

Insecticides influence colonization and toxin accumulation by *Aspergillus* and *Fusarium* in orange and white maize flour varying in susceptibility to mycotoxigenic fungal pathogens

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Research Article

Keywords: Aflatoxin, *Aspergillus*, Fumonisin, *Fusarium*, Insecticide, Resistant varieties

Posted Date: February 9th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2543106/v1>

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Abstract

Mycotoxigenic *Aspergillus* and *Fusarium* species that contaminate cereal crops with aflatoxins and fumonisins are a major threat to food security, human and animal health. Cases of mycotoxin contamination have been on the rise globally, with causes of such surges raising research interests. We conducted factorial experiments in the laboratory to test the hypothesis that some recently produced improved maize varieties that are not purposively bred for resistance to storage insect pests and mycotoxigenic fungi, require protection by synthetic insecticides, which in turn serve as nutrient sources to mycotoxigenic fungi, leading to invasive growth and enhanced toxin production by fungi, especially in conditions of high moisture. Flour from the improved white maize variety *Western 505* that is not purposively bred for resistance to storage insect pests and mycotoxigenic fungi had the highest level of colonisation by *A. flavus* (6,633 CFU g⁻¹) and *Fusarium* sp. (10,870 CFU g⁻¹) with consequent elevated concentrations of aflatoxin (203 ppb) and fumonisin (2.23 ppm); while flour from the local yellow maize variety *Spindi* exhibited the least colonisation and mycotoxin production by *A. flavus* (4,133 CFU g⁻¹) and *Fusarium* sp. (6,145 CFU g⁻¹) with the local white *Rachar* being intermediate, at high moisture conditions. The synthetic insecticides *Super Blue Cross* (BC) and *Actellic Super* (AS) promoted growth and mycotoxin production by *A. flavus* and *Fusarium* sp.; while *Ash* promoted growth of *A. flavus* and *Fusarium* sp., but suppressed mycotoxin production by the two mycotoxigenic fungi (Aflatoxin and Fumonisin: control=114 ppb and 1.46 ppm; *Ash*=114 ppb and 1.46 ppm; AS=270 ppb and 2.31 ppm; BC=320 ppb and 3.55 ppm). It is concluded that flours from the local yellow *Spindi* and local white *Rachar* maize varieties are less susceptible to infection and mycotoxin contamination by *A. flavus* and *Fusarium* sp. compared to the improved white maize variety *WH505*, which accumulates higher levels of the mycotoxigenic fungi and their toxins, especially when treated with the synthetic insecticides *Super Blue Cross* and *Actellic® Super* that act as fungal nutrient sources under high moisture conditions. The implications of these findings are further discussed in the context of some relevant policies.

Introduction

Food contamination by mycotoxigenic fungi of the genera *Aspergillus* (Figure 1a) and *Fusarium* (Figure 1b), with their respective devastating aflatoxins (Figure 2a) and fumonisins (Figure 2b), have been major hazards to human and animal health globally (Ekwomadu et al. 2022; Meneely et al. 2022). Mycotoxins have been associated with health-related complications such as reduced immune functions, cancers, acute liver damage, suppressed growth, abnormal foetal development and even death (Gong et al. 2016; Ostry et al. 2017; Awuchi et al. 2022). Besides, food contamination by mycotoxigenic fungi leads to production of non-marketable grains and hence low economic viability (Udomkun et al. 2017). Mycotoxigenic fungi are therefore a hindrance to food security, human health and an accelerator of poverty (Yahia et al. 2020; Adeyeye et al. 2022). The problem of mycotoxins has been on the rise in Kenya and Uganda among other African countries that heavily consume maize and other cereal crops (Mutiga et al. 2019; Chilaka et al. 2022). For instance, in Kenya, an outbreak of aflatoxin contamination in maize led to 125 human deaths through acute aflatoxicosis in the year 2004 (Omara et al. 2021; Chilaka et al. 2022). This was reported as one of the utmost severe cases of mycotoxin contamination and poisoning in history, and the problem continues to exist (Obonyo and Salano 2018; Dharini et al. 2022; Chilaka et al. 2022).

The underlying causes of the current emerging trends of increased mycotoxin contamination around the world is a subject that has remained to be resolved. There are concerns that some new deadly strains of mycotoxigenic fungi that are resistant to control measures have been emerging (Hamad et al. 2023). There are cases whereby exotic strains of mycotoxigenic fungi and mycotoxin-contaminated foodstuffs have been introduced into new territories

through international trade and humanitarian food aid programs (Chilaka et al. 2022; Nji et al. 2022). A good example is the genetically distinct S strain morphotype of *Aspergillus flavus* (Probst et al. 2012), which was associated with the deadly aflatoxin contamination events in Kenya between the year 2004 and 2006 (Azziz-Baumgartner et al. 2005; Islam et al. 2018; Omara et al. 2021). Climate change and associated variations in temperature and moisture may be linked to the widespread shift in the emergence of mycotoxigenic fungal contaminants on food (Zingales et al. 2022). Besides these explanations, human conflicts and political instability around the world may have accelerated the spread of mycotoxins through their possible use as bioweapons (Venkataramana et al. 2015), with *A. flavus* being among microbes of growing concern as potential bioweapons in Kenya and around the world (UNODA 1972; Nthakanio and Wakhungu 2017).

Management of mycotoxigenic fungi such as *Aspergillus* and *Fusarium* has been a global challenge (Logrieco et al. 2021) because such microbes exhibit both endophytic and saprophytic lifestyles (Ochieno 2020; Akram et al. 2023; Dlamini et al. 2023) with their ubiquitous propagules being capable of surviving in different environments (Abrehame et al. 2023), while infections can occur both in the field and under storage conditions (Lamboni and Hell 2009). Drying of maize grains to 13% moisture content has been the main method that helps reduce mycotoxin contamination (Xu et al. 2022). However, moisture may still reduce gradually in poorly dried produce or build up in storage over time depending on climatic conditions (Fandohan et al. 2006), while damage and contamination of stored food products by post-harvest insect pests promotes contamination by mycotoxigenic fungi (Fandohan et al. 2006; Lamboni and Hell 2009). Currently in a country like Kenya, despite the release of numerous improved maize varieties (KEPHIS 2020), those purposefully bred for resistance towards the mycotoxigenic fungi and storage pests have still not been available (KEPHIS 2020; Krishna et al. 2023; Rock et al. 2023). A wide range of synthetic fungicides are used on-farm and as seed treatments for various crops (Müllenborn et al. 2008; Ochilo et al. 2019; Lamichhane et al. 2020), besides being experimentally assessed for on-farm and post-harvest potential against mycotoxigenic fungi of cereal crops (D'Mello et al. 1998; Masiello et al. 2019). However, some synthetic fungicides may even exhibit stimulatory effects on the production of toxins by mycotoxigenic fungi (D'Mello et al. 1998; Müllenborn et al. 2008). Furthermore, information on post-harvest application of fungicides to control mycotoxigenic fungi on cereal crops in a real-life scenario has been rare. Therefore, maize farmers have been left with the almost inevitable option of applying toxic synthetic insecticides to control storage insect pests associated with mycotoxigenic fungal infections (Bhusal and Khanal 2019; Lalah et al. 2022). The scourge of mycotoxins keeps tormenting consumers (Omara et al. 2021; Miller 2023) amidst an increase in adoption of improved maize varieties and insecticide application.

This scenario leaves an open question on whether the continuous rise in mycotoxigenic fungal contaminant cases (Miller 2023) may be related to the accelerated shift from the more adaptable indigenous local maize landraces due the push for 'varietal turnover' (Abate et al. 2017; Nagarajan et al. 2019; Almekinders et al. 2021; Mathew and Mathew 2023), towards the adoption of new improved maize varieties some among them regarded to be less adaptable (Almekinders et al. 2021; Chivasa et al. 2022; Krishna et al. 2023; Mathew and Mathew 2023), amidst the market-driven increasing demand for synthetic insecticides and other agrochemicals (Hagblade et al. 2022; Lalah et al. 2022).

In the present study, it is hypothesized that some recently produced improved maize varieties that are not purposively bred for resistance to storage insect pests and mycotoxigenic fungi (KEPHIS 2020; Krishna et al. 2023), require protection by synthetic insecticides (Mutiga et al. 2015; Hasnaki et al. 2023), which in turn serve as nutrient sources to mycotoxigenic fungi (Porto et al. 2011; Abd El-Ghany and Masmali 2016; Satish et al. 2017; Bose et al.

2021; Khan et al. 2023), leading to invasive growth and enhanced toxin production by mycotoxigenic fungi (Brzonkalik et al. 2012; Al-Gabr et al. 2013), especially in conditions of high moisture (Manu et al. 2019).

Materials And Methods

Experimental design

Studies were conducted in the Crop Science Laboratory at Masinde Muliro University of Science and Technology (MMUST) within Kakamega County in Kenya, with complementary assays at the Mycotoxin Research Laboratory in Egerton University within Nakuru County in Kenya. The studies comprised *in vitro* and *in vivo* experiments that utilized fungal strains from two genera associated with the production of mycotoxigenic compounds namely, *Aspergillus* and *Fusarium*, interacting with eight types of insecticide treatments. For each of the fungal genera, there were two *in vitro* assays. The first *in vitro* assay of either *Aspergillus* or *Fusarium* respectively, comprised 3×8 full factorial treatments consisting of three fungal strains and eight types of insecticidal compound treatments that included untreated control (24 treatment combinations), having $n=6$ Petri dishes in 4 replicates and repeated once, and hence $N=1152$. The second *in vitro* assay of either *Aspergillus* or *Fusarium* respectively, consisted of $1 \times 2 \times 5$ factorial treatments of one fungal strain and two types of insecticidal compounds at five concentrations that included untreated control (10 treatment combinations), having $n=6$ Petri dishes in 4 replicates and repeated once, and hence $N=480$. The *in vivo* assays of either *Aspergillus* or *Fusarium* respectively, were based on a single fungal strain in incomplete factorial treatments (Table 1), constituting of two levels of moisture content, four insecticide levels including untreated control, four types of maize flour inoculated with the fungus or not, with some possible treatment combinations from a $2 \times 4 \times 4$ complete factorial design not included resulting in the incomplete factorial design (32 treatment combinations) having a sample size (n) of 6 bottles in 3 replicates and hence $N=576$ (Table 1). Data was collected on parameters that included substrate weight change, fungal growth and mycotoxin production.

Fungal strains and inocula

Three strains of *Aspergillus flavus* and three strains of *Fusarium* sp. were obtained from the Mycotoxin Research Laboratory at Egerton University, Nakuru Kenya (Elsie et al. 2016). These fungal strains had originally been isolated from contaminated soils and stored as pure cultures in Potato Dextrose Agar (PDA) at 25°C. The fungal cultures were transported in a cool box to the Crop Science Laboratory (MMUST) and sub-cultured in 19g/L Potato Dextrose Agar (PDA) (HiMedia, Maharashtra, India) at 25°C for 7 days before use in experiments. The *Aspergillus* strains included one non-aflatoxigenic variant *Aspergillus flavus* 1E09602 and two aflatoxigenic variants *A. flavus* 2EMO401 and *A. flavus* 2EMO602, with respect to the mycotoxin production mechanisms (Bhatnagar et al. 2016; Elsie et al. 2016; Kushiro et al. 2018; Lagat et al. 2020). *Fusarium* strains included one non-fumonisin-producing variant *Fusarium* DD6 and two fumonisin-producing variants *Fusarium* CM2 and *Fusarium* MM2, with respect to the mycotoxin production mechanisms (Burgess et al. 2016; Elsie et al. 2016; Munkvold et al. 2021).

