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Technological and community-based methods to reduce mycotoxin exposure

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ABSTRACT

In developing countries the enforcement of compliance to detailed mycotoxin regulations ensures protection of the population from adverse health effects of mycotoxin exposure. In low-income or developing countries mycotoxin regulations are either lacking or poorly enforced which create scenarios where mycotoxin exposures occur above levels set by health regulatory bodies. Population groups that are the worst affected include subsistent maize growing farmer communities where mono-cereal crops are cultivated and locally consumed, and mycotoxin contamination are not monitored. Other factors that aggravate the situation include the consumption of highly mycotoxin contaminated unprocessed maize, the lack of knowledge about the adverse effects as well as traditional uses of maize products not intended for human consumption during periods of food insecurity. These scenarios require ingenious ways to reduce mycotoxin exposure in poor rural communities where access to sophisticated mycotoxins reduction techniques is not available or practically viable. Although community-based and culturally acceptable methods have, to some extent, been adapted the efficacy thereof varies due to the lack of sufficient training. Integration of these methods with more sophisticated technological methods is envisaged, and will be based on a better understanding of mycotoxin biosynthesis and fungus-host interactions on a molecular level. In addition, other methods which include the detoxification of mycotoxins utilising degradation enzymes, clay adsorbents, utilisation of non-toxigenic fungal strains and resistant maize cultivars to fungal infections are just a few approaches under scrutiny. The introduction of good agriculture practices and storage techniques and the identification of critical control points during hazard analyses need to be further explored. Introduction of mycotoxin monitoring programs and validated screening procedures to monitor exposure should be a priority in the future, to facilitate community-based and effective intervention programmes of mycotoxin reduction.

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1. Introduction

Mycotoxins are produced by food borne fungi and are important environmental and carcinogenic agents occurring in many parts of the world. The majority of Africa's grain supplies are at risk to be contaminated by mycotoxins, which is further threatened by food insecurity (Wagacha & Muthomi, 2008). As mycotoxins exhibit a variety of biological effects and are implicated in many human diseases (Wu, Groopman, & Pestka, 2014a), the prevention of chronic exposure, particularly in developing countries such as sub-Saharan Africa and parts of Latin America, is of critical importance (IARC, 2012). Co-contamination of food and co-exposure of especially young children to multiple mycotoxins have been widely documented in low socioeconomic areas in African (Tanzania, Cameroon and Nigeria) and Latin American (Guatemala) countries, and is of particular concern (Ezekiel et al., 2014; Shirima et al., 2015; Torres et al., 2015). A coordinated international response was suggested by an International Agency for Research on Cancer (IARC) Working Group, with emphasis on mycotoxin monitoring; sustained use of intervention technologies for low-income countries; and establishment and enforcement of food regulations (IARC, 2015).

Strict regulations of mycotoxins in food exist in developed

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countries with high standards of monitoring food quality to protect against the adverse effects on human health. The introduction of food safety regulations impact on international trade, as maximum tolerated levels on mycotoxin contamination differ between countries which has important implications on food safety and security measures (Wu, 2004). Globally, separate maize trading communities emerge and nations tend to trade with other nations that have similar food safety standards (Wu & Guclu, 2012). In Africa only 15 countries have mycotoxin regulations, which is mainly related to aflatoxin contamination of major dietary staples (FAO, 2003). As a result of the mycotoxin contamination of staple food, such as grain, high levels often enter the food chain of countries with less strict and/or lacking any regulations such as in sub-Saharan Africa. In addition, the best-quality food products from this region are exported to countries with regulations resulting in the poor quality food contaminated with high mycotoxin levels, being utilised domestically (IARC, 2012). In addition developing countries are often confronted with food insecurity due to severe climatic conditions, poor socioeconomic status and economic instability, and lack of the necessary agricultural expertise regarding crop management pre- and postharvest (Adegoke & Letuma, 2013; Wagacha & Muthomi, 2008). Apart from the economic losses encountered, as it hampers exports to countries with strict mycotoxins regulations, these conditions increase the risk of mycotoxin exposure on a daily basis and the associated adverse health effects.

Contamination of food resources with mycotoxins is widespread, affecting many crops, with maize being one of the major dietary staple in many parts of the world. Maize consumption varies between different regions with the highest consumption encountered in Africa (52–328 g/person/day) and the Americas with Mexico (267 g/person/day) representing the highest intake (Ranum, Pena-Rosas, & Garcia-Casal, 2014). Household consumption of maize in rural subsistent farming communities in parts of Southern Africa often exceeds these intake patterns up to 3–4 fold and could reach intake of 1–2 kg/person/day (Burger et al., 2010). The effects of maize milling on mycotoxin levels also need to be considered as they are generally located in the outer layers of the kernel (Ranum et al., 2014). Although the mycotoxins are not destroyed during milling, they are redistributed across the various milling products. Thus products such as bran, which contain the outer parts of the kernel and are generally used as animal feed, have increased levels and the fine flours much lower mycotoxin levels (Burger, Shephard, Louw, Rheeder, & Gelderblom, 2013). Although this may reduce the exposure of communities utilising sophisticated milling products, the prevalence in subsistence communities of simpler milling processes in which no separation of kernel components is achieved, implies that no such reduction occurs.

