Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination

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Abstract

Maize is consumed world-wide as staple food, livestock feed, and industrial raw material. However, it is susceptible to fungal attack and at risk of aflatoxin contamination under certain conditions. Such contamination is a serious threat to human and animal health. Ensuring that the maize used by food industry meets standards for aflatoxin levels requires significant investment across the supply chain. Good Agricultural Practices (GAP) form a critical part of a broader, integrated strategy for reduction of aflatoxin contamination. We reviewed and summarised the GAP of maize that would be effective and practicable for aflatoxin control within high-risk regions for smallholder farmers. The suggested practicable GAP for smallholder farmers were: use of drought-tolerant varieties; timely harvesting before physiological maturity; sorting to remove damaged ears and those having poor husk covering; drying properly to 13% moisture content; storage in suitable conditions to keep the crop clean and under condition with minimally proper aeration, or ideally under hermetic conditions. This information is intended to provide guidance for maize growers that will help reduce aflatoxin in high-risk regions, with a specific focus on smallholder farmers. Following the proposed guidelines would contribute to the reduction of aflatoxin contamination during pre-harvest, harvest, and post-harvest stages of the maize value chain.

Keywords: *Aspergillus*, maize production chain, smallholder farmers, practical practices.

1. Introduction

Why focus on aflatoxins?

Aflatoxin is by far the most toxic of the eight primary mycotoxins and impacts both human and animal health (Liu *et al.*, 2020). Aflatoxins (B_1 , B_2 , G_1 , G_2) are naturally occurring toxins produced by certain fungi, most importantly *Aspergillus flavus* and *Aspergillus parasiticus* (Bankole *et al.*, 2004; Erdogan, 2004; Pascale *et al.*, 2020). Aflatoxins occur most frequently in dry stress when crops are near maturity, or when high moisture is present during harvest, or when crop drying, and storage conditions are inadequate. Countries in latitudes between 40° North and 40° South are susceptible to aflatoxin contamination (Strosnider *et al.*, 2006). Thus, sub-Saharan Africa, South Asia, and Southeast

Asia are regions of high potential exposure to aflatoxins (Strosnider *et al.*, 2006). Parts of the Americas also have a high intake of aflatoxins posing a particular challenge in the tropics, subtropics and the southern United States (Bunnell and Harvey, 2020; Zuber *et al.*, 1976).

Aflatoxins mainly occur in crops in tropical regions and contaminate a wide variety of food and feed crops and products, including maize (corn), peanuts/groundnuts, and other crops such as sorghum, cassava, macadamia nuts, paprika, melon seed, sesame, rice, yam chips, and chili (Wild and Gong, 2010). A recent report by the World Bank estimates that aflatoxin consumption causes 90,000 cases of liver cancer a year (Jaffee *et al.*, 2018). For this reason, many countries have placed strict limits on the amount of aflatoxin that can be present in food commodities

and animal feed. Aflatoxin is one of the most regulated mycotoxins globally. Nevertheless, there is wide variation in maximum limits (ranging between 1 to 50 µg/kg) depending on the country and regulated material (Anukul *et al.*, 2013). Varying regulations and the difficulty for operators along the value chain to comply with necessary control measures are a reason for many rejections and impact trade volumes.

Impact of aflatoxin contamination on maize for smallholder farmers

About two-thirds of 3 billion rural people from the developing world live in about 475 million small farm households (Rapsomanikis, 2015). In these countries, smallholder farmers produce about 80% of food (FAO, 2004; Suleiman and Rosentrater, 2015). Aflatoxin contamination is a global food safety issue, especially in these regions. Lewis *et al.* (2005) reported that contamination of homegrown maize with aflatoxins ranged from more than 20 to over 1000 µg/kg in rural Kenya in April of 2004, resulting in 317 reported illnesses and 125 deaths. As a result, aflatoxin contamination of homegrown maize presents a significant threat to the health of rural and urban consumers, who are dependent on maize as their staple crop (Azziz-Baumgartner *et al.*, 2005; Daniel *et al.*, 2011; Mahuku *et al.*, 2019; Suleiman and Rosentrater, 2015). Aflatoxin-contaminated maize can also cause economic loss due to low quality/yield (Catholic Relief Services, 2018). Thus, controlling the conditions that result in growth of *Aspergillus* and production of aflatoxins at the farm level will improve food safety and contribute to improved food security, especially for those who are dependent on maize, such as smallholder farmers.

Aflatoxigenic fungi, *A. flavus* and *A. parasiticus*, may invade maize pre-harvest, at harvest time, during post-harvest handling and during storage under unsuitable temperature and humidity conditions (Bryden, 2012; Probst *et al.*, 2014). Pre-harvest infection and contamination of maize by *Aspergillus* is favoured by plant stress factors such as high temperature, drought, insect damage and low soil fertility (Bruns, 2003; Kaaya *et al.*, 2005; Mutunga *et al.*, 2017; PDNA, 2012). The residual inoculum could result in further accumulation of aflatoxin during storage if conditions are favourable for aflatoxin production (Hell *et al.*, 2008). Therefore, a series of measures of prevention and control at these stages would have an impact on preventing contamination with aflatoxins along the maize value chain.

