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Review

Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination

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ABSTRACT

Peanut or groundnut (*Arachis hypogaea* L.) is cultivated in the tropical and warm temperate regions of the world. Its production reaches approximately 39.9 million metric tons per year. The major producers/exporters of peanuts are the United States, Argentina, Sudan, Senegal, and Brazil. One of the major problems in peanut production worldwide is the contamination with *Aspergillus* section *Flavi* and aflatoxins, being these mycotoxins of great concern due to their toxicological effects to human and animals. Different strategies both at pre-harvest and post-harvest stages have been applied to reduce the entry of aflatoxins to the food and feed chains. Nowadays, no single strategy is enough to solve this problem. An integrate management from the field until food or feed processing is necessary to reduce the impact of aflatoxins. This review summarizes the advance in reducing the impact of aflatoxins in different countries where peanuts are cultivated.

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1. Introduction

The cultivated peanut or groundnut (*Arachis hypogaea* L.), originated in South America, is now grown throughout the tropical and warm temperate regions of the world. World peanut production totals approximately 39.9 million metric tons per year, China being the world's largest producer, followed by India and the United States (USDA, 2013). The major exporters of peanuts are Argentina, the United States, Sudan, Senegal, and Brazil. These five countries account for 71% of total world exports. Countries such as India, Vietnam and several African countries periodically enter the world market depending upon their crop quality and world market demand. The major peanut importers are the European Union, Canada and Japan. These three areas account for 78% of the world's imports (Cámara Argentina del Maní, 2013).

All parts of the peanut plant can be used. The peanut, grown primarily for human consumption, has several uses as whole seeds or processed seeds to produce primarily peanut butter and oil; in fact, the seed contains 25 to 32% protein (average of 25% digestible protein) and 42 to 52% oil (Woodroof, 1983).

One of the major problems in peanut production worldwide is aflatoxin (AFs) contamination, which is of great concern as these toxins have toxicological effects, which are dose-dependent; at high doses they are lethal if consumed, causing liver, myocardial and kidney tissue damage. Aflatoxins cause chronic toxicity, e.g. liver cirrhosis, and they are potent human hepatocellular carcinogens at sub-lethal or at low-level exposure doses, respectively (Wild & Turner, 2002). The International Agency for Research on Cancer (IARC) has evaluated AFB1 as a Group 1 carcinogen producing liver cancer in humans (IARC, 1993).

Aflatoxins are produced by several species in *Aspergillus* section *Flavi* (Varga, Frisvad, & Samson, 2011). However, as was reported by Richard and Payne (2003), only two of these species, *Aspergillus flavus* and *Aspergillus parasiticus*, are important in the colonization and contamination of agricultural crops, *A. flavus* being the major producer of aflatoxin. The fungus is isolated from a wide range of climate zones, but is more frequently found between latitudes 16° and 35° in warm climate zones and is not common above 45° latitudes (Klich, 2007). Although *A. flavus* appears to be the dominant species of the section invading peanut seeds, *A. parasiticus* is more frequently found in peanuts than in corn and cottonseed (Asis, Barrionuevo, Giorda, Nores, & Aldao, 2005; Barros, Chiotta, Torres, & Chulze, 2006; Barros, Torres, Palacio, & Chulze, 2003; Horn, 2005; Horn & Dorner, 1998) and can also contribute to aflatoxin contamination in a varying degree (Horn, Dorner, Greene, Blankenship, & Cole, 1994).

Because of human health concerns, many countries have set maximum levels of aflatoxin allowed in food and feed (van Egmond, Schothorst, & Jonker, 2007). The maximum tolerable levels for aflatoxin B_1 in food have a range from 1 to 20 μg/kg, and 2 μg/kg is a limit in force in at least 29 countries, most of these countries belong to the EU (EC, 2006, 2010). Another major limit is 5 μg/kg, followed by 21 countries, spread over Africa, Asia/Oceania, Latin America and Europe. The USA Food and Drug Administration permits maximum aflatoxin levels of 20 ppb in peanut products destined for human consumption; the European Union allows 4 ppb of total aflatoxin and 2 ppb on aflatoxin B_1. The EU regulation standards on aflatoxins have a strong potential impact on nations attempting to export foods that are susceptible to aflatoxin contamination into the EU (Wu, 2008). In a study carried out in 2004, Wu estimated a $ 450 million annual loss, mainly charged to the US, China, Argentina, and sub-Saharan African peanut markets, if the EU aflatoxin standard were adopted worldwide (Wu, 2004).