Of the different mycotoxins, aflatoxin produced by *Aspergillus* spp. and the fumonisins, produced by *Fusarium* spp. are common mycotoxin contaminants of maize and are known to adversely affect human and animal health (IARC, 2012). Implementation of the maximum levels of fumonisin set by the Codex Alimentarius Commission (FAO, 2014) for raw maize and maize flour (including maize meal) of 4 mg/kg and 2 mg/kg, respectively, will dramatically increase fumonisin exposure among southern African maize consumers. This has become apparent as the cooked maize intake among South African consumers range between 475 and 690 g/person/day (Nel & Steyn, 2002) with raw maize intake varying between 100 and 210 g/person/day (Burger, Lombard, Shephard, Danster-Christians, & Gelderblom, 2014). When superimposing these maximum levels to the Mycotoxin Risk Assessment Model (MYCORAM) for fumonisins, depending on the geographical area or Province, 73–97% of South African maize consumers will, under these regulatory conditions, be exposed to levels above the Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 μg/kg bw/day (Burger et al., 2014). Subsistent farmer communities in rural areas will be worst affected with fumonisin exposure levels far above the PMTDI.

The population in low income countries countries is not protected by strict international regulatory measures, underlining the necessity for implementation of mycotoxin control regulations. In this regard the World Health Organisation (WHO) made several recommendations for mycotoxin reduction and control involving an integrated approach including awareness campaigns, strengthened laboratory and surveillance capacities and establishing early warning systems (WHO, 2006). Other approaches such as the implementation of simple and affordable mycotoxin reduction techniques at household level and/or subsistence maize farming communities to effectively reduce exposure are becoming increasingly important. Integration of some of these community-based approaches with recent technological advances to control mycotoxin production and detoxification will be critically discussed in this review.

2. Technological methods for reduction

Technological approaches for mycotoxin reduction are mainly aimed at commercialization and application in areas with established infrastructure, i.e. developed countries. Genetic resources are utilised for breeding of maize cultivars resistant to fungal infection and subsequent mycotoxin contamination, and transgenic maize cultivars resistant to insect infestation and fungal colonisation (Cleveland, Dowd, Desjardins, Bhatnagar, & Cotty, 2003; Duvick, 2001). Information on the role of environmental factors influencing fungal growth and expression of mycotoxin biosynthetic genes could provide more gene targeting strategies to interrupt mycotoxin biosynthesis pre-harvest. The availability of genomic resources are essential for investigations into the biochemical and regulatory pathways of mycotoxin biosynthesis, pathogenesis of fungal-host interactions and the development of targeted and innovative approaches for breeding and engineering crops for resistance (Desjardins & Proctor, 2007). Whole genome sequences and expression sequence tags (ESTs) are important tools for understanding disease caused by fungi, fungal lifecycles and secondary metabolism (Brown, Butchko, & Proctor, 2006). Available genomic resources include genetic maps, genome sequences, EST libraries and integrated gene indexes. Genomic studies on several *Aspergillus* and *Fusarium* fungal species are well underway. Among these are structural, functional and comparative genomics of the toxin-producing species *Fusarium graminearum* (trichothecene producer), *Fusarium verticillioides* (fumonisin producer), and *Aspergillus flavus* (aflatoxin producer) (Xu, Peng, Dickman, & Sharon, 2006). As the number of fungi whose genome sequences have been elucidated continues to rapidly grow, so too does the potential to perform high throughput proteomic analysis on these organisms.

Fanelli, Iversen, Logrieco, and Mulè (2013) studied the effect of environmental conditions (temperature, water activity, pH and salinity) on fumonisin production and *FUM1* and *FUM21* gene expression by *F. verticillioides in vitro*. Gene expression mirrored fumonisin production profiles under all conditions with the exception of temperature: *FUM1* and *FUM21* expression was highest at 15 °C, while maximum fumonisin production was at 30 °C. These data indicate that a post-transcriptional regulation mechanism could account for the different optimal temperatures for *FUM* gene expression and fumonisin production. While transcriptional to translational correlations are often not very strong, it is essential to determine the gene expression to protein translation relationship in fumonisin production to better understand the mechanism

of biosynthesis.