Importance of applying good agricultural practices of maize

Maize (*Zea mays* L.) is one of the main cereals used as a source of food, forage and for processed products in industry. World production of maize is around 790 million tons and it is a staple food providing more than one-third of the calories and protein in some countries (Chulze, 2010). Since

maize is the dietary staple in many developing countries, contamination of maize grain with aflatoxin has been a major issue in these countries (Lewis *et al.*, 2005). Farms in these countries are generally of small size and run traditionally. Under this scenario, aflatoxin contamination of maize presents a significant threat to the health of smallholder farmers, who are dependent on maize as their staple (Mahuku *et al.*, 2019). Good Agricultural Practices (GAP) as broadly defined, 'applies available knowledge to addressing environmental, economic and social sustainability for on-farm production and post-production processes, resulting in safe and healthy food and non-food agricultural products' (FAO, 2003). Appropriate adoption and monitoring of GAP help improve the safety and quality of food and other agricultural products. In addition, GAP provide the added benefit of increased yield and reduced overall post-harvest losses, making it a double win for smallholder farmers, and greatly increasing incentivization and chances of adoption, under the right development models. A multiplicity of GAP codes, standards and regulations have been developed in recent years by the food industry and producer organisations, and by governments and NGOs, aiming to codify agricultural practices at the farm level for a range of commodities (Philippine National Standards, 2008). Their purpose includes fulfilment of trade and government regulatory requirements regarding food safety and quality.

Impact of climate change

Aflatoxin-producing *Aspergillus* species are well adapted to warm and dry climatic conditions (Cotty and Jaime-Garcia, 2007). Modelling of interactions between the host plant and environment during the growing season can enable quantification of aflatoxin risk and its potential management (Boken *et al.*, 2010; Chauhan *et al.*, 2008). Recent studies have shown that there is a direct relationship between the relative gene expression of *Aspergillus* sp. and aflatoxin production under different environmental conditions (Medina *et al.*, 2014).

Changing weather patterns are likely to affect infection of maize with *Aspergillus* and resulting aflatoxin contamination (Battilani *et al.*, 2016). For example, aflatoxin contamination tends to increase when there are drought periods and high temperatures during the pre-flowering stage, followed by heavy rains at harvest (Liu *et al.*, 2016; Massomo, 2020; Njeru *et al.*, 2019). The prevailing global climate change increases aflatoxin risk, especially in tropical and subtropical regions (Joutsjoki and Korhonen, 2021).

Adaptation to climate change will require appropriate approaches to management of aflatoxin (Thornton and Herrero, 2015). Aflatoxins occur most frequently under conditions where dry weather prevails, but high moisture is present during harvest and drying (Magan and Aldred, 2007). Thus, rapid drying of harvested maize to a safe

moisture content within two days can reduce the risk of *Aspergillus* growth and subsequent aflatoxin contamination (Magan and Aldred, 2007). Moreover, reducing tillage, expanding crop rotations and planting cover crops have proven to be good practices for adaptation to climate change (<https://tinyurl.com/46y4dnp2>).

Smallholder farmers dependent on rain-fed agriculture are experiencing the effects of climate change (Nyang'au *et al.*, 2021). It was reported that a decrease of 10% in aggregate yields of maize could occur in smallholder rain-fed farming systems in Africa and Latin America by 2055. The major agricultural practices adopted by farmers to adapt to climate change in these areas included diversification of crops, change of planting time and crop rotation/mixed cropping (Nyang'au *et al.*, 2021).

Importance of good agricultural practices for smallholder farmers

In developed countries levels of aflatoxins in different food commodities are strictly regulated (van Egmond and Jonker, 2008). However, in some developing countries, for example in Tanzania, strict control is difficult for widely scattered smallholder farmers who grow and consume their maize (Massomo, 2020). It is very important to identify practical GAP that can be easily adopted by smallholder farmers to prevent aflatoxin contamination of maize at the farm level. This review will summarise the current agricultural practices specific for smallholder farmers and suggest cost-effective GAP targeting aflatoxin control. Smallholders were the primary target beneficiaries under consideration, however many of these GAP could likewise be applied on larger farms and by different value chain actors.

2. Agricultural practices of maize for smallholder farmers

The traditional maize production chain for smallholder farmers is shown in Figure 1. It separates into pre-harvest, harvest, and post-harvest stages. The pre-harvest stage includes pre-planting, planting, and after-planting steps. The harvest stage includes harvesting, sorting, de-husking and drying of cobs. The post-harvest stage includes shelling, drying the kernels and storage. Aflatoxigenic fungi can infect maize throughout the production chain, thus agricultural practices that can reduce aflatoxin contamination should be undertaken throughout the process.

Pre-harvest practices

Good pre-harvest practices are critical for minimising aflatoxin contamination since fungal infection starts at this stage (Cleveland *et al.*, 2003; Mahuku *et al.*, 2019). Several control strategies for reducing aflatoxin contamination in crops have been investigated, including management/

Figure 1. Overview of maize production chain for smallholder farmers.

prevention of pre-harvest drought stress, breeding programs for heat and pest resistant crop varieties and assessing potential biocontrol agents (Spanjer, 2019).

Environmental factors that favour *A. flavus* infection in the field include high soil and/or air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid the dispersal of conidia during silking (Diener *et al.*, 1987). Factors that influence the incidence of fungal infection include the presence of invertebrate vectors, grain damage, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and

microbiological interactions (Horn, 2003). Crop rotation and management of crop residues also are important in controlling *A. flavus* infection in the field (Hell *et al.*, 2008).