Aspergillus and *Fusarium* inocula for *in vitro* assays comprised of single mycelia from pure sub-cultures of the respective fungal strains in PDA cultured at 25°C for at least 7 days. *Aspergillus* and *Fusarium* inocula for *in vivo* assays consisted of a mycelia-spore suspensions in sterile distilled water with spore densities of $1 \times 10^6 \text{ mL}^{-1}$, respectively (Lagat et al. 2020). *Aspergillus* mycelia and spores were washed off from PDA cultures in Petri dishes by pipetting 2 mL of sterile distilled water into a glass beaker (100 mL). The number of *Aspergillus* spores in the 2 mL supernatant was estimated using a hemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA) at

×100 magnifications under a light microscope (LMU-405), based on the method described by (Lagat et al. 2020). This was followed by serial dilutions to obtain the targeted spore density of $1 \times 10^6 \text{ mL}^{-1}$. *Fusarium* inoculum for *in vivo* assays was obtained in a similar manner, except that the media utilized was Synthetic Nutrient Agar (SNA) comprising of 1.0g potassium dihydrogen phosphate (KH_2PO_4), 1.0g potassium nitrate (KNO_3), 0.5g magnesium sulphate (MgSO_4), 0.5g potassium chloride (KCl), 0.2g D-glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and 20g Agar per litre of distilled water, which facilitates sporulation of *Fusarium* spp. at 25°C within 14 days (Wagacha et al. 2016).

Maize substrates

Grain flour substrate was obtained from three types of insecticide-free grains of maize varieties that included the commercial hybrid *WH505* (Western Seed Company, Kitale, Kenya; KEPHIS 2020), two local landraces *Spindi/Nyamula* (yellow seed) and *Rachar* or Local 8 (white seed), and the fourth being commercial white flour *Dola* of unverified maize variety or insecticide treatment status. *Rachar* and *WH505* have been reported to be relatively less vulnerable to aflatoxin and fumonisin contamination under storage conditions in some parts of western Kenya (Mutiga et al. 2015). Information concerning mycotoxin contamination in *Spindi* maize has been scarce. Commercial maize flour *Dola* (batch number A16436, Kitui Flour Mills Ltd, Kenya) originates from the Eastern region of Kenya where there are high cases of aflatoxin contamination in maize (Asamba 2020; Massomo 2020). The maize grains were milled to Grade II texture flour (0.2 mm mesh size).

Insecticidal compounds

The study utilized six synthetic insecticides commonly used in stored maize targeting insect pests that are endemic in western Kenya such as the Maize Weevil *Sitophilus zeamais* (Curculionidae), the Larger Grain Borer *Prostephanus truncates* (Bostrichidae) and the Angoumois Grain Moth *Sitotroga cerealella* (Gelechiidae) (De Groot et al. 2017; Kagot et al. 2022). The dust formulated insecticides that were selected based on their active ingredients included, *Actellic® Super Dust*, *Actellic® Gold Dust*, *Super Blue Cross Dust*, *Super Grain Dust*, *Skana® Super* and *Sumicombi®* (Table 2). *Ash* was obtained from a mixture of burnt common bean *Phaseolus vulgaris* leaves stems and pods, which is locally utilized for production of traditional salt 'Munyu Musherekha' among the indigenous Luhya people of Kenya (Merchant et al. 2022) as well as an insecticide (Hakbijl 2002), was included as a non-synthetic pest control option.

Insecticide-enriched culture media and fungal inoculation in vitro

Formulation of insecticide-enriched culture media was done based on the method described by Xiang et al. (2020). Potato Dextrose Agar (HiMedia, Maharashtra, India) was mixed with distilled water at a rate of 39g/L in graduated conical flasks. The culture media mixture was then autoclaved at 121 °C for 15 min, and allowed to cool to about 60°C.

For the first *in vitro* assays, insecticide dusts were weighed on a top loading laboratory balance (Ohaus Scout Pro Portable Electronic Balance, Switzerland), to reflect the standard application doses per mass of substrate as per the respective manufacturer's recommendations (Table 2). The insecticide doses were added to the cooled PDA media, swirled gently for 5 min and dispensed into plastic Petri dishes (90 mm diameter). *Ash* was applied in a similar manner based on rates applied by farmers of around 20g of *Ash* per 100g of maize (Jaganathan et al. 2018).

The assays utilized *Aspergillus* spores or *Fusarium* mycelia respectively, which were carefully retrieved using sterile wooden toothpicks from the pure cultures, and inoculated at the center of the solidified insecticide-enriched PDA in

Petri dishes. The Petri dishes were sealed with parafilm and the initial weight recorded using a top loading laboratory balance. The Petri dishes were completely randomized in the dark at room temperature (~ 25°C) for 8 days.

For the second *in vitro* assays, the fungal strain-insecticide treatment combinations that exhibited the strongest positive effect on the growth of *Aspergillus* and *Fusarium* were further assessed at five graduated insecticide concentrations, which ranged from zero (0), 50%, 100%, 150% to 200% of the recommended dose.

Fungal colony growth in vitro

Growth of the fungal strains was recorded using a transparent ruler as the maximum colony diameter at 48 h intervals within 8 days. The biomass of the fungal cultures per Petri dish was also recorded using a top loading laboratory balance at the end of the incubation period as a complementary measure of growth or substrate consumption by the microbes. *Aspergillus* cultures were used for detection of aflatoxin production *in vitro*, while estimation of fumonisin production by *Fusarium in vitro* was visually determined.

Mycotoxin production estimates in vitro

Aflatoxin production by *Aspergillus* strains *in vitro* was assessed at the Crop Science Laboratory (MMUST) by the ammonia vapor test (Shekhar et al. 2017; Rajarajan et al. 2021). The Petri dishes containing the 8-day old *Aspergillus* cultures were turned upside-down to rest on the lids. Some 2 mL of concentrated ammonia solution (NH₄ OH) was pipetted onto the inverted lid and the overturned base holding the fungal culture used as the cover. Ammonia solution was allowed to vaporize for 15 min to have the fungal colonies exposed to the reagent vapor. The extent of color change in the culture media from pale to dark-brown was recorded on a 1-3 scale starting from no clear observable colour difference to the most intense. Colour change in *Fusarium* was visually described without quantification, based on colony colour intensity change from white to the characteristic deep-pink (Leslie and Summerell 2006).

Insecticide-enriched maize flour and fungal inoculation in vivo

Three insecticidal compounds that included two with the strongest effect on growth and one having the weakest growth-promoting effect on the fungal strains *in vitro* were considered in the *in vivo* assays that utilized maize flour substrates. Maize flour substrates contained in 500 mL glass jars were sterilized at 121°C for 15 min in an autoclave (HS-VSE150, Hongshun Medical Instruments, Ahui, China) before cooling to ~40 °C. Insecticidal compounds were applied to match the recommended rates (Table 2), before being thoroughly mixed. The flour was oven-dried at 55 ± 2 °C for 30 min to extremely reduce the moisture content. The flour was weighed in 30 g portions into 50 mL sterile plastic containers. Sterile distilled water (3 mL) was added into flour containers meant for high moisture treatment so as to obtain 100% water activity (1.00_{aw}) (Akbar and Alam 2019). Containers meant for low moisture content were left without addition of water.

Some 1 mL spore suspension (1×10^6 mL⁻¹) inoculum of the selected strains of *Aspergillus* or *Fusarium* were pipetted into the maize flour and mixed thoroughly in the plastic containers as per the experimental design (Table 1). The plastic containers were loosely closed to prevent anaerobic respiration and fermentation of the samples before recording their initial weights. The containers were completely randomized in the dark and incubated for 14 days at room temperature (~25°C).

Fungal colony growth in vivo

Enumeration of fungal propagules as colony forming units (CFUs) in flour was carried out using the dilution plate technique (Cotty 1994; Owuor et al. 2017). Some composite flour sample (1g) obtained from a thorough mixture of the samples per treatment replicate was aseptically mixed with sterilized distilled water (9 mL) to give a 1×10^1 dilution; which was used to prepare a three-fold diluted suspension (1×10^3). Some 0.1 mL of the 1×10^3 solution was dispensed using a micropipette onto 90 mm Petri dishes containing PDA culture media. A flame-sterilized glass rod was used for spreading the solution over the agar. The Petri dishes were sealed with parafilm and incubated at room temperature in the dark for 48 hrs. *Aspergillus* and *Fusarium* colonies that grew on the Petri dishes were counted. Their population was expressed as colony forming units (CFU) per gram based on the mathematical formula:

$$\text{Total fungal colony population} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Weight of flour (g)}}$$

Mycotoxin production estimates in vivo

Mycotoxin concentrations in the *in vivo* assays were determined by Enzyme-Linked Immunosorbent Assay (ELISA) at the Mycotoxin Research Laboratory, Egerton University. This was done for both aflatoxins and fumonisin in the *Aspergillus* and *Fusarium* experiments, respectively.

Aflatoxin quantification by ELISA for in vivo samples

Some 20g of a composite maize flour samples were oven-dried at 30°C for 24 hrs in 500 mL conical flasks. Aflatoxin extraction solvent (100 mL) made of a mixture of 70 mL methanol and 30 mL distilled water was added to the flour samples and mixed by shaking in the sealed conical flasks. The mixture was allowed to sediment and 5 mL filtrate for ELISA test obtained using a Whatman #1 filter paper. Helica Total Aflatoxin Assay based on Direct Competitive ELISA method was applied in quantification of aflatoxins (Helica™ Elisa Total Aflatoxin Kit 941AFL01M-96, Hygiena International, Watford, UK) (Hygiena Catalog# 941AFL01AQ-96; Sigma-Aldrich 2018a). The extraction protocol was as per the manufacturer's instructions. This was followed by quantification of the optical density (OD) of each microwell using a microtiter plate reader (AgileReader™ Köln, Germany) using a 450nm filter. The zero standard was set as 100% binding (Bo). Percentage Binding (%B) was calculated for each standard and sample as a percentage of the zero binding (%B/Bo).

Fumonisin quantification by ELISA for in vivo samples

Some 20g of a composite maize flour samples were oven-dried at 30°C for 24 hrs in 500 mL conical flasks. Sterile distilled water (200 mL) was added to flour samples and vortexed for 5 min. The samples were allowed to sediment for 5 min, and 10 mL of the supernatant carefully filtered of the through a Whatman #1 filter paper into a falcon tube (50 mL). Distilled water (40 mL) was added to the filtrate samples to obtain a ratio 1:4 (sample extract 1:4 in distilled water). Helica Total Fumonisin Assay based on Direct Competitive ELISA method was applied in quantification of fumonisins (Helica™ Fumonisin Hydro ELISA, Hygiena International, Watford, UK) (Hygiena Catalog# 201FUM01AQ-96; Sigma-Aldrich 2018b). The extraction protocol was as per the manufacturer's instructions. This was followed by quantification of the optical density (OD) of each microwell using a microtiter plate reader (AgileReader™ Köln, Germany) using a 450nm filter.