The relevance of safe peanut production, with low aflatoxin content, is therefore mandatory for all producer countries and prevention of seed contamination during crop production is the suitable approach. Therefore, the aim of this review is to present recent advances in methodologies to prevent aflatoxin contamination in peanuts, recommended by different guidelines and code of practices, focusing mainly on pre-harvest strategies.

2. *Aspergillus* section *Flavi* in peanuts

Fungal growth and aflatoxin contamination are the consequences of interactions among the host, the fungus and the environment. The appropriate combination of these factors determines the infection and colonization of the substrate, and the type and amount of aflatoxin produced.

2.1. Crop phenology

Peanut development has been described by Boote (1982) relative to visually identifiable stages, shared between vegetative (V) and reproductive (R), each further subdivided into distinct stages. The V stage is determined by counting the number of developed nodes on the main stem, beginning with the cotyledonary node as zero. The R stages are R1 (beginning bloom), R2 (beginning peg), R3 (beginning pod), R4 (full pod), R5 (beginning seed), R6 (full seed), R7 (beginning maturity), and R8 (harvest maturity). An alternative description is the BBCH scale (edited by the Federal Biological Research Centre for Agriculture and Forestry). The duration of different stage is depending on variety, seasonal conditions and location (Fig. 1).

After aerial fertilization of the peanut flower and gynophore prolongation into the soil, peanuts grow underground. Generally, it is accepted that the peanut plant begins its reproductive stage with the onset of blooms (R1), about 30–45 days after planting. From about 60 days after planting, pods are formed and filled (R3 to R6). Pod weight can increase at a rate of up to 100 kg per hectare per day for the 75–150 days after emergence. Harvest maturity (R8) is reached at 110–170 days (16–24 weeks), and it is also depending on variety, planting time, seasonal conditions and location. The main factor affecting the time to harvest is temperature.

2.2. Infection cycle of Aspergillus section Flavi on peanuts

Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus*, and peanut pods are in direct contact with soil populations of aflatoxigenic fungi (Horn & Pitt, 1997). The disease cycle and epidemiology of *A. flavus* were recently reviewed by Amaike and Keller (2011). *A. flavus* lives in soil as conidia or sclerotia and in plant tissues as mycelia. Sclerotia survive in the soil under severe environmental conditions and produce conidia and possibly ascospores (Horn, Moore, & Carbone, 2009), leading to a population increase under hot and drought weather conditions (Payne, 1998; Wicklow, Wilson, & Nelsen, 1993). Sclerotia germinate as mycelium, which then forms conidiophores and conidia.

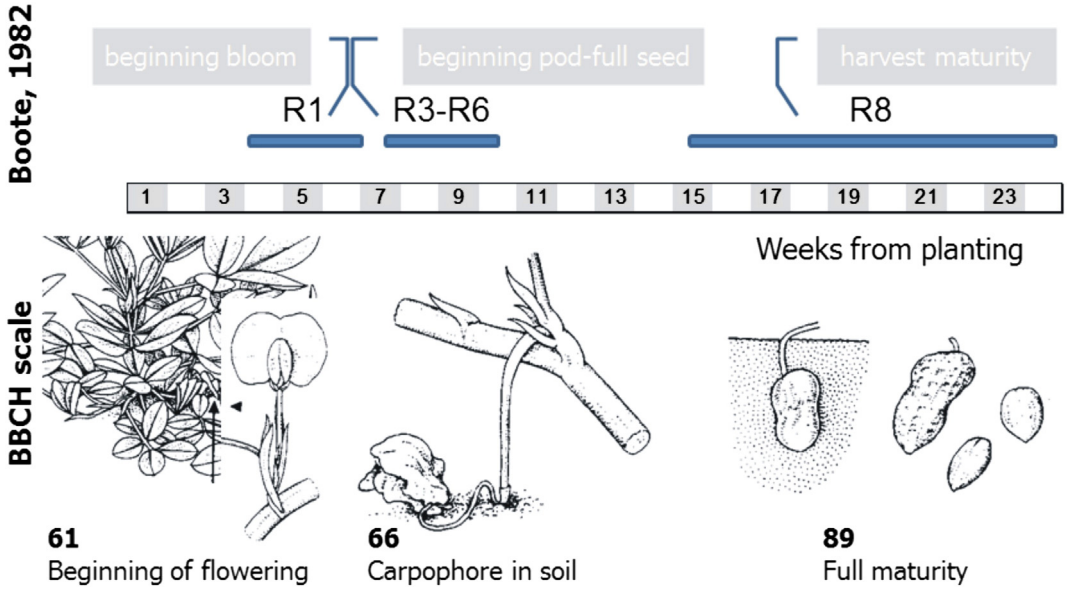

Fig. 1. Peanut crop phenology. Boote (1982) and BBCH scale (edited by the Federal Biological Research Centre for Agriculture and Forestry).