Tillage practices, crop rotation, fertiliser application, weed control, late season rainfall, irrigation, wind, and pest vectors can all affect the source and level of fungal inoculum maintaining the disease cycle in maize (Diener *et al.*, 1987). The likelihood of aflatoxin contamination increased when maize was intercropped with cowpea (Sétamou *et al.*, 1997). In Africa, crops are cultivated under rainfed conditions with low levels of fertiliser and little or no pesticide application. These conditions promote *A. flavus* infection of stressed plants, and any action taken to reduce the probability of silk and kernel infection will reduce aflatoxin contamination (Hell *et al.*, 2008). Preharvest management of aflatoxin contamination is vital to maintain contamination levels below economically feasible and legislated thresholds.

Agricultural practices adopted by farmers for raising maize yield can also reduce the spread and survival of fungi in the field as well as plant susceptibility, and thus mitigate aflatoxin contamination of maize (Liu *et al.*, 2016).

Proper production area

Strategies aiming to minimise aflatoxin should start from the beginning of production to prevent contamination under field conditions, thus a cultivation environment suitable for maize should be chosen. Production areas must always be kept clean. A one to two-month fallow period should be observed before planting if possible (Thai Agriculture Standard, 2010). Critical factors worth evaluation include soil type, soil condition and the availability of viable spores (Horn, 2003). Kaaya *et al.* (2006) demonstrated that areas with high humidity resulted in higher aflatoxin levels in maize samples compared with dry areas.

Crop rotation

Crop rotation (growing different crops sequentially) can improve soil health and enhance soil nutrients, reducing plant stress. Mono-cropping of maize could increase the risk of high aflatoxin levels (Demissie, 2018). Incorporating crop rotations will influence both the quantity of *Aspergillus* in soils and the average aflatoxin producing potential of *Aspergillus* communities (Jaime-García and Cotty, 2006). Inclusion of non-host crops into the rotation will help control *Aspergillus*. For example, rotation of legumes crops (cowpea, soybean) with maize can help to break pest and disease cycles and improve soil fertility (Abbas *et al.*, 2012; Matocha and Richardson, 1990). Moreover, the rotation of maize with non-host crops can effectively reduce plant residues that could allow build-up of inoculum (Jirak-Peterson and Esker, 2011). Rotation schemes may vary geographically based on the local environment or consumption requirement. For example,

double cropping has been one key factor to increase the yield of mature maize in Brazil. This combination of planting two crops in a field in the same year, soybean in the rainy season, and maize in the dry wintertime, has expanded the production of local maize (Logrieco *et al.*, 2021).

Residue management

Fungal spores remain dormant in the soil between crops and from year to year, present in layers of infected crop residues (Hell and Mutegei, 2011). Residue management is critical for reducing the risk of aflatoxin contamination. Previous crop residue on the soil surface can be reduced by various measures including ploughing (Olanya *et al.*, 1997). However, residue management does increase the labour requirements of smallholder farmers. Proper management of the maize residue by removing or burying helps to collapse the chain of fungal infection or eliminate fungal spores, leading to a healthier agricultural ecosystem (Blandino *et al.*, 2008; Liu *et al.*, 2016).

Tillage

Proper soil aeration regulates the availability of water to the crop. Deep tillage has been observed to improve the soil structure and enhance crop vigour (Díaz-Zorita, 2000). This can lead to a significant reduction in infection by the aflatoxin producing fungi. Adapted tillage methods have been identified, such as breaking the hardpan specifically where seeds will be planted, achieve both reduction of drought stress in the maize as well as minimising tillage (IIRR and ACT, 2005).

Intercropping

Intercropping is the planting of two or more crops simultaneously on a given piece of land. This practice produces higher yield by making use of resources or ecological processes, whilst restraining the growth of weeds (Liebman and Dyck, 1993). Maize grown intercropped with legumes, such as cowpea, beans, etc., tended to have lower aflatoxin than maize grown as a sole crop, since this may improve the amount of nitrogen in the soil (Mutiga *et al.*, 2014). In maize farming this agronomic practice can include three components: maize, *Desmodium legume* as the intercrop between maize rows, and *Bracharia* or napier grass (*Pennisetum purpureum*) as the border crop planted around the plot.

Breeding varieties

If the maize variety is not resilient it may be vulnerable to drought, insects, pathogens, etc. (Brown *et al.*, 2003, 2013; Chen *et al.*, 2001; Menkir *et al.*, 2008). Drought-resistant varieties should be used if they are available locally. Alternatives include varieties or hybrids that are adaptable

to the locality and approved by the local country as seed material. Several screening tools have been developed and used to facilitate maize breeding for developing germplasm resistant to fungal growth and/or aflatoxin contamination (Brown *et al.*, 2003). Sources of resistance to *Aspergillus* infection and aflatoxin contamination in maize have been identified, but commercial hybrids have not been developed. This is largely due to the difficulty in finding elite lines that maintain high yields and resistance within multiple environments (Clements and White, 2008). Brown *et al.* (2003) tested aflatoxin resistance in 36 inbred maize lines that were selected in West and Central Africa for moderate to high resistance to maize ear rot. More than half the inbred lines accumulated aflatoxins at levels as low as, or lower than, the resistant US lines. Six tropical maize germplasm lines with resistance to aflatoxin were registered by the same research group in 2008 (Menkir *et al.*, 2008), so that locally adapted hybrids can be developed. Many new biotechnology strategies such as RNA interference, microarray, whole genome sequencing, RNA-sequencing, proteomics, metabolomics have been widely used to deepen understanding of resistance mechanisms and identify genes, proteins, and pathways involved during host-pathogen interactions for aflatoxin contamination in maize. These approaches would allow for the deployment of both conventional and modern breeding tools to facilitate the development of aflatoxin-resistant maize varieties, which reduce the incidence of fungal infection, restrict the growth of aflatoxigenic fungi, or prevent aflatoxin accumulation (Soni *et al.*, 2020).