Data analyses

Data analyses were done using the Statistical Analysis System (SAS 9.1) software (SAS 2004). *PROC MEANS* was applied in generating means for continuous numerical data that included fungal colony diameter (*in vitro*), number of colony forming units (CFU), aflatoxin and fumonisin concentrations, substrate weight (PDA-*in vitro* and maize flour-*in vivo*), as well as categorical data that comprised of colony colour (estimated on a chromogenic scale 1-3) related to aflatoxin concentration. The data were subjected Analysis of Variance (ANOVA) in *PROC GLM*, and post-hoc test for the parameters with continuous data by LSD (Fisher's Least Significant Difference), while Student-Newman-Keuls (SNK) applied to the data on colony color change. Data for fungal colony forming units and mycotoxin concentrations derived from ELISA were described without subjecting them to inferential statistics.

Results

Aspergillus flavus growth in vitro and in vivo

Aspergillus flavus colony growth differences between the insecticide treatments were evident first day ($p < 0.05$), with those grown in PDA enriched with *Actellic® Super* and *Actellic® Gold* being the smallest in diameter, while those grown in PDA enriched with *Ash* or *Super Blue Cross* having the largest diameters after eight days ($p < 0.05$) (Figure 3a). The three *A. flavus* strains did not vary in colony diameter within each insecticide treatment (Figure 3a). In the follow-up *in vitro* experiment the *A. flavus* colony diameters significantly varied ($p < 0.05$), with those grown in higher concentrations of *Ash* and *Super Blue Cross* being larger; but those grown at the highest concentration of *Ash* being the smallest in diameter (Table 3). Cases of microbial contaminants were consistently observed in all petri dishes containing PDA enriched with *Actellic® Super* and *Actellic® Gold* in all replicates and repeats (Figure 4 & 5).

Aspergillus flavus colony forming units (CFU) were more in maize flour containing *Ash*, *Super Blue Cross* and *Actellic® Super* compared to the controls in both low and high moisture conditions (Table 4a). *Aspergillus flavus* colony forming units (CFU) were most in maize flour containing *Ash* followed by *Super Blue Cross* and while flour containing *Actellic® Super* had the fewest CFUs in both low and high moisture conditions (Table 4a). *Aspergillus flavus* CFUs were more in high moisture flour and in the low moisture flour (Table 4a). *Aspergillus flavus* CFUs were most in flour derived from the commercial white maize variety *WH505* followed by the local white maize variety *Rachar* and fewest in the local yellow variety *Spindi*, with this trend appearing consistent between the insecticide treatments under both moisture regimes (Table 4a). The commercial maize flour *Dola* contained morphologically identified colonies of *Aspergillus* spp. whose population increased with moisture (Table 4a).

Aflatoxin estimation by ammonia vapour test and quantification by ELISA

In the ammonia vapour test, the three strains of *A. flavus* including the non-aflatoxigenic IEM2902 exhibited variable levels of colour change towards dark-brown (Figure 4), when subjected to the ammonia vapour test ($p < 0.05$) (Table 5). Colony colours of the three *A. flavus* strains varied between insecticide treatments, with those cultured in PDA enriched with *Super Blue Cross* having the most intense colour change (Figure 4; Table 5). Colonies of *A. flavus* cultured in *Actellic® Super* and *Actellic® Gold* enriched PDA barely expressed detectable colour change (Figure 4; Table 5). Colonies of the three strains of *A. flavus* cultured in *Ash* enriched PDA had lower colour intensity compared to their respective controls (Table 5). In PDA enriched with the four insecticides namely *Super Grain Dust*,

Skana[®] *Super*, *Sumicombi*[®] and *Super Blue Cross*, *A. flavus* strain 2EMO401 had the highest colour intensity followed by strain 2EMO62, while strain IEM2902 had the least colour intensity (Table 5).

In the ELISA test, aflatoxins were detected in all the four maize flour varieties at varying quantities, with their levels tending to increase and the trends being more prominent under high moisture conditions (Table 6a). The concentration of aflatoxins was consistently highest in *A. flavus*-inoculated maize flour containing *Super Blue Cross* followed by *Actellic*[®] *Super*, then *Ash*, no insecticide and lowest in the absolute control comprising sterilized flour without insecticide; with this trend being reflected in all the three types of flour derived from the local white *Rachar*, commercial white *WH505* and local yellow *Spindi* maize varieties (Table 6a). Among the control sterilized flours, the concentration of aflatoxins was highest in the commercial white *WH505*, followed by the local yellow *Spindi* and lowest in local white *Rachar* maize variety (Table 6a). Where *A. flavus* was applied without insecticides, aflatoxin concentration was highest in flour derived from the local yellow *Spindi* followed by commercial white *WH505* and lowest in local white *Rachar* maize variety (Table 6a). In the presence of *Ash*, flour derived from the local white maize *Rachar* variety had the highest concentration of aflatoxins followed by the local yellow *Spindi* and lowest in the commercial white *WH505* maize variety (Table 6a). In the presence of *Actellic*[®] *Super* or *Super Blue Cross*, flour derived from the commercial white *WH505* variety had the highest aflatoxin concentration followed by the local white *Rachar* variety and lowest in the local yellow *Spindi* variety (Table 6a). The commercial processed flour *Dola* contained aflatoxin levels whose quantities increased over 40 times in high moisture with the levels much increased compared to the other three types of flour (Table 6a).

Fusarium growth in vitro and in vivo

Fusarium colony growth differences between the insecticide treatments were evident after the first day ($p < 0.05$), with those grown in PDA enriched with *Actellic*[®] *Super* and *Actellic*[®] *Gold* being the smallest diameters, while those grown in PDA enriched with *Ash* and *Super Blue Cross* having the largest after eight days ($p < 0.05$) (Figure 3b). In the follow-up *in vitro* experiment the *Fusarium* colony diameters significantly varied ($p < 0.05$), with those grown in higher concentrations of *Ash* and *Super Blue Cross* being larger; but those grown at the highest concentration of *Ash* being the smallest in diameter (Table 3b).

Fusarium colony forming units (CFU) were more in maize flour containing *Ash*, *Super Blue Cross* and *Actellic*[®] *Super* compared to the controls in both low and high moisture conditions (Table 4b). *Fusarium* colony forming units were most in maize flour containing *Actellic*[®] *Super* followed by *Super Blue Cross* while flour containing *Ash* had the fewest CFUs in both low and high moisture conditions (Table 4b). *Fusarium* CFUs were more in high moisture flour than in the low moisture flour (Table 4b). *Fusarium* CFUs were the most in flour derived from the commercial white maize variety *WH505* followed by the local white maize variety *Rachar* and lowest in the local yellow variety *Spindi*, with this trend appearing consistent between the insecticide treatments especially in the high moisture regime (Table 4b). The commercial maize flour *Dola* contained culturally identified colonies of *Fusarium* spp. whose population increased with moisture (Table 4b).

Fumonisin quantification visually and by ELISA

In the visual assessment, the three strains of *Fusarium* including the non-fumonisin strain grown in PDA enriched with *Actellic*[®] *Gold*, *Actellic*[®] *Super* and *Super Blue Cross* exhibited increased colour change towards deep pink (Figure 5). In the ELISA test, fumonisins were detected in all the four maize flour varieties at varying quantities, with their levels tending to increase and the trends being more prominent under high moisture conditions (Table 6b). The

concentration of fumonisins was consistently highest in *Fusarium*-inoculated maize flour containing by *Actellic® Super* followed *Super Blue Cross*, no insecticide, *Ash* and lowest in the absolute control comprising sterilized flour without insecticide; with this trend being reflected in all the three types of flour derived from the local white *Rachar*, commercial white *WH505* and local yellow *Spindi* maize varieties (Table 6b). Among all treatments and controls, fumonisin concentration was highest in the commercial white *WH505*, followed by the local white *Rachar* and lowest in local yellow *Spindi* maize variety (Table 6b). The commercial processed flour *Dola* contained fumonisin levels whose quantities increased 5 times more in high moisture with the levels being very high compared to the other three types of flour (Table 6b).

Discussion

The present study tested the hypothesis that, some recently produced improved maize varieties that are not purposively bred for resistance to storage insect pests and mycotoxigenic fungi, require protection by synthetic insecticides, which in turn serve as nutrient sources to mycotoxigenic fungi, leading to invasive growth and enhanced toxin production by mycotoxigenic fungi, especially in conditions of high moisture. Results from *in vitro* and *in vivo* experiments revealed that the growth of *A. flavus* and *Fusarium* sp. was enhanced in substrates enriched with the organophosphate insecticide *Super Blue Cross* that contains Malathion ($C_{10}H_{19}O_6PS_2$) and in substrates enriched with *Phaseolus vulgaris*-derived *Ash*. It is likely that organic carbon (OC) as well as phosphorus (P) and sulphur (S) contained in malathion were utilized as nutrients by the fungal strains (Omar 1998; Leslie and Summerell 2006; Ijadpanahsaravi et al. 2021; Hamad et al. 2023). On the other hand, vegetative ashes including those derived from *P. vulgaris* contain a complex of nutrient elements, especially calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn) and iron (Fe) (Kmiciek et al. 2000; Nwadike et al. 2018), which promote the growth and multiplication of fungi and other microbes (Bang-Andreasen et al. 2017; Walker and White 2018; Singh and Khajuria 2018). However, very high concentrations of *Ash* and *Super Blue Cross* appeared to inhibit the growth of *A. flavus* and *Fusarium* sp. strains *in vitro*. Such fungal growth inhibiting effects of *Ash* and malathion-containing insecticides have been reported (Bang-Andreasen et al. 2017; Srinivasulu and Ortiz 2017), as high concentrations of these two insecticidal compounds become toxic to the microbes (Abd El-Ghany and Masmali 2016), while there still exists mixed information on malathion toxicity to mammals (Jensen and Whatling 2010; Selmi et al. 2018). Although *Actellic® Super* ($C_{11}H_{20}N_3O_3PS + C_{22}H_{20}Cl_2O_3$) and *Actellic® Gold* ($C_{11}H_{20}N_3O_3PS + C_8H_{10}Cl_2N_5$) promoted the growth of *A. flavus* and *Fusarium* sp. *in vivo*, their capacity to promote the growth of these fungal strains could not be verified *in vitro* due to the presence of antagonistic microbial contaminants.