2.3. The environment

Weather conditions during the grain-filling period can significantly affect both crop yield and crop quality due to aflatoxin contamination. Under conditions of adequate rainfall or irrigation, the toxin usually does not occur. However, in the main peanut-growing areas in the world peanut crop is produced under less than ideal conditions (Pitt, Taniwaki, & Cole, 2013). Sanders, Cole, Blankenship, and Dorner (1993) reported aflatoxin contamination in peanut when pods were exposed to drought stress although roots of the crop were well supplied with moisture. Craufurd, Prasad, Waliyar, and Taheri (2006) confirmed that infection and aflatoxin concentration in peanuts can be related to the occurrence of soil moisture stress during pod filling (45 to 70 days after harvest) when soil temperatures are near optimal for *A. flavus*. Pre-harvest aflatoxin contamination of peanuts can also occur after peanuts are dug if they are not quickly harvested, dried and maintained at safe moisture level (Cole, Dorner, & Holbrook, 1995). One of the problems is that the dates for beginning and ending of a reproductive phase are often unknown and are likely to shift each year, depending on the sowing dates and weather conditions (Kumar, 1998).

The main factor influencing *A. flavus* and *A. parasiticus* infection in peanuts is insect damage to the developing seed and plant stress due to drought and high soil temperatures before harvest. It is known that developing peanuts can be infected by different ways, including through flowers or systemically, but the main infection takes place directly from the soil surrounding the pod. The drought stress acts by reducing the plant's natural defenses against infection; by reducing the water activity in the soil, which reduces growth and activity of bacteria, amoebae and competing fungi; and by promoting growth of *A. flavus* and *A. parasiticus*, which are xerophiles (Pitt et al., 2013).

3. Prevention strategies of aflatoxins in peanut

Aflatoxin contamination may occur in the field before harvest, during harvesting, or during storage and processing, thus methods for the prevention of contamination can be divided into pre-harvest, harvesting and post-harvest strategies. Whereas certain treatments have been found to reduce aflatoxin formation in peanuts, the complete elimination of aflatoxin is currently not realistically achievable. Current management practices that reduce the incidence of aflatoxin contamination in the field include timely planting, maintaining optimal plant densities, proper plant nutrition, avoiding drought stress, controlling plant pathogens other than Aspergilli, weeds and insect pests and proper harvesting (Bruns, 2003) and, in post-harvest drying, storage and processing. Code of practice has been developed by Codex Alimentarius for the prevention and reduction of aflatoxin in peanut (CAC, 2004). The recommendations for the reduction of aflatoxins in peanuts are divided into two parts: recommended practices based on Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP); a complementary management system to consider in the future is the use of Hazard Analysis Critical Control Point (HACCP) (Kabak, Dobson, & Var, 2006). HACCP system involves a science-based analysis of potential hazards involved in the production of foods, determination of where the hazards can occur in processing techniques, institution of preventives measures, and corrective actions if they do occur. This system is designed to critically evaluate the effectiveness of controls at each major step involved in processing food. The implementation of HACCP principles to minimize aflatoxins contamination has been successfully applied in Southern Africa in commercially produced peanut butter (FAO, 2003).

3.1. Pre-harvest cropping system

The pre-harvest control of aflatoxin contamination of peanuts must take into consideration all the varied environmental and agronomic factors that influence pod and seed infection by the aflatoxin-producing fungi, and aflatoxin production. These factors can fluctuate considerably from one location to another, and between seasons in the same location. However, using proper agricultural practices, including crop rotation, tillage, planting date, and management of irrigation and fertilization, should reduce aflatoxin contamination in peanuts.

3.1.1. Genetic resistance

Adopting some cultural practices, curing and drying and storage practices can minimize aflatoxin contamination. But these practices may not be suited to small-scale farming in developing countries, especially in tropical areas (Liang, Luo, & Guo, 2006). An important, safe and preventative strategy for aflatoxin minimization is the development of host-plant resistance in order to inhibit the fungal colonization and toxin production. This strategy includes: (1) prevention of fungal infection, which is especially important under stressed environmental

conditions; (2) prevention of subsequent growth of the fungus once infection has occurred; (3) inhibition of aflatoxin production following infection and (4) degradation of aflatoxins by the plant or fungus. Development of aflatoxin-resistant varieties is thus a complex process that may include direct selection for resistance to fungus and aflatoxin accumulation, indirect selection for resistance or tolerance to biotic or abiotic stresses or selection for morphological traits that impede or delay fungal introduction or growth (Mahuku, Warburton, Makumbi, & San Vicente, 2013).