Optimal planting time

Planting at an optimal time in good soil conditions is the foundation for successful maize production, and this practice assists in reducing the incidence of aflatoxin contamination in the field (Bruns, 2003). Maize planting (and maturation time of the selected variety) is suggested to be carefully planned to avoid high temperatures and drought stress during seed development and maturation (Abbas *et al.*, 2009). Early planting can be very beneficial for regions with water stress. Wet periods during early flowering should be avoided if possible (Eeckhout *et al.*, 2013). Cultivation of maize in the rainy season, as occurs by necessity in rain-fed systems in many parts of Africa, can lead to an increase in aflatoxin accumulation, particularly if the plants are treated with low levels of fertiliser or with minimal amounts of pesticides. Action to interrupt the probability of silk and kernel infection will reduce aflatoxin contamination (Diener *et al.*, 1987).

Plant density

Overcrowding of plants may lead to humid and warm conditions which favour insect, pathogen and disease development and it can also result in reduced yield due to

competition for soil nutrients and sunlight (Blandino *et al.*, 2008). It should be noted that high plant density becomes an important risk factor under drought stress (Abbas *et al.*, 2012). By maintaining the recommended row and intra-plant spacing for the species or varieties, overcrowding can be avoided. Seed and breeding companies, as well as extensionists, can provide useful information related to plant spacing. Hybrid crops and inbred lines of maize had significant effects on the severity of ear rot and the level of aflatoxins (Balconi *et al.*, 2014; Blandino *et al.*, 2017). Maize yield and degree of interplant competition were affected by variations in plant density for the specific hybrids (Sangoi *et al.*, 2002). In addition to breeding, it is therefore important to establish planting plans that guarantee optimal numbers to reduce maize contamination by fungi and aflatoxins.

Fertiliser application

Fertilisation is an important practice for aflatoxin control. However, nutrient deficiencies or over-fertilisation, particularly with nitrogen, may lead to greater susceptibility to insect pests and diseases (Tubajika *et al.*, 1999). The appropriate application of fertilisers and soil additions, such as lime (calcium), animal manure, and compost, can minimise plant stress, especially during seed development, by assuring an adequate soil pH and plant nutrition (Mutunga *et al.*, 2017). This can reduce aflatoxin contamination. While application of the fertiliser will introduce additional cost for smallholder farmers, maize cultivation without fertilisation does not bring good economic results. Mineral fertilisers can of course be replaced with natural fertilisers, which are often available for smallholder farmers.

There was a strong correlation between knowledge of maize ear rot and knowledge of aflatoxin for smallholder farmers. Levels of aflatoxin were significantly and positively related to the use of diammonium phosphate (DAP) fertiliser at planting (Njeru *et al.*, 2019). Thus, the use of DAP fertiliser is considered one of the critical agronomic factors in maize production (Ndemera *et al.*, 2018).

Insect control

Insects propagate fungi and cause damage that allows the fungi to access the maize, increasing the chances of aflatoxin contamination (McMillian *et al.*, 1985; Sétamou *et al.*, 1997). The maize borer, *Ostrinia nubilalis*, the maize earworm, *Heliothis zea*, and the fall armyworm, *Spodoptera frugiperda*, have been identified as the major insects implicated in *A. flavus* infection and subsequent aflatoxin contamination of preharvest maize (Widstrom, 1979; Windham *et al.*, 1999). When insect damage occurred simultaneously with favourable climatic conditions, such as drought stress and high temperature, high aflatoxin concentrations were usually found in maize grains (Magagnoli *et al.*, 2021). Appropriate insect management

techniques will reduce formation of holes and damage to the cobs which, in turn, will reduce the entry points for the fungus (Hell and Mutegei, 2011; Sétamou *et al.*, 1998). Thus, the application of registered insecticides especially when drought and heat stress frequently occur during the reproductive stages of the plants, can mitigate the risk of fungal growth. However, smallholder farmers often lack access to appropriate insecticides, proper information or the means to properly and safely apply them, so health risks must also be taken into consideration (further addressed in the paragraph below on fungicide application). Pests and diseases must be controlled early enough to avoid epidemics that can lead to extreme plant stress, quality damage, and economic losses (Hell and Mutegei, 2011; Hingley and Pedigo, 1993). The incidence of the insect borer *Mussidia nigrivenella*, was positively correlated with aflatoxin contamination of maize samples collected in Benin (Hell *et al.*, 2008). The chance of insect damage and aflatoxin contamination increases when loose-husked maize hybrids are used (Dowd, 2003).