Aflatoxin detection by ammonia vapour test *in vitro* indicated that *A. flavus* strains grown on PDA-enriched with *Super Blue Cross* had the highest colour intensity, which is an indication of elevated production of aflatoxins (Dufossé et al. 2005; Rajarajan et al. 2021). Enhanced production of aflatoxins may be linked to nutritive components of the insecticide in the form of organic carbon, phosphorus and sulphur in *Super Blue Cross* ($C_{10}H_{19}O_6PS_2$), which are consumed by the fungi for growth and mycotoxin production (Hamad et al. 2023). This observation in *A. flavus* appeared similar to visual assessment in *Fusarium* sp., whereby colonies grown in PDA-enriched with *Super Blue Cross* were large with bright pink colour, indicating enhanced accumulation of secondary metabolites including fumonisins and various pigments (Soumya et al. 2018; Singh and Khajuria 2018; Menezes et al. 2020; Rajarajan et al. 2021). Conversely, *Actellic® Super*, *Actellic® Gold* and the *Ash* appeared to suppress mycotoxin production by the *A. flavus* strains in the ammonia vapour test. Only *Ash* appeared to inhibit fumonisin production by *Fusarium* sp. based on limited colour change as per the visual assessment. Mechanisms behind

aflatoxin and fumonisin inhibition by *Ash* may be explained in terms its alkalinity due to the presence of Ca^{2+} and K^{+} cations (Ayo et al. 2018), which facilitate the breakage of lactone rings resulting in incomplete synthesis of the toxins (Bozoğlu 2011; Ayo et al. 2018; Odukoya et al. 2021). ELISA results in the *in vivo* experiments that utilized maize flour substrates indicated that *Super Blue Cross* and *Actellic® Super* treatments had the highest aflatoxin and fumonisin concentrations. On the other hand, maize flour enriched with *Ash* had the lowest aflatoxin and fumonisin concentrations. In the case of *A. flavus*, trends in the *in vivo* results corroborated the *in vitro* ones, except for *Actellic® Super*, whereby aflatoxin detection *in vitro* may have been impeded by microbial contaminants. Methodological differences between ELISA *in vivo* and ammonium vapour test *in vitro* also make the results not to have common bases for analysis, and hence producing inconsistent interpretation of aflatoxin concentrations among the *Actellic® Gold* and *Actellic® Super* treatments (Shekhar et al. 2017). Dichlorvos-Ammonia (DV-AM) method for determining aflatoxigenicity would have produced results that are more consistent with ELISA (Kushiro et al. 2018; Lagat et al. 2020).

Flour substrates from the four maize varieties expressed varying levels of colonization by the mycotoxigenic fungi and mycotoxin accumulation. Generally, flour from the improved white maize variety *WH505* under high moisture conditions exhibited the highest levels of *A. flavus* and *Fusarium* sp. colonization in terms of colony forming units as well as aflatoxin and fumonisin concentrations. Flour from the local yellow maize variety *Spindi* appeared to exhibit the least levels of colonization and mycotoxin production by *A. flavus* and *Fusarium* when compared to the white local *Rachar* and improved maize varieties *WH505*. Yellow grain maize varieties including *Spindi* express beta-carotenes (LaPorte et al. 2022), which are antioxidants that quench reactive oxygen species (ROS) produced in response to fungal pathogen invasion to limit the production of mycotoxins (Díaz-Gómez et al. 2016). The concentrations of aflatoxin (2.35 ppb) and fumonisin (1.22 ppm) in the retailed maize flour *Dola* were within the recommended safe levels as per the Kenya Bureau of Standards (KEBS) and the European Commission (EC) (Njeru et al. 2018). However, addition of moisture into *Dola* maize flour led to increase in aflatoxins and fumonisin concentration 40-fold and 5-fold, respectively. This is because moisture has a great influence on the transcriptional activation of mycotoxin biosynthetic genes and mycotoxin production (Kolawole et al. 2021). Despite these observations, there needs to be more critical interpretation of the *in vivo* aflatoxin data, especially the fact that flour from the artificially inoculated local yellow maize *Spindi* variety had the highest concentration of the toxin (135 ppb) when inoculated with *A. flavus* without insecticidal compounds, even when compared to the commercial *Dola* (100 ppb) that was already naturally contaminated with the fungus.

Various observations have been made in this study in relation to mycotoxigenic fungi and their interaction with maize varieties, insecticides and moisture. However, there are some issues that could form basis for future studies. For instance, the present study utilized maize flour instead of whole grain and found that insecticides promote mycotoxin contamination. Mutiga et al. (2019) did not observe an increase in mycotoxin concentrations in whole maize grains treated with insecticides in farmers' stores. Furthermore, besides *Dola* maize flour that contained *Aspergillus* and *Fusarium* among other microbes, inclusion of non-sterile maize flours from the improved white *WH505*, local white *Rachar* and local yellow *Spindi* maize varieties would have contributed more information on the diverse natural microbial communities in maize grains (Liu et al. 2020; Ekpakpale et al. 2021), and their interactions with postharvest insecticides (Ngwenyama et al. 2022). Therefore, future studies may compare the effects of insecticides on mycotoxin contamination between whole maize grain and their respective flours through controlled experiments and using samples from farmers or retailers. Microbial contaminants were consistently detected in the synthetic insecticides *Actellic® Super* and *Actellic® Gold*. The identity of such microbes, their source and effects on the synthetic insecticides need to be established, as the pesticides could be sources of microbial contaminants with

unknown effects to human and animal health (Ng et al. 2005). Little information is available on this emerging perspective of microbe-contaminated synthetic pesticides and the wider pest-contaminated agrochemicals. Information on such microbes could help improve on policies and procedures related to pesticide registration (PCPB 2022), as the current regulations for insecticide labels do not adequately capture such details (PCPB 2018b). Flour from the local indigenous yellow *Spindi* and white *Rachar* maize varieties had relatively lower levels of mycotoxigenic fungal colonization and their toxins. These local landrace maize varieties, especially the yellow one that is a natural source of Vitamin A (Kean et al. 2008; Hwang et al. 2016), need to be conserved for improvement of the commercial ones (Ortiz et al. 2010), particularly towards resistance against mycotoxigenic fungi (Williams 2006; Chalivendra et al. 2020; Ortega-Beltran et al. 2022), which is yet to be actualized by maize breeding programs in a country like Kenya (KEPHIS 2020; Okolo et al. 2023; Rock et al. 2023). Non-mycotoxigenic strains of *Aspergillus* and *Fusarium*, including commercially available products such as Aflasafe™ (Atehnkeng et al. 2022; Chang 2022), may need to be further investigated for their possible roles in promoting food safety through biodegradation of synthetic insecticides in stored food or during microbial-based food processing (Jin et al. 2022). There is need for continuous review and development of general public health policies and strategies to mitigate the possible triple-threat arising from interactions between pest-susceptible maize varieties, mycotoxigenic fungi and toxic synthetic insecticides (Othieno 2018; Kilonzo 2019; Aleke 2020; Chilaka et al. 2022). This would help Kenya and Uganda among other African nations achieve food security and nutrition (SDG2), good health and well-being (SDG3) through sustainable food consumption and production (SDG12), while building networks such as the Partnership for Aflatoxin Control in Africa (PACA) (SDG17) in line with the United Nations Sustainable Developments Goals (SDGs) and Agenda 2063 of the African Union (AUC 2015; ICSU 2017).

To summarize, this study was based on the hypothesis that some recently produced improved maize varieties that are not purposively bred for resistance to storage insect pests and mycotoxigenic fungi, require protection by synthetic insecticides, which in turn serve as nutrient sources to mycotoxigenic fungi, leading to invasive growth and enhanced toxin production by fungi, especially in conditions of high moisture. Flour from the improved white maize variety *Western 505* that is not purposively bred for resistance to storage insect pests and mycotoxigenic fungi had the highest level of colonisation by *A. flavus* and *Fusarium* sp. with consequent elevated aflatoxin and fumonisin concentrations. Flour from the local yellow maize variety exhibited the least colonisation and mycotoxin production by *A. flavus* and *Fusarium* sp., especially under high moisture conditions. The synthetic insecticides *Super Blue Cross* (C₁₀H₁₉O₆PS₂) and *Actellic® Super* (C₁₁H₂₀N₃O₃PS; C₂₂H₂₀Cl₂O₃) promoted growth and mycotoxin production by *A. flavus* and *Fusarium* sp. The *P. vulgaris*-derived *Ash* promoted growth of *A. flavus* and *Fusarium* sp., but suppressed mycotoxin production by the two mycotoxigenic fungi. Based on these findings, it is concluded that flours from the local yellow *Spindi* and local white *Rachar* maize varieties are less susceptible to infection and mycotoxin contamination by *A. flavus* and *Fusarium* sp. compared to the improved white maize variety *WH505*, which accumulates higher levels of the mycotoxigenic fungi and their toxins, especially when treated with the synthetic insecticides *Super Blue Cross* and *Actellic® Super* that act as fungal nutrient sources under high moisture conditions.

Declarations

Acknowledgements

The work presented in this article was delivered through partial support from the University Innovation Fund (UIF) of Masinde Muliro University of Science and Technology (MMUST) under the CAMNFF project '*Development of Fungal Culture Media from Alternative Materials*'; the National Research Fund (NRF) of Kenya through the '*Aflatoxin*

Mitigation Project of the Mycotoxin Research Laboratory in Egerton University; and the East African Community (EAC), the Inter-University Council for East Africa (IUCEA) and German Development Bank-KfW (EAC KfW Scholarship) through Mbarara University of Science and Technology (MUST). We greatly appreciate the technical support that was offered by Samuel Mulama and Kelvin Kiprotich of CAMNFF and the Department of Biological Sciences (MMUST), and Peter Kipruto Rono of the Mycotoxin Research Laboratory (Egerton University).

Conflicts of Interest: None

References

1. Abate T, Fisher M, Abdoulaye T, Kassie GT, Lunduka R, Marennya P, Asnake W (2017) Characteristics of maize cultivars in Africa: How modern are they and how many do smallholder farmers grow? *Agric Food Secur* 6:30 (2017). <https://doi.org/10.1186/s40066-017-0108-6>
2. Abd El-Ghany TM, Masmali IA (2016) Fungal biodegradation of organophosphorus insecticides and their impact on soil microbial population. *J Plant Pathol Microbiol* 7(5):1000349. <https://doi.org/10.4172/2157-7471.1000349>
3. Abrehame S, Manoj VR, Hailu M, Chen Y-Y, Lin Y-C, Chen Y-P (2023) Aflatoxins: Source, detection, clinical features and prevention. *Processes* 11(1):204. <https://doi.org/10.3390/pr1101020>
4. Adeyeye SAO, Ashaolu TJ, Idowu-Adebayo F (2022) Mycotoxins: Food safety, consumer health and Africa's food security. *Polycycl Aromat Compd* 42(8):5779–5795. <https://doi.org/10.1080/10406638.2021.1957952>
5. Akbar F, Alam S (2019) Influence of water activity and time duration on fusarium mycotoxins production in maize grains during post-harvest storage. *Sarhad J Agric* 35(4):1326–1335. <http://dx.doi.org/10.17582/journal.sja/2019/35.4.1326.1335>
6. Akram S, Ahmed A, He P, He P, Liu Y, Wu Y, Munir S, He Y (2023) Uniting the role of endophytic fungi against plant pathogens and their interaction. *J Fungi* 9(1):72. <https://doi.org/10.3390/jof9010072>
7. Aleke M (2020) Prevention of aflatoxin contamination key for food and livestock feed safety. In: Kamau J (ed) *The Benchmark*. Kenya Bureau of Standards (KEBS), Nairobi, Kenya. <https://www.kebs.org/images/newsletters/Benchmark-Newsletter-2020.pdf>
8. Al-Gabr HM, Ye C, Zhang Y, Khan S, Lin H, Zheng T (2013) Effects of carbon, nitrogen and pH on the growth of *Aspergillus parasiticus* and aflatoxins production in water. *J Environ Biol* 34(2 Spec No):353–358 PMID: 24620605
9. Almekinders CJ, Hebinck P, Marinus W, Kiaka RD, Waswa WW (2021) Why farmers use so many different maize varieties in West Kenya. *Outlook Agric* 50(4):406–417. <https://doi.org/10.1177/00307270211054211>
10. Asamba M (2020) 17 maize flour brands banned over high aflatoxin levels. *The Standard Newspaper, Kenya* (retrived on January 22nd 2023). <https://www.standardmedia.co.ke/business/article/2001358551/17-maize-flour-brands-banned-over-high-aflatoxin-levels>
11. Atehnkeng J, Ojiambo PS, Ortega-Beltran A, Augusto J, Cotty PJ, Bandyopadhyay R (2022) Impact of frequency of application on the long-term efficacy of the biocontrol product Aflasafe in reducing aflatoxin contamination in maize. *Front Microbiol* 13:1049013. <https://doi.org/10.3389/fmicb.2022.1049013>
12. AUC (2015) Agenda 2063. *The Africa We Want*. Addis Ababa, Ethiopia. https://au.int/sites/default/files/documents/36204-doc-agenda2063_popular_version_en.pdf

