Wageningen Academic Publishers

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Atoxigenic-based technology for biocontrol of aflatoxin in maize and groundnuts for Tanzania

G. Mahuku[1*], A. Mauro[1], B. Pallangyo[2], E. Nsami[3], S.B. Boni[1,4], E. Koyano[1], O. Mponda[5], A. Ortega-Beltran[6], J. Atehnkeng[6], F. Aquiline[1], R. Samuel[1], J. Njela[1], P.J. Cotty[7] and R. Bandyopadhyay[6]

[1]International Institute of Tropical Agriculture (IITA) Eastern Africa Hub, Plot 25, Mwenge Coca-Cola Road, Mikocheni B, P.O. Box 34441, Dar es Salaam, Tanzania; [2]Plant Health Services, Ministry of Agriculture, P.O. Box 2182, Dodoma, Tanzania; [3]National Biological Control Center, P.O. Box 30031, Kibaha, Tanzania; [4]World Vegetable Center, Eastern and Southern Africa, P.O. Box 10, Duluti, Arusha, Tanzania; [5]Department of Research, Ministry of Agriculture, Livestock and Fisheries, P.O. Box 9192, Dar es Salaam, Tanzania; [6]IITA Headquarters, PMB 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria; [7]USDA-ARS, 416 West Congress Street, Tucson, AZ 85701, USA; g.mahuku@cgiar.org

Received: 2 December 2021 / Accepted: 6 May 2022
© 2022 Wageningen Academic Publishers

OPEN ACCESS **RESEARCH ARTICLE**

Abstract

Application of biocontrol products containing atoxigenic isolates of *Aspergillus flavus* to reduce aflatoxin content in crops is an effective strategy for managing aflatoxin in several regions throughout the world. We report the development and validation of two aflatoxin biocontrol products, Aflasafe TZ01 and Aflasafe TZ02, for use in maize and groundnut in Tanzania, a country frequently affected by aflatoxin contamination. Each product contains four atoxigenic *A. flavus* genotypes native and widely distributed in Tanzania. Efficacy tests on maize and groundnut were conducted over two years and in four regions of Tanzania where aflatoxin contamination is prevalent. Application of both products significantly ($P<0.05$) reduced aflatoxin levels in maize and groundnut in both years and in all districts. No differences were observed in total *Aspergillus* section *Flavi* population in treated and untreated fields, revealing that application of the biocontrol products do not alter overall *Aspergillus* populations in the environment. The results indicate that both products are effective tools for aflatoxin mitigation in groundnut and maize. The products were officially registered in 2018. Currently, there are scale-out and-up efforts of aflatoxin biocontrol products in Tanzania through a private sector company that is making the products available to farmers. Protecting maize and groundnut from aflatoxin contamination in Tanzania can result in health, income, and trade benefits.

Keywords: *Aspergillus flavus*, aflatoxin, biocompetitive exclusion, biological control, mycotoxigenic fungi

1. Introduction

Agricultural productivity enhancement, local procurement, and robust market access efforts are being scaled up in sub-Saharan Africa (SSA) in light of recent calls for sustainable development goals and population growth (FAO, 2018; Thompson *et al.*, 2017). However, food quality, food safety and postharvest losses issues resulting from aflatoxin contamination negatively affect programs designed to improve nutrition, increase agricultural production, and reduce postharvest losses while linking smallholder farmers to markets. Aflatoxins are naturally occurring toxic compounds produced by fungi belonging to *Aspergillus* section *Flavi* that are highly dangerous even at minute concentrations (Cotty *et al.*, 1994).

Aflatoxin producers are ubiquitous in warm agricultural areas. They occupy soil, colonise diverse organic matter, and produce conidia that associate with crops leading to aflatoxin contamination in both fields and stores (Horn, 2003). *Aspergillus flavus* is the major aflatoxin producer. There are other aflatoxin producers, such as *Aspergillus parasiticus* and *Aspergillus nomius,* but they are less frequently implicated. *A. flavus* is subdivided into two distinct morphotypes, the L and the S, based on morphological, physiological, and genetic criteria (Cotty,

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

1989). Both morphotypes can produce B aflatoxins but there are many L morphotype genotypes that cannot produce aflatoxins (hereafter referred as atoxigenic) due to natural defects in genes necessary for aflatoxin biosynthesis (Chang *et al.*, 2012). The S morphotype of *A. flavus* is not very common in African countries, but fungi belonging to diverse species resembling this morphotype (e.g. *Aspergillus aflatoxiformans*, *Aspergillus minisclerotigenes*) are relatively common across Africa (Frisvad *et al.*, 2019; Singh *et al.*, 2020). Some of these species produce both B and G aflatoxins.

Aflatoxins are a global health threat because of their acute and chronic health effects on humans and domesticated animals. Aflatoxin exposure is frequent and widespread in most African countries where key staples, maize and groundnut, are particularly vulnerable (Meijer *et al.*, 2021). Exposure to high doses of aflatoxins cause serious illnesses such as acute liver cirrhosis and death as reported in Kenya (Lewis *et al.*, 2005) and Tanzania (Kamala *et al.*, 2018). Chronic exposure is widespread, leading to potential negative nutritional and immunological effects, and cancer (Udomkun *et al.*, 2017). Aflatoxins interact in an adverse way (i.e. exhibit co-morbidity) with Hepatitis B virus (HBV). Combined exposure to aflatoxin and HBV increases rates of liver cancer to 60 times more than either factor alone (Liu *et al.*, 2012). A health economics study sponsored by the Partnership for Aflatoxin Control in Africa (PACA) in Tanzania estimated that a median total of 546,000 disability-adjusted life years (DALYs) are lost because of morbidity and mortality from liver cancer resulting from synergistic effects of chronic aflatoxin and HBV exposure. The median total cost of illness was estimated at US$ 32,540 (range: US$ 10,230 to 90,180). Monetary loss at a median of US$ 332.5 million (range: US$ 93 to 758 million) would accrue mostly from these DALYs associated with mortality and morbidity. Health related economic cost of aflatoxin exposure would be even higher if DALYs associated with stunting and immune system suppression were included in the analysis (Abt Associates, 2013).

Maize is the single most important staple food in Tanzania with an estimated annual per capita consumption of 128 kg (Mboya *et al.*, 2011; Suleman and Kurt, 2015). Maize and groundnut account for >40% of the calorie intake, and on average each Tanzanian consumes 520 g of maize and groundnut per day (Abt Associates, 2013). In addition, both maize and groundnut are important basic ingredients in complementary weaning foods (Kimanya *et al.*, 2008; Magoha *et al.*, 2014; Mboya *et al.*, 2011). Thus, even small aflatoxin levels in maize and groundnut could present a high risk of aflatoxin exposure. In Tanzania, most of the population are subsistence agricultural farmers who consume most of what they produce. Therefore, the highest impact of aflatoxin is on health of the local population, as was observed in 2016, when 68 people fell ill after consuming aflatoxin contaminated maize products and 20 lost their lives (Kamala *et al.*, 2018). Several studies have documented both high exposure and adverse effects (stunting, underweight, and wasting) in Tanzanian children <5 years (Routledge *et al.*, 2014; Shirima *et al.*, 2013, 2015). Up to 95% of maize samples collected from Kilosa district had detectable aflatoxin levels and up to 1,080 μg/kg (Kamala *et al.*, 2015); thus, many people in Tanzania are chronically exposed to aflatoxins, putting their health and lives at severe risk.

Economic growth, increased farmers' income, and poverty reduction occurs when farmers can reach export markets or premium domestic markets (Diao *et al.*, 2013). However, aflatoxin contamination limits domestic, regional, and international trade of affected commodities. Many countries have established regulations to limit human and animal exposure to aflatoxins, expressed in μg/kg or parts per billion (EC, 2007, 2010; Matumba *et al.*, 2017; Van Egmond, 2002). Producers, traders, and processors incur operating costs as they strive to meet the standards of the markets they intend to reach, either domestically or internationally. Rejection of crop lots exceeding aflatoxin tolerance thresholds imposed by the importing countries, or local industries, results in substantial economic losses. Recently, Kenya banned the importation of maize from Tanzania and Uganda due to aflatoxin (The Independent, 2021) and this resulted in huge economic losses for farmers. Even with regulations in place, food that does not move through formal export market channels (i.e. almost all food sold in local markets) is effectively unregulated. To make matters worse, products are pre-screened based on aflatoxin levels prior to export, without destruction of contaminated material, effectively concentrating contaminated crops in the local food chain. Therefore, the local population is exposed to unacceptably high levels of aflatoxins.

Minimising aflatoxin contamination of maize and groundnut is required to protect Tanzanian populations from adverse effects of aflatoxins and opening regional and international markets. The use of native atoxigenic *A. flavus* strains to mitigate aflatoxin contamination has been demonstrated as the most effective technology for reducing aflatoxin in various crops (Mehl *et al.*, 2012). Aflatoxin content in treated crops is generally 80% less (sometimes 100% less) compared to adjacent untreated crops (Agbetiameh *et al.*, 2020; Bandyopadhyay *et al.*, 2016; Lewis *et al.*, 2019; Senghor *et al.*, 2020). Biocontrol products based on atoxigenic *A. flavus* strains are already commercially available in USA (AF36 and afla-guard®) (Cotty *et al.* 2007; Dorner 2004), and in several African countries under the tradename Aflasafe (Moral *et al.* 2020; Schreurs *et al.* 2019). To pave way for large scale commercial use, a biocontrol product must be registered with the biopesticide authorities in the country for intended use. To be able to do this, data are required on efficacy of the product to minimise aflatoxin

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

contamination with no significant adverse effects, nor alteration in overall population densities of *Aspergillus* spp. in the soil. Therefore, this study was conducted to evaluate the efficacy of two experimental products, Aflasafe TZ01 and Aflasafe TZ02, each containing four atoxigenic *A. flavus* isolates belonging to distinct vegetative compatibility groups (VCGs) native to Tanzania as active ingredients in reducing aflatoxin contamination in maize and groundnut in different agroecological zones (AEZs) in Tanzania. Evaluations were conducted over a two-year period under farmer-field conditions on fields planted with maize and groundnut. The results demonstrate that both experimental products are highly effective in reducing pre-harvest aflatoxin contamination in maize and groundnut across different AEZs of Tanzania.