Studies into molecular mechanisms involved in the secondary metabolism of microorganisms have experienced great advances in recent years. This is mainly due to the development of modern, high-throughput and information-rich "omic" techniques, together with genome sequencing tools and the development of mutants and reverse genetic approaches. Proteomic studies have shown to be effective for deciphering toxin production mechanisms as well as for identifying potential pathogenic factors (Houterman et al., 2007) in *Fusarium* and *Aspergillus*. Intracellular proteins potentially involved in *F. graminearum* virulence were up-regulated under *in vitro* trichothecene-inducing conditions as well as under infection conditions *in planta* (Taylor et al., 2008). Gel-based profiling analysis of *A. flavus* proteome and 2D proteome mapping using 2DE and MALDI-TOF-MS/MS, identified 538 mycelial proteins of the aflatoxigenic strain NRRL 3357 (Pechanova, Pechan, Rodriguez, Paul Williams, & Brown, 2013). The majority of these proteins were functionally annotated as related to various cellular metabolic and biosynthetic processes as well as a few enzymes from the aflatoxin synthesis pathway. Extracellular proteins during *F. graminearum* growth *in vitro* and infection of wheat heads *in planta* have been identified (Paper, Scott-Craig, Adhikari, Cuomo, & Walton, 2007). The proteins identified in the *in planta* study could play an important role in pathogenicity since secreted proteins constitute the first contact between a pathogen and its host and are virulence or avirulence effectors in various fungal diseases. The secretome analysis is also important since fungi secrete numerous extracellular enzymes which can also be used in early detection of contamination. Hou, Zheng, Xu, Chen, and Zhou (2013) conducted the first proteomic analysis of *F. graminearum* treated with the fungicide JS399-19 showing the effect on energy metabolism, the synthesis and transport of proteins and DNA as well as its effect on signal transduction in different degrees. This study provides a useful insight into the mode of action of the fungicide against the fungus.

The investigation of fungal-host interactions may provide important information on the changes in gene expression as well as changes in protein abundance in order to identify those proteins that are essential during such interactions. A proteomic approach was utilised to investigate the rachis tissue from aflatoxin accumulation resistant (Mp313E and Mp420) and susceptible (B73 and SC212m) maize inbred lines (Pechanova, Pechan, Williams, & Luthe, 2011). The differential expression of many stress/defence proteins during rachis juvenility, maturation and after *A. flavus* infection demonstrated that resistance relies on constitutive defences, while susceptibility is more dependent on inducible defences. Proteomic analysis, therefore has become a powerful tool in studying molecular and cellular mechanisms in plant-microbe interactions and in identifying gene products that play a key role in pathogenicity, virulence and host-resistance. This could potentially provide a better understanding of events of mycotoxin biosynthesis at a molecular level (Zhou et al., 2015) and assist in identifying key protein targets in the development of agrochemicals, which may open new ways for crop disease diagnosis and protection. In this regard novel strategies to control mycotoxin contamination could be developed to reduce the risk of mycotoxins exposure (Bhatnagar et al., 2008; Gašo-Sokač, Kovač, & Josić, 2010; Giacometti, Tomljanovíc, & Josić, 2013).

In the future, new methods will be required for identification, monitoring and assessment of foodborne hazards during production, storage, delivery and consumption (Giacometti et al., 2013). Integration of these new technologies in strategies of mycotoxin reduction in developing countries remains a major challenge, but should be eagerly pursued, further developed and made accessible.

2.1. Transgenic Bt maize

Genetically modified *Bt* maize contains *cry* genes from the bacterium *Bacillus thuringiensis*, which upon expression *in planta*, produce proteins toxic to various Lepidopteran pests, including the maize stalkborer, *Busseola fusca*, which is the predominant stalkborer on maize in southern Africa. This pest causes wounding in maize, which invariably leads to increased infection by certain *Fusarium* species, particularly *F. verticillioides*. Previous studies have shown that *Bt* maize has the potential to reduce insect damage and fumonisin levels compared to non-*Bt* hybrids (Abbas, Bellaloui, & Bruns, 2016; Munkvold, Hellmich, & Rice, 1999), but the effectiveness of *Bt* in reducing aflatoxin contamination is inconclusive (Abbas et al., 2013).

Insect resistant, transgenic (*Bt*) maize have seen impressive adoption rates since its introduction in South Africa in the 1998/1999 crop season (Gouse, Kirsten, & Van Der Walt, 2008). Results from a study conducted in rural areas in northern KwaZulu-Natal Province of South Africa, from 2004 to 2008 (Pray et al., 2013), demonstrated a clear advantage of *Bt* maize over conventional hybrids and traditional maize seed. *Bt* maize had 40% less fumonisin than traditional varieties. Relative to the non-*Bt* commercial maize hybrids, *Bt* maize had on average 16% less fumonisin contamination. A similar study of fumonisin in maize in Argentina and the Philippines showed that although the use of *Bt* maize reduced fumonisin levels considerably, location and weather factors had much more influence on the overall fumonisin data than the *Bt* trait itself (De la Campa, Hooker, Miller, Schaafsma, & Hammond, 2005). Field trials conducted in 2002 and 2003 at two locations in a commercial maize-growing area of South Africa showed that the fumonisin levels in the *Bt* hybrids were generally between 39 and 83% lower than their respective non-*Bt* isolines (Rheeder et al., 2005). The study also highlighted the considerable diversity in results among the different hybrids/isolines, locations and cropping seasons.

