize aflatoxin levels significantly in shelled groundnuts (Dickens and Whitaker, 1975). Technological advancement (infrared and UV sorting coupled with colour-detection technology) are now available to enable inspection of aflatoxin-contaminated products on a large scale (Womack et al., 2014).

CONCLUSION

of aflatoxin The increasing rate contamination has become a worldwide challenge that requires immediate action. The many diseases and cankers this toxigenic mycotoxin have resulted in is alarming. Reducing aflatoxin contamination agricultural commodities especially in groundnut means bringing the population of the aflatoxin producing fungi under proper control. Finally, the involvement of all major research institutions and groundnut producers will aid in combating this mycotoxin.

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