2. Materials and methods

Atoxigenic active ingredients

Atoxigenic *A. flavus* strains native to Tanzania were identified from initially over 5,000 isolates of *Aspergillus* section *Flavi* recovered from naturally infected maize and groundnut seed collected from fields and farmer stores in different AEZs of Tanzania. Several *A. flavus* VCGs composed entirely by atoxigenic isolates were detected and the genetic basis of their atoxigeniciy determined. Based on the relative adaptation to maize and groundnut cropping systems, frequency of occurrence, and competitive potential to move to crops and limit crop aflatoxin content (unpublished results), eight atoxigenic *A. flavus* isolates were selected as active ingredient fungi for the formulation of two aflatoxin biocontrol products: Aflasafe TZ01 and Aflasafe TZ02. Each biocontrol product contains four atoxigenic isolates as active ingredient fungi. Information of the origin of the eight atoxigenic isolates composing Aflasafe TZ01 and Aflasafe TZ02 is given in Table 1. The atoxigenic isolates of the two biocontrol products developed for use in Tanzania are maintained in the fungal culture collection of the Pathology and Mycotoxin laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan-Nigeria.

Biocontrol product preparation

Biocontrol products containing the atoxigenic *A. flavus* strains were produced according to the method described by Garber *et al.* (2012). Roasted sorghum grains were used as the inoculum carrier and coated with a suspension containing the atoxigenic isolates. To make the suspensions, mother cultures of the atoxigenic *A. flavus* isolates were first grown on 5-2 agar (5% V8 Juice [Campbell Soup Company, Camden, NJ, USA] and 2% Bacto-agar [Difco Laboratories, BD, Franklin Lakes, NJ, USA], pH 6.0) and incubated at 31 °C for 5 d. Then, large quantities of spores were produced on sorghum grains placed in flasks with moisture levels adjusted to 20% using a suspension of spores from the 5-d-old cultures. Flasks were sealed with sterile Tyvek® membrane (Dupont, Wilmington, DE, USA) to control humidity levels but allow gas exchange, and incubated (7 d, 35 °C). The spore suspension for biocontrol production was prepared by harvesting spores with 100 ml sterile 0.1% Tween80, and concentration adjusted to 1×10^8 spores/ml using a haemocytometer. The final suspension was mixed with sterile sorghum grains, a polymer, and a dye. The dye was used to differentiate sorghum coated with atoxigenic *A. flavus* isolates from regular sorghum. This process has been described in detail (Senghor *et al.*, 2020).

Table 1. Origin of isolates of native atoxigenic *Aspergillus flavus* Vegetative Compatibility Groups (VCGs) used as active ingredients in biocontrol products developed for aflatoxin mitigation in Tanzania.

Product/VCG	Isolate[1]	Origin substrate	Region	District	Basis for selection[2]
Aflasafe TZ01					
TMS199-3	TMS 199-3	maize soil	Iringa	Iringa Rural	wide geographic distribution of atoxigenic members
TMH104-9	TMH 104-9	maize grain	Rukwa	Nkasi	wide geographic distribution of atoxigenic members
TGS364-2	TGS 364-2	groundnut	Manyara	Kiteto	wide geographic distribution of atoxigenic members
TMH30-8	TMH 30-8	maize grain	Manyara	Babati	wide geographic distribution of atoxigenic members
Aflasafe TZ02					
TMS64-1	TMS 64-1	maize	Morogoro	Kilosa	large deletions in aflatoxin genes, wide geographic distribution in maize fields
TGS55-6	TGS 55-6	groundnut	Manyara	Babati	large deletions in aflatoxin genes
TMS205-5	TMS 205-5	maize	Iringa	Makambako	wide geographic distribution in both maize and groundnut fields
TMS137-3	TMS 137-3	maize	Rukwa	Sumbawanga Rural	isolated from both maize and groundnut

[1] Representative isolates of VCGs used as the active atoxigenic isolate for the biocontrol products.
[2] Unpublished results.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Quality control of the atoxigenic biocontrol products

The quality of the products (purity, sporulation, and composition of the active ingredient fungi) was measured for both Aflasafe TZ01 and Aflasafe TZ02. Approximately 100 g of inoculated sorghum grains were collected per each 20 kg of finished product and transferred to sterile plastic bags. For each sample 100 biocontrol sorghum grains were placed on two plates each of 5-2 agar, Nutrient Agar (Lam M; 28 and 20 g/l glucose), and Violet Red Bile Agar (VRBA; Difco Laboratories; 41.5 g/l, pH 7.4) in a biosafety cabinet. Plates were incubated in a closed plastic container at 31 °C for 7 d and examined to count the number of grains colonised by *A. flavus* and presence/absence of any other microorganism, including faecal coliforms on VRBA. Spore production was evaluated by placing 24 grains from each batch in individual wells in 24-well plates and incubating as above. After incubation, three replicates of two seeds in the 24-well plates were rinsed three times with 10 ml 100% ethanol. The resulting wash from each replicate was mixed with 10 ml distilled water and poured into a turbidimeter vial. Spore yield was quantified by turbidity using an Orbeco-Helling digital direct reading turbidimeter (Orbeco Analytical Systems Inc., Farmingdale, NY, USA) and a nephelometric turbidity unit (NTU) versus colony-forming unit (cfu) standard curve (y = 49,937x; x = NTU; y = spores/ml). From each subsample, 20 isolates were examined to assess membership in VCGs to which Aflasafe TZ01 and Aflasafe TZ02 isolates belong. This was done using nitrate non-utilising (*nit*) mutants, which were generated following previously described protocols (Atehnkeng *et al.*, 2014, 2016). All recovered mutants were tested for membership in one of the four Aflasafe TZ01 of Aflasafe TZ02 VCGs using vegetative compatibility assays. Fungal suspensions (15 μl containing ~150 spores) of each VCG tester pair and the mutant of interest were seeded into 3-mm diameter wells 1 cm apart (in a triangular pattern) in starch agar (36 g/l dextrose, 20 g/l soluble starch, and 2% Bacto-agar, pH 6.0) (Cotty and Taylor, 2003) and incubated for 7 d at 31 °C. Mutants of isolates complementing a tester pair of a VCG were assigned to that VCG. Complementation was observed as a zone of dense prototrophic growth where complementary mutants met and fused.

Site and field selection

Districts for testing the effectiveness of the biocontrol products in maize and groundnut were selected based on information obtained during a national survey conducted in 2012 (Boni *et al.*, 2021). Eleven districts in four maize and/or groundnut producing regions and known for high aflatoxin contamination (Boni *et al.*, 2021) were selected (Figure 1). Stakeholders composed mainly of farmers and Agricultural Extension Agents (AEAs) of the Ministry

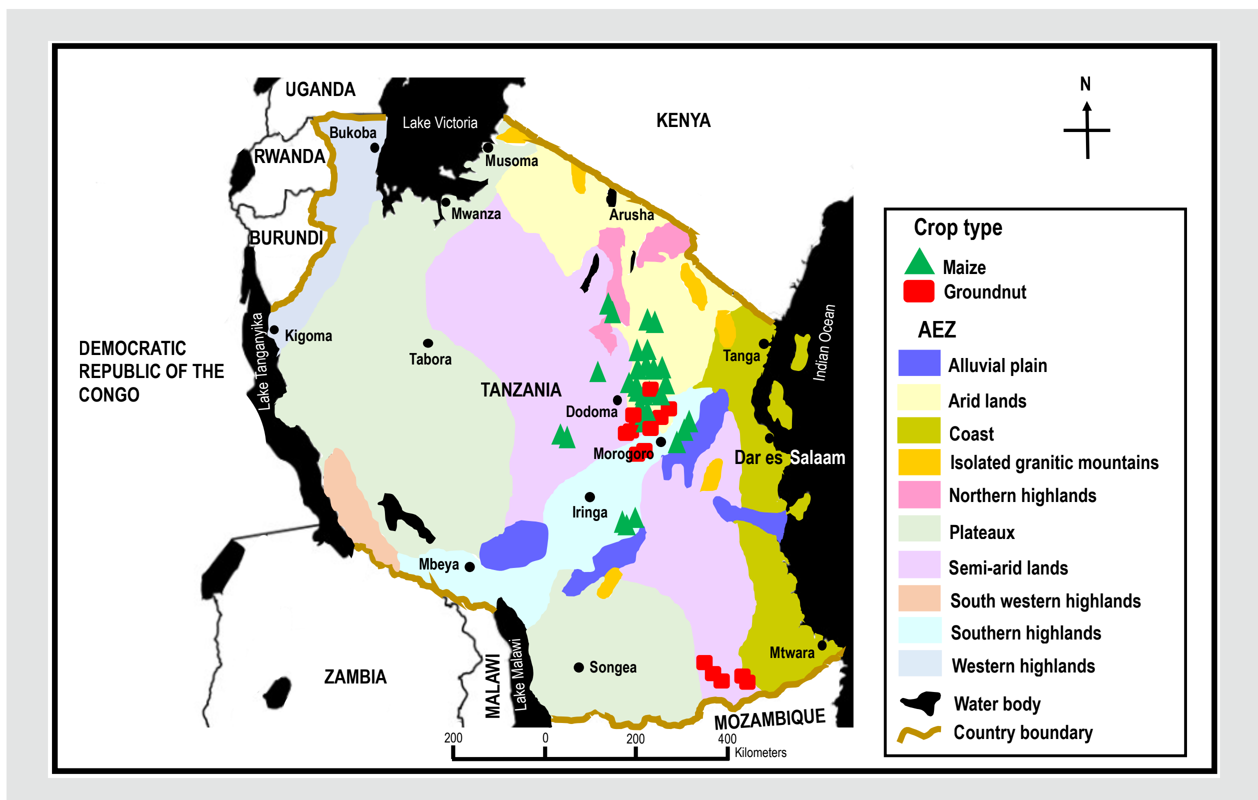

Figure 1. Map of Tanzania indicating regions where efficacies of two biocontrol products were tested for aflatoxin mitigation in maize and groundnut during 2016 and 2017 seasons.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

of Agriculture (MoA) were sensitised and trained on the basics of crop aflatoxin contamination and its management including use of biocontrol products. Farmer and farmer-field selection was done in collaboration with officials from the District Agriculture, Irrigation and Cooperative Officer (DAICO) of MoA. The products were evaluated in 216 maize and 118 groundnuts smallholder farmer fields in 2016 and 2017 (Table 2). Agronomic management of both crops followed practices recommended by agricultural specialists in their respective areas without any other special intervention. An untreated field of the same crop, separated by a minimum of 25 m from treated fields, served as a control for each treated field. The size of experimental fields ranged from 0.5 to 2 ha. All farmers voluntarily agreed to host efficacy trials. Farmers' fields were weeded by hand and top-dressed with urea prior to biocontrol application. No other agronomic interventions were made.