In South African rural areas, farmers are more likely to purchase herbicide tolerant transgenics than *Bt* hybrids. The highly variable incidence of stalkborer infestation due to the influence of seasonal and climatic conditions does not appear to warrant the purchase of expensive *Bt* seed (Gouse, 2012). As a result many poor subsistence farmers do not make use of transgenic hybrids due to its high cost and that it is more beneficial in planting herbicide tolerant hybrids and controlling weeds in their fields. In some areas farmers have been supplied with starter transgenic seed packs for one or two seasons, but are then expected to purchase the seed in the following years. These farmers prefer to revert back to their open pollinated or traditional varieties which they can grow from year to year without purchasing commercial seed each season (Fischer, Van den Berg, & Mutengwa, 2015).

2.2. Biocontrol with non-toxigenic microbial strains

Biocontrol of aflatoxin producing *Aspergillus* strains in the field relies on competitive exclusion, whereby large quantities of non-toxigenic inoculum (*A. flavus* and *Aspergillus parasiticus*) are introduced into the soil around growing crops and these then compete with toxigenic strains for infection sites on the developing crop (IARC, 2015). In Africa, the commercial use of AFLASAFE™ is now widely used in both West and East Africa, mainly for maize cultivation. The use of such a biocontrol agent would be beneficial when dealing with pre- and postharvest aflatoxin problems as this technology also brings benefits into storage (Bandyopadhyay, 2010). Thus, competitive exclusion in the field translates into a decreased risk of contamination during storage and transport due to fewer aflatoxin-producers moving into the store and the

biocontrol agents stay with the crop until use. This is of particular interest as the main contamination in Africa occur due to inadequate storage practices. Recent cases of aflatoxin poisoning in humans in Kenya have attributed the problem to ingestion of maize that was contaminated after harvest by improper storage (Ehrlich, 2014). In such circumstances, control strategies need to include pre-harvest and postharvest measures.

A number of environmental factors have been identified that affect the efficacy of biocontrol with non-toxigenic strains of *A. flavus*, including dew, moisture levels, very dry soils, heavy rains and timing of the application (Bock & Cotty, 1999). However, several other concerns regarding biocontrol need to be highlighted:

(i) The effect of non-toxigenic fungal strains on fungal dynamics in a specific host could affect the dominance of other toxigenic fungi such as *Fusarium*. This is of importance as it is known that fumonsins and aflatoxin co-occur.
(ii) Horizontal gene transfer of toxigenicity between toxigenic and non-toxigenic strains as a function of time.
(iii) Although non-toxigenic strains are introduced, continued mycotoxin monitoring is still required to confirm the reduction from a food safety perspective.
(iv) Does the application of the biocontrol agent pose any health risks (inhalation allergies, infectious potential) to field workers and surrounding communities, especially children and immune-compromised patients?
(v) Biocontrol strains must be carefully selected that are suitable for the environment and lack production of other mycotoxins such as cyclopiazonic acid produced by *A. flavus*.
(vi) Damage to crops by non-toxigenic strains under unfavourable climatic conditions could further contribute to food insecurity in poor subsistent farming communities.

Other bacterial and fungal strains showing potential as biocontrol agents by effectively reducing *Aspergillus* and *Fusarium* growth, include *Trichoderma* spp. (Calistru, McLean, & Berjak, 1997; Yates, Meredith, Smart, Bacon, & Jaworski, 1999) and *Bacillus subtilis* (Bacon, Yates, Hinton, & Meredith, 2001; Luongo et al., 2005). These organisms are mainly applied to soil or as seed treatments pre-harvest, but could also be applied postharvest during storage. Rhizobacterial strains of *Enterobacter cloacae, Arthrobacter globiformis, Azotobacter armeniacus, Pseudomonas solanacearum, Microbacterium eoleovorans, B. subtilis* and certain mixtures thereof proofed effective against *F. verticillioides* infection of maize seedlings at root level (Hinton & Bacon, 1995; Cavaglieri, Andrés, Ibáñez, & Etcheverry, 2005a, Cavaglieri, Orlando & Etcheverry, 2005b, Cavaglieri, Orlando, Rodríguez, Chulze & Etcheverry, 2005c). Biocompetitive inhibition of aflatoxigenic fungi by bacteria naturally occurring in the geocarposphere (zone around the groundnut pod) effectively protect developing groundnut pods (Chaurasia, 1995). In this regard *Flavobacterium odoratum* have shown effective inhibition of *A. flavus* growth and aflatoxin production. Although certain biocontrol microorganisms have been commercialized and are successfully being applied in African countries (South Africa, Kenya and Zambia), the potential for integration in rural subsistence farming communities still needs to be explored.