Mitigation of aflatoxin contamination through breeding has been attempted in several peanut-producing countries since the late 1960s. In different regions in the world, as sub-Saharan Africa the conditions are highly conducive to *A. flavus* infection and toxin production and prevention in such conditions is a complex task. In this region the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and its partners have integrated a research program to mitigate aflatoxin contamination, the different approaches were described by Waliyar, Kumar, Ntare, Diarra, and Kodio (2008).

The development of cultivars which resist preharvest aflatoxin contamination has been limited by the lack of genes for resistance. Several attempts to develop aflatoxin resistant varieties have been carried out leading to the development of elite resistant varieties, which were eventually released as improved germplasm in some countries (Upadhyaya, Bramel, Ortiz, & Singh, 2003). However, resistance in peanuts to aflatoxin contamination under all conditions has still not been achieved and breeding efforts continue.

Recently, with the objective of understanding the molecular basis of host resistance to aflatoxin contamination, a large-scale project was carried out developing expressed sequence tags (ESTs) from peanut seeds to identify resistance-related genes involved in defense response against *A. parasiticus* infection and subsequent aflatoxin contamination (Guo et al., 2008). Based on these and other EST sequences, Guo et al. (2011) developed a peanut microarray to identify candidate genes that confer resistance to *A. flavus* infection due to up-expression in response to fungal infection using a resistant peanut line vs. a susceptible line. This interesting work was a first step towards a comprehensive genome-scale platform for developing *Aspergillus*-resistant peanut cultivars through targeted marker-assisted breeding and genetic engineering.

Quantitative trait loci (QTL) for both fungal and aflatoxin accumulation resistance have been mapped, and the transfer of these QTL into elite germplasm is underway. Final confirmation of genomic regions providing improved resistance using near isogenic lines is nearing completion for several QTL and gene sequences at International Maize and Wheat Improvement Center (CIMMYT) and the US Department of Agriculture's Agriculture Research Service (USDA-ARS). Finally, new techniques involving RNA interference (RNAi) gene silencing may allow transgenic plant to resist infection by *A. flavus*, using DNA sequences from the fungus itself to allow recognition and prevent growth of the fungus in the plant (Brown, Bhatnagar, Cleveland, Chen, & Menkir, 2013).

3.1.2. Crop rotation

The continued cultivation of peanuts on the same land may contribute to a built-up of high *A. flavus*/*A. parasiticus* population in the soil, with the consequent increase of infection and aflatoxin contamination (Ortiz et al., 2011). There are some studies that demonstrated the effect of crop rotation on aflatoxin contamination, but it depends on the environment, for example in semi-arid environment populations of *Aspergillus* may be very high, and crop rotations may have little influence on the fungal activity (CAC, 2004).

3.1.3. Soil type

There is evidence that peanuts grown in different soil types may have significantly different levels of mold infection. Light sandy soil, for example, favors the rapid proliferation of fungi, particularly under dry conditions. Heavier soils have a higher water-holding capacity and, therefore, there is less likelihood of drought stress occurring, which may be partly responsible for the lower than average level of aflatoxin contamination in peanuts grown on such soils. It is important to note that soil tests, to determine if there is a need to apply fertilizer and/or soil conditioners to avoid plant stress, especially during seed development, should ideally be conducted, prior to application (CAC, 2004).

3.1.4. Water stress

While many factors are known to influence the production of mycotoxins in the field, of these, drought stress during plant growth is among the most important. In general, prolonged moisture deficit during the seed filling period and elevated soil temperatures (>22 °C) enhances aflatoxin production (Cole, Hill, Blankenship, Sanders, & Garren, 1982; Cole, Sanders, Dorner, & Blankenship, 1989; Horn, 2005; Nageswara Rao, Wright, & Krosch, 2002). Cole, Sanders, Hill, and Blankenship (1985) suggested that even after kernel infected by fungi *A. flavus* or *A. parasiticus*, aflatoxin production does not occur into kernel until the natural resistance mechanism of plant broke down as a result of environmental stress (water deficit and elevated temperature). Maintaining high kernel water activity until the time of harvest preserves the natural defense mechanism (phytoalexin production) of peanuts against growth by aflatoxigenic fungi, even if fungal invasion occurs (Dorner, Cole, Sanders, & Blankenship, 1989; Dorner, 2008). For this reason, late season irrigation is recommended to help combat heat and drought stress, but this cultural practice seems to be impractical in some areas, especially in semi-arid and arid areas where water supplies are limited.