Product application and sample collection

Prior to biocontrol application, sub-samples of topsoil (~2 cm depth) were taken randomly from 50 different spots to compose a sample of about 150 g for each treated and corresponding untreated field. Similarly, soil samples were collected three months after harvest. Biocontrol products were broadcasted by hand on the soil surface 35 to 40 d after planting for groundnut and 2-3 weeks before flowering for maize and at a rate of 10 kg/ha as described by Agbetiameh *et al.* (2019). Farmers were advised to finalise agronomic operations before treatment and reduce movement in the field for 7 to 10 d after treatment so that the product remained on the soil surface. At harvest, five quadrants measuring 5 m^2 were identified for each field (treated and untreated). For maize, five cobs were randomly selected from each quadrant to give a total of 25 cobs per field. The cobs were hand shelled, and the grain thoroughly mixed to form a composite sample per field. The moisture content of harvested grain was measured and where the crop had

Table 2. Number of groundnut and maize fields treated with Aflasafe TZ01 and TZ02 and accompanying untreated fields in different districts of Tanzania in 2016 and 2017.

Year/Region	District	Crop	Aflasafe TZ01				Aflasafe TZ02			
			Maize		Groundnut		Maize		Groundnut	
			U[1]	T[1]	U	T	U	T	U	T
2016										
Manyara	Babati	maize	12	12	–[2]	–	12	12	–	–
Dodoma	Kibaigwa	maize	10	10	–	–	10	10	–	–
Morogoro	Kilombero	maize	10	10	–	–	10	10	–	–
Morogoro	Kilosa	maize	9	9	–	–	10	10	–	–
Mtwara	Masasi	groundnut	–	–	9	9	–	–	10	10
Dodoma	Mpwapwa	groundnut	–	–	11	11	–	–	11	11
Mtwara	Nanyumbu	groundnut	–	–	7	7	–	–	9	9
Total			41	41	27	27	42	42	30	30
2017										
Dodoma	Chamwino	maize	10	10	–	–	10	10	–	–
Dodoma	Chemba	maize	9	9	–	–	10	10	–	–
Dodoma	Kibaigwa	maize	5	5	–	–	5	5	–	–
Morogoro	Kilombero	maize	10	10	–	–	10	10	–	–
Morogoro	Kilosa	maize	10	10	–	–	10	10	–	–
Manyara	Kiteto	maize	8	8	–	–	9	9	–	–
Dodoma	Kondoa	maize	11	11	–	–	9	9	–	–
Dodoma	Kongwa	grundnut	–	–	9	9	7	7	–	–
Mtwara	Masasi	groundnut	–	–	8	8	–	–	8	8
Dodoma	Mpwapwa	groundnut	–	–	10	10	–	–	10	10
Mtwara	Nanyumbu	groundnut	–	–	8	8	–	–	8	8
Total			72	72	26	26	70	70	26	26
Grand total			113	113	53	53	112	112	56	56

[1] U = untreated; T = treated.

[2] Efficacy trials were not conducted.

not sufficiently dried to less than 14% moisture content, the grain was dried on plastic mats. A 1 kg sample was drawn from treated and untreated fields for subsequent aflatoxin quantification. All crop and soil samples were sent to IITA-Dar es Salaam for aflatoxin and microbiological analyses. Samples were stored in a deep freezer (-20 °C) until processed. A similar procedure was followed for groundnuts; plants from each quadrant were harvested and a 5 kg (in-shell) sample collected.

Aflatoxin quantification

Total aflatoxins in maize and groundnut samples were extracted and analysed using the Reveal® Q+ kit (Reveal Q+ for aflatoxin with AccuScan testing system, Neogen Corporation, Lansing, MI, USA) following the manufacturer's instruction. For groundnut, 50 g grain sample were combined with 100 ml 80% methanol (Cole and Dorner, 1994), and milled using a laboratory blender (Waring Commercial, Springfield, MO, USA) for 1 min in a 1 l stainless steel blending jar (MC-2). The blending jar was washed between samples with 80% ethanol to prevent aflatoxin cross contamination. Maize grains were pulverised to a fine powder in a coffee grinder (Bunn Company, Springfield, IL, USA), 50 g of maize flour drawn and mixed with 250 ml 65% ethanol and shaken for 30 min at 400 rpm using an orbital shaker [HS501, IKA Werke Company, Staufen, Germany) or a Roto-Shake Genie (Scientific Industries, Bohemia, NY, USA)]. The mixture was passed through filter paper (Whatman paper No. 1, Buffalo Grove, IL, USA) and the filtrate (100 µl) was added to a diluent (500 µl) and mixed by inverting tubes 5 times. The mixture (100 µl) was transfer to a new tube and an aflatoxin strip was placed into the tube and kept for 6 min before reading using AccuScan testing system. Reveal Q+ kit quantifies aflatoxins in the range of 2-150 µg/kg. Values above the upper limit were diluted and read again in order to bring the quantification inside the range.

Mycoflora analysis in soils and grains

Densities and composition of communities of *Aspergillus* section *Flavi* were determined in soil prior to application of the biocontrol products, grains at harvest, and soil collected three months after harvest for both treated and untreated fields. Briefly, soil samples were dried in a forced air oven (50 °C, 48 h), aseptically pulverised and sieved through 2 mm wire mesh to remove gravels and large particles. *Aspergillus* section *Flavi* fungi in soils and grains were isolated using dilution plate technique on modified rose Bengal Agar (MRBA) as described by Atehnkeng *et al.* (2014). A 1 g of sample was suspended in 10 ml sterile distilled water, mixed by shaking for 20 min (100 rpm) on an orbital shaker. Aliquots (100 µl per plate) of the resulting suspension were spread on three MRBA Petri plates. Adjustments to aliquot volume or sample quantity were made to obtain less than 10 *Aspergillus* section *Flavi* colonies per plate. Plates were incubated at 31 °C in the dark for 3 d. Incidences of *Aspergillus* spp. in soils and grains were calculated as cfu/g of sample. From each sample, 16 discrete *Aspergillus* colonies were sub-cultured on 5-2 agar (31 °C, 7 d). Isolates were assigned to their corresponding species/morphotype (*A. flavus* L morphotype, fungi with S morphotype, *A. parasiticus*, or *Aspergillus tamarii*) based on colony characteristics and spore morphology (Cotty, 1989). There are several species with phenotype similar as to that of the *A. flavus* S morphotype (Frisvad *et al.*, 2019); since molecular characterisation was not conducted, we refer to all those morphologically similar isolates as fungi with S morphotype. Isolations were performed at least twice for each sample. Isolates were saved and stored as agar plugs (3 mm diameter) of sporulating cultures in 4 ml vials containing 2 ml sterile distilled water and maintained at room temperature.

Data analysis

Data analysis of soil population densities (cfu/g), frequency of members of *Aspergillus* section *Flavi*, and aflatoxin concentrations were transformed using the equation $y = \log_{10}(\text{response variable} + 1)$ to stabilise the variance prior to data analysis. Data were subjected to statistical analysis using the mixed procedure (PROC MIXED) of SAS software (version 9.2, Cary, NC, USA). The experiments were conducted in completely randomised designs and each farmer was considered as a replicate. Differences in all response variables between treated and untreated fields were tested by analysis of variance (ANOVA) using the PROC GLM procedure in SAS. Means of the response variables obtained from treated and untreated were separated using Student's t-test (α=0.05). All field data were analysed using paired *t*-tests (PROC TTEST) of SAS.

3. Results

Quality test check for formulated products

All grains from batches of Aflasafe TZ01 and Aflasafe TZ02 examined were colonised only by *A. flavus*. All *A. flavus* isolates recovered from the products belonged to one of the VCG of the constituent active ingredients of the respective biocontrol product. Other genotypes of *A. flavus* were never detected. In each biocontrol product, each of the four-active ingredient VCGs was found on 25±3% carrier grains of the examined batches. The spore yield per g of product following incubation in 24-well plates for 7 d was, on average, 3,500±300 cfu.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Aflatoxin levels in treated and untreated maize and groundnut grains

Aflatoxin levels in maize and groundnut varied markedly in both years. Average total aflatoxin content of Aflasafe TZ01 untreated crops ranged from 7.6 to 36 μg/kg (avg. = 15.9 μg/kg) in maize and from 41.8 to 368.6 μg/kg (avg. = 254.4 μg/kg) in groundnut (Table 3). For treated fields, aflatoxin ranged from 0.9 to 4.7 μg/kg (avg. = 2.3 μg/kg) in maize and 4.4 to 145.8 μg/kg (avg. = 52.1 μg/kg) in groundnuts. For Aflasafe TZ02, aflatoxin content in untreated maize ranged from 2.1 to 37.3 μg/kg (avg. = 14.4 μg/kg), while in groundnut it ranged from 11.5 to 1,304.5 μg/kg (avg. = 421.6 μg/kg). Aflatoxin content in grain treated with Aflasafe TZ02 ranged from 0.9 to 4.6 μg/kg (avg. = 2.5 μg/kg) for maize and from 1.6 to 116.5 μg/kg (avg. = 31.7 μg/kg) for groundnut. In both years, aflatoxin levels were higher in groundnut than in maize (Table 3). In both years and across regions, significant reductions in aflatoxin content were observed for both maize and groundnut from fields treated with a biocontrol product compared to grains from corresponding untreated fields (Table 3). Maize grain from fields treated with Aflasafe TZ01 contained on average 80.5 and 87.1% less aflatoxins than grain from untreated fields in 2016 and 2017, respectively. Aflatoxin reductions ranged from 75.8% (Dodoma) to 88.2% (Morogoro) in 2016, and from 80.4% (Morogoro) to 92.5% (Dodoma) in 2017. Maize grain from fields treated with Aflasafe TZ02 contained on average 52.1 and 94.8% less aflatoxins than crops from untreated fields in 2016 and 2017, respectively. Aflatoxin reductions ranged from 30.4% (Dodoma; aflatoxin in untreated plots was 4.6 μg/kg) to 87.7% (Morogoro) in 2016 and from 69.7% (Manyara) to 91.5% (Dodoma) in 2017 (Table 3). On average, aflatoxin concentrations were less in maize treated with either product (73-90.8% less).