13. Awuchi CG, Ondari EN, Nwozo S, Odongo GA, Eseoghene IJ, Twinomuhwezi H, Ogbonna CU, Upadhyay AK, Adeleye AO, Okpala COR (2022) Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: A review. *Toxins* 14(3):167. <https://doi.org/10.3390/toxins14030167>
14. Ayo EM, Matemu A, Laswai GH, Kimanya ME (2018) An *in vitro* evaluation of the capacity of local Tanzanian crude clay and ash-based materials in binding aflatoxins in solution. *Toxins* 10(12):510. <https://doi.org/10.3390/toxins10120510>
15. Azziz-Baumgartner E, Lindblade K, Giesecker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, Rubin C (2005) Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environ Health Perspect* 113(12):1779–1783. <https://doi.org/10.1289/ehp.8384>
16. Bang-Andreasen T, Nielsen JT, Voriskova J, Heise J, Rønn R, Kjølner R, Hansen HCB, Jacobsen CS (2017) Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. *Front Microbiol* 8:1400. <https://doi.org/10.3389/fmicb.2017.01400>
17. Baranyi N (2013) Current trends in aflatoxin research. *Acta Biologica Szegediensis* 57(2):95–107. <https://abs.bibl.u-szeged.hu/index.php/abs/article/view/2799>
18. Bhatnagar D, Cary JW, Ehrlich K, Yu J, Cleveland TE (2006) Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development. *Mycopathologia* 162(3):155–166. <https://doi.org/10.1007/s11046-006-0050-9>
19. Bhusal K, Khanal D (2019) Role of maize weevil, *Sitophilus zeamais* Motsch. on spread of *Aspergillus* section *flavi* in different Nepalese maize varieties. *Adv Agric* 2019:7584056. <https://doi.org/10.1155/2019/7584056>
20. Bose S, Kumar PS, Vo DVN, Rajamohan N, Saravanan R (2021) Microbial degradation of recalcitrant pesticides: a review. *Environ Chem Lett* 19(4):3209–3228. <https://doi.org/10.1007/s10311-021-01236-5>
21. Bozoğlu F (2011) Inactivation mechanisms of different mycotoxins. In: Hefnawy M (ed) *Advances in Food Protection*. NATO Science for Peace and Security Series A: Chemistry and Biology. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1100-6_11
22. Brzonkalik K, Hümmer D, Syltatk C et al (2012) Influence of pH and carbon to nitrogen ratio on mycotoxin production by *Alternaria alternata* in submerged cultivation. *AMB Expr* 2:28. <https://doi.org/10.1186/2191-0855-2-28>
23. Burgess KM, Renaud JB, McDowell T, Sumarah MW (2016) Mechanistic insight into the biosynthesis and detoxification of fumonisin mycotoxins. *ACS Chem Biol* 11(9):2618–2625. <https://doi.org/10.1021/acschembio.6b00438>
24. Chalivendra S, Huang F, Busman M, Williams WP, Ham JH (2020) Low aflatoxin levels in *Aspergillus flavus*-resistant maize are correlated with increased corn earworm damage and enhanced seed fumonisin. *Front Plant Sci* 11:565323. <https://doi.org/10.3389/fpls.2020.565323>
25. Chang PK (2022) *Aspergillus flavus* La3279, a component strain of the Aflasafe™ biocontrol product, contains a partial aflatoxin biosynthesis gene cluster followed by a genomic region highly variable among *A. flavus* isolates. *Int J Food Microbiol* 366:109559. <https://doi.org/10.1016/j.ijfoodmicro.2022.109559>
26. Chilaka CA, Obidiegwu JE, Chilaka AC, Atanda OO, Mally A (2022) Mycotoxin regulatory status in Africa: A decade of weak institutional efforts. *Toxins* 14(7):442. <https://doi.org/10.3390/toxins14070442>
27. Chivasa W, Worku M, Teklewold A, Setimela P, Gethi J, Magorokosho C, Davis NJ, Prasanna BM (2022) Maize varietal replacement in Eastern and Southern Africa: Bottlenecks, drivers and strategies for improvement. *Glob Food Sec* 32:100589. <https://doi.org/10.1016/j.gfs.2021.100589>

28. Cotty PJ (1994) Comparison of four media for the isolation of *Aspergillus flavus* group fungi. *Mycopathologia* 125:157–162. <https://doi.org/10.1007/BF01146521>
29. De Groote H, De Groote B, Bruce AY, Marangu C, Tefera T (2017) Maize storage insects (*Sitophilus zeamais* and *Prostephanus truncatus*) prefer to feed on smaller maize grains and grains with color, especially green. *J Stored Prod Res* 71:72–80. <https://doi.org/10.1016/j.jspr.2017.01.005>
30. Dharini V, Halder N, Selvam SP, Mikunthan G, Kumar MM (2022) Potential threat of mycotoxins to food safety and security. In: Mishra SK, Goyal MR, Gaare M (eds) *Biological and Chemical Hazards in Food and Food Products*. Apple Academic Press, New York, pp 3–26. <https://doi.org/10.1201/9781003189183>
31. Díaz-Gómez J, Marín S, Nogareda C, Sanchis V, Ramos AJ (2016) The effect of enhanced carotenoid content of transgenic maize grain on fungal colonization and mycotoxin content. *Mycotoxin Res* 32(4):221–228. <https://doi.org/10.1007/s12550-016-0254-x>
32. Dlamini SP, Akanmu AO, Fadiji AE, Babalola OO (2023) Maize rhizosphere modulates the microbiome diversity and community structure to enhance plant health. *Saudi J Biol Sci* 30(1):103499. <https://doi.org/10.1016/j.sjbs.2022.103499>
33. D'Mello FJP, Macdonald AM, Postel D, Dijksma WT, Dujardin A, Placinta CM (1998) Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. *Eur J Plant Pathol* 104:741–751. <https://doi.org/10.1023/A:1008621505708>
34. Dufossé L, Galaup P, Yaron A, Arad SM, Blanc P, Murthy KNC, Ravishankar GA (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends Food Sci Technol* 16(9):389–406. <https://doi.org/10.1016/j.tifs.2005.02.006>
35. Ekpakpale DO, Kraak B, Meijer M, Ayeni KI, Houbraken J, Ezekiel CN (2021) Fungal diversity and aflatoxins in maize and rice grains and cassava-based flour (Pupuru) from Ondo State, Nigeria. *J Fungi* 7(8):635. <https://doi.org/10.3390/jof7080635>
36. Ekwomadu T, Mwanza M, Musekiwa A (2022) Mycotoxin-linked mutations and cancer risk: A global health issue. *Int J Environ Res Public Health* 19(13):7754. <https://doi.org/10.3390/ijerph19137754>
37. Elsie NS, Meshack AO, Faith JT, Benard OO, Bonaventure OA (2016) Diversity of putatively toxigenic *Aspergillus* species in maize and soil samples in an aflatoxicosis hotspot in Eastern Kenya. *Afr J Microbiol Res* 10(6):172–184. <https://academicjournals.org/journal/AJMR/article-full-text-pdf/741DC9357109>
38. Fandohan P, Gnonlonfin B, Hell K, Marasas WFO, Wingfield MJ (2006) Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. *Afr J Biotechnol* 5(7):546–552. <https://www.ajol.info/index.php/ajb/article/view/137899>
39. Fujimoto H (2011) Yeasts and Molds | Mycotoxins: Classification, Occurrence and Determination. *Encyclopedia of Dairy Sciences* (Second Edition) pp. 792–800. <https://doi.org/10.1016/B0-12-227235-8/00350-3>
40. Gong YY, Watson S, Routledge MN (2016) Aflatoxin exposure and associated human health effects, a review of epidemiological studies. *Food Saf* 4(1):14–27. <https://doi.org/10.14252/foodsafetyfscj.2015026>
41. Haggblade S, Diarra A, Traoré A (2022) Regulating agricultural intensification: Lessons from West Africa's rapidly growing pesticide markets. *Dev Policy Rev* 40(1):12545. <https://doi.org/10.1111/dpr.12545>
42. Hakbiji T (2002) The traditional, historical and prehistoric use of ashes as an insecticide, with an experimental study on the insecticidal efficacy of washed ash. *Environ Archaeol* 7(1):13–22. <https://doi.org/10.1179/env.2002.7.1.13>