2.3. Enzymatic detoxification

Enzymatic detoxification of mycotoxins in food has become a promising approach and could find application in a far wider context including low socioeconomic subsistence farmer communities. The focus is on targeted modification of the chemical structures of mycotoxins by enzymatic cleavage or conversion of chemical bonds/groups that play a key role during cytotoxicity (Gelderblom, Cawood, Snyman, Vleggaar, & Marasas, 1993; Heinl et al., 2010). A knowledge base on reduction of mycotoxin levels by bacterial and fungal cultures has been established over the years (Alberts, Gelderblom, Botha, & van Zyl, 2009, 2006; Blackwell, Gilliam, Savard, Miller, & Duvick, 1999; Ciegler, Lillehoj, Peterson, & Hall, 1996; Duvick, Rood, & Wang, 1998b, 1998a; Hartinger et al., 2011; Heinl et al., 2010; Mann & Rehm, 1977; Teniola et al., 2005; Wu et al., 2015; Yehia, 2014). This information was further developed by identifying microbial enzymes responsible for detoxification; characterization of genes encoding the enzymes; expression of genes in food-grade microorganisms; and development of culture and recombinant enzyme preparations for commercial application. Enzymatic detoxification of AFB_1 mainly focuses on cleavage of the double bond of the furofuran ring through oxidation. The furofuran ring is actively involved in oxidation of AFB_1 to AFB_1-8,9-epoxide, a highly reactive precursor of malignant transformation (Minto & Townsend, 1997; Mishra & Das, 2003). Microbial manganese peroxide and oxidase enzymes catalyse cleavage of the 8,9-unsaturated carbon-carbon bond of AFB_1 through oxidation and hydrolysis to AF_1-8,9-dihydriol (Wang, Ogata, Hirai, & Kawagishi, 2011; Wu et al., 2015; Yehia, 2014). Treatment with laccase enzymes resulted in effective reduction of the mutagenic properties of AFB_1 (Alberts et al., 2009). With regards to the fumonisins, the free amino group plays a pivotal role in its toxicological effects *in vitro* and *in vivo,* while the tricarballylic acid moiety is required for effective absorption of the fumonisins from the gut of rats (Gelderblom et al., 1993). The fumonisins disrupt sphingolipid biosynthesis by inhibiting the enzyme ceramide synthase (Wang, Norred, Bacon, Riley, & Merrill, 1991), and the tricarballylic acid moiety is required for maximal effect (Van der Westhuizen, Shephard, Abel, Swanevelder, & Gelderblom, 1998). Complete detoxification of fumonisin B_1 (FB_1) is achieved through de-esterification by carboxylesterases and subsequent deamination of hydrolysed FB_1 (HFB_1) by aminotransferases, with the formation of 2-keto HFB_1 (Heinl et al., 2010). Recombinant enzymes for detoxification of mycotoxins are mainly produced by expressing bacterial genes, which are not necessarily considered “generally regarded as safe” (GRAS) by the United States Food and Drug Administration (US FDA), utilising *E. coli* (not GRAS) and *Pichia pastoris* (GRAS) expression systems.

Although certain enzyme technologies for detoxification have been commercialized and are considered safe for humans, animals and the environment by the European Food Safety Authority (EFSA), application is presently mainly directed towards the animal feed industry (Duvick, Maddox, & Gilliam, 2003, 1998b; Moll, Hartinger, Grießler, Binder, & Schatzmayr, 2011). Development of enzyme preparations with GRAS status could hold advantages for application in humans. Enzymatic methods could in future be applied in combination with community-based reduction methods in rural subsistence farming communities, to further reduce exposure to mycotoxins. However, integration of this kind of methodology in established communities could be a challenge due to strong cultural beliefs, traditions and practices.

2.4. Adsorbents

Another postharvest reduction approach involves the irreversible binding of mycotoxins in food by natural clay adsorbents, thereby disrupting the adsorption from the gastro-intestinal tract of humans and animals. The phyllosilicate clay montmorillonite tightly and selectively binds aflatoxins and fumonisins, thereby decreasing their bioavailability and associated toxicities in the gastrointestinal tract of experimental animals (Aly, Abdel-Galil, & Abdel-Wahhab, 2004; Adegoke, & Letuma, 2013; Mitchell et al., 2014; Phillips, Sarr, & Grant, 1995). Dietary calcium

montmorillonite (Uniform particle size, Novasil; UPNS) effectively reduces urinary biomarkers of AFB_1 and FB_1 in rats (Mitchell et al., 2014), while reduction of these biomarkers in human participants in study groups in Ghana has also been demonstrated (Phillips et al., 2008; Robinson et al., 2012). Montmorillonite clays are generally regarded as safe by the United States Food and Drug Administration [US FDA, GRAS substances evaluated by the Select Committee on GRAS substances (SCOGS)] and common African cooking practices do not affect the efficacy of its adsorption to aflatoxins (Elmore et al., 2014). Clay adsorbents are mainly applied in the food and beverage, and animal feed industries, while enterosorbents in the form of capsules could present a promising and sustainable public health intervention for application in African rural communities (Robinson et al., 2012).