Groundnut from fields treated with Aflasafe TZ01 contained on average 73.6 and 90.8% less aflatoxins than grain from untreated fields in 2016 and 2017, respectively. Aflatoxin reductions ranged from 57.3% (Dodoma) to 89.9% (Mtwara) in 2016 and from 89.5 to 92.1% in 2017 (Table 3). Groundnut from fields treated with Aflasafe TZ02 contained on average 94.8 and 87.3% less aflatoxins than crops from untreated fields, in 2016 and 2017, respectively. Aflatoxin reductions ranged from 86.1 to 98.5% in 2016 and from 86.1 to 88.4% in 2017. On an average, aflatoxins concentrations were reduced by 82.8% in fields treated with Aflasafe TZ01 and by 91% in fields treated with Aflasafe TZ02.

Community composition of *Aspergillus* section *Flavi* in soil and grains

In both years and across districts, the *A. flavus* L-morphotype dominated all soils before inoculation (range = 31.3 to 93.1%) (Tables 4 and 5). Other *Aspergillus* section *Flavi* fungi included fungi with S morphotype (range = 4.3 to 39.4%), *A. parasiticus* (range = 0 to 15.6%), and *A. tamarii* (range = 0 to 11.1%) (Tables 4 and 5). There were no

Table 3. Aflatoxin concentrations in maize and groundnuts from fields untreated and treated with Aflasafe TZ01 and Aflasafe TZ02 in various districts of Tanzania in 2016-2017.

Year/Region	Crop	Aflasafe TZ01				Aflasafe TZ02			
			Aflatoxin (μg/kg)[1]				Aflatoxin (μg/kg)		
		n	Untreated[2]	Treated	Reduction (%)[3]	n	Untreated	Treated	Reduction (%)
2016									
Manyara	maize	12	8.5***	1.9	77.6	12	2.1	1.3	38.1
Dodoma	maize	10	9.1***	2.2	75.8	10	4.6	3.2	30.4
Dodoma	groundnut	11	341.4	145.8	57.3	11	1,304.5***	116.5	91.1
Morogoro	maize	19	7.6***	0.9	88.2	20	37.3***	4.6	87.7
Mtwara	groundnut	16	368.6***	37.4	89.9	19	340.4***	5.1	98.5
2017									
Dodoma	maize	44	36.0***	2.7	92.5	41	21.3***	1.8	91.5
Dodoma	groundnut	42	265.7***	20.9	92.1	36	30.1***	3.5	88.4
Morogoro	maize	25	24.0***	4.7	80.4	23	18.5***	3.3	82.2
Manyara	maize	8	10.4***	1.2	88.5	9	2.8	0.9	67.9
Mtwara	groundnut	16	41.8***	4.4	89.5	16	11.5***	1.6	86.1

[1] Values in the aflatoxin column are the sum of aflatoxin B_1, B_2, G_1, and G_2.

[2] In each region, aflatoxin values from untreated samples with asterisks (*) significantly differed from those found in its corresponding treated field using Student's *t*-test (α=0.05). A single asterisk (*) indicates $P<0.05$, while three asterisks (***) indicate $P<0.0001$.

[3] Percent reduction was calculated for each district as follows: ([mean of control – mean of Aflasafe TZ01 or Aflasafe TZ02 treated]/mean of control) × 100.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

significant differences (*P*>0.05) in frequencies of each fungal type in treated and untreated soils, regardless of year and district (Tables 4 and 5). The L-morphotype continued to dominate in grain samples with an average of 85.5% (range = 72 to 93%) in treated fields compared to 83.5% (range = 63 to 96%) in untreated fields. Frequencies of fungi with S morphotype were 20.9% in treated fields compared to 21% in untreated fields. Frequencies of *A. parasiticus* and *A. tamarii* were low in grains across districts and years regardless of treatment (Tables 4 and 5). In soils collected three months after harvest from both maize and groundnut fields across all districts, the application of the biocontrol products generally resulted in increased proportions of *A. flavus* L morphotype and reduced incidences of fungi with S morphotype, *A. parasiticus*, and *A. tamarii* (Tables 4 and 5). Compared to grains from untreated fields, significantly (*P*<0.05) lower L morphotype incidences were observed in a few cases. Conversely, higher proportions of fungi with S morphotype, *A. parasiticus*, and *A. tamarii* were generally recovered from untreated grains in comparison to treated grains (Tables 4 and 5).

Densities of *Aspergillus* section *Flavi* in soils and grains

In both years, fungal densities varied in soils and grains across regions, districts, and treatments irrespective of biocontrol treatments (Table 6). Fungal densities were generally lower in soils prior to treatment and soil collected three months after harvest, and highest in grains at harvest. Densities of *Aspergillus* section *Flavi* ranged from 159 to 8,985 cfu/g in soils prior to application of either biocontrol product in both years and no significant (*P*>0.05) differences were observed within treatments in any of the comparisons (Table 6). In soils collected three months after harvest,

Table 4. Frequencies of *Aspergillus* species and strains in soil and grain samples collected from untreated and Aflasafe TZ01-treated fields before biopesticide application, at harvest, and 3 months after harvesting in four regions of Tanzania in 2016 and 2017.

Year/Region	Crop/ Treatment[1]		n	*Aspergillus* species/strain distribution (%)[1,2]											
				Soil before application				Grain at harvest				Soil 3 months after harvesting			
				L	S	P	T	L	S	P	T	L	S	P	T
2016															
Manyara	M	U	12	51.1	39.1	7.3	2.6	99.0***	1.1***	0	0***	45.8	32.3	11.0***	10.9
		T	12	66.2	26.6	4.2	3.1	90.3	6.1	0	3.7	60.7	27.9	2.6	8.9
Dodoma	M	U	10	47.5	35.6	7.5	9.4	84.4*	10.0**	0.6	5.0*	30.7	40.0	25.7	3.8**
		T	10	43.8	39.4	10.0	6.9	70.0	27.5	0.6	1.9	43.6	46.3	18.1	1.9
Dodoma	G	U	11	85.8	5.7**	3.4***	5.1	85.8	14.2	0	0	92.5*	6.9**	0.6	0
		T	11	77.3	14.8	0.6	7.4	87.5	12.5	0	0	98.1	1.9	0	0
Morogoro	M	U	20	62.2	26.3	5.9	5.6	67.5	31.9	0.3***	0.3***	44.1	42.2	7.2***	6.6
		T	20	65.3	25.0	3.8	6.3	81.6	18.4	0	0	65.6	27.2	1.3	6.0
Mtwara	G	U	16	84.4	7.8	1.2	6.7	92.2	7.1	0.8***	0***	89.9	5.5	3.9	0.8
		T	16	86.7	4.3	2.7	6.3	93.8	5.9	0	0.4	93.8	3.5	2.4	0.4
2017															
Dodoma	M	U	24	59.9	38.1	0***	2.1**	73.5	23.5	0	3.1**	53.2	41.0	3.4**	2.5
		T	24	64.4	34.1	0.8	0.8	74.1	19.4	0	6.6	69.1	27.5	1.6	1.9
Dodoma	G	U	10	92.4	6.3	0	1.4***	93.8	6.3	0	0	95.9	4.2	0	0
		T	10	93.1	7	0	0	86.5	13.5	0	0	94.8	5.2	0	0
Manyara	M	U	4	81.3	17.2	0	1.8	81.3	18.8**	0	0	81.3	15.7	0	3.2
		T	4	76.6	21.9	0	1.8	93.8	6.3	0	0	89.1	9.4	0	1.8
Morogoro	M	U	18	75.4	21.9	0	2.8	88.0	9.6	0***	2.4**	44.7	51.0	0	4.3***
		T	18	73.3	25.0	0	1.7	82.2	10.1	1	6.7	64.0	26.0	0	10.1
Mtwara	G	U	8	86.1	12.5	0	1.4	75.8	24.3	0	0	57.8	41.4	0	0.8***
		T	8	82.7	16.0	0	1.4	82.8	17.2	0	0	76.6	23.5	0	0

[1] M = maize; G = groundnut; U = untreated; T = treated.

[2] L = *Aspergillus flavus* L morphotype; S = *Aspergillus flavus* S morphotype; P = *Aspergillus parasiticus*; T = *Aspergillus tamarii*.