43. Hamad GM, Mehany T, Simal-Gandara J, Abou-Alella S, Esua OJ, Abdel-Wahhab MA, Hafez EE (2023) A review of recent innovative strategies for controlling mycotoxins in foods. *Food Control* 144:109350. <https://doi.org/10.1016/j.foodcont.2022.109350>
44. Hasnaki R, Ziaee M, Mahdavi V (2023) Pesticide residues in corn and soil of corn fields of Khuzestan, Iran, and potential health risk assessment. *J Food Compos Anal* 115:104972. <https://doi.org/10.1016/j.jfca.2022.104972>
45. Jensen IM, Whatling P (2010) Malathion: a review of toxicology. Hayes' Handbook of Pesticide Toxicology. In: Krieger R et al (eds) Hayes' Handbook of Pesticide Toxicology. Academic Press, Elsevier, London, UK, pp 1527–1542. <https://doi.org/10.1016/B978-0-12-374367-1.00071-9>
46. Hwang T, Ndolo VU, Katundu M, Nyirenda B, Bezner-Kerr R, Arntfield S, Beta T (2016) Provitamin A potential of landrace orange maize variety (*Zea mays* L.) grown in different geographical locations of central Malawi. *Food Chem* 196:1315–1324. <https://doi.org/10.1016/j.foodchem.2015.10.067>
47. ICSU A Guide to SDG Interactions: from Science to Implementation [D.J. Griggs M, Nilsson A, Stevance D, McCollum (eds)(2017)].International Council for Science (ICSU), Paris. <https://doi.org/10.24948/2017.01>
48. Ijadpanahsaravi M, Punt M, Wösten HA, Teertstra WR (2021) Minimal nutrient requirements for induction of germination of *Aspergillus niger* conidia. *Fungal Biol* 125(3):231–238. <https://doi.org/10.1016/j.funbio.2020.11.004>
49. Islam M-S, Callicott KA, Mutegi C, Bandyopadhyay R, Cotty PJ (2018) *Aspergillus flavus* resident in Kenya: High genetic diversity in an ancient population primarily shaped by clonal reproduction and mutation-driven evolution. *Fungal Ecol* 35:20–33. <https://doi.org/10.1016/j.funeco.2018.05.012>
50. Jaganathan GK, Jiajin L, Yashu Y, Baolin L (2018) Efficacy of Ash in Post-Harvest Seed Storage of Three Species of Cucurbitaceae. *J Plant Sci Res* 5(2):181
51. Jin F-J, Wang B-T, Wang Z-D, Jin L, Han P (2022) CRISPR/Cas9-based genome editing and its application in *Aspergillus* species. *J Fungi* 8(5):467. <https://doi.org/10.3390/jof8050467>
52. Kagot V, De Boevre M, Landschoot S, Obiero G, Okoth S, De Saeger S (2022) Comprehensive analysis of multiple mycotoxins and *Aspergillus flavus* metabolites in maize from Kenyan households. *Int J Food Microbiol* 363:109502. <https://doi.org/10.1016/j.ijfoodmicro.2021.109502>
53. Kean EG, Hamaker BR, Ferruzzi MG (2008) Carotenoid bioaccessibility from whole grain and degermed maize meal products. *J Agric Food Chem* 56(21):9918–9926. <https://doi.org/10.1021/jf8018613>
54. KEPHIS (2020) National Crop Variety List-Kenya <https://kephis.org/images/pdf-files/UPDATED%202020%20August%20NATIONAL%20VARIETY%20LIST1.pdf>
55. Khan N, Muge E, Mulaa FJ et al (2023) Mycelial nutrient transfer promotes bacterial co-metabolic organochlorine pesticide degradation in nutrient-deprived environments. *ISME J*. <https://doi.org/10.1038/s41396-023-01371-7>
56. Kilonzo R, International Food Policy Research Institute (IFPRI) (2019) Overview and Implementation of Aflatoxin Policy in Kenya. Catalyzing the Use of Aflatoxin Control Technologies in Kenya and Ghana. A Webinar Presentation, 26th November 2019. <https://www.slideshare.net/ifpri/overview-and-implementation-of-aflatoxin-policy-in-kenya>
57. Kmiecik W, Lisiewska Z, Jaworska G (2000) Content of ash components in the fresh and preserved broad bean (*Vicia faba* v *major*). *J Food Compos Anal* 13(6):905–914. <https://doi.org/10.1006/jfca.2000.0918>

58. Kolawole O, Meneely J, Petchkongkaew A, Elliott C (2021) A review of mycotoxin biosynthetic pathways: Associated genes and their expressions under the influence of climatic factors. *Fungal Biol Rev* 37:8–26. <https://doi.org/10.1016/j.fbr.2021.04.003>
59. Krishna VV, Lantican MA, Prasanna BM, Pixley K, Abdoulaye T, Menkir A, Bänziger M, Erenstein O (2023) Impact of CGIAR maize germplasm in Sub-Saharan Africa. *Field Crops Res* 290:108756. <https://doi.org/10.1016/j.fcr.2022.108756>
60. Kushiro M, Hatabayashi H, Yabe K, Loladze A (2018) Detection of aflatoxigenic and atoxigenic Mexican *Aspergillus* strains by the dichlorvos–ammonia (DV–AM) method. *Toxins* 10(7):263. <https://doi.org/10.3390/toxins10070263>
61. Lagat MK, Toroitich FJ, Obonyo MA (2020) Development of an ELISA-based method for testing aflatoxigenicity and aflatoxigenic variability among *Aspergillus* species in culture. *Sci Afr* 7:e00266. <https://doi.org/10.1016/j.sciaf.2020.e00266>
62. Lalah JO, Otieno PO, Odira Z, Ogunah JA (2022) Pesticides: Chemistry, manufacturing, regulation, usage and impacts on population in Kenya. *Intech Open Journal*. <https://doi.org/10.5772/intechopen.105826>
63. Lamboni Y, Hell K (2009) Propagation of mycotoxigenic fungi in maize stores by post-harvest insects. *Int J Trop Insect Sci* 29:31–39. <https://doi.org/10.1017/S1742758409391511>
64. Lamichhane JR, You MP, Laudinot V, Barbetti MJ, Aubertot JN (2020) Revisiting sustainability of fungicide seed treatments for field crops. *Plant Dis* 104(3):610–623. <https://doi.org/10.1094/PDIS-06-19-1157-FE>
65. LaPorte MF, Vachev M, Fenn M, Diepenbrock C (2022) Simultaneous dissection of grain carotenoid levels and kernel color in biparental maize populations with yellow-to-orange grain. *G3 (Bethesda)* 12(3):jkac006. <https://doi.org/10.1093/g3journal/jkac006>
66. Leslie JF, Summerell BA (2006) *The Fusarium Laboratory Manual*. John Wiley & Sons, New Jersey, NY. <https://doi.org/10.1002/9780470278376>
67. Liu Y, Yan H, Zhang X et al (2020) Investigating the endophytic bacterial diversity and community structures in seeds of genetically related maize (*Zea mays* L.) genotypes. *3 Biotech* 10:27. <https://doi.org/10.1007/s13205-019-2034-8>
68. Logrieco A, Battilani P, Leggieri MC, Jiang Y, Haesaert G, Lanubile A, Mahuku G, Mesterházy A, Ortega-Beltran A, Pasti M, Smeu I (2021) Perspectives on global mycotoxin issues and management from the MycoKey Maize Working Group. *Plant Dis* 105(3):525–537. <https://doi.org/10.1094/PDIS-06-20-1322-FE>
69. Manu N, Opit GP, Osekere EA, Arthur FH, Mbata G, Armstrong P, Danso JK, McNeill SG, Campbell JF (2019) Moisture content, insect pest infestation and mycotoxin levels of maize in markets in the northern region of Ghana. *J Stored Prod Res* 80:10–20. <https://doi.org/10.1016/j.jspr.2018.10.007>
70. Masiello M, Somma S, Ghionna V, Logrieco AF, Moretti A (2019) *In vitro* and in field response of different fungicides against *Aspergillus flavus* and *Fusarium* species causing Ear Rot Disease of Maize. *Toxins* 11(1):11. <https://doi.org/10.3390/toxins11010011>
71. Massomo SM (2020) *Aspergillus flavus* and aflatoxin contamination in the maize value chain and what needs to be done. *Tanzan Sci Afr* 10:e00606. <https://doi.org/10.1016/j.sciaf.2020.e00606>
72. Mathew E, Mathew L (2023) Conservation of landraces and indigenous breeds: An investment for the future. In: Sukumaran ST, Keerthi TR (eds) *Conservation and Sustainable Utilization of Bioresources*. Sustainable Development and Biodiversity. Springer, Singapore. https://doi.org/10.1007/978-981-19-5841-0_12

73. Meneely JP, Kolawole O, Haughey SA, Miller SJ, Krska R, Elliott CT (2022) The challenge of global aflatoxins legislation with a focus on peanuts and peanut products: A systematic review. *Expo Health*. <https://doi.org/10.1007/s12403-022-00499-9>
74. Menezes BS, Solidade LS, Conceição AA, Santos MN, Leal PL, de Brito ES, Canuto KM, Mendonça S, de Siqueira FG, Marques LM (2020) Pigment production by *Fusarium solani* BRM054066 and determination of antioxidant and anti-inflammatory properties. *AMB Express* 10(1):1–12. <https://doi.org/10.1186/s13568-020-01054-y>
75. Merchant EV, Odendo M, Ndinya C, Nyabinda N, Maiyo N, Downs S, Hoffman DJ, Simon JE (2022) Barriers and facilitators in preparation and consumption of African Indigenous Vegetables: A qualitative exploration from Kenya. *Front Sustain Food Syst* 6:801527. <https://doi.org/10.3389/fsufs.2022.801527>
76. Miller JD (2023) Mycotoxins: Still with us after all these years. In: Knowles ME, Anelich LE, Boobis AR, Popping B (eds) *Present Knowledge in Food Safety. A Risk-Based Approach through the Food Chain*. pp. 62–78. Academic Press. <https://doi.org/10.1016/B978-0-12-819470-6.00009-3>
77. Muimba-Kankolongo A (2018) Cereal production (Chap. 8). In: *Food Crop Production by Smallholder Farmers in Southern Africa. Challenges and Opportunities for Improvement*. Academic Press. <https://doi.org/10.1016/B978-0-12-814383-4.00008-6>
78. Müllenborn C, Steiner U, Ludwig M, Oerke E-C (2008) Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. *Eur J Plant Pathol* 120:157–166. <https://doi.org/10.1007/s10658-007-9204-y>
79. Munkvold GP, Proctor RH, Moretti A (2021) Mycotoxin production in *Fusarium* according to contemporary species concepts. *Annu Rev Phytopathol* 59:373–402. <https://doi.org/10.1146/annurev-phyto-020620-102825>
80. Mutiga SK, Hoffmann V, Harvey JW, Milgroom MG, Nelson RJ (2015) Assessment of aflatoxin and fumonisin contamination of maize in western Kenya. *Phytopathology* 105(9):1250–1261. <https://doi.org/10.1094/PHYTO-10-14-0269-R>
81. Mutiga SK, Mushongi AA, Kangéthe EK (2019) Enhancing food safety through adoption of long-term technical advisory, financial, and storage support services in maize growing areas of East Africa. *Sustainability* 11(10):2827. <https://doi.org/10.3390/su11102827>
82. Nagarajan L, Naseem A, Pray C (2019) Contribution of policy change on maize varietal development and yields in Kenya. *J Agribusiness Dev Emerg Econ* 9(1):4–21. <https://doi.org/10.1108/JADEE-01-2018-0013>
83. Ng PJ, Fleet GH, Heard GM (2005) Pesticides as a source of microbial contamination of salad vegetables. *Int J Food Microbiol* 101(2):237–250. <https://doi.org/10.1016/j.ijfoodmicro.2004.11.009>
84. Ngwenyama P, Mvumi BM, Stathers TE, Nyanga LK, Siziba S (2022) How different hermetic bag brands and maize varieties affect grain damage and loss during smallholder farmer storage. *Crop Prot* 153:105861. <https://doi.org/10.1016/j.cropro.2021.105861>
85. Njeru NK, Midega CAO, Muthomi JW, Wagacha JM, Khan ZR (2018) Population of *Aspergillus flavus* and *Fusarium verticillioides* and contamination of maize with associated mycotoxins under push-pull cropping system in Siaya County, Western Kenya. *RUFORUM Working Document Series* (ISSN 17(1):1607–9345
86. Nji QN, Babalola OO, Ekwomadu TI, Nleya N, Mwanza M (2022) Six main contributing factors to high levels of mycotoxin contamination in African foods. *Toxins*. 2022; 14(5):318. <https://doi.org/10.3390/toxins14050318>
87. Nthakanio NP, Wakhungu J (2017) Emergence of Bio/Agro-Terrorism in Kenya. *Annu Res Rev Biol* 15(6):1–12. <https://doi.org/10.9734/ARRB/2017/34167>