3.1.5. Chemical control

Several chemical control agents have been reported to inhibit aflatoxigenic mold growth and subsequent aflatoxin biosynthesis (Kabak et al., 2006). In relation to chemical agents, the results of studies on the application of fungicides on freshly harvested or windrowed peanuts are ambiguous. While some studies suggested that pesticides and fungicides may be useful in controlling mycotoxin production under field conditions, other results have found that pesticides were ineffective in controlling mycotoxin production by *Aspergillus* species.

An additional concern is that fungicides resistance development compromises effective control and mycotoxin contamination indirectly, through the impact of resistance mutations on the mycotoxigenic ability of the strains. In the case of aflatoxigenic fungi, Markoglou, Doukas, and Malandrakis (2011) reported laboratory *A. parasiticus* strains resistant to anilinopyrimidine that produced aflatoxins in higher quantities than the wild type strains.

Indeed, it might be expected that the primary effect of the use of pesticides during plant growth in the field is in the control of insect damage, thereby reducing the risk of mycotoxigenic fungi invasion (EC, 1999).

3.1.6. Biological control

One strategy that has been developed for reducing preharvest aflatoxin contamination of crops is biological control, which is achieved by applying competitive non-toxigenic strains of *A. flavus* and/or *A. parasiticus* to the soil of developing crops. This approach is based on the premise that when high number of spores of the nontoxigenic strains is added to soil, they will compete with naturally occurring toxigenic strains for infection sites for growth on peanut and for essential nutrients. Also, it has been demonstrated that soil inoculation with nontoxigenic strains has a carryover effect and may protect peanuts from contamination during storage (Dorner, 2004; Dorner & Cole, 2002).

The biological control of aflatoxin using the competitive exclusion approach has been demonstrated under field conditions in cotton (strain AF36) and maize (*A. flavus* K49) in the United States

(Abbas, Zablotowicz, et al., 2011; Cotty, 2006; Cotty & Antilla, 2003; Horn, Greene, & Dorner, 2000). Also, in Nigeria using four non-aflatoxigenic *A. flavus* strains formulated into a biocontrol product named Aflasafe™ (Atehnkeng et al., 2008). Specifically in peanuts, a non-toxigenic *A. flavus* strain NRRL 21882 has been successfully commercialized as Afla-Guard™ brand biological control agent (Dorner & Cole, 2002; Dorner, Cole, & Blankenship, 1998; Horn & Dorner, 2009). The first commercial use of Afla-Guard® in USA, resulted in an aflatoxin reduction averaging 85% in farmers' stock peanuts and as high as 98% in shelled stock (Dorner, 2009). The same strategy was applied to prevent the aflatoxin contamination in peanuts from Australia and Argentina (Alaniz Zanon, Chiotta, Giaj-Merlera, Barros, & Chulze, 2013; Pitt & Hocking, 2006).

Multi-agency, multi-state, and large-scale field trials are underway to determine the extent of aflatoxin reduction that is possible and how to best incorporate biocontrol into standard agricultural practice (Abbas, Weaver, et al., 2011).

A sexual stage was recently described for *A. flavus* (Horn et al., 2009) and the potential for sexual recombination in nature and its consequences are being explored (Moore et al., 2009; Olarte, Horn, Monacell, Stone, & Carbone, 2010). The repeated application over many years of non-aflatoxigenic strains could result in a shift in the genetic composition of native strains because of the transfer of genes from the biocontrol strain. The capacity of these new genotypes to produce mycotoxins and their competitiveness in invading crops merit close evaluation (Olarte et al., 2012).

4. Harvest time

It is very important to harvest the crop at optimum maturity, as excessive numbers of overmature or very immature pods at harvest can be reflected in high levels of aflatoxin in the final product. Also delays in harvesting will result in poor quality seed due to mold infections and subsequent aflatoxin contamination of the seeds/pods (CAC, 2004).

The peanut harvest involves many activities designed to deliver the highest quality peanut to the consumer. These include digging peanuts in which the pods are exposed to air and sunshine; threshing peanuts from the vine with little mechanical damage and removing foreign material are important factors to take into account in order to prevent mycotoxin contamination. Mechanical damage to kernels makes them much more vulnerable to invasion by storage molds, including *A. flavus*. Aflatoxin concentrations in seeds from pods injured by insects can be dramatically higher than those in the seeds from uninjured pods. Insect injury to peanut pods may result in aflatoxin contamination in seeds under conditions that normally do not favor fungal infection and aflatoxin production (Sobolev, Gou, Holbrook, & Lynch, 2007). After peanuts are dug and harvested, contamination can be prevented by rapidly drying peanuts to or below a water activity = 0.83, a condition that prevent aflatoxin synthesis (Diener & Davis, 1970). In many developing countries, the combination of insufficient drying equipment coupled with high atmospheric humidity results in unacceptable levels of aflatoxin in harvested peanuts (Kabak et al., 2006).