[3] In each region, species/strain frequencies from treated samples with an asterisk (*) significantly differed from those found in its corresponding control treatment using Student's *t*-test (α=0.05). A single asterisk (*) indicates *P*<0.05; two asterisks (**) indicate *P*<0.001; while three asterisks (***) indicate *P*<0.0001.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

fungal densities were generally higher in treated soils and ranged from 306 to 62,267 cfu/g across regions and years, compared to 277 to 55,580 for the untreated soil. Higher fungal densities were detected in grains at harvest, but these varied between treatments. In certain regions, fungal densities in grains from untreated fields were higher than in the corresponding treated fields while in others the opposite occurred. In 2017 for instance, groundnut grains treated with Aflasafe TZ01 in Dodoma had 657,011 cfu/g compared to 726,482 cfu/g in grains from corresponding untreated fields. Overall, densities in grains treated with Aflasafe TZ01 ranged from 174 to 3.5×10^6 cfu/g while that from untreated fields ranged from 263 to 726,482 cfu/g. In comparison to grains treated with Aflasafe TZ01, fungal densities in Aflasafe TZ02-treated grains were relatively higher and ranged from 3 to 3.03×10^6 cfu/g while those from paired untreated fields ranged from 2 to 1.7×10^6 cfu/g (Table 6).

4. Discussion

This study evaluated two aflatoxin biocontrol products for their potential to minimise aflatoxin contamination of maize and groundnut for two successive years in Tanzania. In both years and across crops, the two products effectively reduced aflatoxin contamination by >85%. Even though the two products contain different active ingredients, they were equally effective in reducing aflatoxin levels across regions. The robustness of the atoxigenic-based biocontrol approach is now demonstrated for the first time in East Africa. Our results are in agreement with those that have been reported in other parts of West Africa (Atehnkeng

Table 5. Frequencies of *Aspergillus* species and strains in soil and grain samples collected from untreated and Aflasafe TZ02-treated fields before application, at harvest, and 3 months after harvesting in four regions of Tanzania in 2016 and 2017.

Year/Region	Crop/Treatment[1]		n	*Aspergillus* species/strain distribution (%)[2,3]											
				Soil before application				Grain at harvest				Soil 3 months after harvesting			
				L	S	P	T	L	S	P	T	L	S	P	T
2016															
Manyara	M	U	13	51.4	31.6	10.1***	6.9	98.5*	1.6	0	0	45.7	24.5	17.8	12.0
		T	13	50.8	38.9	4.0	6.3	96.7	3.4	0	0	63.0	14.9	6.7	15.4
Dodoma	M	U	10	70.7	13.1	8.2	8.1	76.4*	19.6**	0.7	3.5	35.0	43.8	20.0	1.3
		T	10	63.2	21.9	9.4	5.6	90.6	8.1	0.6	0.6	31.9	50.0	15.6	2.5
Morogoro	M	U	13	58.2	30.7	2.5*	8.8*	56.0	44.1	0***	0	40.7	44.7	6.0**	8.8***
		T	13	63.8	24.1	6.6	5.6	76.9	22.8	0.3	0	61.6	35.3	1.6	1.6
Mtwara	G	U	19	82.3	9.6	2.3	5.9	88.5	10.6	0.3	0.6***	87.8	9.9	1.6	0.6***
		T	19	84.9	8.2	2.0	4.9	88.8	10.9	0.3	0	89.8	8.2	2	0
Dodoma	G	U	11	79.6	14.2	2.8***	3.4	80.1	19.9	0	0***	80.7***	13.7***	0	5.7***
		T	11	71.3	8.5	8.5	5.7	85.8	13.1	0	1.1	94.9	4.6	0	0.6
2017															
Dodoma	G	U	10	88.9	10.4	0	0.7	92.7	7.3	0	0	94.8	5.2	0	1.1***
		T	10	91.7	7.6	0	0.7	85.4	14.6	0	0	93.8	6.3	0	0
Dodoma	M	U	26	66.4	30.1	2.4***	1.2	82.2	15.6	0	2.2*	56.6	36.3	3.1***	4.1
		T	26	67.3	30.3	0	2.4	86.3	12.2	0	0.9	77.5	18.8	0.6	3.1
Manyara	M	U	4	62.5	32.9	0	4.7***	81.3	6.3	0	12.5	71.9	28.2	0	0
		T	4	75.0	25.0	0	0	84.4	3.1	0	12.5	70.3	29.7	0	0
Morogoro	M	U	18	70.2	22.2	0	7.3	97.7	2.3	0	0***	32.4	60.8	0	6.8
		T	18	91.9	23.3	0	4.9	93.8	2.3	0	4.0	47.2	43.8	0	9.1
Mtwara	G	U	8	85.1	14.6	0	0.4***	75.8	24.2	0	0	72.7	27.4	0	0
		T	8	84.0	16.0	0	0	77.4	22.7	0	0	88.3	11.7	0	0

[1] M = maize; G = groundnut; U = untreated; T = treated.

[2] L = *Aspergillus flavus* L morphotype; S = *Aspergillus flavus* S morphotype; P = *Aspergillus parasiticus*; T = *Aspergillus tamarii*.

[3] In each region, species/strain frequencies from treated samples with an asterisk (*) significantly differed from those found in its corresponding control treatment using Student's *t*-test (α=0.05). A single asterisk (*) indicates P<0.05; two asterisks (**) indicate P<0.001; while three asterisks (***) indicate P<0.0001.

Table 6. Densities of *Aspergillus* section *Flavi* in soil and grain samples collected from untreated and Aflasafe-treated fields before application, at harvest, and 3 months after harvesting in four regions of Tanzania in 2016 and 2017.

Year/Region	Crop/Treatment[1]		n	Aflasafe TZ01 (cfu/g)[2]			Aflasafe TZ02 (cfu/g)[2]		
				Soil before	Grain	Soil after	Soil before	Grain	Soil after
2016									
Manyara	M	U	12	1,825***	51,281	1,335*	1,605	83,515***	1,716**
		T	12	5,261	76,635	770	1,652	619,274	4,340
Dodoma	M	U	10	2,041***	6,488	249	4,209	35,891**	277
		T	10	8,370	7,139	306	5,169	95,843	353
	G	U	16	6,015**	726,482	55,580	6,231*	1,659,523	16,010*
		T	16	3,850	657,011	62,267	8,985	1,667,252	35,232
Morogoro	M	U	19	6,224***	36,899***	299**	1,028***	105,558***	243**
		T	19	1823	432,488	535	2,696	703,093	422
Mtwara	G	U	16	4,462**	662,333***	4,865***	1,454**	257,356***	2,648**
		T	16	1,662	3,493,637	4,546	2,548	3,033,240	7,214
2017									
Dodoma	M	U	10	1,711	869**	1,999***	3,465	632***	2482
		T	10	2,283	1,610	4,860	4,697	19,897	3,483
	G	U	10	2,072	92,414	3,647*	1,573**	27,072	5,815
		T	10	2,306	37,725	6,715	3,185	21,517	8,359
Manyara	M	U	4	792	263	364*	1,013	2	356*
		T	4	364	174	356	819	3	703
Morogoro	M	U	4	1,317	28,546	2,064	1,460***	176,365***	4,568
		T	4	1,038	25,746	2,895	4,128	69,745	3,348
Mtwara	G	U	8	264	61,679	397	159	37,691***	534
		T	8	186	75,863	1111	291	11,177	582

[1] M = maize; G = groundnut; U = untreated; T = treated.

[2] cfu = colony forming units. In each region, cfu/g values from treated samples with an asterisk (*) significantly differed from those found in its corresponding untreated fields using Student's *t*-test (α=0.05). A single asterisk (*) indicate $P<0.05$; two asterisks (**) indicate $P<0.001$; while three asterisks (***) indicate $P<0.0001$.

et al., 2014; Bandyopadhyay *et al.*, 2019; Senghor *et al.*, 2020) and the world at large (Alaniz Zanon *et al.*, 2016; Dorner, 2004, 2010; Doster *et al.*, 2014; Mauro *et al.*, 2018; Savić *et al.*, 2020; Weaver *et al.*, 2015) where the technology has been tested. Although efficacy of the two products varied from year to year and from region to region, they were equally effective in minimising levels of aflatoxins in the two crop commodities. Results from the current study provide evidence that the products utilised are highly effective and potentially sustainable tools for reducing aflatoxin contamination of maize and groundnut throughout Tanzania. Therefore, the adoption and use of these biocontrol products can help farmers produce aflatoxin-safe food thereby improving health and increasing chances for greater income for smallholder farmers across the country.

The active ingredients for the two biocontrol products tested were selected following a country wide survey to document prevalence of aflatoxin in Tanzania that was conducted in 2012 (Boni *et al.*, 2021; TFDA, 2012). Characterisation of L-morphotype of *A. flavus* identified 12 distinct atoxigenic strains that were formulated into four products for testing under field conditions. Following initial testing conducted in 2015, two products were selected for expanded country-wide testing in regions that had previously been identified as hot spots of aflatoxin contamination of maize and groundnut (Boni *et al.*, 2021; TFDA, 2012). The active ingredients of Aflasafe TZ01 and Aflasafe TZ02 were selected based on the extent of aflatoxin reductions in laboratory, inability to produce cyclopiazonic acid (CPA), preliminary field studies, the extent of their VCG distribution within the country and deletion patterns in the aflatoxin biosynthesis gene cluster. This rigorous selection process allowed identification of active ingredients that are competitive and adapted to Tanzania's agricultural environments.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Registration of bioprotectants with regulatory authorities must satisfy various requirements. A condition from the Environmental Protection Agency of Tanzania is that the applied active ingredients do not alter the overall population of *Aspergillus* section *Flavi* in the environment. To test this, soil samples were collected before application of the products and three months after harvesting, and the samples analysed to monitor the incidence and prevalence of *Aspergillus* species. *Aspergillus* section *Flavi* species detected included *A. flavus* L morphotype, fungi with S morphotype, *A. parasiticus*, and *A. tamarii*. All members of *Aspergillus* section *Flavi* were recovered in soils and grains across regions and districts in both years. These results are consistent with previous observations which indicate that *Aspergillus* communities in agricultural fields consist of individuals with diverse morphological and phenotypic characteristics (Cardwell and Cotty, 2002; Cotty *et al.*, 1994). Incidences and prevalence varied across districts in both years, but there were no significant differences in the overall populations between fields treated and those not treated with biocontrol products. Across study sites, *A. flavus* L morphotype was the dominant section *Flavi* species in soils prior to product application, in grains and in soil collected three months after harvest (Tables 4 and 5). *Aspergillus flavus* L morphotype is recognised as the most common coloniser of crop substrates (Atehnkeng *et al.*, 2014; Dorner and Horn, 2007). Our results are consistent with this assertion. The high incidence of *A. flavus* L morphotype in soils and crop samples was consistent with reports from other studies conducted in Africa (Agbetiameh *et al.*, 2020; Atehnkeng *et al.*, 2014; Bandyopadhyay *et al.*, 2019; Senghor *et al.*, 2020).