88. Nwadike C, Okere A, Nwosu D, Okoye C, Vange T, Apuyor B (2018) Proximate and nutrient composition of some common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp.) accessions of Jos-Plateau, Nigeria. *J Agr Ecol Res Int* 15(1):1–9. <https://doi.org/10.9734/JAERI/2018/42138>
89. Obonyo MA, Salano EN (2018) Perennial and seasonal contamination of maize by aflatoxins in eastern Kenya. *Int J Food Contam* 5(1):1–5. <https://doi.org/10.1186/s40550-018-0069-y>
90. Ochieno DM (2020) Towards consensus on the transfer of *Fusarium oxysporum* V5w2-enhanced tissue culture banana technology to farmers through public-private partnerships in East Africa. *Sci Afr* 10:e00605. <https://doi.org/10.1016/j.sciaf.2020.e00605>
91. Ochilo WN, Nyamasyo GN, Kilalo D, Otieno W, Otipa M, Chege F, Karanja T, Lingeera EK (2019) Characteristics and production constraints of smallholder tomato production in Kenya. *Sci Afr* 2:e00014. <https://doi.org/10.1016/j.sciaf.2018.e00014>
92. Odukoya JO, De Saeger S, De Boevre M, Adegoke GO, Audenaert K, Croubels S, Antonissen G, Vermeulen K, Gbashi S, Njobeh PB (2021) Effect of selected cooking ingredients for nixtamalization on the reduction of *Fusarium* mycotoxins in maize and sorghum. *Toxins* 13(1):27. <https://doi.org/10.3390/toxins13010027>
93. Okolo JC, Adejumo BA, Amedu JE (2023) Genetically modified crops in Africa: Perspectives and safety concerns. In: Babalola OO, Ayangbenro AS, Ojuederie OB (eds) *Food Security and Safety*, vol 2. Springer, Cham. https://doi.org/10.1007/978-3-031-09614-3_5
94. Omar S (1998) Availability of phosphorus and sulfur of insecticide origin by fungi. *Biodegradation* 9:327–336. <https://doi.org/10.1023/A:1008310909262>
95. Omara T, Kiprop AK, Wangila P, Wacoo AP, Kagoya S, Nteziyaremye P, Odero MP, Nakiguli CK, Obakiro SB (2021) The scourge of aflatoxins in Kenya: A 60-year review (1960 to 2020). *J Food Qual* 2021:8899839. <https://doi.org/10.1155/2021/8899839>
96. Ortega-Beltran A, Jaime R, Cotty PJ (2022) Resistance of maize landraces from Mexico to aflatoxin contamination: influence of aflatoxin-producing fungi genotype and length of incubation. *Eur J Plant Pathol* 162:237–246. <https://doi.org/10.1007/s10658-021-02385-7>
97. Ortiz R, Taba S, Tovar VHC, Mezzalama M, Xu Y, Yan J, Crouch JH (2010) Conserving and enhancing maize genetic resources as global public goods—a perspective from CIMMYT. *Crop Sci* 50(1):13–28. <https://doi.org/10.2135/cropsci2009.06.0297>
98. Ostry V, Malir F, Toman J, Grosse Y (2017) Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res* 33(1):65–73. <https://doi.org/10.1007/s12550-016-0265-7>
99. Othieno RO (2018) EAC Policy Briefs on Aflatoxin Prevention and Control launched in Nairobi. East African Community Headquarters, Arusha, Tanzania. <https://www.eac.int/documents/category/aflatoxin-prevention-and-control>
100. Owuor MJ, Midega CAO, Obonyo M, Khan ZR (2017) Distribution of *Aspergillus* and *Fusarium* ear rot causative fungi in soils under push-pull and maize monocropping system in Western Kenya. *Afr J Microbiol Res* 11(37):1411–1421. <https://doi.org/10.5897/AJMR2017.8685>
101. PCPB (2018a) Pest Control Products Registered for Use in Kenya. Pest Control Products Board (PCPB), Nairobi, Kenya. https://pcpb.go.ke/listofregproducts/List%20of%20Registered%20Products%20%20Version%201_2018.pdf
102. PCPB (2018b) Pest Control Products (Labelling, Advertising and Packaging) Regulations. Pest Control Products Board (PCPB), Nairobi, Kenya. <https://www.pcpb.go.ke/wp-content/uploads/2020/03/Draft-labelling->

103. PCPB (2022) Pest Control Products (Registration) Regulations, 2022. Pest Control Products Board (PCPB), Nairobi, Kenya. <https://www.pcpb.go.ke/wp-content/uploads/2022/04/REGISTRATION-REGULATIONS.pdf>
104. Porto ALM, Melgar GZ, Kasemodel MC, Nitschke M (2011) Biodegradation of pesticides. In: Stoytcheva M (ed) Pesticides in the Modern World–Pesticides Use and Management. IntechOpen. <https://doi.org/10.5772/17686>
105. Probst C, Callicott KA, Cotty PJ (2012) Deadly strains of Kenyan *Aspergillus* are distinct from other aflatoxin producers. Eur J Plant Pathol 132:419–429. <https://doi.org/10.1007/s10658-011-9887-y>
106. Rajarajan P, Sylvia K, Periasamy MP, Subramanian M (2021) Detection of aflatoxin producing *Aspergillus flavus* from animal feed in Karnataka, India. Environ Anal Health Toxicol 36(3):e2021017–e2021010. <https://doi.org/10.5620/eaht.2021017>
107. Rock JS, Schnurr MA, Kingiri A, Glover D, Stone GD, Ely A, Fischer K (2023) Beyond the Genome: Genetically modified crops in Africa and the implications for Genome Editing. Dev Change 54(1):117–142. <https://doi.org/10.1111/dech.12750>
108. SAS (2004) SAS/STAT ® 9.1 User's Guide. Cary, NC: SAS Institute Inc. https://support.sas.com/documentation/onlinedoc/91pdf/sasdoc_91/stat_ug_7313.pdf
109. Satish GP, Ashokrao DM, Arun SK (2017) Microbial degradation of pesticide: a review. Afr J Microbiol Res 11(24):992–1012. <https://doi.org/10.5897/AJMR2016.8402>
110. Selmi S, Rtibi K, Grami D, Sebai H, Marzouki L (2018) Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicol Rep 5:189–195. <https://doi.org/10.1016/j.toxrep.2017.12.021>
111. Shekhar M, Singh N, Dutta R, Kumar S, Mahajan V (2017) Comparative study of qualitative and quantitative methods to determine toxicity level of *Aspergillus flavus* isolates in maize. PLoS ONE 12(12):e0189760. <https://doi.org/10.1371/journal.pone.0189760>
112. Sigma-Aldrich (2018a) Total Aflatoxin ELISA Kit for grains, cereals, nuts, and animal feed. Technical Bulletin, Catalog Number SE120006. Sigma-Aldrich Co. LLC., US. <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/199/900/se120006bul.pdf>
113. Sigma-Aldrich (2018b) Fumonisin ELISA Kit for maize (corn). Technical Bulletin, Catalog Number SE120010. Sigma-Aldrich Co. LLC., US. <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/225/375/se120010bul.pdf>
114. Singh S, Khajuria R (2018) Regulation by metal ions. In: New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 151–164). In: Gupta VK, Anita Pandey A (eds) New and Future Developments in Microbial Biotechnology and Bioengineering. Microbial Secondary Metabolites Biochemistry and Applications. Elsevier. <https://doi.org/10.1016/C2014-0-00306-1>
115. Soumya K, Murthy NK, Sreelatha GL, Tirumale S (2018) Characterization of a red pigment from *Fusarium chlamydosporum* exhibiting selective cytotoxicity against human breast cancer MCF-7 cell lines. J Appl Microbiol 125(1):148–158. <https://doi.org/10.1111/jam.13756>
116. Srinivasulu M, Ortiz DR (2017) Effect of pesticides on bacterial and fungal populations in Ecuadorian tomato cultivated soils. Environ Process 4(1):93–105. <https://doi.org/10.1007/s40710-017-0212-4>
117. Udomkun P, Wiredu AN, Nagle M, Bandyopadhyay R, Müller J, 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>

118. UNODA (1972) Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. The Biological Weapons Convention. United Nations Office of Disarmament Affairs (UNODA), New York, USA. <https://treaties.unoda.org/t/bwc>
119. Venkataramana M, Chandranayaka S, Prakash HS, Niranjana SR (2015) Mycotoxins relevant to biowarfare and their detection. In: Gopalakrishnakone P, Balali-Mood M, Llewellyn L, Singh BR (eds) Biological Toxins and Bioterrorism. Toxinology. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-5869-8_32
120. Wagacha JM, Njeru NK, Okumu OO, Muthomi JW, Mutegei CK (2016) Occurrence of *Fusarium* head blight of wheat and associated mycotoxins in Narok and Nakuru Counties, Kenya. World J Agric Res 4(4):119–127. <https://hdl.handle.net/10568/77778>
121. Walker GM, White NA (2018) Introduction to fungal physiology (pp. 1–35). In: Kavanagh K. (ed) Fungi: Biology and Applications. John Wiley & Sons, Inc. <https://doi.org/10.1002/9781119374312.ch1>
122. Williams WP (2006) Breeding for resistance to aflatoxin accumulation in maize. Mycotox Res 22:27–32. <https://doi.org/10.1007/BF02954554>
123. Xiang F, Zhao Q, Zhao K, Pei H, Tao F (2020) The efficacy of composite essential oils against aflatoxigenic fungus *Aspergillus flavus* in maize. Toxins 12(9):562. <https://doi.org/10.3390/toxins12090562>
124. Xu F, Baker RC, Whitaker TB, Luo H, Zhao Y, Stevenson A, Boesch CJ, Zhang G (2022) Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination. World Mycotoxin J 15(2):171–186. <https://doi.org/10.3920/WMJ2021.2685>
125. Yahia EM, de Jesús Ornelas-Paz J, Bridgemohan P, Vergara S (2020) Food losses during production of agricultural commodities. In: Yahia E (ed) Preventing Food Losses and Waste to Achieve Food Security and Sustainability. Burleigh Dodds Science Publishing, London, UK, pp 249–286. <https://doi.org/10.1201/9780429266621>
126. Zingales V, Taroncher M, Martino PA, Ruiz M-J, Caloni F (2022) Climate change and effects on molds and mycotoxins. Toxins 14(7):445. <https://doi.org/10.3390/toxins14070445>

Tables

Tables 1 to 6 are available in the Supplementary Files section

Figures



Figure 1

Symptoms of maize cob infection by mycotoxigenic fungi, **(a)** *Aspergillus* species characterized by olive-green powdery mould that produces aflatoxins (Baranyi 2013); and **(b)** *Fusarium* ear rot that is associated with the accumulation of fumonisins in maize (Muimba-Kankolongo 2018).