Fungicide application

Air dispersal of fungi is associated with infection of maize (Anitha *et al.*, 2019). Fungal infection can occur through airborne spores in the field during pre-harvest or during storage. The fungus usually colonises the silk and kernels when the silk is cut or the cob is damaged due to excessive drought (Amaike and Keller, 2011). Keep plants healthy to avoid fungal infection. Fungicides currently remain our most potent weapon against fungal maize pathogens (Steinberg and Gurr, 2020). However, no chemical control against *Aspergillus* is authorised in maize to date (Ferrigo *et al.*, 2019). Fungicides have not been widely used for aflatoxin management in maize. Fungicides have been shown to be effective for controlling deoxynivalenol in small grain cereals, so this success has promoted similar research in maize (Logrieco *et al.*, 2021). Several azole-based fungicides have proved to effectively reduce *A. flavus* growth and aflatoxin production, but stimulation of aflatoxin production was found under particular conditions and low-dosage treatments (Ferrigo *et al.*, 2019; Mateo *et al.*, 2017). Azole-based fungicides have potential to be included in a multi-strategy approach to control *Aspergillus* infection in maize, in the context that sustainable use of pesticides is ensured (Ferrigo *et al.*, 2019).

Pesticide misuse considerations

In the case of smallholder farmers in developing countries, the capacity of the agricultural system to inform and support the proper and safe application of the correct pesticides (insecticides and fungicides) is greatly lacking. This must be a critical consideration before pesticides are recommended as an approach. A meta-analysis by

Boedeker *et al.* (2020) aimed to check the World Health Organization's 1990 estimate that one million global pesticide (fungicide and insecticide) unintentional, acute pesticide poisonings (UAPP) occur annually; the study estimated that approximately 44% of farmers globally suffer from UAPP, or ~385 million with ~11,000 fatalities. Without substantial and sustained capacity building in many developing country agricultural systems, targeted to key actors, other GAP methods are much safer overall in those systems as they exist currently. The GAPs recommended in this review include these types of important alternatives, with developing country smallholder farming systems as the key focus. For example, hermetic bags and cocoons obviate the need for pesticide application during storage; some of these have been developed with slow-release safe insecticide (Paudyal *et al.*, 2017), gaining regulatory approval by developing country governments such as Ghana, and effectively bringing pesticides safely into the smallholder farming system without placing the responsibility of appropriate and safe application in the hands of the smallholder farmers and input suppliers. In another example of a possible alternative, Zhang *et al.* (2016) estimated that wider adoption of GM crops which reduce pesticide use in China and globally may increase farmer health. While pesticides are an important tool to consider in addressing mycotoxin contamination, a holistic and sustainable approach to effectively empower proper use in national systems by resource-poor farmers must be a strong part of any initiative recommending this strategy.

Weed control

Timely control of weeds should be undertaken to avoid them competing with the crop. Appropriate weed control measures such as proper land preparation, off-barring at 12 to 15 days after planting (DAP) and/or hilling-up at 25 to 30 DAP should be undertaken (Philippine National Standards, 2008). Care should be taken during cultivation to avoid damage to the plant. Weed control can be done manually with a hoe, bull, tractor, or with an herbicide. Safety considerations around misuse of herbicides are similar to those discussed above.

Water requirement

An adequate water supply should be maintained, especially at the stage of seed-germination. Irrigation and water management in maize planting could be ranked as one of the top three critical factors (Logrieco *et al.*, 2021). Irrigation systems should be designed to provide sufficient water against drought stress, while the water requirement should also be met to avoid moisture stress particularly during flowering up to the maturation stage. However, excessive moisture increases contamination risk in the crop canopy, since the crop is more susceptible to aflatoxin contamination at these stages (Philippine National

Standards, 2008). Unfortunately, this is often not readily available as an option to smallholders growing maize in high-risk areas as they rarely have access to irrigation. In these situations, it is suggested to build soil organic matter for greater water retention (Ankenbauer and Loheide, 2017), and breaking the hardpan at the point of seed planting (Farm Input Promotions Africa strategy). Research has proven that aflatoxin levels in maize could be reduced by 99% when a combination of appropriate irrigation and insecticide were applied, compared to non-irrigated, non-treated maize in the US. Nevertheless, accessibility to irrigation systems and the cost of this combination appears to be challenging for smallholder farmers (Khangwiset and Wu, 2010; Smith and Riley, 1992).

Practices during harvest

Reduction of fungal infection during pre-harvest is not enough to prevent aflatoxin contamination. Aflatoxin management must continue at the harvest and post-harvest periods. Practices during harvest can play a key role in preventing or arresting aflatoxin contamination.

Timely harvesting

Extended field drying of maize could result in serious crop losses during storage and timely harvesting is recommended to combat aflatoxin problems (Borgemeister *et al.*, 1998; Kaaya *et al.*, 2006). An increase of aflatoxins of up to seven times was observed when maize harvesting was delayed for a month in Uganda (Kaaya *et al.*, 2006). Another report noted that aflatoxins in maize increased 4- to 7-fold after 3-4 weeks delay in harvest after maturity (Demissie, 2018). Physiological maturity is approximately 100 to 120 days after planting for yellow maize and 90 to 100 days after planting for white maize (Philippine National Standards, 2008); as another reference, there are varieties of maize in the Northern USA which mature at 80 and 120 days (Ashley, 2021). The exact day depends on the varieties or hybrids. Harvesting of maize at physiological maturity is recommended in low risk regions (Mejía, 2003). Fields with higher risk for aflatoxin formation should be treated differently from those fields with a lower risk. Earlier harvesting is recommended in high-risk regions, as fungal pathogens will have less time to develop and potentially produce aflatoxins (Bruns, 2003; Dovenyi-Nagy *et al.*, 2020; Monda *et al.*, 2020; Udoh *et al.*, 2000).