Incidences of other members of *Aspergillus* section *Flavi* were low. The proportions of fungi with S morphotype in soil collected before product application ranged from 3.5 to 59.0%, that of *A. parasiticus* ranged from 0 to 10% while that of *A. tamarii* ranged from 0 to 10.4% (Tables 4 and 5). The fungi with S morphotype are known to produce high concentrations of aflatoxins in crops (Cardwell and Cotty, 2002; Singh and Cotty, 2019). Similarly, *A. parasiticus* is among the most consistent aflatoxin producing species of *Aspergillus* section *Flavi* (Kachapulula *et al.*, 2017; Probst *et al.*, 2014). However, in this study, the incidence of *A. parasiticus* was very low, ranging from 0 to 10% in soil before application, 0 to18% in grain collected at harvest and from 0 to 25.6% in soil collected three months after harvest. Similarly, incidence of *A. tamarii* was equally low, ranging from 0 to 20.3%. Our results reveal that *A. flavus* is the dominant *Aspergillus* section *Flavi* spp. in soils in Tanzania. Although *A. parasiticus* has been reported as a major aflatoxin producer in groundnut (Kachapulula *et al.*, 2017; Probst *et al.*, 2014), this was not the case in the current study. Recovery of *A. parasiticus* in soil and crop grain samples was low, even in regions where groundnuts are a dominant crop. Similarly, in Ghana, *A. parasiticus* is infrequently associated with groundnut (Agbetiameh *et al.*, 2018). Further studies are needed to understand the relatively low frequencies of *A. parasiticus* in Tanzanian soils.

Members of the VCGS to which the atoxigenic isolates composing either Aflasafe TZ01 or Aflasafe TZ02 are relatively common across Tanzania. Indeed, the natural widespread occurrence of these competitive atoxigenic isolates in soils across Tanzania was a criterion for their selection as active ingredients. However, natural frequencies of these atoxigenic isolates are insufficient to reliably result in aflatoxin safe food and feeds; thus, it is essential that the population of these isolates is artificially enhanced through biocontrol treatment. Treatment with atoxigenic isolates early in the season, enables them to multiply and establish as a founding population on the treated crop before other, potentially toxigenic, *Aspergilli* have the opportunity to increase (Cotty and Mellon, 2006; Ortega-Beltran *et al.*, 2019). Successful establishment of the atoxigenic isolates was observed as increased frequencies of L morphotype on crops from treated fields and the low frequencies of fungi with S morphotype in treated fields compared to the non-treated fields. Similar reductions in frequencies of aflatoxin-producers and increases in L morphotypes has been reported in other studies (Agbetiameh *et al.*, 2020; Atehnkeng *et al.*, 2014; Bandyopadhyay *et al.*, 2019; Ezekiel *et al.*, 2019; Senghor *et al.*, 2020).

Applications of atoxigenic biocontrol products can be done without increasing densities of *A. flavus* on treated crops (Agbetiameh *et al.*, 2020; Cotty, 1994; Doster *et al.*, 2014; Ezekiel *et al.*, 2019; Mehl *et al.*, 2012; Senghor *et al.*, 2020). The results from our study agree with previous studies, as there were no significant differences in overall populations of *Aspergillus* section *Flavi* between treated and untreated fields. Fungal densities in grains at harvest and soil collected three months after harvest did not vary between treated and untreated fields in both years irrespective of biocontrol product. However, there were a few instances where this was not the case. For example, 144 times higher fungal densities were detected on grains from fields treated with Aflasafe TZ01 compared to untreated fields from Masasi in 2016 (Table 6). However, the contrary was observed in Dodoma in 2017, where grain from untreated fields contained 3-fold higher fungal densities on grains from untreated fields than from fields treated with Aflasafe TZ02. Similar findings were recently reported from Nigeria (Bandyopadhyay *et al.*, 2019) and Senegal (Senghor *et al.*, 2020) where in most cases, fungal densities in grains from treated fields did not differ significantly from untreated grains. In a previous study, Atehnkeng *et al.* (2014) consistently detected higher fungal densities in grains from treated compared to untreated fields with an application rate of 40 kg/ha. In the current study, both biocontrol products were applied at a rate of 10 kg/ha and this resulted in the production

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

of grains with both significantly less aflatoxin and similar quantities of fungal propagules compared to grains from untreated fields.

Treated and untreated fields should be separated by a relatively large distance (>200 m) to minimise/prevent movement of atoxigenic biocontrol products into untreated areas and prevent confounding results when comparing aflatoxin levels from treated and untreated fields (Agbetiameh *et al.*, 2019; Bandyopadhyay *et al.*, 2019; Senghor *et al.*, 2020). Spores of *A. flavus* are dispersed by wind, rain, and insects (Bock *et al.*, 2004; Horn, 2003; Stephenson and Russell, 1974), thus when fields are in close proximity, atoxigenic spores might move from treated to untreated fields and confound the results. In cases where a farmer had a larger farm that could be separated into two plots with a buffer zone of 20 m, both treated and untreated plots were administered. It has been reported that a buffer zone of 20 m between treated and untreated plots might not be sufficient to prevent movement of biocontrol product active ingredients, thus resulting in low levels of aflatoxin in untreated plots. Observations on movement of inoculum from treated plots to adjoining (20 m distance) untreated plots have been made in the USA (Weaver and Abbas, 2019). This observation should be used to streamline and redefine protocol for testing efficacy of atoxigenic based biocontrol products to not confound the results. Untreated plots must be sufficiently separated from treated plots to avoid underestimation of efficacy. These observations also suggest biocontrol products will have positive influences not only on treated fields but also nearby untreated fields and supports the concept of area-wide application for effective management of aflatoxin contamination (Cotty *et al.*, 2007). On the other hand, determining the appropriate maximum and minimum distance to conduct field efficacy trials of aflatoxin biocontrol products (and biocontrol products in general) deserves further investigation.

Recently, Kenya banned importation of maize from Tanzania due to high levels of aflatoxin (The Independent, 2021). These reports scare people and creates diplomatic rows between nations. In this study, application of the biocontrol products resulted in production of crops containing permissible aflatoxin levels. Therefore, this technology has a real potential to help solve the aflatoxin menace that is frequently observed in Tanzania and that sometimes results in loss of lives. The results from the field efficacy trials reported in the current study were used to prepare dossiers for registration of Aflasafe TZ01 and Aflasafe TZ02 with regulatory authorities in Tanzania. In October 2018, the Register General of Tanzania approved the unrestricted use of both products for aflatoxin mitigation in groundnut and maize throughout Tanzania. Subsequently, a technology transfer and licensing agreement (TTLA) was signed that authorised a private company A to Z Textile Mills Ltd., to manufacture and distribute Aflasafe TZ01 widely in Tanzania. A to Z Textile Mills Ltd. used its own capital to construct the manufacturing plant and is using its own resources to manufacture and distribute Aflasafe TZ01 in Tanzania. The commercial use of only Aflasafe TZ01 was deliberate and currently Aflasafe TZ02 is not being used. A potential strategy to further reduce the risk of contamination is to rotate mixtures of atoxigenic VCGs between seasons and crops to promote a more diverse, stable atoxigenic community with a large repertoire of adaptive traits (e.g. host adaptation, climate change resilience, prevalence under changing soil and cropping systems, increased sporulation) for long-term persistence in a target area (Mehl *et al.*, 2012). Communities dominated by one or a few VCGs may not be stable over the long-term (Ortega-Beltran and Cotty, 2018) and therefore rotation of multi-genotype biocontrol products could be beneficial. Rotating Aflasafe TZ01 and Aflasafe TZ02 in Tanzania could serve to test if a more robust aflatoxin control strategy is achieved with more complex atoxigenic VCG communities. Large-scale use of either Aflasafe TZ01 or Aflasafe TZ02 can help farmers produce crops with greatly reduced aflatoxin content, thereby reducing dietary exposure and concomitant health effects while improving trade opportunities and income of the Tanzanian people. Ultimately, large-scale use of these biocontrol products, which solve an invisible problem, will result when appropriate technological, social, and institutional approaches converge into a holistic approach to address the frequent detriment of crop aflatoxin contamination in Tanzania. Adoption of Aflasafe TZ01 throughout Tanzania can help farmers to produce aflatoxin-safe food thereby improving health and increasing the income of the Tanzanian people. In addition, this can significantly increase farmers' chances to meet the stringent aflatoxin thresholds imposed by both local and international premium markets.

5. Conclusions

Application of aflatoxin biocontrol products did not increase densities of *Aspergillus* spp. in treated fields in comparison to the untreated fields. However, the applied fungi successfully displaced toxigenic strains and in so doing, there were lower aflatoxin levels in grain from treated fields compared to untreated fields. In some cases, reductions of toxigenic fungi were not significant. This revealed that the resident toxigenic population was high; thus, application in more than one season would be required to reduce frequencies of those toxigenic groups to safe levels. Application of Aflasafe products significantly reduced aflatoxin levels in both maize and groundnut by an average of 95.6 and 98.1% for maize and groundnut in 2016 and 2017, respectively. Thus, the use of Aflasafe products in maize and groundnut would significantly increase farmers' chances to meet the stringent aflatoxin thresholds imposed by both local and international premium markets. Adoption and use of Aflasafe throughout Tanzania can help farmers

to produce aflatoxin-safe food thereby improving health and increasing the income of the Tanzanian people.