Aflatoxin B_1 (AFB_1) is also effectively removed from the intestines of animals by binding to probiotic bacteria and the formation of aflatoxin-bacteria complexes (Peltonen, El Nezami, Salminen, & Ahokas, 2000). Bacteria capable of binding to aflatoxin include *Lactobacillus rhamnosus, Propionibacterium freudenrichii* and *Bifidobacterium* sp. The mechanism of binding involves hydrophobic and electrostatic interactions as well as the formation of hydrogen bonds. Application of dietary adsorbents in impoverished communities which are nutritionally compromised with respect to vitamins needs to be further investigated.

2.5. Good agricultural practices – rural versus commercial

Good agricultural practice (GAP), as with good manufacturing practise (GMP), is a prerequisite and complementary approach to the hazard analysis and critical control point (HACCP) system. GAP is an essential element in the agricultural industry in order to guarantee and maintain the lowest mycotoxin levels possible in cultivated food crops. These harvested crops are then transported from the farming environment to the food processing facilities, where GMP measures should be equally well entrenched.

The essence and details of GAP and HACCP are extensively covered and concisely summarised in several accessible sources (FAO, 2001; FAO, 2002; IARC, 2012). For the purpose of this publication, which is focussed mostly on African agriculture and its food industries (Adegoke & Letuma, 2013), a summary of key GAP activities is presented.

2.5.1. Pre-harvest strategies

- Implementation of a crop rotation schedule. Wheat and maize are particularly susceptible to *Fusarium* pathogens and these two crops should not be used in rotation with each other. Rotation crops such as potato, vegetables and dry beans, should rather be used to reduce the pathogen's inoculum in the field (commercial application mostly).
- Adequate preparation of the seed bed for each new crop by ploughing under or by removing old crop debris that may potentially serve as substrate for the growth of mycotoxin-producing fungi. Areas vulnerable to soil erosion, no-till and other integrated pest management practices may be required in the interests of soil conservation (commercial; subsistence application).
- Utilize soil tests to determine if there is need to apply fertilizer and/or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development (commercial application).
- Subsistence farmers should apply livestock manure to their fields to enhance stubble breakdown, improve the soil microbiome and aid in plant nutrition (subsistence application).
- Cultivate seed varieties developed for resistance to seed-infecting fungi and insect pests, and recommended for a specific area (commercial application mostly).
- Crop planting should optimally be timed to avoid high temperatures and drought stress during the period of seed development and maturation (commercial; subsistence application).
- Avoid overcrowding of plants by maintaining the recommended row and plant spacing (commercial application mostly).
- Minimize insect damage and fungal infection by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management programme (commercial; subsistence application).
- Control weeds in the crop mechanically or with the use of registered herbicides or other safe and suitable weed eradication practices (commercial; subsistence application).
- Minimize mechanical damage to plants and fruit during cultivation (commercial; subsistence application).
- Ensure that irrigation is applied evenly and that all plants in the field have an adequate supply of water. Excess moisture during flowering makes conditions favourable for the dissemination and infection by *Fusarium*; thus irrigation during flowering and crop maturation should be avoided (commercial application mostly).

2.5.2. Postharvest strategies

- Harvest grain at low moisture content and full maturity, unless under extreme heat, rainfall or drought conditions (commercial; subsistence application).
- Ensure that farm personnel are adequately trained and that all farming equipment is kept clean and functioning properly, to minimize damage to plants and the harvested crop (commercial; subsistence application).
- Containers and vehicles to be used for collecting and transporting the harvested crop from the field to drying and/or storage facilities, should be clean, dry and free of insects, soil and visible fungal growth (commercial; subsistence application).
- Determine crop moisture levels immediately after harvest. Where applicable, dry the crop to the recommended moisture content for storage. Cereals should be dried in such a manner that damage to the grain is minimized and moisture levels are lower than those required to support mould growth during storage (generally less than 14%). Sun drying of some commodities in high humidity may result in fungal infection. Avoid piling or heaping of wet, freshly harvested commodities (commercial; subsistence application).
- Freshly harvested cereals and nuts should be cleaned or sorted, where possible, to remove damaged kernels/nuts and other foreign matter (commercial; subsistence application).
- Storage facilities must be dry, well-vented structures that provide protection from rain, surface or ground water, protection from vermin and birds, and protected from extreme temperature fluctuations (commercial; subsistence application).
- Ensure that bags are clean and dry. Filled bags should be stacked on pallets or a system must incorporate a water impermeable layer between the bags and the floor (commercial; subsistence application).
- The relevant mycotoxin levels in harvested crops should be monitored when warranted, using appropriate sampling and testing programmes (commercial application).