Proper harvesting techniques

Harvesting should be completed in the shortest time possible especially during the rainy season. Care must be taken during harvesting to prevent damage, as damage to the cobs permits easy entrance of fungi (Sumner and Lee, 2017). Harvesting of maize with the husk could reduce

aflatoxin contamination (Demissie, 2018). Fungal attack occurs during harvest mostly because of dropping and drying cobs on bare ground, allowing easy transfer of the fungus from the soil to the storage facilities (Mutiga *et al.*, 2019). Cobs should not be left to dry in the field on bare soil after harvesting (Massomo, 2020). Field stacking (heaping) does not provide enough aeration for the ears and could lead to colonisation of maize by aflatoxigenic fungal strains (Manu *et al.*, 2019). Thus, harvested products should be collected into clean baskets or onto clean underlayers. Maize ears should not be left in containers for more than 6 hours between harvesting and drying (Sumner and Lee, 2017).

Sorting to remove poor quality ears

Damaged cobs should not be mixed with healthy ones, to reduce the spread of spores and subsequent infection. Sorting and removing visibly mouldy, damaged, or irregularly shaped cobs and cobs having poor husk covering before storage is an effective method for reducing aflatoxins (Afolabi *et al.*, 2006; Fandohan *et al.*, 2005; Mutiga *et al.*, 2014; Park, 2002; Turner *et al.*, 2005). This was also ranked as an important practice by Logrieco *et al.* (2021). For example, it has been suggested that maize ears having greater than 10% ear damage should be removed (Sétamou *et al.*, 1998), although even those with less than 10% damage can serve as a source of mycotoxigenic fungal inoculum during storage, especially under poor storage conditions. Either simple physical (e.g. manual sorting) for smallholder farmers (Kagot *et al.*, 2019) or flotation and density separation methods for middle/larger scale farmers can be done for sorting purpose (Khangwiset and Wu, 2010); however, wetting the grains for flotation is not recommended since any fungi present will have ideal conditions to rapidly multiply and produce aflatoxin. It was reported that physical sorting alone has been found to reduce aflatoxin levels by 40-80% (Kumar *et al.*, 2016). Separate maize ears with poor quality away from the production area.

De-husking

Manual harvesting is the most convenient and popular method. Extension efforts with smallholder farmers typically recommend that maize ears are suggested to be removed from the plants by hand without husking (Shee *et al.*, 2019). The basis of this recommendation is that the husk will protect the cobs by husk during transportation. Removal of its leafy outer covering can be done manually before drying. This harvesting method can be done quickly and will help protect the kernels from exposure to soil during harvesting, and damage or cracking during harvest and transportation.

Immediate drying after harvesting

Aflatoxin contamination can increase 10-fold in a three day period when harvested maize is stored with a high moisture content (Hell *et al.*, 2008). Leaving ears in containers for more than six hours between harvesting and drying can increase risk of aflatoxin accumulation, and drying maize on the cob before shelling is a good practice (Chulze, 2010); the generally accepted recommendation is that harvested commodities should be dried as quickly as possible. The cobs are tougher and can overcome damage brought about by the shelling at this moisture content (Philippine National Standards, 2008). This was ranked as the most important postharvest management action by Logrieco *et al.* (2021). Achieving this through simple sun-drying is the traditional practices for smallholder farmers. It is advised that cobs should be dried on polyethylene sheets laid on the ground instead of directly drying them on the soil. Alternatively, a range of solar and mechanical dryers have been developed and are already scaling with smallholder farmers in a number of countries. For example, the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss developed the BAU-STR dryer in Bangladesh, and it now scaling with smallholder farmers (Alam *et al.*, 2020).

Practices after harvest

Post-harvest strategies are critical in preventing aflatoxin contamination, given *Aspergillus*' ability to grow and spread during this period (Kamala *et al.*, 2018). Appropriate sorting, drying, and storage techniques can significantly reduce aflatoxin contamination by 63-88% or more (Unnevehr and Grace, 2013).

Shelling and sorting

It is important to avoid damage and remove broken kernels during shelling. Care should be taken during manually shelling if that is the only option available to the smallholder farmers. The containers used for the shelled maize should also be clean and dry. Shi *et al.* (2014) found that removing fines resulted in aflatoxin levels in the removed fines 46-50 times that in the cleaned fraction. Although other studies failed to observe significant reduction of aflatoxin by manually sorting out discoloured and broken kernels, they still found reduction of fumonisin (Mutiga *et al.*, 2014), further supporting this as a best practice.