Acknowledgements

Special thanks to the maize and groundnut farmers in Tanzania for willingly allowing the trials to be conducted in their fields and participating in the current study. The authors would like to thank Tanzania Food and Drugs Authority (TFDA) for aflatoxin analysis, Sokoine University of Agriculture (SUA), Tropical Pesticide Research Institute (TPRI) and the Ministry of Agriculture in Tanzania for help with efficacy trials. This study was jointly funded by the USAID's Feed the Future Africa Rising initiative and the Bill & Melinda Gates Foundation (OPP1007117) as part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH).

Conflict of interest

The authors receive no direct financial benefit from the manufacturing and marketing of any of the aflatoxin biocontrol products mentioned in this article. The Aflasafe name is a Trademark of the International Institute of Tropical Agriculture (IITA). IITA used to manufacture and commercialise Aflasafe for use in Nigeria, Senegal, Burkina Faso, The Gambia, and Ghana. Manufacturing and distribution responsibilities have been licensed to the private or public sector. IITA charges a small licensing fee to manufacturers for use of the Aflasafe name and cost associated with technology transfer and technical backstopping. G.M., A.O.-B., J.A., F.A., R.S., J.N. and R.B. are employed by IITA.

References

Abt Associates Inc., 2013. Country and economic assessment for aflatoxin contamination and control in Tanzania. Abt Associates Inc., Bethesda, MD, USA. Available at: https://tinyurl.com/2fccvvyw

Agbetiameh, D., Ortega-Beltran, A., Awuah, R.T., Atehnkeng, J., Cotty, P.J. and Bandyopadhyay, R., 2018. Prevalence of aflatoxin contamination in maize and groundnut in Ghana: population structure, distribution, and toxigenicity of the causal agents. Plant Disease 102: 764-772. https://doi.org/10.1094/PDIS-05-17-0749-RE

Agbetiameh, D., Ortega-Beltran, A., Awuah, R.T., Atehnkeng, J., Elzein, A., Cotty, P.J. and Bandyopadhyay, R., 2020. Field efficacy of two atoxigenic biocontrol products for mitigation of aflatoxin contamination in maize and groundnut in Ghana. Biological Control 150: 104351. https://doi.org/10.1016/j.biocontrol.2020.104351

Agbetiameh, D., Ortega-Beltran, A., Awuah, R.T., Atehnkeng, J., Islam, M.-S., Callicott, K.A., Cotty, P.J. and Bandyopadhyay, R., 2019. Potential of atoxigenic *Aspergillus flavus* vegetative compatibility groups associated with maize and groundnut in Ghana as biocontrol agents for aflatoxin management. Frontiers in Microbiology 10: 2069. https://doi.org/10.3389/fmicb.2019.02069

Alaniz Zanon, M.S., Barros, G.G. and Chulze, S.N., 2016. Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. International Journal Food Microbiology 231: 63-68. https://doi.org/10.1016/j.ijfoodmicro.2016.05.016

Atehnkeng, J., Donner, M., Ojiambo, P.S., Ikotun, B., Augusto, J., Cotty, P.J. and Bandyopadhyay, R., 2016. Environmental distribution and genetic diversity of vegetative compatibility groups determine biocontrol strategies to mitigate aflatoxin contamination of maize by *Aspergillus flavus*. Microbial Biotechnology 9: 75-88. https://doi.org/10.1111/1751-7915.12324

Atehnkeng, J., Ojiambo, P.S., Cotty, P.J. and Bandyopadhyay, R., 2014. Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link:Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.). Biological Control 72: 62-70. https://doi.org/10.1016/j.biocontrol.2014.02.009

Bandyopadhyay, R., Atehnkeng, J., Ortega-Beltran, A., Akande, A., Falade, T.D.O. and Cotty, P.J., 2019. 'Ground-truthing' efficacy of biological control for aflatoxin mitigation in farmers' fields in Nigeria: from field trials to commercial usage, a 10-year study. Frontiers in Microbiology 10: 2528. https://doi.org/10.3389/fmicb.2019.02528

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., Senghor, A.L., Adhikari, B.N. and Cotty, P.J., 2016. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. World Mycotoxin Journal 9: 771-789. https://doi.org/10.3920/WMJ2016.2130

Bock, C.H., Mackey, B. and Cotty, P.J., 2004. Population dynamics of *Aspergillus flavus* in the air of an intensively cultivated region of south-west Arizona. Plant Pathology 53: 422-433.

Boni, S.B., Beed, F., Kimanya, M.E., Koyano, E., Mponda, O., Mamiro, D., Kaoneka, B., Bandyopadhyay, R., Korie. S. and Mahuku, G. 2021. Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. World Mycotoxin Journal 14: 553-564. https://doi.org/10.3920/WMJ2020.2646

Cardwell, K.F. and Cotty, P.J., 2002. Distribution of *Aspergillus* section *Flavi* among field soils from the four agroecological zones of the Republic of Benin, West Africa. Plant Disease 79:1039-1045.

Chang, P.K., Abbas, H.K., Weaver, M.A., Ehrlich, K.C., Scharfenstein, L.L. and Cotty, P.J., 2012. Identification of genetic defects in the atoxigenic biocontrol strain *Aspergillus flavus* K49 reveals presence of a competitive recombinant group in field populations. International Journal of Food Microbiology 154: 192-196. https://doi.org/10.1016/j.ijfoodmicro.2012.01.005

Cole, R.J. and Dorner, J.W., 1994. Extraction of aflatoxins from naturally contaminated peanuts with different solvents and solvent/peanut ratios. Journal of AOAC International 77: 1509-1511. https://doi.org/10.1093/jaoac/77.6.1509

Cotty, P.J. and Mellon, J.E., 2006. Ecology of aflatoxin-producing fungi and biocontrol of aflatoxin contamination. Mycotoxin Research 22: 110-117.

Cotty, P.J. and Taylor, D.R., 2003. Influence of complementation medium composition on vegetative compatibility analyses of *Aspergillus flavus*. Phytopathology 93: S18.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Cotty, P.J., 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 79: 808-814.

Cotty, P.J., 1994. Comparison of four media for the isolation of *Aspergillus flavus* group fungi. Mycopathologia 125: 157-162. https://doi.org/10.1007/BF01146521

Cotty, P.J., Antilla, L. and Wakelyn, P.J., 2007. Competitive exclusion of aflatoxin producers: farmer-driven research and development. In: Vincent, C., Goettel, M.S. and Lazarovits, G. (eds.) Biological control: a global perspective. CAB International, Wallingford, UK, pp. 242-253.

Cotty, P.J., Bayman, P., Egel, D.S. and Elias, K.S., 1994. Agriculture, aflatoxins and *Aspergillus*. In: Powell, K. (ed.) The genus *Aspergillus*. Plenum Press, New York, NY, USA, pp. 1-27. https://doi.org/10.1007/978-1-4899-0981-7_1

Diao, X., Kennedy, A., Mabiso, A. and Pradesha, A., 2013. Economy wide impact of maize export bans on agricultural growth and household welfare in Tanzania. A dynamic computable general equilibrium model analysis. IFPRI Discussion Paper 01287. International Food Policy Research Institute, Washington, DC, USA. Available at: http://www.ifpri.org/sites/default/files/publications/ifpridp01287.pdf

Dorner, J.W. and Horn, B.W., 2007. Separate and combined applications of nontoxigenic *Aspergillus flavus* and *A. parasiticus* for biocontrol of aflatoxin in peanuts. Mycopathologia 163: 215-223.

Dorner, J.W., 2004. Biological control of aflatoxin contamination of crops. Journal of Toxicology: Toxin Reviews 23: 425-450.

Dorner, J.W., 2010. Efficacy of a biopesticide for control of aflatoxins in corn. Journal of Food Protection 73: 495-499. https://doi.org/10.4315/0362-028x-73.3.495

Doster, M.A., Cotty, P.J. and Michailides, T.J., 2014. Evaluation of the atoxigenic *Aspergillus flavus* strain AF36 in pistachio orchards. Plant Disease 98: 948-956.

European Commission (EC), 2007. Commission Regulation (EC) No. 1126/2007 of 28 September 2007 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of European Union L 255: 14-17.

European Commission (EC), 2010. Commission Regulation (EU) No. 165/2010 of 26 February 2010, amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin. Official Journal of the European Union L 50: 8-12.

Ezekiel, C.N., Ortega-Beltran, A., Oyedeji, E., Atehnkeng, J., Kössler, P., Tairu, F., Hoeschle-Zeledon, I., Karlovsky, P., Cotty, P.J. and Bandyopadhyay, R. 2019. Aflatoxin in chili peppers in Nigeria: extent of contamination and control using atoxigenic *Aspergillus flavus* genotypes as biocontrol agents. Toxins 11: 429.

Food and Agriculture Organisation (FAO), 2018. Transforming food and agriculture to achieve the SDGs. FAO, Rome, Italy. Available at: http://www.fao.org/3/i9900en/i9900en.pdf

Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.B., Nováková, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W. and Samson, R.A., 2019. Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology 93: 1-63. https://doi.org/10.1016/j.simyco.2018.06.001

Garber, N.P., Ortega-Beltran, A., Barker, G., Probst, C., Callicott, K.A., Jaime, R., Mehl, H.L. and Cotty, P.J., 2012. Brief protocols for research on management of aflatoxin-producing fungi, 1st edition. School of Plant Sciences, The University of Arizona, Tucson, AZ, USA, pp. 95-100.

Horn, B.W., 2003. Ecology and population biology of aflatoxigenic fungi in soil. Journal of Toxicology: Toxin Reviews 22: 351-379.

Kachapulula, P.W., Akello, J., Bandyopadhyay, R. and Cotty, P.J., 2017. *Aspergillus* section *Flavi* community structure in Zambia influences aflatoxin contamination of maize and groundnut. International Journal of Food Microbiology 261: 49-56.