3. Community-based methods for mycotoxin reduction

3.1. Hand-sorting and washing of grains

In sub-Saharan Africa, 80% of farms are smallholdings that likewise provide subsistence crops to their households (Mboya & Kolanisi, 2014). Hand-sorting or segregation of crops prior to storage or cooking, is a common practice in many African countries such as West Africa (Benin), Nigeria, Tanzania and Southern Africa (Fandohan et al., 2005.; Afolabi, Bandyopadhyay, Leslie, & Ekpo, 2006; Kimanya et al., 2008:; Van der Westhuizen et al., 2011, 2010; Matumba et al., 2015). Traditional food processing methods ideally form a sustainable, practical and inexpensive postharvest prevention strategy to reduce mycotoxin contamination and exposure. Several studies conducted in Africa among maize-subsistence farming communities indicated that the separation of visibly damaged (including broken), discoloured and obviously mouldy maize kernels from perceived good-for-eating kernels, decreased aflatoxin and fumonisin contamination levels. Compared to developed market economies utilising mechanical maize grain cleaners, with a reduction in aflatoxin and fumonisin levels of between 50 and 60% (Malone, Richard, Romer, Johansson, & Whitaker, 1998; Pacin & Resnik, 2012), hand-sorting proves to be just as effective. Van der Westhuizen et al. (2010; 2011) developed and validated a simple and culturally acceptable hand-sorting and washing intervention method in a community-based study. An overall decrease of 84% in FB_1 was observed, resulting in a reduction of 62% in the probable daily intake and 52% urinary excretion of FB_1. Effective separation of aflatoxin and fumonisin contaminated maize kernels from non-contaminated kernels, based on differences in density, can also be achieved through floating in water; 30% sucrose; or saturated sodium chloride solutions (Grenier, Loureiro-Bracarense, Leslie, & Oswald, 2014). The fate of rejected food is however a concern, as it might still be consumed by humans in times of food insecurity (IARC, 2015; Johnson, Atherstone, & Grace, 2015). Normally rejected food is used either as animal feed or in some circumstances for the preparation of traditional beer (Shephard et al., 2005).

3.2. Winnowing, shelling, dehulling and milling

Important postharvest practices include shelling, winnowing, dehulling and milling of maize. Shelling consists of the removal of the maize kernels from the cob and winnowing is a chaff-removal step to clean the kernels. Dehulling and milling removes parts of the kernel itself, with dehulling the pericarb (outer layers) and during milling the outer layers, hull, pericarp (bran), germ and tip cap are removed to expose the endosperm. In many African cultures such as in West and South Africa, these physical processes are traditionally conducted by women by hand, using mortar and pestle, grinding rocks and/or wooden sticks to stamp the maize (Abass et al., 2014; Fandohan et al., 2006; Grobbelaar & Bateman, 1991; Rose, 1972). In some areas mechanical dehulling and milling technologies are available (Abass et al., 2014). In a study conducted in Benin, West Africa, the cumulative use of sorting, winnowing, washing crushing and dehulling of maize resulted in a reduction of between 30 and 40% in aflatoxin and fumonisin levels (Fandohan et al., 2005). The efficacy of dehulling and milling to reduce mycotoxin levels are related to the higher susceptibility of the outer layers (hull, germ and pericarp) of the maize kernels to fungal colonisation and mycotoxin production and the physical removal of these (Brera, Debegnach, Grossi, & Miraglia, 2004; Bullerman & Bianchini, 2007; Burger et al., 2013; Kent & Evers, 1994; Scudamore, Banks, & MacDonald, 2003).

4. Diet/crop diversification

Dietary diversification as a strategy to reduce mycotoxin exposure is focused on the quality of food, its micronutrient sufficiency and available energy (Arimond & Ruel, 2004; Hoddinott & Yohannes, 2002; Moursi et al., 2008). Whether this dietary strategy can be grouped under community-based methods to reduce mycotoxin exposure in Sub-Saharan Africa is a contested matter, although improving food preferences in any population is known to have a wholesome effect on both nutritional and health issues (FAO, 1997, Chap. 5; Ruel, 2003; Frison, Smith, Johns, Cherfas, & Eyzaguirre, 2006; Lovo & Veronesi, 2015; Wu, Mitchell, Male, & Kensler, 2014b). The main threat to diet diversity, excluding important environmental factors, comes from food insecurity, cultural traditions and poverty facing in Sub-Saharan Africa (Mugendi Njeru, 2013). However, in countries such as China where agricultural reform was introduced in the 1980's, the trading of other crops besides maize and the inclusion of other crops (such as rice) and foods in the population diet, reduced aflatoxin exposure and liver cancer mortality (Chen et al., 2013; Lin, 1992). The challenges associated with introducing diet diversity as a part of public health policy in most developing countries will be enormous when considering the scarcity of food and unfavourable climate changes. Therefore, despite strong evidence of improvement of health, dietary diversification as an intervention strategy is faced with major challenges with respect to the practical implementation in poor subsistent mono-cereal diet populations (IARC, 2015).

Crop diversification is a known agricultural strategy and perceived as a "risk coping" practice by farmers during inadequate climate conditions (Di Falco & Veronesi, 2013; Seo & Mendelsohn, 2008; Wang, Mendelsohn, Dinar, & Huang, 2010). Insufficient crop diversification practices are also linked to a lack of dietary diversity (Arimond & Ruel, 2004). The impact of crop diversification on child growth, utilising dietary survey data from Tanzania (Tanzania National Panel Survey) have shown a positive and significant impact on child nutritional status, particularly for girls, as well as on child height (Lovo & Veronesi, 2015).