5. Post-harvest management

Pre-harvest peanut seeds contain mycelia and spores of aflatoxigenic fungi, which can result in a significant decrease in grain quality when they are stored. If the storage conditions are not good, these seeds may cause serious damage and aflatoxin accumulation at higher than international accepted levels. The first studies carried out by Dickens (1977) recommended that storage of peanut must be done under clean, dry conditions with low kernel moisture content (about 8%) and at low temperature, and with protection from insect infestation to avoid molding of peanut and consequent risk of aflatoxin contamination. This approach is still the base for a proper storage even if further tools were later developed.

5.1. Segregation

The first step of correct post-harvest management is lot segregation, which means that lots with visible molds, confirming the presence of *A. flavus* or *A. parasiticus*, or with aflatoxin contamination above the legal limit must be stored separately and not used for edible purposes. After this step, post-harvest screening to remove contaminated seeds is the most effective way to remove off-color, and suspect kernels by means of electronic color sorting. When aflatoxin contamination occurs, there are usually only a few highly contaminated seeds irregularly distributed in the peanut lots while most of the harvested seeds are free of contamination. Kernels that differ substantially in color (i.e., are darker, or lighter or molded) from the standard for the particular cultivar or cultivars being examined, should be discarded.

Applicable methods include manual sorting, seed size and density separation, or electronic color sorting. The most effective technique for managing aflatoxin contamination in commercial shelling plants is electronic color sorting (Dorner, 2008), reported to produce a 70% reduction for aflatoxin (Cole et al., 1995). This approach has a disadvantage: the altered kernels are linked to aflatoxin contamination, but they are not necessarily contaminated. It means that yield losses due to different sorting approaches are not always justified by aflatoxin reduction (Waliyar et al., 2008). In recent years, continued advances in electronic color sorting technology have improved sorter efficiency; however, not all aflatoxin-contaminated kernels are discolored, so this technology is never 100% effective in aflatoxin removal. Finally to reduce effectively the aflatoxin concentration in shelled peanut lots the best method is blanching followed by photoelectric color sorting and hand-picking (Dorner, 2008). The major disadvantage to this form of aflatoxin reduction is the cost, including direct charges, the weight loss during blanching, and the loss of kernels by sorting (Dorner & Lamb, 2006).

5.2. Moisture control

According to the guide of Codex, to prevent an increase in aflatoxin contamination occurring during storage and transportation, it is important to control the moisture content, the temperature in the environment, and the hygienic conditions. Post-harvest aflatoxin contamination is most attributable to improper storage of the pods and seed. It is well established that mold invasion is facilitated because of increased moisture levels of stored commodities (Abramson, 1998). Inappropriate kernel moisture during storage can proceed from leaky roofs, condensation because of improper ventilation in the warehouse, high-moisture foreign material associated with stored peanuts, and high-moisture peanuts initially going into storage (Davidson, Hill, Cole, Mixoon, & Henning, 1982).

The minimum moisture content for *A. flavus* growth on peanut is 8–10% at around 82% relative humidity, and aflatoxin production is generally correlated with kernel moisture contents of 10% or higher (Diener & Davis, 1970). It is well known also that stock piling of peanuts can cause heat built-up and moisture accumulation, resulting in mold growth and aflatoxin contamination. Other studies reported that the maximum moisture content for storage of groundnuts (unshelled) is 9% while that for shelled peanut is 7%. At these moisture contents, if the environment relative humidity is maintained at 70% and temperature at 25–27 °C, safe storage of nuts is guaranteed for approximately one year (Odogola, 1994; Waliyar, Ntare, Diallo, Kodio, & Diarra, 2007; Waliyar et al., 2008).

It is then necessary to maintain safe storage moisture until peanuts are processed, but this can be difficult or impossible to accomplish

because of environmental conditions during the storage period in some areas.

5.3. Cleaning

It is recommended to thoroughly clean, with water and compressed air, all dust, dirt and peanut residue from the entire warehouse (inside and out) and handling equipment included, but not limited to conveyor belts, boots, elevators and farmer stock peanut cleaners. Prior to admission of farmer stock peanuts into warehouse storage facilities, or into the shelling system, those loads with excessive troublesome foreign material (>4%) or with excessive (>5% suggested) Loose-Shelled Kernels (LSKs) should be passed through the cleaning system. Special attention should be given to the removal of LSKs, high moisture components and dirt since these materials will likely increase the risk of insect damage and mold contamination leading to aflatoxin development during storage. In fact, LSKs removed from the farmer stock peanuts at this point may meet edible quality requirements; however, after a period of storage, they may not.