Kamala, A., Ortiz, J., Kimanya, M., Haesaert, G., Donoso, S., Tiisekwa, B. and De Meulenaer, B., 2015. Multiple mycotoxin co-occurrence in maize grown in three agro-ecological zones of Tanzania. Food Control 54: 208-215. https://doi.org/10.1016/j.foodcont.2015.02.002

Kamala, A., Shirima, C., Jani, B., Bakari, M., Sillo, H., Rusibamayila, N., De Saeger, S., Kimanya, M., Gong, Y.Y. and Simba, A., 2018. Outbreak of an acute aflatoxicosis in Tanzania during 2016. World Mycotoxin Journal 11: 311-320. https://doi.org/10.3920/wmj2018.2344

Kimanya, M.E., De Meulenaer, B., Tiisekwa, B., Ndomo-Sigonda, M., Devlieghere, F., Van Camp, J. and Kolsteren, P., 2008. Cooccurrence of fumonisins with aflatoxins in home stored maize for human consumption in rural villages of Tanzania. Food Additives and Contaminants Part A 25: 1353-1364. https://doi.org/10.1080/02652030802112601

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., DeCock, K., Rubin, C. and Kenya Aflatoxicosis Investigation Group, 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environmental Health Perspectives 113: 1763-1767. https://doi.org/10.1289/ehp.7998

Lewis, M.H., Carbone, I., Luis, J.M., Payne, G.A., Bowen, K.L., Hagan, A.K., Kemerait, R., Heiniger, R. and Ojiambo, P.S., 2019. Biocontrol strains differentially shift the genetic structure of indigenous soil populations of *Aspergillus flavus*. Frontiers in Microbiology 10: 1738. https://doi.org/10.3389/fmicb.2019.01738

Liu, Y., Chang, C.C.H., Marsh, G.M. and Wu, F., 2012. Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. European Journal of Cancer 48: 2125-2136.

Magoha, H., Kimanya, M., De Meulenaer, B., Roderfroid, D., Lachat, C. and Kolsteren, P., 2014. Association between aflatoxin M1 exposure through breast milk and growth impairment in infants from Northern Tanzania. World Mycotoxin Journal 7: 277-284.

Matumba, L., Van Poucke, C., Njumbe Ediage, E.N. and De Saeger, S., 2017. Keeping mycotoxins away from the food: does the existence of regulations have any impact in Africa? Critical Reviews in Food Science and Nutrition 57: 1584-1592. https://doi.org/10.1080/10408398.2014.993021

Mauro, A., Garcia-Cela, E., Pietri, A., Cotty, P.J. and Battilani, P., 2018. Biological control products for aflatoxin prevention in Italy: commercial field evaluation of atoxigenic *Aspergillus flavus* active ingredients. Toxins 10: 30. https://doi.org/10.3390/toxins10010030

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

Mboya, R., Tongoona, P., Derera, J., Mudhara, M. and Langyintuo, A., 2011. The dietary importance of maize in Katumba ward, Rungwe district, Tanzania, and its contribution to household food security. African Journal of Agricultural Research 6: 2617-2626. https://doi.org/10.5897/AJAR10.891

Mehl, H.L., Jaime, R., Callicott, K.A., Probst, C., Garber, N.P., Ortega-Beltran, A., Grubisha, L.C. and Cotty, P.J., 2012. *Aspergillus flavus* diversity on crops and in the environment can be exploited to reduce aflatoxin exposure and improve health. Annals of the New York Academy of Sciences 1273: 7-17. https://doi.org/10.1111/j.1749-6632.2012.06800.x

Meijer, N., Kleter, G., De Nijs, M., Rau, M.L., Derkx, R. and Van der Fels-Klerx, H.J., 2021. The aflatoxin situation in Africa: systematic literature review. Comprehensive Reviews in Food Science and Food Safety 20: 2286-2304. https://doi.org/10.1111/1541-4337.12731

Moral, J., Garcia-Lopez, M.T., Camiletti, B.X., Jaime, R., Michailides, T.J., Bandyopadhyay, R. and Ortega-Beltran, A., 2020. Present status and perspective on the future use of aflatoxin biocontrol products. Agronomy 10: 491. https://doi.org/10.3390/agronomy10040491

Ortega-Beltran, A. and Cotty, P.J., 2018. Frequent shifts in *Aspergillus flavus* populations associated with maize production in Sonora, Mexico. Phytopathology 108: 412-420. https://doi.org/10.1094/PHYTO-08-17-0281-R

Ortega-Beltran, A., Moral, J., Picot, A., Puckett, R.D., Cotty, P.J. and Michailides, T.J., 2019. Atoxigenic *Aspergillus flavus* isolates endemic to almond, fig, and pistachio orchards in California with potential to reduce aflatoxin contamination in these crops. Plant Disease 103: 905-912. https://doi.org/10.1094/PDIS-08-18-1333-RE

Probst, C., Bandyopadhyay, R. and Cotty, P.J., 2014. Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. International Journal of Food Microbiology 174: 113-122. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010

Routledge, M.N., Kimanya, M.E., Shirima, C.P., Wild, C.P. and Gong, Y.Y., 2014. Quantitative correlation of aflatoxin biomarker with dietary intake of aflatoxin in Tanzanian children. Biomarkers 19: 430-435. https://doi.org/10.3109/1354750X.2014.924998

Savić, Z., Dudaš, T., Loc, M., Grahovac, M., Budakov, D., Jajić, I., Krstović, S., Barošević, T., Krska, R., Sulyok, M., Stojšin, V., Petreš, M., Stankov, A., Vukotić, J. and Bagi, F., 2020. Biological control of aflatoxin in maize grown in Serbia. Toxins 12: 162. https://doi.org/10.3390/toxins12030162

Schreurs, J., Bandyopadhyay, R., Kooyman, C., Ortega-Beltran, A., Akande, A., Konlambigue, M. and Van den Bosch, N., 2019. Commercial products promoting plant health in African agriculture. In: Neuenschwander, P. and Tamò, M. (eds.) Critical issues in plant health: 50 years of research in African agriculture. Burleigh Dodds Science Publishing, Cambridge, UK, pp. 345-363. https://doi.org/10.19103/AS.2018.0043.14.

Senghor, L.A., Ortega-Beltran, A., Atehnkeng, J., Callicott, K.A., Cotty, P.J. and Bandyopadhyay, R., 2020. The atoxigenic biocontrol product Aflasafe SN01 is a valuable tool to mitigate aflatoxin contamination of both maize and groundnut cultivated in Senegal. Plant Disease 104: 510-520.

Shirima, C.P., Kimanya, M.E., Kinabo, J.L., Routledge, M.N., Srey, C., Wild, C.P. and Gong, Y.Y., 2013. Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. Molecular Nutrition and Food Research 57: 1874-1881.

Shirima, C.P., Kimanya, M.E., Routledge, M.N., Srey, C., Kinabo, J.L., Humpf, H.U., Wild, C.P., Tu, Y.K. and Gong, Y.Y., 2015, A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environmental Health Perspectives 123: 173-178. https://doi.org/10.1289/ehp.1408097

Singh, P. and Cotty, P.J., 2019. Characterization of *Aspergilli* from dried red chilies (*Capsicum* spp.): insights into the etiology of aflatoxin contamination. International Journal of Food Microbiology 289: 145-153. https://doi.org/10.1016/j.ijfoodmicro.2018.08.025

Singh, P., Mehl, H.L., Orbach, M.J., Callicott, K.A. and Cotty, P.J., 2020. Phenotypic differentiation of two morphologically similar aflatoxin-producing fungi from West Africa. Toxins 12: 656. https://doi.org/10.3390/toxins12100656

Stephenson, L.W. and Russell, T.E., 1974. The association of *Aspergillus flavus* with hemipterous and other insects infesting cotton bracts and foliage. Phytopathology 64: 1502-1506.

Suleiman, R.A. and Kurt, R.A., 2015. Current maize production, postharvest losses and the risk of mycotoxins contamination in Tanzania. In: 2015 ASABE Annual International Meeting. Paper No. 152189434. American Society of Agricultural and Biological Engineers, St. Joseph, MI, USA, pp. 1-128 https://doi.org/10.13031/aim.20152189434

Tanzania Food and Drugs Authority (TFDA), 2012. Aflatoxin contamination and potential solutions for its control in Tanzania. A summary of the country and economic assessment conducted in 2012 and the aflatoxin stakeholder workshop held on December 3 and 4, 2012, in Dar es Salaam. TFDA, Mwanza, Tanzania.

The Independent, 2021. Kenya's ban on Uganda, Tanzania maize threatens EAC treaty. The Independent, March 9, 2021. Available at: https://tinyurl.com/ybev4p76

Thompson, H., Shepherd, B., Welch, G.H. and Anyimadu, A., 2017. Developing businesses of scale in sub-Saharan Africa: insights from Nigeria, Tanzania, Uganda and Zambia. Available at: https://tinyurl.com/2fnmemzd

Udomkun, P., Wiredu, A.N., Nagle, M., Bandyopadhyay, R., Muller, J. and Vanlauwe, B., 2017. Mycotoxins in sub-Saharan Africa: present situation, socio-economic impact, awareness, and outlook. Food Control 72: 110-122. https://doi.org/10.1016/j.foodcont.2016.07.039

Van Egmond, H.P., 2002. Worldwide regulations for mycotoxins. Advances in Experimental Medicine and Biology 504: 257-269. https://doi.org/10.1007/978-1-4615-0629-4_27

Weaver, M. and Abbas, H.K., 2019. Field displacement of aflatoxigenic *Aspergillus flavus* strains through repeated biological control applications. Frontiers in Microbiology 10: 1788. https://doi.org/10.3389/fmicb.2019.01788

Weaver, M.A., Abbas, H.K., Falconer, L.L., Allen, T.W., Pringle, H.C. and Sciumbato, G.L., 2015. Biological control of aflatoxin is effective and economical in Mississippi field trials. Crop Protection 69: 52-55.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2021.2758 - Friday, August 12, 2022 1:53:09 AM - IP Address:77.248.0.158

View publication stats