5. Biomarkers of exposure – measurement of human exposure to mycotoxins

The quantitative measurement of human exposure to mycotoxins is challenging. Yet the development and implementation of the reduction methods described in this manuscript need to be assessed and verified by suitable analytical methods. Intake of mycotoxins via the diet is a function of both the contamination level of the food and the quantities consumed. The non-homogeneous distribution of mycotoxins in food is a source of experimental error, especially in analyzing individual raw food components. Although this can be minimized by duplicate diet analysis, in dishes other than simple cereal porridges, the diversity of ingredients in a prepared food dish can give rise to analytical problems caused by dilution of the contaminant and from analytical recovery from complex food matrices. This is further complicated by possible mycotoxin-food component interactions and any mycotoxin loss that may occur during food preparation. In certain foods the presence of modified or "masked" mycotoxins, which are not detected in traditional analytical methods and whose fate after ingestion is unclear, adds to the uncertainty of actual exposure (Dall'Asta & Berthiller, 2015). Similarly, the measurement of food intake by traditional methods such as food frequency questionnaires and 24-h recalls is subject to various biases. For these reasons, the development of a biological marker (biomarker) of exposure to a specific mycotoxin provides a single measurement that represents mycotoxin exposure from all intake sources and can

be related to health impacts (Paustenbach & Galbraith, 2006). Such a measure circumvents problems in assessing contaminant levels in foods consumed and individual food intakes. In addition, it accounts for mycotoxin loss during food preparation for individual variation in mycotoxin absorption from the gut and, ideally, for differences in physiological responses of consumers. It can also take into account all forms of the mycotoxin ingested and available for absorption, including modified or masked forms now known to be present in addition to the basic toxin.

To date, the development of biomarkers has been most successfully pursued for aflatoxin. AFM_1 (a hydroxylated AFB_1 metabolite) in urine, the aflatoxin-N7-guanine adduct in urine and the AFB_1-albumin adduct in blood plasma have all found application in linking aflatoxin exposure to adverse health outcomes such as primary liver cancer and early childhood stunting (Kensler, Roebuck, Wogan, & Groopman, 2011). Of these three measures, the urinary biomarkers represent relatively recent intake, whereas, due to the long half-life of albumin, this biomarker represents moderate to long term exposure of several months. A successful intervention study in West Africa, which reduced aflatoxin exposure by introducing a package of drying and storage measures in subsistence farming villages, was monitored using an aflatoxin biomarker (Turner et al., 2005).

Initial attempts to develop a FB_1 biomarker involved analysis of sphingoid bases in plasma or urine, or alternatively measurements of FB_1 in faeces and hair (Shephard, van der Westhuizen, & Sewram, 2007). These have been supplanted by measurement of urinary FB_1, which was shown to be correlated with tortilla consumption in a study in Mexico (Gong et al., 2008). Subsequently, urinary FB_1 has been used to validate an intervention method to reduce fumonisin exposure in a rural population by a culturally acceptable maize sorting and washing technique (Van der Westhuizen et al., 2011). Although these biomarkers can be determined individually, recent advances in HPLC-MS enables the urinary biomarkers to be analyzed by multi-mycotoxin methodology, which can also include urinary markers for exposure to other mycotoxins such as deoxynivalenol, ochratoxin A and zearalenone (Shephard et al., 2013).

6. Conclusions

Challenges regarding the integration of technological and community-based mycotoxin reduction strategies into remote rural subsistence farming communities include the availability of maize (food insecurity); the traditional use of mouldy maize (beer-making, specific maize-based dishes or animal feed); cultural beliefs and practices; education level; and the socioeconomic status of the specific population (Fandohan et al., 2005; Kimanya et al., 2008). Therefore, a partnership between researchers, funders, agricultural- and public health stakeholders will be necessary to drive the implementation and sustainability of mycotoxin reduction strategies in low-income maize-subsistence farming countries. Superimposed on this are differences that exist in mycotoxin regulations on maximum levels that determine the international trade of major food commodities which could be detrimental to the health of people residing in low-income countries. Apart from this, there is also a lack of scientific field testing, validation and continued monitoring of compliancy regarding mycotoxin reduction strategies in developing countries that hinders large-scale implementation and integration into health policy. Country-specific data on exposure to mycotoxins are lacking in developing countries, due to a lack of resources and sophisticated analytical techniques and large differences that exist in the quality and quantity of surveillance data generated by laboratories in developed countries and developing countries (IARC, 2015). To ensure local sustainability of interventions, continued interaction and education of local stakeholders such as agricultural extension officers, health- and community workers and traditional leadership will be critical. The lack of effective and sustained awareness and education of the threat of mycotoxins to human health in high-risk areas in sub-Saharan Africa will hinder any reduction strategy.

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