Peanuts in storage should be routinely checked for evidence of mold. If mold is evident, the reason for mold formation should be immediately determined and corrected. Moldy peanuts must be removed from edible use.

5.4. Air gas composition

Since mycotoxin-producing molds are obligate aerobes, it seems likely that mycotoxin production could be prevented or at least reduced by modification of atmospheric gasses in storage silos; such as by using carbon dioxide, nitrogen, carbon monoxide, and sulfur dioxide (Kabak et al., 2006). Heathcote and Hibbert (1978) reported that increases in the concentration of CO_2 in storage silo resulted in significant reductions in aflatoxin production.

5.5. Biological control agents

Biocontrol based on competitive exclusion by non-toxigenic strain of *A. flavus* has also demonstrated that field application of the strains had a carry-over effect and reduced aflatoxin contamination that occurred in storage (Dorner & Cole, 2002).

A strain of marine *Bacillus megaterium* isolated from the Yellow Sea of East China was evaluated for its activity in reducing postharvest decay of peanut kernels caused by *A. flavus* in in vitro and in vivo tests. The results showed that the antagonist had a significant effect on biocontrol effectiveness in vivo significantly reducing the biosynthesis of aflatoxins and expression of aflR gene and aflS gene (Kong, Shan, Liu, Wang, & Yu, 2010).

5.6. Detoxification

Despite improved handling, processing and storage, aflatoxin contamination remains a problem in the peanut industry. Therefore, new ways to detoxify contaminated products are needed to limit economic/health impacts and add value to the peanut industry. A study was done by Proctor, Ahmedna, Kumar, and Goktepe (2004) to evaluate the effectiveness of ozonation and mild heat in breaking down aflatoxins in peanut kernels and flour, and to quantify aflatoxin destruction compared with untreated samples. It was demonstrated that ozonation efficiency increased with higher temperatures and longer treatment times. Regardless of treatment combinations, aflatoxins B_1 and G_1 exhibited the highest degradation levels, with a more efficient degradation achieved in peanut kernels than in flour. The temperature effect lessened as the exposure time increased, suggesting that ozonation at room temperature for 10–15 min could yield degradation levels similar to those achieved at higher temperatures with a shorter exposure time, while being more economical.

More recently de Alencar, Faroni, Soares, da Silva, and Carvalho (2012) demonstrated the fungicidal and detoxifying effects of ozone on aflatoxins in peanuts. Peanut kernels were ozonated at concentrations of 13 and 21 mg L^{-1} for periods of 0, 24, 48, 72 and 96 h. Ozone was effective in controlling total fungi and potentially aflatoxigenic species in peanuts, with a reduction in colony-forming units per gram greater than 3 log cycles at the concentration of 21 mg L^{-1} after 96 h of exposure. A reduction in the percentage of peanuts with internal fungal populations was also observed, particularly after exposure to ozone at 21 mg L^{-1}. A reduction in the concentrations of total aflatoxins and aflatoxin B_1 of approximately 30 and 25% respectively was observed for kernels exposed to ozone at 21 mg L^{-1} for 96 h.

5.7. Chemical control strategies

From human health perspectives, the antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl paraben (PP) are allowed for use as antimicrobial agents by the US Food and Drug Administration (FDA) and are regarded as safe (GRAS) chemical. The use of formulations containing these antioxidants is effective in preventing oxidation of peanuts by delaying the development of oxidative rancidity and have been proposed to control total mycoflora and *Aspergillus* section *Flavi* populations in natural and inoculated stored peanuts. The development of natural peanut mycoflora, and *Aspergillus* section *Flavi* populations particularly, was inhibited and no aflatoxin was detected in stored peanut treated with the ternary mixtures of food grade antioxidants (Passone, Ruffino, Ponzio, Resnik, & Etcheverry, 2009). Besides, there is a growing considerable interest by the food industry and in consumer preferences for different natural phytochemicals focused on controlling growth and aflatoxin synthesis by *Aspergillus* section *Flavi* (Etcheverry, Nesci, & Passone, 2011).