Drying

Maize should be dried after harvest to halt fungal growth and aflatoxin production during postharvest storage (Hawkins *et al.*, 2005). Natural direct sunlight is the most feasible option for many smallholder farmers for drying their crops, and is the traditional practice. Extensionists often advise that smallholder farmers should dry on polyethylene

sheets laid on the ground (however some studies have failed to find that these make a statistical difference in aflatoxin levels, e.g. Bauchet *et al.*, 2020). Farmers should make sure the layers are not too deep when drying the cobs or shelled kernels. Cobs or kernels at the bottom will take longer to dry and high moisture maize is more likely to become contaminated with aflatoxin. Improper drying of cobs leads to retention of moisture. It is imperative to ensure that crops have reached optimal moisture levels within 48 hours after shelling, in order to discourage mould growth and toxin production while in storage (Philippine National Standards, 2008). The moisture content for maize kernels is recommended to be below 13% (Manu *et al.*, 2019). The measurement of the moisture content of the maize should be determined in a representative sample randomly taken from the whole batch. Clean suitable containers should be used for the dried kernels. It is strongly suggested that dry maize should not be mixed with wet maize or any foreign matter.

However, outdoor sun drying is the traditional method that smallholder farmers can use for reducing the moisture content of maize, so it can be challenging to achieve the safe moisture content in time, potentially favouring fungal infection, and aflatoxin contamination. It is a common practice among farmers that they estimate maize moisture content by puncturing kernels with their thumbnail or biting kernels with their teeth (Liu *et al.*, 2016).

Proper storage

Proper storage and moisture management have been suggested as critical aflatoxin contamination control interventions (Walker *et al.*, 2018). It should be situated in areas where there is no flooding (Kaaya and Kyamuhangire, 2006). Proper condition should be undertaken and minimally with proper aeration, or ideally under hermetic conditions. Moisture in stored maize should be kept below 13% to stop the development of aflatoxin (Shepherd, 1999). Maize should not be heaped in stores, unless in appropriate hermetic bags; it should be packed in clean, sealed containers to avoid exposure to excessive moisture and humidity. Maize should be packaged into manageable weights (up to 25 kg) in clean containers or bags. The temperature of the stored maize should be checked periodically. Visual checks should also be conducted for evidence of fungal growth and to allow separation of the infested or infected portion. Cleanliness to prevent insect infestation and disease infection should always be maintained. Traditional practice in India is to use the jute bags for storage (Bari *et al.*, 2012). Recent data indicated that it is better to use new jute bags; if this is not possible bags should be cleaned as much as possible and free of aflatoxigenic fungi (Wang *et al.*, 2021). The first-in first-out principle should be followed during storage and use of the maize.

3. Practicable good agricultural practices for smallholder farmers

Based on the reported information specific for smallholder farmers, the following agricultural practices were identified as having high impact on the reduction of aflatoxins: residue management, crop rotation, variety, plant density, fertiliser use, insect control, water requirement in the pre-harvest stage; timely harvesting, proper harvesting techniques, sorting, immediate drying, in the harvest stage; drying and proper storage in the post-harvest stage (Catholic Relief Services, 2018; Demissie, 2018; Hell *et al.*, 2011; ICRISAT, 2016; Mahuku *et al.*, 2019; Sumner and Lee, 2017; Udomkun *et al.*, 2018). Most smallholder farmers indicated that the use of residue management, crop rotation, and irrigation are the most expensive of the potential control methods (Mutiga *et al.*, 2019; Udomkun *et al.*, 2018). Only some farmers accept new production technology such as fertilising and pest control (Semple *et al.*, 1989). Additionally, residue management requires more labour for smallholder farmers. Smallholder farmers are also unwilling to change their manner of crop rotation and decrease plant density (Moswetsi *et al.*, 2017; Udomkun *et al.*, 2018). These changes may need more communication and education with the support of local governments. In summary, the suggested practicable GAP for smallholder farmers in high-risk regions are use of drought resistant varieties, timely harvesting, sorting, drying and proper storage. These GAP provide a balance between impact on aflatoxin prevention and practical feasibility that can help smallholder farmers reduce aflatoxin contamination at a farm level in high-risk regions.

4. Emerging technologies for smallholder farmers

Biological control using atoxigenic strains of *A. flavus* during preharvest was reported to reduce infection by toxigenic strains (Bandyopadhyay *et al.*, 2016; Cotty and Bhatnagar, 1994; Pitt, 2019). This type of biocontrol was achieved by spreading non-aflatoxigenic *A. flavus* spores onto the field at particular times prior to harvest. It was reported that atoxigenic strains could out-compete endemic, toxigenic strains, thus reducing the aflatoxin levels in kernels (Ehrlich, 2014). Biological control using non-toxigenic fungi, although applied in the field, can reduce aflatoxin contamination for as long as six months post-harvest (Khlangwiset and Wu, 2010). The International Institute of Tropical Agriculture (IITA) and the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), as well as local national institutes, successfully adapted and improved this technology for use in controlling aflatoxin contamination in sub-Saharan Africa under the trade name Aflasafe (Bandyopadhyay *et al.*, 2016; Logrieco *et al.*, 2021). Several countries in Africa have registered Aflasafe products (<https://aflasafe.com>). In

Kenya, Aflasafe is manufactured at a government-owned facility and sells for US\$ 2.00 per kg (Keyser and Sela, 2020). Reports showed that a single application of Aflasafe two to three weeks before maize flowering can prevent aflatoxin contamination throughout the production process, even when grains are stored. It has been suggested that local atoxigenic *Aspergillus* strains that potentially competitively exclude aflatoxigenic strains are used where possible, to avoid the introduction of 'foreign' fungi (Khlangwiset and Wu, 2010). Evaluation of the affordability and cost-effectiveness of biological control products needs to be addressed for smallholder farmers (Kagot *et al.*, 2019; Massomo, 2020; Pitt, 2019). The promotion of biological products as a strategy to reduce aflatoxin contamination has raised concerns about potential long-term environmental risks and long-term efficacy in the field conditions (Joutsjoki and Korhonen, 2021; Kagot *et al.*, 2019; Pitt, 2019). Moreover, the allergenic and human health aspects of the atoxigenic strain need to be evaluated (Atehnkeng *et al.*, 2008; Kagot *et al.*, 2019).