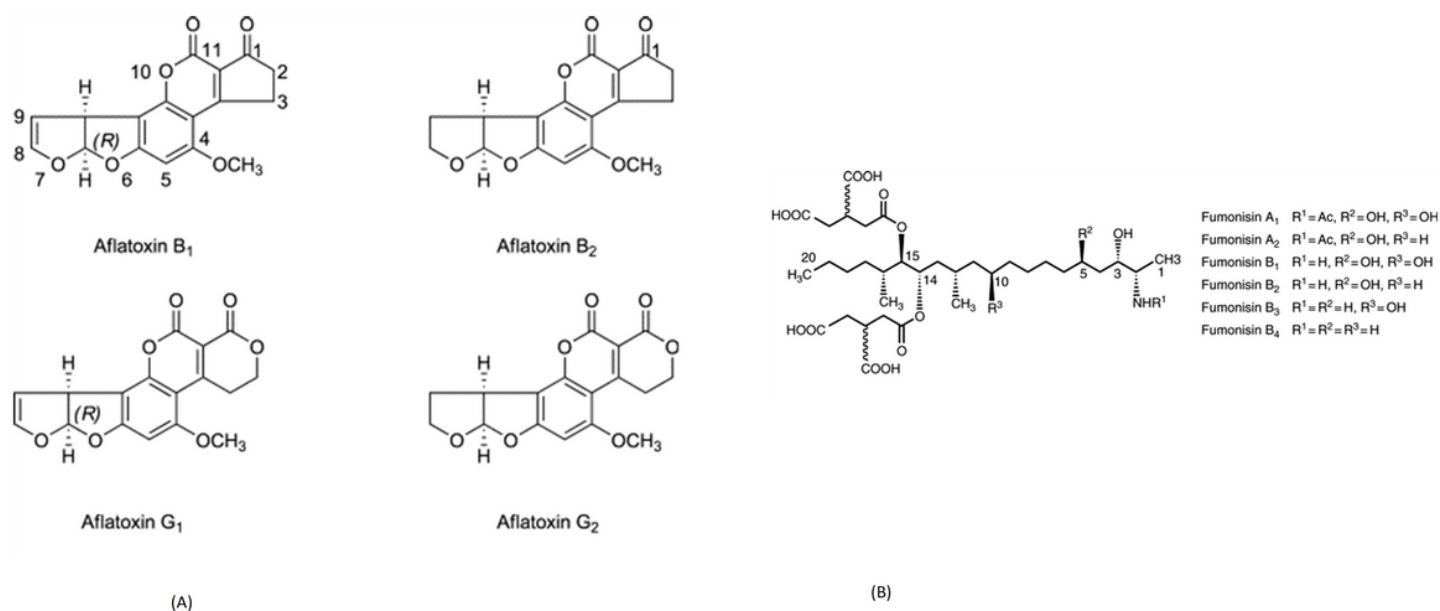


Figure 2

Molecular structures of Mycotoxins, **(a)** Aflatoxin B₁, B₂, G₁ and G₂ produced by mycotoxigenic strains of *Aspergillus*; and **(b)** Fumonisin A₁, A₂, B₁, B₂, B₃ and B₄ produced by mycotoxigenic strains of *Fusarium* (Fujimoto 2011).

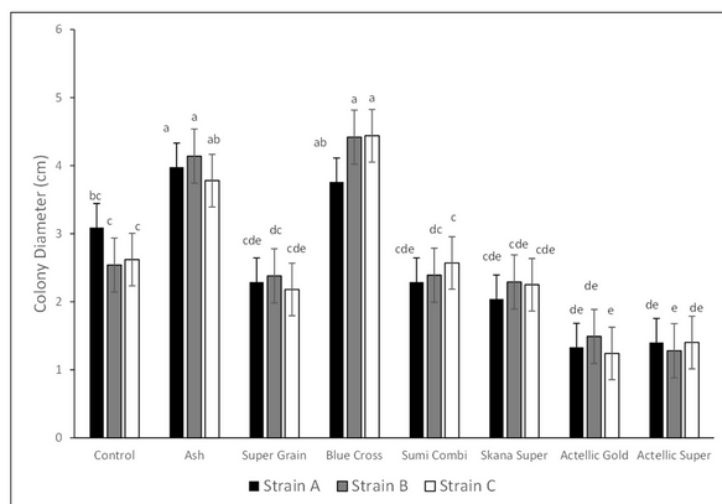


Figure3A

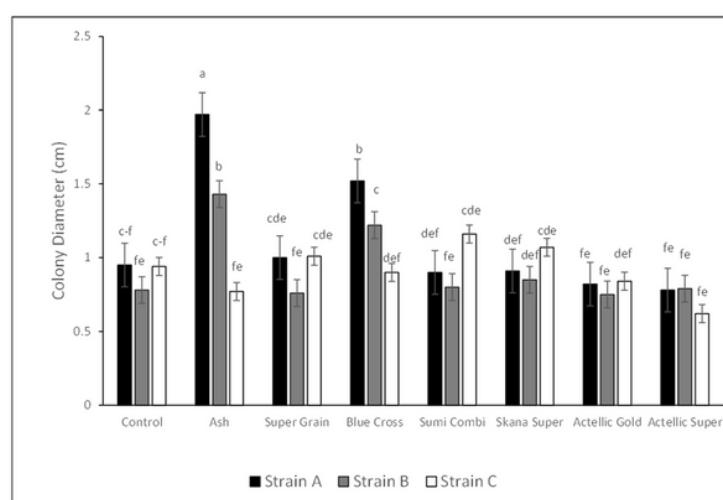


Figure3B

Figure 3

Colony diameters (mean $\pm$ stderr cm) of Mycotoxigenic fungal strains as influenced by insecticidal compounds in Potato Dextrose Agar; **(a)** *Aspergillus flavus* Strain A (accession number 2EM0401) and Strain B (accession number 2EM062) are aflatoxigenic while Strain C (accession number 1E09602) is non-aflatoxigenic; **(b)** *Fusarium* Strain A (accession number MM2) and Strain B (accession number CM2) are fumonisin-producing while Strain C (accession number DD6) is non-fumonisin producing. These fungal strains were obtained from the Mycotoxin Research Laboratory at Egerton University, Kenya. Means having the same letter within a bar are not significantly different ($p>0.05$).

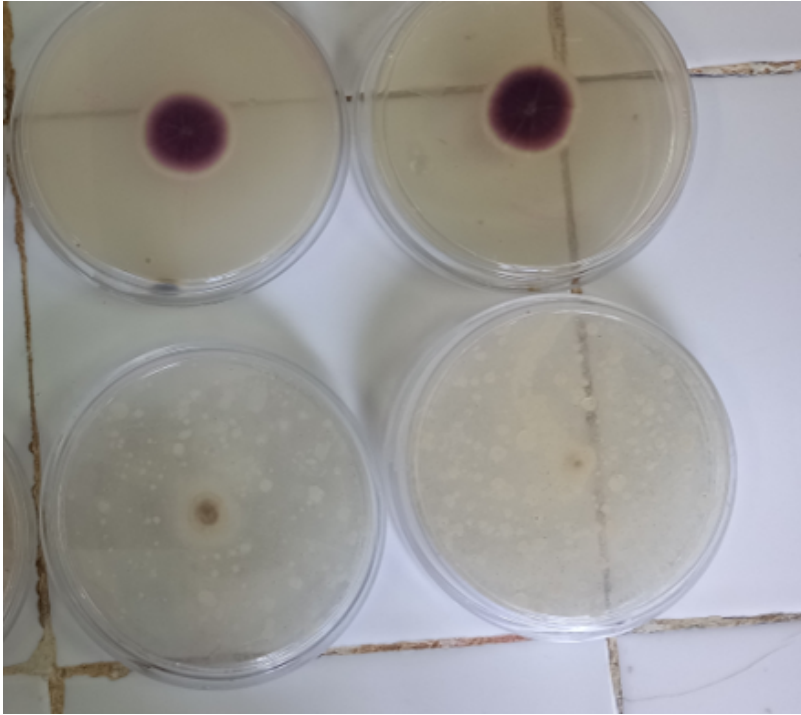


Figure 4

Aspergillus flavus colony colour change when exposed to fumes of ammonium solution (NH_4OH). The upper two Petri dishes contained PDA enriched with *Super Blue Cross* insecticide, while the two lower two Petri dishes contained PDA enriched with *Actellic[®] Super* insecticide revealing the presence of cream-coloured colonies of microbial contaminants.

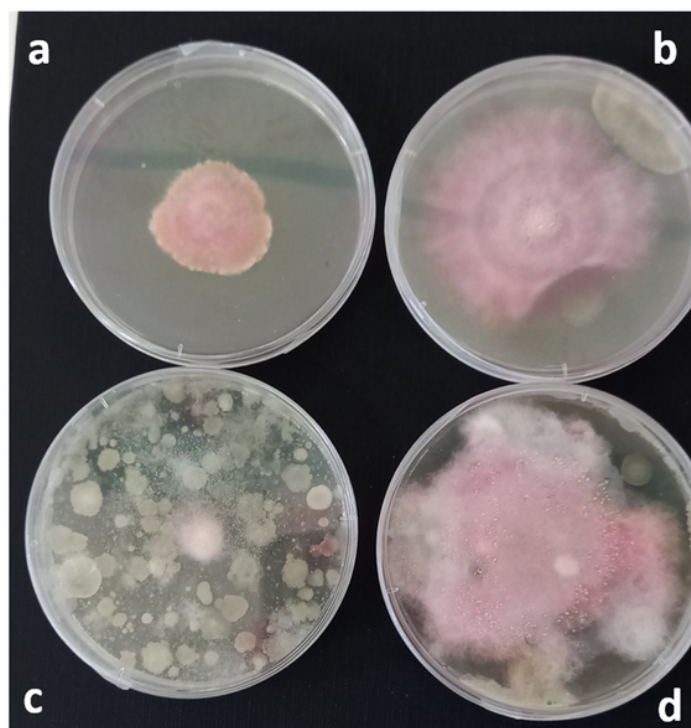


Figure5A

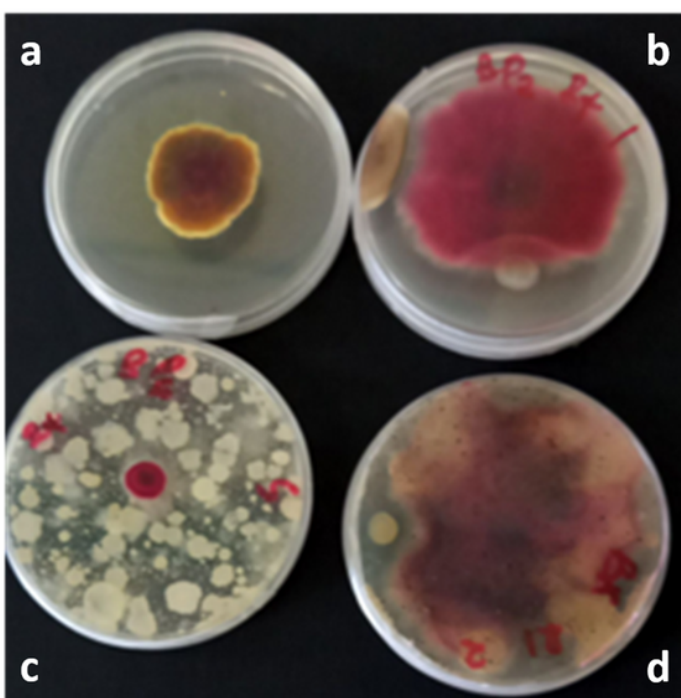


Figure5B

Figure 5

Top view **(A)** and Bottom view **(B)** of *Fusarium* colony colour change in Petri dishes containing PDA without any additive **(a)**, or PDA enriched with *Super Blue Cross* insecticide **(b)**, *Actellic® Super* insecticide treatment revealing the presence of pale-coloured colonies of microbial contaminants **(c)**, and *Ash* derived from common bean *Phaseolus vulgaris* **(d)**.

Supplementary Files

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