6. Aflatoxin risk forecasting

Meteorological and soil conditions in the peanut growing area are the main factors able to influence the risk of aflatoxin contamination; therefore, preventive actions in pre-harvest are the main tools available for farmers to reduce contamination. Nevertheless, a proper harvest time and post-harvest management can help tentatively to reduce toxin content and consumer exposure, even if involving additional costs. Since, the occurrence of water stress over the last days of growth has a very substantial influence on aflatoxin contamination; the risks can be evaluated for farmers from the simulation data, if the timing of stress in the drought years is provided. The time under late season drought conditions that is necessary for aflatoxin contamination to occur varies and is dependent on numerous factors, the most important being soil temperature. Forecasting of the risk posed by these conditions can assist in minimizing pre-harvest contamination and especially in optimizing harvest post-harvest management.

The USA National Peanut Research Laboratory developed aflatoxin prediction models that could be used to predict when aflatoxin contamination is likely to occur in farmers' fields (Parmar et al., 1997; Thai, Blankenship, Cole, Sanders, & Dorner, 1990). Farmers could use that information to include aflatoxin risk in making harvest decisions. However, that technology never has been seriously utilized in the USA because the marketing system for FS peanut does not allow for economic penalties based on a measure of aflatoxin, the penalties are for incoming loads containing visible *A. flavus*. Therefore, harvest decisions are still primarily made to achieve the highest yield possible (Dorner, 2008).

In Australia, Nageswara Rao, Wright, Krosch, and Tatnell (2004), developed a model to evaluate the risk of contamination using the crop simulation model of The Agricultural Production Systems Simulator (APSIM) to 'count' the number of stress days, which is then related to the risk of contamination. These simple empirical relations

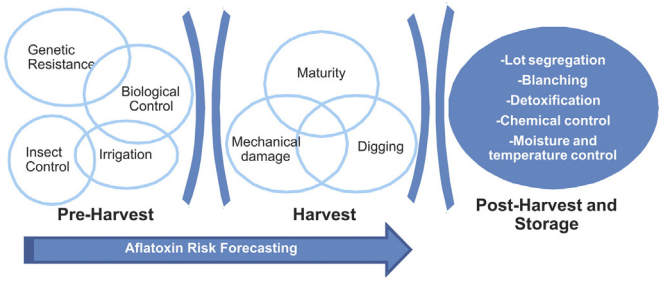

Fig. 2. Pre- and post-harvest management of peanuts to minimize aflatoxin contamination.

provided the basis for a decision support system (DSS) that can be used by farmers; this ultimately led to the development of 'AFLOMAN', a user-friendly internet-based aflatoxin monitoring and DSS for growers (www.apsim.info/afloman). Farmers input information on daily rainfall and soil and ambient temperatures via the internet, the APSIM peanut aflatoxin model is then run for the specific field with results uploaded back to the website showing aflatoxin risk. This information can then be taken into account to apply possible ameliorating practices such as earlier harvesting or even supplementary irrigation, when possible. Recently, to facilitate routine monitoring of aflatoxin risk in peanut by growers in near real time, a web interface of the model was developed and the aflatoxin risk index ARI simulated by the model resulted in a reliable indicator of aflatoxin contamination (Chauhan et al., 2010).

CROPGRO-peanut is a mechanistic crop growth model that can simulate water balance, pod zone soil temperatures, foliar temperature and plant water deficits in response to weather inputs, soil traits, plant growth traits, and crop management practices (Boote, Jones, & Hoogenboom, 1998; Williams, & Boote, 1995). This model could be particularly suited to predicting aflatoxin and has been used in some African countries to predict aflatoxin risk (Boken et al., 2008, Craufurd et al., 2006).

7. Conclusions

Research efforts in prevention and management of aflatoxin contamination in peanuts gave a very good base for the development of GAP and GMP in the production chain (Fig. 2). Nevertheless, weak points are still present and suggestions for future research can be stressed:

- More studies on genetic resistance of peanut to *Aspergillus* section *Flavi* infection and aflatoxin accumulation are needed.
- Biocontrol as a possible strategy that reduces aflatoxins both at pre-harvest and post-harvest is a good tool to be considered to reduce the impact of aflatoxins in the food and feed chains.
- Ozonization is a promising treatment to reduce aflatoxin contamination in peanuts, but further studies are requested, including legislative aspects in different countries.
- Predictive systems are available, but they should be improved and cover not only pre-harvest, including crop phenology models or in-season observations to consider variability across regions. The post-harvest period of the peanut chain should be also included in modeling for a better support to optimize peanuts management for toxin mitigation. Therefore, it is important to have in mind that *A. flavus*/*parasiticus* isolates from the same agricultural field may vary widely in aflatoxin producing ability, making it difficult to assess impacts of climate on the average aflatoxin-producing ability of fungal producer-communities.

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