During the preharvest stage mechanical technologies, such as soil testing devices, controlled irrigation systems, soil irrigation sensors, and gypsum and fertiliser spreaders, can reduce aflatoxin contamination (Kamau *et al.*, 2018), and all these also help to increase crop yield and net return (Parimi *et al.*, 2018). However, application of these technologies will introduce additional cost, limiting their usefulness for most smallholder farmers.

Shellers can contribute to reduction of aflatoxin contamination by shelling pods faster and keeping them dry. However, in some cases water may be sprinkled onto pods during hand shelling to make them softer, increasing the risk of aflatoxin contamination (Kamau *et al.*, 2018).

For drying, moisture monitoring can be undertaken with a calibrated moisture meter. Alternatively, the DryCard is a new technology developed by the Horticulture Innovation Lab at University of California, Davis targeted at smallholder farmers (Thompson *et al.*, 2017). This uses colour changes to indicate whether the kernels are dried to the safe level (Catholic Relief Services, 2018). For middle/larger scale farmers, mobile grain-drying equipment have been tested in field trials (Hoffmann and Jones, 2018). But these dryers have not been adopted smallholder farmers largely owing to their high investment and operating costs and unattainability (Hoffmann and Moser, 2017; Joutsjoki and Korhonen, 2021). In industrial nations, drying with forced air and supplemental heat is common to control moisture levels. It is reported that at 70 °C, *A. flavus* infection is significantly reduced compared to that in the maize dried at 40 °C. But this high temperature may potentially reduce seed germination and increase stress cracks (Hawkins *et al.*, 2005; Khlangwiset and Wu, 2010).

During storage, many farmers store maize in bags, especially polypropylene bags which are not airtight. However, there is evidence that this method of storage facilitates fungal contamination and aflatoxin development (Hell *et al.*, 2000; Udoh *et al.*, 2000). Hermetic storage technologies to prevent weevil damage and subsequent accumulation of aflatoxins have been developed, such as improved hermetic storage bags, Purdue Improved Crop Storage (PICS), and GrainSafe® bags (Ben *et al.*, 2009; Murdock *et al.*, 1997; Walker *et al.*, 2018; Williams *et al.*, 2014). The AflaSTOP project determined that PICS bags were the most successful solution for smallholder maize farmers (ACDI/VOCA, 2017; ICRISAT, 2016). The Post-Harvest Loss Innovation Lab (PHLIL) worked with Vestergaard on ZeroFly Hermetic bags – which also contain slow-release insecticide. They are very effective and being scaled in several developing countries.

5. Challenges

Risks and contingencies

Farmers might not be willing or able to change their current paradigms. This could be due to the fear of change and potential impact on family income. In some cases, GAP implementation will increase production costs. Also, limited awareness of the smallholder farmers on risks at all levels and insufficient knowledge on the options to reduce aflatoxin contamination through the production to consumption chain influence their ability to change.

Agricultural practices summarised in this review are mainly focused on high-risk regions, such as sub-Saharan Africa, India, Thailand, and Southeast Asia (Benkerroum, 2020; CAST, 2003; Khlangwiset and Wu, 2010). It is unlikely that there is a single solution for all regions due to different geographic locations and climate.

Suggestions

Awareness of ‘win-win’ practices which lead to improvements of yield and aflatoxin reduction, as well as the environment and health and safety of farmers, needs to be raised. If applicable, creating financial incentives to encourage smallholder farmers to adapt these GAP. Communication to, and education of the key stakeholders is imperative. Continued efforts by relevant international and regional agencies (e.g. FAO, World Food Program (WFP), The Partnership for Aflatoxin Control in Africa (PACA), etc.) in collaboration with local government and in partnership with the food business actors and the scientific community will be necessary to educate smallholder farmers on the danger of commercialising and consuming contaminated foods. We can change agricultural practices through education and demonstration, to help them reduce aflatoxin contamination.

6. Conclusions

This review summarised the current knowledge of GAP of maize throughout the maize production chain. Practicable GAP to reduce aflatoxin contamination of maize in high-risk areas were identified. These GAP extended across the pre-harvest, harvest, and post-harvest stages of maize cultivation, and were prioritised based on their likely impact on the level of aflatoxin contamination. The five main GAP were: use of drought-tolerant varieties; early harvest before physiological maturity; sorting to remove damaged ears and those having poor husk covering; drying properly; proper storage. This information is intended to provide guidance for maize growers that will help reduce aflatoxin in high-risk regions, with a specific focus on smallholder farmers.

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Conflicts of interest

All authors declare no conflict of interest.

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